Molecular mechanism for oscillation in flagella and muscle

(cross-bridges/computer simulation)

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ABSTRACT   Two-state models for muscle cross-bridges, of the type originally detailed by Andrew Huxley, were examined. Rate functions for cross-bridge attachment and detachment can be chosen which yield a steady-state force-velocity relationship appropriate for the spontaneous generation of oscillatory contractions. Computer simulations have been used to demonstrate oscillation of such cross-bridge systems, and to demonstrate that distribution of this type of local shear oscillation along the length of a flagellum is sufficient for the generation of propagated bending waves.

Many biological movement rhythms are generated by controlling elements such as the nervous system, for many locomotory and respiratory rhythms in animals, or the cell membrane, for the rhythmic beating of vertebrate heart muscle. Here, I am concerned with cases where oscillation results from intrinsic properties of the molecular mechanisms for generating movement, when coupled to an appropriate load. An intrinsic origin of oscillatory contraction has been demonstrated in insect fibrillar flight muscle, by observation that the contractions are not synchronized with nerve or muscle cell membrane excitation (1) and that glycerol-extracted muscles supplied with ATP oscillate when coupled to an appropriate load (2). The oscillatory capability of insect fibrillar muscle has been ascribed to a delayed stretch-activation of actin-myosin interaction (1, 3, 4), but the molecular mechanisms for such activation have not been detailed. Evidence for similar properties has been found in several other striated muscles (5–7).

An intrinsic origin of oscillatory bending and bending wave propagation has been demonstrated in flagella, by the observation that isolated, glycerol-demembranated, flagella supplied with ATP generate all the components of normal movement—bending, oscillation, and coordinated propagation of bending waves (8, 9). Distribution of oscillatory capability throughout the length of a flagellum has been demonstrated by observation of localized oscillatory bending in short regions locally activated with ATP (10). A possible similarity between the oscillatory mechanisms of flagella and insect fibrillar muscle was suggested at an early stage in the investigation of fibrillar muscle (11). Machin (12, 13) explored this possibility in quantitative terms, with an analysis of wave propagation by a flagellar model in which bending was generated by stretch-activated contractile elements on either side of the flagellum. Machin's model contains a feedback loop, with active contraction causing bending of the flagellum and stretch of the antagonistic contractile elements, coupled to stretch-activation of active contraction. If stretch-activation includes a time delay, spontaneous oscillations should result.

There is now firm evidence (14, 15) that flagellar bending is generated not by an active contraction mechanism, but by an active sliding process involving interaction between adjacent elements in the ring of nine microtubular doublets which is the dominant structural feature of the flagellum. Active sliding is believed to be brought about by the activity of the flagellar ATPase, dynein, which is located in "arms" or "bridges" between the microtubular doublets (16). An extension of Machin's ideas to a sliding microtubule mechanism for generating flagellar bending indicated that oscillation and coordinated bend propagation should result from control of active sliding by bending (17). A feedback loop involving proportional control of active sliding by the curvature of the flagellum, and bending as a result of differences in the amount of active sliding at different positions along the length, automatically contains a phase shift, so that no time delay is required for oscillatory behavior (17). A computer simulation procedure developed to test this control mechanism demonstrated that realistic-appearing bending waves could be generated from a very minimal set of assumptions about internal flagellar mechanisms (18). This computer simulation procedure has recently been used to examine models in which the curvature of the flagellum controls the rate of attachment of cross-bridges which generate active sliding (19). These models show similar oscillation and wave propagation, but have not turned out to provide a more successful interpretation of experimental results.

The inclusion of the curvature of the flagellum as a control variable in a feedback loop responsible for flagellar oscillation is not based on any direct experimental evidence. Observation of oscillation in very short activated regions (10) has stimulated a search for oscillatory properties of an active sliding system which do not depend on a feedback control by bending. I will show that such properties can arise naturally in simple cross-bridge models for generating active sliding, of the type first detailed by Andrew Huxley (20).

In these models, cross-bridges based at intervals along one type of filament (the myosin or A-band filament of muscle; the A-microtubule of flagella) form transitory attachments to sites located at intervals along a parallel filament (the actin or I-band filament of muscle; the B-microtubule of flagella). In muscle, the cross-bridges correspond to the heavy-meromyosin portion of the myosin molecule, which is known to contain the ATPase and actin-binding sites. Much less is known about the structure of flagellar cross-bridges, but there is evidence that they contain much of the flagellar ATPase activity (16). Excellent reviews of the evidence for, and the current status of, cross-bridge models for muscular contraction have recently appeared (21, 22). I will consider here only "two-state" models, in which there is one unattached state, in which a cross-bridge exerts no force, and one attached state, in which a cross-bridge exerts a force F along the direction of the filaments. The force F is a function *only* of the position of the base of the cross-bridge relative to the

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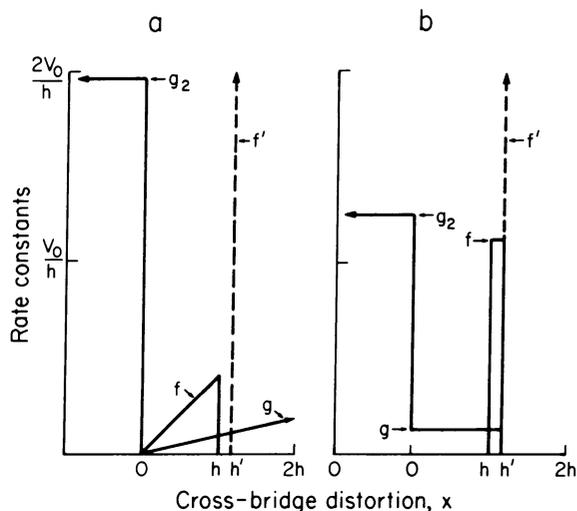*Proc. Nat. Acad. Sci. USA* 72 (1975)   3103

FIG. 1. Rate functions for cross-bridge models. The attachment rate (f) and detachment rate (g) functions shown by solid lines in a are those used by Huxley (20). The dashed line (f′) indicates rapid breakage of cross-bridges by reversal of the attachment reaction, when the cross-bridge distortion is greater than h′.

position of its current site of attachment. It is convenient to measure these relative positions by a variable, x, such that $x=0$ when $F=0$, and to refer to x as the "distortion" of the cross-bridge (22). For the present discussion, it is sufficient to assume that $F(x)$ is a simple linear function containing a force constant, k:

$$F(x) = kx \qquad [1]$$

This appears to be a reasonably good approximation for skeletal muscle (21). In order to obtain a net force from an ensemble of cross-bridges, the rates of attachment and detachment of cross-bridges, which will be functions of x, must be specified in a way which will produce a biased distribution of distortions of attached cross-bridges. Huxley (20) proposed a simple specification of rate functions, illustrated in Fig. 1a, which generated a model with properties closely matching experimental measurements of the steady-state behavior of frog skeletal muscle. The force-velocity behavior calculated for Huxley's model is illustrated by curve A in Fig. 2.

Huxley noted that the calculated force-velocity behavior agreed well with experimental observations for shortening, and for stretching at low velocities, but not for stretching at higher velocities. His model predicted that with increasing velocities of stretch, the force would increase to a maximum value equal to 5.33 P_0, whereas a maximal force of between 1.7 and 2.0 P_0 was measured experimentally (23). His model also predicted a high cross-bridge cycle rate during stretch, which would correspond to a (heat + work) output much greater than observed experimentally. Huxley suggested that more realistic behavior during stretch might be obtained from a model in which cross-bridges were broken during stretch by a reversal of the attachment reaction, rather than by the normal detachment reaction involving dephosphorylation of ATP (20).

A rigorous theoretical framework for discussion of cross-bridge models has now been developed by Terrell Hill (24, 25). For a two-state cross-bridge model which is able to generate work from a coupled dephosphorylation of ATP, a rigorous analysis must deal with a minimum of *four* rate functions, as identified in the diagram in Fig. 3. If $F(x)$, $f(x)$, and
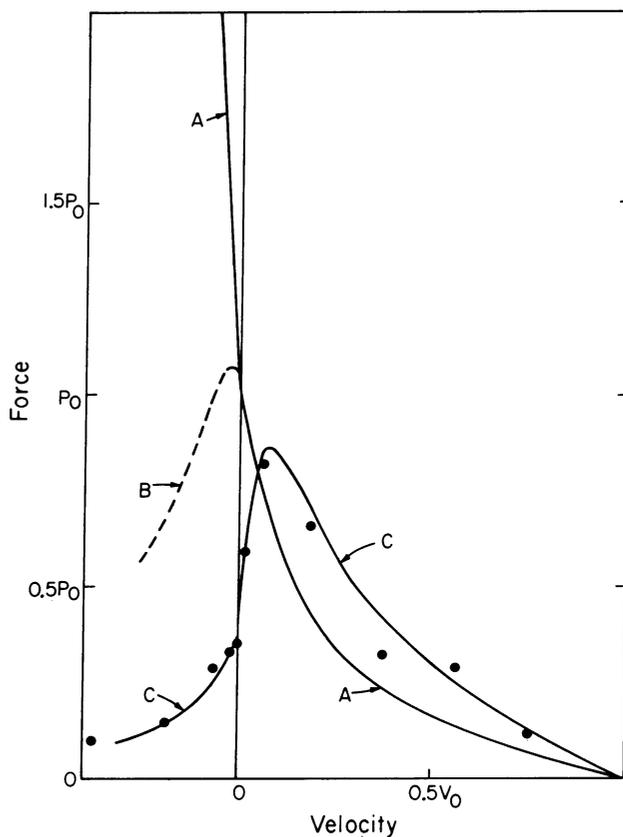

FIG. 2. Force-velocity behavior of cross-bridge models. Curve A is the behavior of the original model of Huxley (20). Curve B is the behavior of the same model, after inserting the reverse attachment rate (f′) shown in Fig. 1a. Curve C is the behavior of the model with rate functions shown in Fig. 1b.

$g(x)$ are specified, as in Huxley's formulation, the forms of $f'(x)$ and $g'(x)$ are determined, and the magnitudes of these two reverse rate functions are determined by the free energy available from the coupled dephosphorylation of an ATP molecule and the relative energy levels (at $x=0$) of the attached and unattached states of a cross-bridge. Of particular interest is the reversal of cross-bridge attachment, governed by $f'(x)$. Hill's analysis demonstrates that outside of a limited range of x, the ratio $f'(x)/f(x)$ will rapidly become much larger than 1, so that no significant fraction of attached cross-bridges could exist at equilibrium. The critical value of x is likely to be close to the value which will make the potential energy of cross-bridge distortion $(kx^2/2)$ equal to the energy available from dephosphorylation of an ATP molecule. With the specification used by Huxley, this critical value of x will be greater than h, and therefore in a region where $f(x)$ is assumed to be 0. This will make $f'(x)$ also equal to 0,
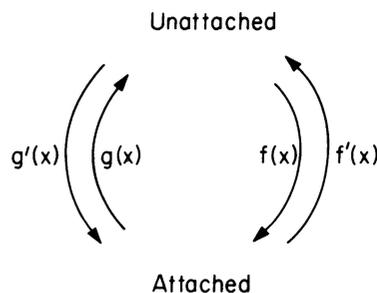
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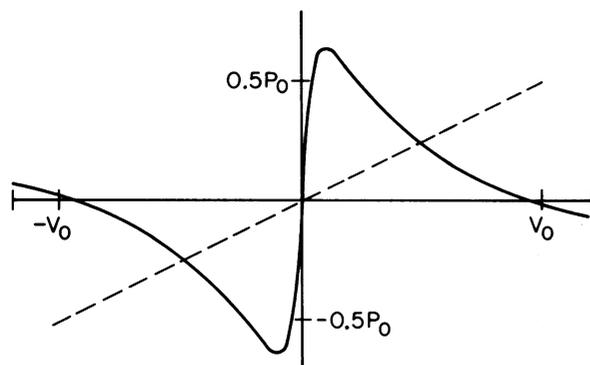FIG. 3. Rate functions required for a self-consistent two-state cross-bridge model (24, 25).

FIG. 4. Force-velocity behavior of an antagonistic pair of cross-bridge models with properties shown in Fig. 1b and curve C of Fig. 2. P_0 and V_0 are the same as in Fig. 2.
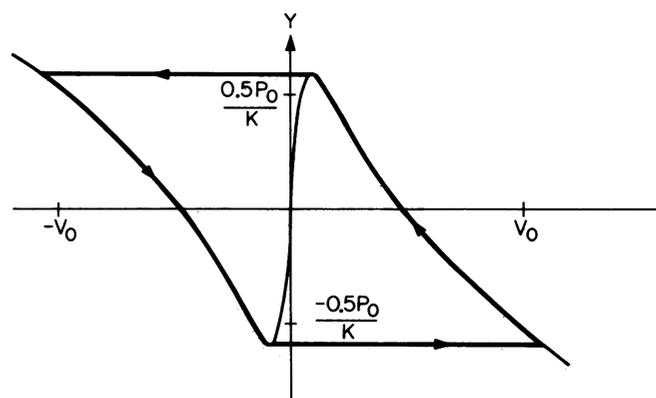

FIG. 5. Displacement-velocity plot for the pair of models described by Fig. 4, when combined with a viscous load (represented by the dashed line in Fig. 4, with a slope of 0.5 P_0/V_0) and an elastic restoring force = $-Ky$. The system will oscillate in the stable limit cycle represented by the trajectory shown by the heavy line with arrows.

but this is a somewhat artificial conclusion, resulting from the arbitrary form chosen for $f(x)$. It would seem to be appropriate to consider the behavior of the model in a case where $f'(x)$ becomes large for $x > h'$. An appropriate choice for h' might be $h' = h(4/3)^{1/2}$, since this is the value of x which makes the energy of cross-bridge distortion equal to the energy supplied by 1 ATP, according to Huxley's specifications (20). This $f'(x)$ function is represented by the vertical dashed line in Fig. 1a. Its effect on the force-velocity relationship during stretch can be obtained from Huxley's analysis by terminating the force integration at $x = h'$, and is shown by the dashed curve (curve B) in Fig. 2. The real behavior of muscle lies between the two extremes represented by curves A and B in Fig. 2 (23, 26). The force measured during stretch at a given velocity is always greater than the force measured during shortening at the same velocity (26); curves A and B both have this property. This is an important property for the stable operation of striated muscle (27). If the active sliding system could generate more force during shortening than during stretching, it would be possible for adjacent sarcomeres in an activated muscle to contract at each other's expense, leading to a very unstable situation.

This line of thought suggests that a system for generating oscillatory movement might be obtained by a further modification of cross-bridge properties in the same direction, so that the system would produce a smaller force during stretching than during shortening. A specification of rate functions which will lead to this type of behavior is shown in Fig. 1b. The same type of analysis used by Huxley (20) can be applied to these rate functions to obtain the steady-state behavior of a cross-bridge ensemble. During stretch at a velocity $-V$, the average force per cross-bridge is

$$P(-V) = (kh^2 f/(f + g)\alpha^2 d)\{\alpha^2(\rho^2 - 1)/2 -$$
$$(1 - \alpha) + (1 - \alpha\rho) \exp (\alpha\rho - \alpha)\}. \quad [2]$$

During shortening at a velocity V, the average force per cross-bridge is

$$P(V) = (kh^2 f/(f + g)\alpha^2 d)\{\alpha^2(\rho^2 - 1)/2 -$$
$$(1 - \alpha) \exp (\alpha - \alpha\rho) + (1 - \alpha\rho) -$$
$$\beta^2 (1 - \exp (\alpha - \alpha\rho))(1 - \alpha/\beta -$$
$$(1 - (g/g_2)^2) \exp (-\alpha/\beta))\}. \quad [3]$$

In these equations, $\alpha = h(f+g)/V$, $\beta = (f+g)/g$, $\rho = h'/h$, and d is the distance between the sites for cross-bridge attachment. These functions are illustrated by curve C in Fig. 2.

The shape of curve C, Fig. 2, appears to be determined by two features of the rate-function specification given in Fig. 1b. One feature is the high value of f' for all values of x beyond the region of cross-bridge attachment defined by f. This feature prevents the force during stretch from rising above the isometric force, P_0. As the velocity of stretch increases, the number of attached cross-bridges in the attachment region will fall below the number for $V=0$, and the force will fall below P_0. Together, these effects can be referred to as a stretch-deactivation of the cross-bridge system. The second feature is the restriction of cross-bridge attachment to a narrow region well to the right of $x=0$. During shortening, many cross-bridges will remain attached with values of x between 0 and h, and the total number of attached cross-bridges, and the total force, can be greater than at $V = 0$. In combination, these features produce a $P(V)$ relationship with a positive slope in the region around $V = 0$, rather than the negative slope required for stability.

If two cross-bridge systems with these properties are connected together to form an antagonistic pair, the net steady-state force-velocity behavior of the pair will be given by $P(V)-P(-V)$, as illustrated in Fig. 4. A paired system of this type might provide a model for an antagonistic set of muscles, such as the indirect flight muscles of insects. For a flagellum, nine such cross-bridge systems arranged around the circumference of a circle should be considered. With respect to a particular plane of bending, some of these systems—perhaps those with microtubular doublets on one side of a bending plane passing through the center of the flagellum—will be oriented to generate maximum force while sliding to produce bending in one direction. The remainder—perhaps those with microtubular doublets on the other side of the plane of bending—will be oriented so that they are stretch-deactivated. The roles of these systems will reverse when the direction of sliding reverses during the other half of the bending wave cycle. Qualitatively, the net effect will be similar to the situation illustrated by Fig. 4, but a more detailed analysis is required to determine to what extent the quantitative behavior is influenced by the particular geometrical arrangement of cross-bridge systems within flagella.

If a force generator with properties illustrated by Fig. 4 is connected to an appropriate load, spontaneous oscillatory motion can result. In the simplest case, where the load consists of linear elastic and viscous resistances, the system will
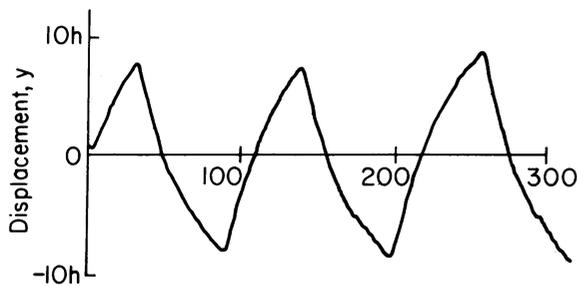
FIG. 6. Displacement-time plot obtained by numerical simulation of the paired cross-bridge system described in Figs. 1b and 4. For this simulation, each system of the pair had a population of 400 cross-bridges and 1000 time steps were made. Time is measured in units of h/V_0.
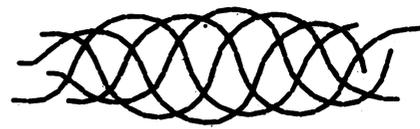

FIG. 7. Output from a computer simulation of the movement of a flagellar model, using 30 length intervals and 48 time steps/beat cycle. The method is similar to that used for earlier models (18), except that the active moment is determined according to Eqs. 4 and linear elastic resistances were used.

be a familiar type of relaxation oscillator (28). Its behavior can be described by a trajectory in the V,y plane shown in Fig. 5, where $V = dy/dt$. Standard methods are available for numerical or graphical integration of this trajectory to obtain $y(t)$. However, this approach will only give a valid description of the oscillatory behavior of the cross-bridge systems if the time-scale of the oscillations is long compared to the time-scale required for the cross-bridge populations to reach a steady state at a particular value of y. In both insect fibrillar muscle and flagella, the time scale of interest is such that the frequency of oscillation is of the same order of magnitude as the frequency of the cross-bridge attachment-detachment cycle (29, 30). It cannot, therefore, be expected that the steady-state behavior will be more than a rough guide to the possible oscillatory behavior of cross-bridge systems of this type.

For further study of these cross-bridge systems, a computer program has been developed which follows the time history of individual cross-bridges in a large population in order to obtain the average behavior of the cross-bridge population. This procedure, which will be described in detail elsewhere, is expensive, but is advantageous because it is not restricted to easily integrable rate functions such as those shown in Fig. 1, and can easily examine transient behavior as well as steady-state behavior of a cross-bridge system. Some results obtained with this program are shown in Figs. 2 and 6. The points accompanying curve C in Fig. 2 are computed steady-state forces for a population of 800 cross-bridges. Fig. 6 shows the time history of a paired cross-bridge system with the steady-state behavior shown in Fig. 4, when connected to a simple linear viscoelastic load. In this example, the elastic resistance (K) was equivalent to 0.05 P_0/h and the viscous resistance was equivalent to 0.5 P_0/V_0. Under these conditions, the peak-to-peak amplitude of oscillation was approximately 17 h, the cross-bridge turnover rate was approximately 1.75 per cycle of oscillation, and the work done against the viscous resistance was equivalent to 40–50% of the energy input from ATP dephosphorylation. These results suggest that a simple two-state cross-bridge system with the specifications shown in Fig. 1b has oscillatory properties appropriate for flagellar oscillation. This particular specification was chosen for ease of integration, and no attempt has yet been made to determine an optimal specification of cross-bridge properties. Further study is required to see whether the properties of this oscillatory system can be correlated with experimental observations on the effects of viscosity and ATP concentration on the frequency and amplitude of flagellar oscillation (31) and whether these systems have any utility for explaining oscillation in fibrillar

muscle. Previous explanations of oscillation in fibrillar muscle have emphasized the transient behavior of the muscle, whereas the present ideas emphasize the steady-state behavior.

Are oscillatory properties of an active sliding system distributed along a flagellum sufficient to explain the initiation and propagation of bending waves? This question can be approached by combining the computer program for analysis of a cross-bridge population with the earlier program for simulation of flagellar movement. As a less expensive preliminary step, the program for computer simulation of flagellar movement (18) has been modified to contain a force generator having a relationship between shear moment, m, and shear rate, $\dot{\sigma}$, similar to the $P(V)$ relationship shown in Fig. 4. A simple piecewise-linear approximation was used, given by the following specification:

$$m = m_0(-1 - \dot{\sigma}/\dot{\sigma}_{max}) \quad \text{for } \dot{\sigma} \text{ less than } -0.2\dot{\sigma}_{max},$$
$$m = 4m_0\dot{\sigma} \quad \text{for } -0.2\dot{\sigma}_{max} \leq \dot{\sigma} \leq 0.2\dot{\sigma}_{max},$$
$$m = m_0(1 - \dot{\sigma}/\dot{\sigma}_{max}) \quad \text{for } \dot{\sigma} > 0.2\dot{\sigma}_{max}$$

$$[4]$$

As might be expected, computer simulations with this flagellar model readily show synchronous shear oscillation, in which bending is restricted to the basal end of the flagellum, similar to Fig. 8 of ref. 18. However, with appropriate values of internal viscous and elastic resistances, propagated bending waves requiring metachronal shear oscillation (32) can be obtained, as shown in Fig. 7. This result demonstrates that local oscillatory properties of flagellar cross-bridges might be a sufficient explanation for propagated flagellar bending waves. Further study with the computer simulation method is required to define the conditions for stable bending wave propagation and the factors which determine bending wave parameters, to define experiments which might discriminate between local shear oscillator models and the earlier models involving control by curvature, and to relate experimental data on flagellar oscillation and bending wave propagation to molecular properties of flagellar cross-bridges.

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