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Flagellar Movement: A Sliding Filament Model

An explanation is suggested for the spontaneous propagation of bending waves by flagella.

Charles J. Brokaw

The movements of cilia and flagella have fascinated many generations of microscopists. In the last 20 years, advances in techniques for the photographic recording of flagellar movement, electron microscopy, and biochemical studies have provided a wealth of new data on cilia and flagella. Out of this a reasonable working model is now appearing which brings us to a level of understanding that approaches our understanding of the contractile mechanisms of muscle. In this article I survey this progress and discuss some of the problems which remain.

Only the cilia and flagella of eukaryotic cells are considered; the much smaller and very different flagella of bacteria remain very much a mystery.

The focus of this article is the "simple" flagellum, exemplified by the tail of a sea urchin spermatozoon. These flagella generally occur alone or in small numbers, and may be employed for the propulsion of small cells, such as spermatozoa and small protozoa, or the production of fluid currents, as in sponge choanocytes. Their movement is generally characterized by the propagation of undulatory bending waves along the length of the flagellum, so that a propulsive thrust is developed more or less along the line of the flagellum. Their internal structure, shown by the electron microscope, almost always consists of an axoneme containing the familiar "9 + 2" pattern of filaments running along the length of the flagellum. The two central filaments appear to be typical microtubules, while the nine filaments of the outer ring appear to be microtubular doublets (*1, 2*).

Cilia are similar in structure to simple flagella, but commonly occur in large numbers on a surface and generate a flow of fluid parallel to the surface. They may be used for locomotion, as in ciliated protozoa or flatworms; or they may cause movements of fluids over a "stationary" surface, as in the movement of mucus by cilia in the esophagus. The movements of cilia are often very asymmetrical, and are coordinated with the movements of neighboring cilia to produce metachronal waves. These features make their movements considerably more difficult to observe and photograph than the movements of simple flagella, but some significant progress has been made in recent years (*3, 4*).

Mammalian spermatozoa are the best-known examples of what may be called "complex" flagella, in which the 9 + 2 axoneme of simple flagella is supplemented by additional components within the flagellar membrane. In mammalian spermatozoa, the axoneme is typically supplemented by nine "outer fibers" which are electron-dense and much larger than microtubules (*5*). In insect spermatozoa, an additional ring of nine microtubules is commonly found outside the axoneme (*6*). Other types of complex flagella are common among amphibian spermatozoa. The functions of these supplementary components are still unknown.

Flagella, and other structures that move like flagella, have also been found which do not have the typical 9 + 2 axoneme. Variations in the number of central microtubules within the ring of nine microtubular doublets have been described in spermatozoa of insects (*6*) and flatworms (*7*). Other insect spermatozoa have been found with more than nine microtubular doublets (*8*), and others with only singlet microtubules (*9*). Undulatory bending is also generated by the microtubular arrays forming the axostyles of several species of termite symbionts (*10*).

From all of these studies, one can conclude that the ability to generate propagated bending waves is not exclusively associated with a particular structural pattern. The 9 + 2 pattern may be particularly advantageous, but is not essential. Although cilia and flagella can generate a wide variety of patterns of bending movements, attempts to correlate these variations in movement pattern with variations in internal structure have so far been conspicuously unsuccessful.

Movement of a Simple Flagellum

The movements of cilia and flagella can be recorded by high-speed cinematography or, more simply, by multiple-exposure dark-field photomicrography, as illustrated by Fig. 1. This photograph of a swimming sea urchin spermatozoon was taken with four flashes of light from a lamp flashing at 100 flashes per second. The spermatozoon is swimming close to the surface of the microscope slide, which prevents it from rotating and keeps the plane of bending of its flagellum in the plane of optical focus. The bending waves of sea urchin sperm flagella are easy to study because they are uniform, planar, and nearly symmetrical, and because

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The author is professor of biology at the California Institute of Technology, Pasadena 91109.

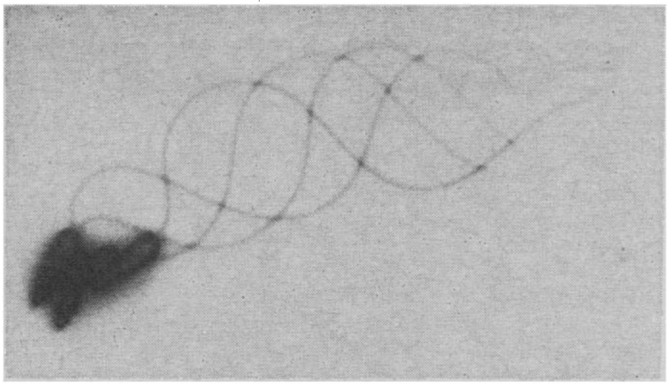


Fig. 1. A multiple-flash photomicrograph of a spermatozoon from the sea urchin, *Lytechinus pictus,* obtained with four light flashes separated by 10-millisecond intervals. This is a negative print of a photomicrograph obtained by dark-field illumination.

the spermatozoa can be easily observed in isolation from the effects of the movement of other spermatozoa.

Photographs such as Fig. 1 show that undulatory bending waves are propagated along the sperm flagellum at a uniform velocity of nearly 1 millimeter per second. These waves are not sinusoidal waves such as those which can be produced on a vibrating string. The bends, or bent regions, of the bending waves have a much more constant curvature than the bent regions of sinusoidal waves. Flagellar bending waves can be closely approximated by waves constructed of circular arcs and straight lines (*11*) or by "sine-generated" waves, in which the curvature of the flagellum is a sinusoidal function of distance along the flagellum (*12*). These are waves which are defined "biologically" as a function of distance measured along the flagellum, rather than as a function of distance along a hypothetical x-axis.

Bending Mechanisms

The old idea that cilia and flagella might be passive cellular appendages, which are waved back and forth as a result of active processes located within the cell, has now been soundly rejected. Such a mechanism cannot explain the ability of flagella to swim around with nearly normal movements, when completely separated from a cell body (*11, 13*). Theoretical calculations (*14*) and experimental observations (*15*) have also demonstrated that the propagated waves which can be generated by oscillating one end of a passive elastic filament immersed in a viscous medium will rapidly decrease in amplitude. The bending waves which can be propagated along flagella with essentially constant amplitude must be maintained by active processes distributed along the flagellum.

Flagella which have had their surface membranes damaged or removed can be reactivated by adenosine triphosphate (ATP) (*16*), and in favorable cases, the reactivated movements are very similar to those shown by normal flagella (*13, 17*). Flagella, like muscle, appear to contain a mechanochemical mechanism that converts the chemical energy of ATP into movement, and this mechanism appears to be distributed fairly uniformly along the flagellum. Adenosine triphosphate can apparently be supplied to the flagellum at a sufficient rate by simple diffusion through the interior of the flagellum, from a source within the cell (*18*).

The active mechanochemical process in flagella might take the form of a localized contraction at the sides of the flagellum, which would cause bending if other parts of the flagellum can resist compression (*14, 15*). The bending movements used for swimming by many higher organisms, such as worms, eels, or snakes, originate in this manner. A "local contraction" mechanism of this sort has the property that the bending observed at a particular point along the length of the flagellum is a direct reflection of the active mechanochemical processes occurring within the flagellum at that point. A propagated bending wave is associated with a propagated wave of contraction, and both waves have the same phase.

Alternatively, flagellar bending could arise from a shearing or "sliding filament" mechanism. Figure 2 illustrates the relationship between shear and bending in a simple two-filament model of a flagellum (*19*). If these two filaments lie in a plane and are separated by a constant distance, r, and if the filaments are completely resistant to changes in their length, the relationship between bending and shear can be formulated as

$$du/ds = r d\theta/ds = r\kappa \qquad (1)$$

The shear displacement of one filament relative to the other is measured by u, the curvature of the filaments is measured by κ, s is a measure of position along the filaments, and θ is a measure of the angle of bend of the filament. For a more generalized flagellum containing an arbitrary number and arrangement of inextensible filaments, it is useful to use a parameter σ to indicate the amount of internal shear without reference to a particular arrangement of internal filaments. Then,

$$d\sigma/ds = \kappa \qquad (2)$$

In the sliding filament model suggested for muscle, the filaments are interconnected in a large array, so that there must be uniform shear along the filaments ($d\sigma/ds = 0$) and no bending occurs. A sliding filament process similar to that suggested for muscle could cause flagellar bending, if there are no structural constraints which require uniform shear along the length of the flagellum. In that case, a propagated bending wave will be associated with a propagated wave of shear, but as required by Eq. 2, these two waves will differ in phase by a quarter of a cycle (Fig. 3). With a sliding filament mechanism, the bending observed at a particular point on the flagellum is not the direct reflection of a local mechanochemical process, but is the result of shear processes distributed throughout adjacent regions of the flagellum.

Evidence for a Sliding Filament Mechanism

A sliding filament mechanism for flagellar bending originates, of course, from the suggestion by Hanson and Huxley (*20*) that muscle contraction might be explained by a sliding filament mechanism. This explanation for muscle contraction has now become widely accepted. Speculation that a similar mechanism might be responsible for the movements of cilia and flagella began soon after the discovery of the "arms" projecting from the outer microtubular doublets (*1*).

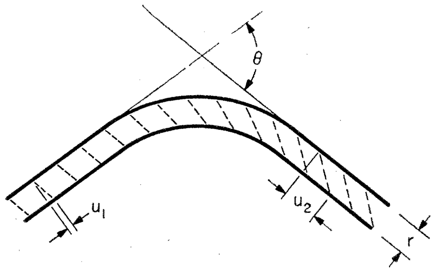
Direct evidence for sliding of ciliary filaments was first provided by a series of electron microscope studies by Satir (*21*). He developed techniques for the fixation of molluscan gill cilia in different positions of bending, so that the ultrastructure of the cilia could be compared between these states. By a careful analysis of the termination of the ciliary filaments (the outer microtubular doublets) he was able to obtain evidence that the filaments retained constant relative lengths during the bending cycle, so that bending was accompanied by interfilament sliding, rather than by contraction or elongation of the filaments.

Supplementary evidence for interfilament sliding in cilia was provided by Horridge's study (*22*) of the ultrastructure of the macrocilia found on a ctenophore, *Beroë*. These macrocilia contain several thousand axonemes within a single membrane. Examination of the ultrastructure of bent macrocilia indicates that interfilament sliding must occur within the macrocilium. The location of cross-bridges between the outer microtubular doublets of adjacent axonemes implies that interfilament sliding is likely to occur within the axonemes, in addition to or instead of between the axonemes.

A mechanism for generating bending by active sliding between filaments is particularly attractive for cilia, because a cilium is usually overcoming the greatest viscous resistance to bending during its effective stroke, when its bending is restricted to a relatively short region near its base. A local contraction mechanism would require an unreasonably high, localized, conversion of chemical to mechanical work in the bending region, while a sliding filament mechanism allows this conversion to occur throughout most of the length of the cilium (*4*).

Evidence for interfilament sliding in sperm flagella was first provided by an observation of the movement of sea urchin spermatozoa in solutions containing thiourea (*11*). One of the effects of this inhibitor is a selective inhibition of the distal end of the sperm flagellum, so that it no longer bends either actively or passively, and appears stiff. At the same time, the movements of the active, proximal, portion of the flagellum are restricted so that they occur without altering the angle between the basal and distal ends of the flagellum. Photographs such as Fig. 1 show that this restriction is not a feature of normal movement. The spermatozoa inhibited by thiourea behave as if they contain a bundle of filaments of constant length which are tightly tied together at the base and in the tip region of the flagellum, but which are free to slide, and generate bending, in the active region.

Much more direct and substantial evidence for an active sliding process in flagella has been provided by observations by Summers and Gibbons (*23*). They isolated sea urchin sperm flagella, and used the detergent Triton X-100 to remove the flagellar membranes so that bending wave movements could be reactivated by addition of ATP. After a brief treatment with trypsin, the addition of ATP caused a disintegration of the flagellar axoneme into isolated microtubular doublets. The disintegration process was observed with dark-field microscopy, and was seen to involve the extrusion of filaments from the axoneme by a gradual sliding process. The ATP requirement and ionic specificity of these results closely matched the requirements for normal movements and ATP dephosphorylation activity. Their observations also indicated that the presence of the normal basal end of the flagellum interfered with sliding apart of the filaments, and thus confirmed the assumption that the flagellar filaments are normally connected together at the base of the flagellum.



$$\theta = \frac{u_2 - u_1}{r} = \Delta\sigma$$

$$\kappa = \frac{d\theta}{ds} = \frac{d\sigma}{ds}$$

Fig. 2. Relationship between shear and bending in a simple flagellar model consisting of two inextensible filaments. The total angle of bend in the bent region is θ. The dashed lines illustrate shear strain imposed on material between the filaments, measured by the shear displacement, u. A dimensionless measure of shear is denoted by σ, the curvature of the flagellum by κ, and position along the filaments by s.

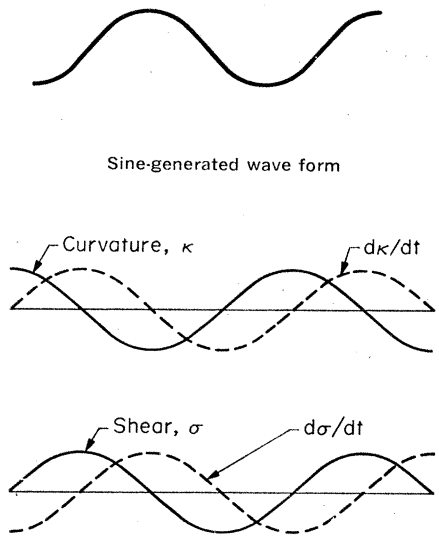


Fig. 3. A sine-generated wave form, and its generating functions, which show the distribution of curvature and shear as functions of distance, s, measured along the length of the flagellum. If the waves are propagating from left to right, $d\kappa/dt$ and $d\sigma/dt$ will be given by the dashed curves, as indicated.

Control Mechanisms

In addition to a mechanism for bending, flagella and cilia must contain control mechanisms that generate a rhythmic cycle of bending and maintain phase differences between the bending of different parts of the flagellum, in order to generate propagated bending waves. The control mechanisms must also be an intrinsic part of the flagellum, since they appear to operate nearly normally in isolated and demembranated flagella (*13, 17*). It seems unlikely that each element of the flagellum is controlled by signals from a central computer, and rather more likely that the system operates by local feedback processes (*14*).

A bending wave on a flagellum can be conveniently described by a "wave generating function," which is simply a function giving the curvature, κ, as a function of length measured along the flagellum (*12*). An example is shown in Fig. 3, where the wave generating function is a sinusoidal wave. The wave form that results from this wave generating function is known as a "sine-generated" wave. It is the curve of minimum energy for bending of an elastic filament, and has been used to describe the form of river meanders (*24*). It is also a close approximation to the wave forms found on flagella (*12*).

If a sine-generated wave is being propagated uniformly along a flagellum, the rate of bending of the flagellum, $d\kappa/dt$, must vary according to the dashed curve shown in Fig. 3. This is simply another sinusoidal function which is displaced a quarter of a cycle ahead of the curvature. A feedback mechanism in which the rate of bending is controlled by the curvature could provide a control mechanism for flagellar bend propagation if it included some means for introducing the quarter-cycle phase difference between κ and $d\kappa/dt$.

Machin (*14, 25*) was the first to describe a control mechanism of this type for flagellar movement. In his proposal, the phase shift was provided by a time delay in the feedback loop between the measurement of negative curvature and the active process which generated a positive $d\kappa/dt$. If τ is the magnitude of this time delay, the beat frequency of the flagellum must be $1/4\tau$. Any experimental variable that causes a change in the beat frequency must therefore change the time delay. Since it is difficult to see how an external variable such as the viscosity of the surrounding fluid, which causes large changes in the beat frequency, could alter the internal time delay, this control mechanism has not been considered satisfactory (*26*). This difficulty was circumvented by a rather different mechanism for introducing a phase difference between κ and $d\kappa/dt$, suggested by Brokaw (*27*). He suggested that the active process which caused $d\kappa/dt$ was activated by the passive bending which would result from internal shear near an abrupt change in curvature; this mechanism has been developed further by Lubliner and Blum (*28*). Both of these mechanisms, in which the active process is assumed to be equivalent to a local contraction process, have difficulty in explaining the uniform velocity at which bends are propagated along a flagellum (*29*). A sliding filament mechanism appears to offer a more attractive possibility for introducing the necessary phase shift into the control process.

With a sliding filament process, the rate of shearing or sliding, rather than the rate of bending, can be controlled by the curvature of the flagellum (*19*). A linear relationship which expresses this control process is:

$$d\sigma/dt = -\mu\kappa \qquad (3)$$

where μ is a constant characteristic of the control process. Inspection of Fig. 3 shows that Eq. 3 represents the relationship between shear rate and curvature which is required for propagation of a sine-generated bending wave. More generally, from Eqs. 2 and 3 we get:

$$\begin{aligned} d\kappa/dt &= d(d\sigma/ds)/dt \\ &= d(d\sigma/dt)/ds \\ &= -\mu d\kappa/ds \qquad (4) \end{aligned}$$

A general expression for propagation of a wave of bending in the positive direction along s is one in which κ is a periodic function of $(s-vt)$, where v is a propagation velocity. In such a case, $d\kappa/dt = -v d\kappa/ds$, which is equivalent to Eq. 4. Consequently Eq. 3 will always generate the rate of bending that is required to propagate bending waves. In other words, the sliding filament mechanism automatically introduces a quarter-cycle phase shift, independent of the beat frequency, into the feedback loop involving κ and $d\kappa/dt$. Rhythmic bending waves will therefore be generated automatically by a control process in which active sliding is a function of the curvature of the flagellum. This feature of the sliding filament model overcomes most of the objections that have been raised to simple control systems based on local contraction models for the mechanism of active bending (*19*).

Control of Bending Moments

The mechanism suggested for the control of bend propagation by a sliding filament model for flagella, as expressed by Eq. 3, is incomplete, because it involves the unreasonable assumption that the rate of sliding generated by the active process is determined only by the control function, and not by the resistance, or load, encountered in trying to bend the flagellum. It seems much more reasonable to expect that the rate of active sliding will decrease if the viscous loading on the flagellum is increased. A more realistic model can be developed by assuming that the force generated by the active process, rather than the rate of movement, is determined by the control process. This approach was used by Machin in his original analysis of a control mechanism for bend propagation by a local contraction model of a flagellum. Machin's proposal was equivalent to stating that the active bending moment at a particular point on the flagellum, $M_a(s)$, was controlled by the curvature of the flagellum at that point (*14*). The time delay of a quarter cycle was then required to cause the phase of the active bending moment to match the phase of the bending moment resulting from viscous resistances to movement of the flagellum.

With the sliding filament model, the local curvature of the flagellum can control the intensity of the sliding forces generated at each point along the length. These forces can be measured by a variable, m, which represents the active moment generated per unit length of flagellum. The total active bending moment at any point, $M_a(s)$, is then obtained by adding up all the moment produced by active sliding processes along the flagellum, or,

$$dM_a/ds = -m = m_0\kappa \qquad (5)$$

where m_0 is a characteristic constant for the active process for the case of a linear control process. If κ is a sinusoidal function of length, as in Fig. 3, Eq. 5 indicates that $M_a(s)$ will also be a sinusoidal function of s, but will be displaced by a quarter cycle, so that it will match the dashed curve for $d\kappa/dt$ in Fig. 3.

For small-amplitude bending waves on a long flagellum, the bending moment resulting from the resistance of the external viscous medium to movement of the flagellum will be proportional to $d\kappa/dt$, and will therefore be in phase with $M_a(s)$. The quarter-cycle phase shift introduced into the control process by the sliding filament process makes it possible for the viscous bending moment to be exactly balanced by the active bending moment, at any beat frequency (*19*).

The flagellum is also likely to have elastic resistances to bending which will vary in phase with κ. These must also be overcome by the active bending moment. This can be accomplished if there is a small time delay in the relationship between κ and $-m$, so that M_a is phase shifted and can be resolved into two components, one in phase with the viscous bending moment and the other in phase with the elastic bending moment (*19*).

These relationships between active and resistive bending moments can be calculated for small-amplitude bending waves on a long flagellum, where a simple sinusoidal function is a reasonable approximation for the viscous bending moments. For real flagella, which generate large-amplitude bending waves and are short enough so that the viscous bending moments are strongly influenced by end effects, the computation of viscous bending moments is more difficult, but can be carried out numerically (*29*). Examination of the bending moments appropriate for real flagella suggests that the sliding filament mechanism should be able to provide the proper match between active and resistive bending moments (*19*), but a conclusive demonstration that the mechanism will work depends on the methods that are outlined in the next section.

Computer Simulation of Flagellar Movement

Because the distribution of viscous bending moments on a moving flagellum is a complicated function of the bending of the flagellum, a detailed analysis of the balance of active and resistive bending moments in a sliding filament model for a flagellum is difficult. The problem can be approached more conveniently by building a model flagellum, in the form of a computer program that simulates the movement of the flagellum. Results from such a program have already demonstrated the power of this approach and confirmed that the sliding filament model for a flagellum can explain the generation of propagated bending waves in a very simple manner (*30*).

Models constructed of 25 to 35 straight segments have been found to give sufficient accuracy with minimum computing cost. The model is allowed to bend at each of the joints between the straight segments. At each of these joints, an equation is set up for the balance of active, viscous, and elastic bending moments, in terms of the unknown values for the rate of bending at each joint, the known initial values for the angle of bend at each joint, and several constant parameters. Viscous bending moments are obtained by numerical integration, starting with the simple hydrodynamic assumptions that have been used for most recent analyses of flagellar movement (*29*). After addition of the unknowns and equations required to satisfy boundary conditions at the ends of the model, a set of simultaneous linear equations is obtained, which can be solved to obtain the rate of bending at each joint. A forward time integration step is then performed, in which the rate of bending at each joint is used to find a new value for the angle of bend at that joint after a small time interval. The whole process is then repeated to generate successive images of the model over an extended time period.

Results obtained from computations with the simplest form of the model are illustrated by the example in Fig. 4a. For these computations, the model contained 32 straight segments of equal length, and 24 time integration steps were performed for each beat cycle. The model generates propagated bending waves and swims forward through its hypothetical viscous environment until the computer run is terminated.

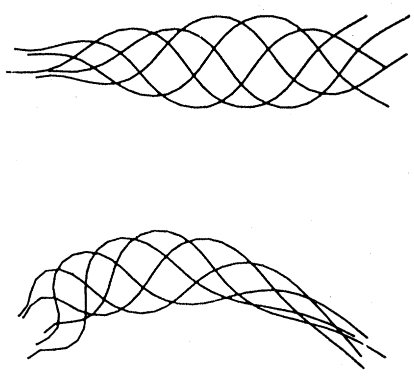


Fig. 4. Examples of stable movement patterns generated by the computer program which simulates the movement of the sliding filament model for a flagellum. Each pattern shows one beat cycle, at quarter-cycle intervals.

The parameters of the stable, periodic bending pattern illustrated in Fig. 4a are independent of the starting configuration (*30*).

In order to obtain the stable movement illustrated in Fig. 4, two additions to the model were required (*30*). Cubic nonlinearities were incorporated into the internal elastic resistance, in order to stabilize the amplitude of bending. An internal viscous bending resistance was included to restrict the movements of the ends of the model, since the external viscous resistance near the ends approaches zero more rapidly than the active bending moments. The relationship between internal and external viscosities has been found to determine the wavelength at which the movement stabilizes. The beat frequency of the movement is determined by the relationship between the characteristic parameter for the active process, m_0, and the internal and external viscosities.

The computer simulation approach is a powerful method for studying the relationship between the internal parameters of a flagellum and the parameters of movement, regardless of the particular model used. The results illustrated have been obtained from a model that incorporates the two basic features of the sliding filament model: first, that the active bending moment is obtained by integration of the moment generated by the active sliding process along the length of the flagellum, and second, that the magnitude of the active sliding process is proportional to the local curvature of the flagellum. These assumptions are therefore sufficient to explain both the initiation and the propagation of bends by flagella.

By varying the internal parameters of the model, a variety of wave patterns of different wavelength, amplitude, and frequency can be obtained, and any particular set of movement parameters from real flagella can be matched. However, there are some minor differences between the movements of the model illustrated in Fig. 4a and the movements of real flagella. The most importance difference is found near the basal end, where, as shown in Fig. 1, real flagella form bends of a smaller radius of curvature, which then enlarge to the radius of curvature that is maintained along most of the flagellum. No combination of internal parameters which are constant along the length of the model will cause the model to reproduce the behavior of real flagella in the basal region. More realistic bending behavior near the base of the model can be obtained if a region of reduced internal viscosity is introduced near the base. This modification, and the introduction of asymmetry into the relationship between m and κ, leads to the generation of swimming patterns, such as that illustrated in Fig. 4b, which more nearly resemble the movements of real sperm flagella. Further study of these factors, and comparison between the behavior of the model and the behavior of real flagella under various experimental conditions, has been carried out (*31*).

Internal Organization of a Flagellum

In speculation about the generation of flagellar bending by a sliding filament process it is most often assumed that the "arms" that extend from each outer microtubular doublet are in some way analogous to the arms or bridges of heavy meromyosin which, in striated muscle, are believed to generate sliding by interaction with actin filaments. This assumption has been supported by evidence that the arms contain a high-molecular-weight protein which appears to be the major ciliary adenosine triphosphatase (*32*). The arms could interact with the adjacent microtubular doublet, or with some other fibrillar structure such as the "secondary filaments," or with the matrix or core surrounding the central pair of microtubules. The observations of Summers and Gibbons (*23*) have now provided some direct evidence for interaction between adjacent microtubular doublets. The active sliding which they observed after adding ATP to trypsin-treated and demembranated flagella could sometimes cause extrusion of filaments to a total length equal to or greater than five times the original length. This result would not be expected if all the filaments were interacting with a common core or matrix, instead of with each other.

The generation of planar bending waves by the sliding filament model requires that at each point along the flagellum, active sliding can occur in either direction, depending on the curvature of the flagellum. This could mean that each active site (each arm?) can slide actively in either direction, depending on the curvature. A more likely possibility, which is more consistent with our knowledge of the properties of the active sites in striated muscle, is that the active sites are polarized and only slide actively in one direction, and that the sites differ in their orientation with respect to the plane of bending. Curvature in one direction would activate the sliding of one set of sites, and curvature in the other direction would activate the sliding of the other set of sites.

In any event, the overall bending of the flagellum would appear to be the result of the activity of several interfilament sliding systems, oriented at various angles. Generation of planar bending waves implies some degree of coordination between these pairs of sliding filaments. In a completely linear system, each pair of interacting filaments would generate its own pattern of bending independently of the other pairs, and some form of supplementary control system would be required to coordinate them to produce a particular overall movement. However, the system must contain nonlinearities that limit the amplitude of bending or sliding or both. In a nonlinear system, interactions between the pairs of filaments will tend to cause synchronization between their movements. This synchronization is formally equivalent to the synchronization that occurs between two spermatozoa swimming close to each other (*25*). If there is a sufficient degree of interaction between the pairs of filaments, they will probably become synchronized so that their bending movements add up to generate a planar bending wave. Other things being equal, the bending plane might be the plane of least resistance defined by the orientation of the central pair of microtubules. Attempts to correlate the orientation of the central pair, as seen in electron micrographs, with the bending plane have supported this suggestion. Under other conditions, the interactions between filament pairs might produce a metachronal coordination, in which there is a phase difference between the bending of adjacent filament pairs, with the result that a three-dimensional pattern of bending may be maintained.

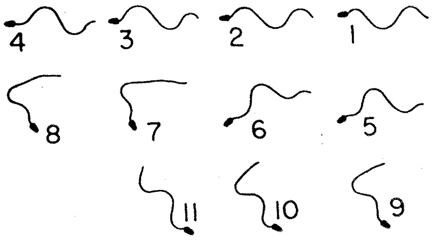


Fig. 5. Tracings from a multiple-exposure photograph of a *Tubularia* spermatozoon during a chemotactic response. The time interval between each image corresponds to approximately two beat cycles. The images have been separated for clarity. [From Miller and Brokaw (*43*)]

The computer model for simulation of flagellar movement can be extended to deal with the interactions between two or more sliding filament systems, so that the conditions for synchronization between their movements can be studied. This approach should provide additional information about the factors that determine the form of flagellar bending waves, and perhaps help us to find an explantion for the ability of some flagella to make either planar or helical bending waves, depending on the conditions (*17*).

Characteristics of the Active Process

A complete understanding of the sliding filament model for generating flagellar bending waves must include an understanding of the mechanochemistry of the active sites at the molecular level. The basic mechanochemical process by which energy from ATP dephosphorylation is used to produce mechanochemical work may be very similar to the active sliding process responsible for the contraction of muscle. The second part of the model, involving the activation of the mechanochemical process by bending the flagellum, may be closely related to the stretch activation which may be a general property of striated muscle (*33*) and which has been developed into a mechanism for an autonomous rhythm of contraction in insect fibrillar flight muscle. Further progress toward a molecular understanding of flagellar movement will rely heavily on progress in understanding the mechanochemical events in muscle at the molecular level, but it is possible that studies on cilia and flagella may offer some unique insights into these mechanochemical mechanisms.

Some information has been accumulated about the chemical characteristics of the active process in flagella. The beat frequency of the ATP-reactivated movements of glycerinated sea urchin spermatozoa varies with ATP concentration, according to Michaelis-Menten kinetics, with a Michaelis constant near 0.4 millimolar ATP (*34*). This suggests that at low ATP concentrations the reaction between ATP and a specific binding site is a rate-limiting step in the mechanochemical process.

Silvester and Holwill (*35*) used absolute reaction rate theory to calculate that, at low ATP concentrations, the collision frequency between ATP molecules and an active site could be no more than two or three times the beat frequencies observed with ATP-reactivated flagella. This calculation implies that it would be impossible for each active site to turn over many molecules of ATP during the fraction of the beat cycle in which it was contributing to active sliding. Silvester and Holwill proposed a molecular model for the behavior of the active site in which they assumed that reaction between ATP and an active site occurred during an inactive portion of the beat cycle, to convert the site to an "activated" site. A mechanical signal associated with the propagated bending wave would then turn on the active process which converts the potential energy of the "activated" site into movement. With this model, each active site would probably use only one ATP molecule per beat cycle.

This model has been supported by measurements of the adenosine triphosphatase activity of the ciliary adenosine triphosphatase, dynein (*34, 36*), by measurements of the movement-coupled ATP dephosphorylation of suspensions of glycerinated sea urchin spermatozoa (*37*), and by measurements of the movement-coupled metabolism of *Ciona* spermatozoa (*38*). These measurements suggest that there is a relatively tight coupling between movement and ATP dephosphorylation, and that the average number of ATP molecules dephosphorylated by a flagellum per beat cycle is approximately equal to the estimated number of dynein molecules, or arms on the microtubular doublets, in a flagellum.

The assumption that each active site uses one ATP molecule per beat cycle, irrespective of the parameters of the propagated wave, is somewhat difficult to reconcile with the assumption of a linear controlling relationship between curvature and the active process. However, computer simulation experiments have shown that stable bending waves can also be generated by a model incorporating a nonlinear controlling relationship (*30*). In this model, the maximum active moment generated by the sliding process was turned on by relatively small amounts of curvature, and remained constant during the active phase of the beat cycle. The bending waves generated by this model had bent regions of more constant curvature, which may more closely resemble the bent regions in real flagellar bending waves. Further study of the computer simulation of the movement of flagellar models containing various forms for the active process may provide some additional information about the active process. However, this approach is at present limited by the low resolution of methods for measuring the curvature of flagellar bending waves.

Extrinsic Control

In the mechanism for the control of bend propagation by a sliding filament model for flagella, described in this article, each active site along the flagellum contributes equally to the autonomous rhythm of propagated bending. No special process is required for initiation of bends at the base of the flagellum, and there is little indication that the boundary conditions at the base of the flagellum can have a major influence on the movement. However, there are numerous observations which suggest that this is too simple a view, and that some additional mechanisms may be involved in the control of movement in cilia and flagella. A comprehensive review of extrinsic control of ciliary movement has been published (*39*); only a few observations will be mentioned here.

1) Rikmenspoel and Sleigh (*40*) analyzed the distribution of bending moments during a ciliary beat cycle, and concluded that a simultaneous activation of the active sliding process throughout a cilium would be required at the beginning of the effective stroke. They suggested that a rapidly propagated signal would be required to activate all of the cilium simultaneously.

2) Some flagella can propagate bending waves in either direction along their length (*41*). According to the control mechanism described here, a reversal of the propagation direction would require a reversal of the sign relationship between $-m$ and κ (Eq. 5), throughout the entire flagellum. There is at the moment no obvious mechanism which could explain such a reversal, although possibly such a mechanism would be easier to see if we had a detailed molecular model for the activation of the sliding process by curvature of the flagellum.

3) There are many indications that the initiation of the beat cycle in cilia can be determined by external factors. Most cilia beat in a metachronal rhythm. Although much of the coordination implied by such rhythms may simply result from mechanical and hydrodynamic interactions between adjacent cilia there is evidence which suggests that additional control mechanisms are involved (*42*).

4) Some cilia and flagella can alter their pattern or direction of beating in response to external stimuli. For example, the spermatozoa of the hydroid, *Tubularia*, are chemically attracted to the eggs (*43*). Their chemotactic response involves changes in the pattern of beating of the sperm tail, illustrated in Fig. 5, which are superimposed on the basic pattern of bending which continues at its normal frequency.

5) When sea urchin sperm flagella are irradiated near their base with a laser microbeam, the distal portion of the flagellum continues to propagate the bends which have already formed, but does not initiate any new bends (*44*). Application of target theory to data from experiments on the radiation sensitivity of bull spermatozoa has suggested the presence of a rather small "control center" which was much more sensitive to radiation damage than the flagellum as a whole (*45*). These results, and some other observations, point to the presence of some form of specialized control region, probably at the base of the flagellum, which might be involved in the normal initiation of the beat cycle in some flagella.

Taken together, these observations indicate that, even though a simple feedback relationship between curvature and the active process might form the fundamental basis for the rhythmic generation and propagation of bends along cilia and flagella, there remain many unanswered questions about the control of bending in cilia and flagella. Additional work, involving both experimental study of real flagella and continued analysis of theoretical models by the computer simulation method, will be required before these questions can be resolved.

Summary

A sliding filament mechanism appears to provide the most satisfactory basis for a simple feedback mechanism for the control of bend propagation and bend initiation by flagella, and is supported by strong experimental evidence. A computer simulation of the movements of a flagellar model based on the sliding filament mechanism demonstrates that this mechanism offers a sufficient explanation for the automatic generation of flagellar bending waves. Further computer simulation studies may provide insight into questions such as the applicability of the sliding filament mechanism to the generation of the more complicated asymmetrical bending patterns of cilia, and the control of the bending pattern by interaction between several sliding filament systems within a flagellum.

Unanswered questions remain in two important areas. Very little is known about the detailed molecular mechanisms which are responsible for active sliding and its control by flagellar bending, although these mechanochemical processes are assumed to be closely related to those occurring in muscle. A variety of evidence points to the presence in flagella of control processes in addition to the basic mechanism suggested for spontaneous bend initiation and propagation; further work is required before mechanisms for these additional control processes can be suggested.

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Metallocarboranes That Exhibit Novel Chemical Features

A virtually unlimited variety of structural and dynamic features are observed in metallocarborane chemistry.

M. Frederick Hawthorne and Gary B. Dunks

In 1964 it was reported (*1*) that icosahedral 1,2-dicarba-*closo*-dodecaborane-12 (hereafter referred to as 1,2-$B_{10}C_2H_{12}$) and its carbon-substituted derivatives, formed in the reaction of an acetylene with $B_{10}H_{14}$ in the presence of a ligand catalyst, could be degraded with base to form anions having the general formula $B_9C_2H_{10}R_2^-$ (where R stands for hydrogen, alkyl, or aryl). Later work (*2*) proved that the more thermodynamically stable 1,7-dicarba-*closo*-dodecaborane-12 (1,7-$B_{10}C_2H_{12}$), a carbon atom position isomer of 1,2-$B_{10}C_2H_{12}$ formed by thermal rearrangement (*3*) of the 1,2- isomer, was degraded under similar conditions to yield an isomeric $B_9C_2H_{12}^-$ ion. Both reactions proceed by the formal extraction of a BH^{2+} vertex from the corresponding isomeric $B_{10}C_2H_{12}$ icosahedral carboranes followed by proton addition to the resulting $B_9C_2H_{11}^{2-}$ ion. The removed vertex proved to be one of two equivalent 3- or 6-BH vertices located as nearest neighbors of the two CH vertices of both $B_{10}C_2H_{12}$ isomers (Fig. 1). At the time, it was suspected that the twelfth, or one "extra," hydrogen atom present in the isomeric $B_9C_2H_{12}^-$ ions was bonded to the open pentagonal face of the icosahedral fragment formed by BH^{2+} removal. The mode of such bonding has probably been established (*4*) by ^1H and ^{11}B nuclear magnetic resonance and selective deuteration experiments. Thus, the "extra" hydrogen atom (Fig. 1) is present in one (1,7- isomer) or two equivalent (1,2- isomer) B-H-B bridge positions accompanied by rapid equilibration in the latter isomer.

With these results, the stage was set for the discovery of an entirely new class of extremely stable organometallic complexes, now known as metallocarboranes. The ligands present in these new complexes were formed by the removal of the "extra" B-H-B bridge hydrogen atom from the (3)-1,2- and (3)-1,7-$B_9C_2H_{12}^-$ ions as a proton, thus producing a new type of ligand, the ions (3)-1,2- and (3)-1,7-$B_9C_2H_{11}^{2-}$, respectively [where (3) indicates the vacant position in the icosahedron]. Each $B_9C_2H_{11}^{2-}$ ligand is capable of donating six delocalized electrons to a transition metal electron acceptor as in the well-known case of ferrocene, $(\pi\text{-}C_5H_5)_2Fe$, which has two similarly disposed $C_5H_5^-$ ligands. The first report of this new transition metal bonding scheme appeared in 1965 (*5*), and the general scope of metallocarborane chemistry is still expanding at a rapid rate. The initial research developed syntheses of the transition metal complexes $[(B_9C_2H_{11})_2M^n]^{n-4}$ (Fig. 2), where $n-4$ denotes the charge of the complex and M represents a transition metal ion such as formal Fe(II), Fe(III), Co(II), or Co(III) complexed with a pair of (3)-1,2- or (3)-1,7-$B_9C_2H_{11}^{2-}$ ions (*6*), hereafter termed "dicarbollide ions."

An early x-ray diffraction study (*7*) proved that the iron atom in [(3)-1,2-$B_9C_2H_{11}](C_5H_5)Fe^{III}$ was symmetrically located between the open face of the (3)-1,2-$B_9C_2H_{11}^{2-}$ ligand and the π-bonding face of the $C_5H_5^-$ ligand, thus completing the icosahedron. Many other structural studies with a variety of transition metal dicarbollide ion complexes have proved this to be the general mode of ligand to metal bonding. Facial "sandwich" bonding of the $B_9C_2H_{11}^{2-}$ ligand with transition metal moieties such as $M(CO)_3$ metal carbonyl and $M(\pi\text{-}C_5H_5)$ metal cyclopentadienide units suggests that the metal bonding orbitals of the $B_9C_2H_{11}^{2-}$ ligands (*6, 8*) closely resemble those found in the simple metallocenes de-

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Dr. Hawthorne is a professor of chemistry and Dr. Dunks is a research associate at the University of California, Los Angeles 90024.