Functional Significance of the Outer Dense Fibers of Mammalian Sperm Examined by Computer Simulations With the Geometric Clutch Model

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The flagella of mammalian sperm possess certain structural characteristics that distinguish them from simple flagella. Most notable of these features are the sheath (surrounding the axoneme), the outer dense fibers or ODFs (that are attached to the outer doublets), and the connecting piece (which anchors the ODFs at the base of the flagellum). In this study, the significance of these specialized axonemal elements is explored. Their impact on microtubule sliding and force production within the axoneme is specifically analyzed. A working hypothesis is developed based on the premise that forces produced by interdoublet sliding are transferred to the ODFs. In this way, the torque required to bend the flagellum is developed between the ODFs, which are anchored in the connecting piece. This working hypothesis was incorporated into the pre-existing "geometric clutch" model that earlier simulated only cilia and simple flagella. The characteristic length and stiffness of bovine sperm flagella were specified as modeling parameters. Additionally, the inter-ODF spacing of bull sperm was incorporated to calculate doublet sliding and bending torque. The resultant computer-simulated pattern of flagellar beating possesses many of the attributes of the beat of a live bull sperm flagellum. Notably, this life-like simulation can be produced using parameters for the central axonemal "motor" that are comparable to those effective in modelling a simple flagellum. In the proposed scheme, the accessory structures of the mammalian sperm axoneme provide increased stiffness while at the same time providing a means to proportionately raise the bending torque to overcome that additional flexural rigidity. This capacity is due to the inter-ODF distances being larger than the corresponding interdoublet spacings. If force is transmitted to the flagellar base by way of the ODFs, then the larger effective diameter generates both a greater bending torque and increased interdoublet sliding. This has the interesting effect of consolidating the energy from more dynein cross-bridges into the production of a single bend. Consequently, greater bending torque development is permitted than would be possible in a simple flagellum. In this way, the same 9 + 2 organization of a simple flagellum can power a much larger (and stiffer) version than would otherwise be possible.

Key words: outer dense fibers, fibrous sheath, connecting piece, dynein, t-force, outer doublets, axoneme, stiffness

INTRODUCTION

The flagella of mammalian sperm are possessed of a common set of distinctive features that together modify the flagellar axoneme from the pattern seen in other,
more simple flagella. In mammalian sperm, each of the outer doublets of the $9 + 2$ arrangement is paired with an accessory fiber (ODF). The ODFs join together at the basal end of the flagellum and unite with the connecting piece. This connecting piece provides a basal anchor for the ODFs, and also for the nine outer doublets that do not terminate in a basal body. In addition to the ODFs, the mammalian sperm flagellum has a sheath surrounding most of the flagellar length. The basal portion of the sheath is composed of circularly arranged mitochondria (which in bull sperm cover the first 12 to 14 µm of the flagellum). Distal to the mitochondrial sheath is a proteinaceous sheath of keratin-like material that forms the fibrous sheath. This fibrous sheath is a tapered cylinder surrounding all but the last few microns of the axoneme. Most of the available evidence supports the view that these modifications provide the structural and mechanical support to stabilize larger and stronger flagella. The ODFs are modified intermediate filaments composed of a heavily disulfide linked keratin-like protein [Bedford and Calvin, 1974; Olson and Sammons, 1980]. This could ideally act to stiffen the axoneme, and the ODFs have been assigned this function by many investigators (Phillips and Olson, 1973; Gibbons, 1973; Woolley, 1979). Likewise, the mitochondrial and fibrous sheaths would reasonably be expected to add stiffness to the flagellum, making it stronger and less prone to kinking or breaking under stress. These modifications may have become necessary in order to sustain the long flagella observed in most mammalian sperm, for as the flagellar length increases so too does the number of contributing dynein motor molecules. Consequently, a larger flagellum is capable of harnessing the power of a proportionately larger number of motor molecules, but only if the basic $9 + 2$ structure is sufficiently reinforced to withstand the additional stresses produced internally. In this study, a hypothetical scheme is examined that brings into play many of the special features of the mammalian sperm axoneme to explain how the mammalian sperm flagellum may function. This theoretical working mechanism is modelled by incorporating the structural modifications of a bovine sperm axoneme into the "Geometric Clutch" model, previously developed to model the motility of simple cilia and flagella [Lindemann, 1994a,b; Linde- mann and Kanous, 1995].

**ANALYSIS AND MODEL**

At the core of the mammalian flagellum is a complete $9 + 2$ microtubule axoneme. Currently, it is assumed that the central axoneme retains the same functional mechanisms that are present in simple cilia and flagella. The "Geometric Clutch Model" of the axoneme [Lindemann, 1994a,b; Lindemann and Kanous, 1995] was used as the starting point of this study. To produce a model of a mammalian sperm flagellum, the special features of bull sperm flagella were integrated into the Geometric Clutch Model, and the resultant behavior of the simulated flagellum was compared to that of living bull sperm.

**SPECIAL FEATURES OF THE MAMMALIAN SPERM FLAGELLUM**

Mammalian sperm flagella tend to be longer than those of simple cilia and flagella. The average length of bull and human sperm flagella is approximately 60 µm [Cummins and Woodall, 1985], while rat and hamster sperm flagella are 150–250 µm in length [Cummins and Woodall, 1985].

The main accessory structures in a mammalian sperm axoneme are the ODFs that are attached to the outer doublets over much of their length [Lindemann and Gibbons, 1975; Olson and Linck, 1977]. Figure 1 shows a cross-section of a bull sperm axoneme with the ODF/outer doublet combinations numbered [per Aifczelius, 1959]. Serial sectioning of mammalian sperm flagellum has shown that all the ODFs taper to a termination in the principal piece [Telkka et al., 1961], and in the case of human sperm the longest of them (typically ODF 1) extends through 60% of the length of the principal piece [Serres et al., 1983]. Rough estimates of the ODF lengths in bull sperm have arrived at the same conclusion [Lindemann and Gibbons, 1975].

At the basal end of the mammalian sperm flagellum the ODFs are anchored into a large cap-like structure, the connecting piece. The outer doublets and central pair microtubules of the central axoneme do not penetrate into the connecting piece region, appearing to terminate without contacting the basal centriole. The proximal centriole is usually within the connecting piece and is at right angles to the axis of the flagellum [Fawcett, 1975]. The distal centriole disintegrates during spermatogenesis and is not present in the functional sperm [Fawcett, 1975]. It appears that the anchoring mechanism usually provided by the distal centriole (basal
Fig. 1. A transmission electron micrographic cross-section of a bovine sperm flagellum. This section shows the typical appearance of an intact axoneme from a bull sperm flagellum with the outer dense fibers (ODFs) numbered for reference purposes. Note that the simultaneous presence of the fibrous sheath and ODFs number 3 and 8 pinpoint the location of this cross-section along the flagellum as being near the midpiece/principal piece junction (as the fibrous sheath is indigenous to the principal piece, while ODFs 3 and 8 are found in the midpiece and do not extend far into the principal piece). Comparison of this section with others having strategically identified locations makes it possible to estimate the inter-ODF spacing at known positions along the flagellum. Extrapolating this information between these points was useful in determining the inter-ODF distances for modeling purposes. (Reproduced from Kanous et al., 1993, with permission of the publisher.) ×110,000.

... body) has been relegated to the connecting piece in mammalian sperm. Subsequently, forces produced by the dynein-tubulin interaction between the outer doublets are ultimately transferred to the ODFs that are anchored to the connecting piece at the flagellar base. A schematic illustration of this concept is displayed in Figure 2.

While this is conceptually a simple idea, it has some very interesting consequences that have not previously been focused upon. If the forces from the dynein-tubulin interaction are first transferred to the ODFs before transmission to the flagellar base, then the bending torques that power the motility must be based on the center-to-center spacing of the ODFs rather than the interdoublet spacing. Since the center-to-center distance between the ODFs is larger than the interdoublet spacing, this results in a greater sliding displacement between doublets when the flagellum bends. Initially, this study recognized that greater torques are produced in larger flagella. Now, it is also obvious that more interdoublet sliding occurs to create a bend in a big flagellum. Of course, more sliding may also equate to more cycles of dynein attachment/detachment, requiring more ATP hydrolysis to bend the larger structure. It also infers that more power output is possible. The situation is analogous to running a car in low gear, making it possible to push a heavier load, but at a sacrifice of speed. Is there evidence for such a geared down operation? In comparing flagellar size and speed, simple sea urchin sperm typically beat at 50 cycles per second, whereas the bigger bull sperm average 15 cycles per second and the even larger rat sperm beat at 5–10 cycles per second.

The second major structural modification of mammalian sperm flagella is the presence of a sheath enclosing the entire axoneme. Again, the major assumption is that the sheath exists to provide additional stiffening and structural support for large flagella that would otherwise be prone to damage or instability. Measurements of the flagellar stiffness of bull sperm confirm the belief that it is much stiffer than a sea urchin flagellum [Lindemann et al., 1973]. At the base of the bovine sperm flagellum, the stiffness equals 4.0 × 10⁻¹² dyne cm², which is approximately twenty times that of a sea urchin sperm flagellum.

Both the sheath and the ODFs taper over the flagellar length. Consequently, the flexibility of a mammalian sperm flagellum is not uniform, but increases as a func-
Fig. 2. A functional schematic diagram of the mammalian sperm axoneme. A: In the mammalian sperm axoneme, the outer microtubule doublets are not anchored into a basal body at the flagellar base, but are attached to the ODFs along much of their length. However, the ODFs are affixed to the striated columns of the connecting piece. Consequently, when the doublets slide by the action of the dynein motors, the resultant force is transmitted through the ODFs to the connecting piece (arrows). The connecting piece assumes the role of a basal anchor replacing the basal body which disassembles during spermatogenesis. B: The sliding induced by the dynein-tubulin cross-bridge cycle during formation of principal and reverse bends results in maximal sliding displacement between elements 1 and 5–6. Unlike in simple flagella, the magnitude of interdoublet sliding is not determined by interdoublet spacing, but is dictated by the inter-ODF distances in the bending plane (identified as Ds in the diagram). Force will also be transmitted to the ODFs, causing the greatest torque to develop from the force imparted to elements 1 and 5–6 because they exhibit the greatest separation (the effective diameter, DE). Os and Xs indicate thrust in the directions into (O) and out of (X) the page if the axoneme were being viewed baseward.

In a previous report [Lindemann, 1994b], a simulation of a simple axoneme was devised that was capable of producing a good facsimile of both a 10 μm cilium and a 30 μm simple flagellum. In this study, it is presumed that the axoneme at the core of the more complex mammalian sperm flagellum utilizes the same working mechanism as that of simple flagella and cilia. If this assumption is correct, adding the special features of a mammalian sperm flagellum to that identical core should enable one to convert the simple axoneme model into a simulated bull sperm flagellum. A conservative approach was taken to test this premise.

The earlier computer simulation, described by Lindemann [1994b], is used without alteration, except for those modifications necessary to incorporate characteristics derived from experimental studies of bull sperm. Modifications were implemented as described below.
Length

The Geometric Clutch simulation is a program written in QBasic that utilizes a structure composed of thirty calculation segments. Each segment is assigned a length of 2 μm, resulting in a 60 μm simulated flagellum consistent with actual measurements of bull sperm flagella.

Stiffness

The experimentally estimated bull sperm stiffness of $4 \times 10^{-12}$ dyne cm$^2$ [Lindemann et al., 1973] was incorporated in the simulated flagellum as the stiffness at the midpiece/principal piece junction (consistent with the site of elasticity determination used in the source report). Because the flagellar sheath and ODFs taper over the length of the principal piece, the stiffness assigned to each of the successive thirty segments was determined as a linear function defined by the $4 \times 10^{-12}$ dyne cm$^2$ midpiece value and $0.4 \times 10^{-12}$ dyne cm$^2$ assigned to the endpiece. Thus, the last segment of the model uses a stiffness value that approaches the range of $0.3-1.5 \times 10^{-12}$ dyne cm$^2$ determined for sea urchin sperm [Okuno and Hiramoto, 1979], and is only a factor of two from the $2 \times 10^{-12}$ dyne/cm$^2$ reported for *Subellaria* cilia [Rikmenspoel and Rudd, 1973]. The following linear relation was used to assign the stiffness at each segment:

$$IE_n = \text{SLOPE} \times (n \times \Delta s) + I_0E \quad (1)$$

Where the slope equals $-7.3 \times 10^{-10}$, $\Delta s$ (the segment length) equals $2 \times 10^{-4}$ cm, and the basal stiffness ($I_0E$) equals $4.8 \times 10^{-12}$ dyne cm$^2$. In this way each segment has its own assigned stiffness, rather than a single, uniform value for the entire flagellum (as would be appropriate for a simple flagellum or cilium). Each assigned stiffness value is used to calculate the elastic moment at that segment by multiplying the local stiffness ($IE$) times the local curvature ($\frac{d\theta}{ds}$).

$$M_{\text{ELASTIC}} = IE \times \frac{d\theta}{ds} \quad (2)$$

At each iteration of the model, an elastic moment is calculated that exactly balances the active moment acting on the segment in question. This theoretical balance point defines an equilibrium curvature at each segment, toward which the curvature at each segment is allowed to decay at a rate dictated by the viscous drag.

Effective Diameter

As discussed above, and illustrated in Figure 2, the geometry of the mammalian sperm axoneme transmits the active shear force to the flagellar base through the ODFs. Therefore, the sliding displacement of one doublet relative to its neighbor depends on the distance between the two associated ODFs and not on the interdoublet spacing. This concept was introduced into the model by estimating the center-to-center distances between ODF numbers 2 and 4, and between 7 and 9 using electron micrograph cross-sections of bovine sperm axonemes. These distances were then used to determine the effective diameter for calculating the sliding displacements of doublets 2, 3, and 4, and doublets 7, 8, and 9. The diminution in size of the ODFs toward the endpiece of the flagellum required that inter-ODF distances be estimated from electron microscopic cross-sections near the flagellar base, and at or near the midpiece-principal piece junction. (Fig. 1 exhibits an example of the latter.) A profile of spacing was created to estimate the tapering of the ODFs by using these two reference points. These inter-ODF distances (IOD), when multiplied by the curvature ($\frac{d\theta}{ds}$) and length of the segment ($\Delta s$), yield the local contribution to interdoublet sliding:

$$\text{LOCAL SLIDING} = \frac{d\theta}{ds} \times \Delta s \times IOD \quad (3)$$

Adding each local contribution, starting from the flagellar base, yields the total sliding at any segment $n$.

$$\text{SLIDING}_n = \sum_{i=1}^{n} \text{LOCAL SLIDING} \quad (4)$$

The individual sliding displacements are then used to calculate the local and global $f$-forces involved in dynein bridge switching, using the same method given in the previous studies [Lindemann, 1994a,b].

This treatment is, of necessity, a simplification. In order to bend a composite structure, some structural distortion is required. Since the ODFs, over much of their length, are larger and denser than the outer doublets, the assumption has been made that the compliance will be in the outer doublet length, and not the ODF length. In reality, it may be more complex, but the adopted simplification is probably workable for the following reason. The ODFs are smallest where they taper in the principal piece. There the simplification would be least valid, since the outer doublets might be stronger and less deformable than the ODFs in that region. However, in that same transitional region, the inter-ODF spacing converges toward the inter-doublet spacing and the required amount of distortion therefore becomes minimal.

In a complete axoneme, the interdoublet force contributed by dyneins can be distributed to the outermost ODFs, numbers 1 and 5–6. Doublets 5 and 6 are permanently linked, and therefore can be expected to act as
a single entity [Lindemann and Gibbons, 1975]. Consequently, the distance between ODF 1 and the linked ODFs 5–6 would appear to be the correct effective diameter for calculating the active moments in a mammalian sperm. Estimates of the inter-ODF center-to-center distances between ODFs 1 and 5–6 were also measured from bovine sperm micrographs. Again, distances were measured near the flagellar base, and at or near the midpiece-principal piece junction, to extrapolate a matrix of effective diameter approximations for use in calculating the active moment at each segment. The two matrices used in modeling the bull sperm are appended.

Bull sperm nuclei are often observed adhering to the microscopic slide, causing partial immobilization of the cell due to the fact that the cell is no longer free to pivot as the head encounters drag from contact with the slide surface. In order to permit a more direct comparison of the model output with the actual motility of live sperm, the program was slightly modified from the earlier version. The new algorithm solves for a fractional offset of the total drag torque at the base, instead of solving for the boundary condition where the drag torque equals 0 at the base (a free-pivoting version.) This new version allows the simulation to represent the condition where the base can provide some viscous drag to counter the viscous moment provided by the flagellum. The basal drag is specified by a number between 1 and 0, where 1 is completely fixed, and 0 is completely free to pivot. Using this new formulation, the simulation is now very successful at mimicking the behavior of bull sperm attached by their heads to a slide or Petri dish.

RESULTS

The modified flagellar simulation, incorporating the length, stiffness, and inter-ODF spacing as estimated for a bull sperm, is able to develop a stable repetitive pattern of beating. Simulations were computed for the equivalent of twenty beat cycles to insure that a stable periodic pattern was obtained. Figure 3 presents plotted output from the revised model side by side with tracings of the beat of live bull sperm. The modeled behavior is compared with live sperm under conditions where the sperm head is relatively free to rotate (Fig. 3A and D), and also where the flagellar base (or head) is firmly attached to the slide (Fig. 3C and F), and for an intermediate condition where a substantial fraction (40%) of the basal torque is offset by surface drag (Fig. 3B and E). The modeling parameters utilized in the displayed computer output are given in Table I. Note that the force per dynemin head was kept at $1 \times 10^{-7}$, which is in the same range as the values that produced good facsimiles of ciliary and flagellar motion when modeling a simple axoneme ($1.2 \times 10^{-7}$ dyne was used for the cilium, and $4 \times 10^{-8}$ was used for the simple flagellum in Lindemann [1994b]). The same is true of the nexin link elasticity constant; it is identical to the value used in the earlier model of a simple flagellum. The drag value used is based on the estimation method given by Rikmenspoel [1965] for bull sperm, utilizing a viscosity of 0.012 poise. The resultant beat frequency averages 7.4 hertz when the head is fixed, and 13 hertz when the head is pivoting. This is somewhat reduced from measured frequencies for live bull sperm at 37°C, which beat at an average frequency of 13.8–23.8 hertz [Rikmenspoel, 1965], but is higher than the traced beats shown in Figure 3, which are from live sperm at 23°C. The live sperm displayed in Figure 3 have frequencies of 3.5 hertz (head fixed), 4.6 (head partially free), and 6.0 hertz (head pivoting).

Figure 4 displays a series of simulations conducted to explore the model’s performance under the individual influences of modified stiffness and modified working diameters. In order to provide the best comparisons between conditions, all of the simulations were run using the same bridge force value ($8 \times 10^{-8}$ dyne), the same specific resting probability of attachment for P and R bridges (0.06 and 0.03, respectively), and the same nexin link elasticity (0.03 dyne/cm). In Figure 4A, the complete bull sperm simulation is run with one change: a uniform stiffness of $2 \times 10^{-15}$ dyne cm$^2$ has been substituted, a value typical of a simple axoneme. In Figure 4B, the simulation maintains the stiffness value of a bull sperm, but the torque and t-force calculations have not been corrected for the effect of the ODFs. Figure 4C exhibits output from the bull sperm simulation which combines both the correct stiffness values and the correct working diameter contributed by the presence of the ODFs. The combination of both effects results in a bull sperm-like beat pattern. It also yields an intermediate frequency as a result of better balance between torque and elastic resistance. The unstiffened flagellum in Figure 4A displays an irregular, uncoordinated beat with a substantial variance from one beat to the next (see insets Fig. 4A). This instability does not appear when the stiffness is increased (Fig. 4B). However, without the amplified torque provided by the ODFs, there is little wave propagation and a very sluggish beat. The combination of both elevated stiffness and the amplification of the torque restores stable beating and bull sperm-like wave propagation. The same values of bridge force ($8 \times 10^{-8}$ dyne) and the same resting probabilities of bridge attachment (0.06/0.03) will also yield stable beating in the simple axoneme simulation as published earlier [Lindemann, 1994b], and displayed here in Figure 4D and E for a 10 μm cilium and a 30 μm flagellum. This confirms that the different patterns of motion are not dependent on unique selections of the bridge force, nor are
Fig. 3. Tracings of live bull sperm and computed simulations. A, B, and C are traced positions of live bull sperm at 23°C from videotaped images using strobed illumination. Each tracing is at 0.0166 seconds. D, E, and F are computed simulations using the version of the Geometric Clutch Model that has been modified to use the dimensions and stiffness of a bull sperm. All three of the live sperm were partially immobilized by having the head stuck to a microscope slide. In A, the head is fairly free to pivot (basal drag = 0.2). In B, it is somewhat they the result of varying the ratios of any of the individual modelling parameters. Using common parameters for bridge force, nexin elasticity, and bridge attachment probabilities, the resultant simulations of a simple flagellum (using a 30 µm flagellum with the base free to pivot) and a complete bull sperm are shown side by side in Figure 4E and F. Stable beating, good bend propagation, and similar wave form are demonstrated in both cases. The bull sperm model modifications allow the flagellum to be scaled to twice the size of a simple flagellum without developing mechanical instabilities, and without necessitating fundamental changes in the t-force switching mechanism.

Many important features of the mammalian sperm beat are spontaneously manifested in the modified simulation. The beat frequency of the revised model is slower than a simple flagellum. The simple flagellum version of the same model displayed a beat frequency of \( \approx 59 \) hertz [Lindemann, 1994b], which is comparable to the 46 hertz reported for live sea urchin sperm [Gibbons and Gibbons, 1972]. This value drops to 13 hertz in the revised bull sperm model, which falls near the range previously reported by Rikmenspoel [1965]. Additionally, the bends have a smaller maximal curvature than those of simple cilia or flagella. A result of this lesser curvature is that the bends are longer and involve a greater number of dyneins pulling together in the same bend (Fig. 5). The participation of the additional dyneins allows the torques produced to be proportionately larger as well (Fig. 6). So, more dyneins are pulling together to create a single bend, generating a force that acts across a greater effective diameter to yield a very high torque. Figure 6 presents an adjusted comparison of the torques developed in the bull sperm model shown in Figure 4C to
### TABLE I. Modeling Parameters for Ciliary/Bull Sperm Simulation

<table>
<thead>
<tr>
<th></th>
<th>Simple Axoneme</th>
<th>Bull Sperm</th>
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<tbody>
<tr>
<td></td>
<td>10 μm</td>
<td>30 μm</td>
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<tr>
<td>Length (cm)</td>
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<td></td>
</tr>
<tr>
<td>No. of segments</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Functional diameter (D) (cm)²</td>
<td>— 1.0 × 10⁻⁵</td>
<td>[1.00 to 1.95] × 10⁻⁵</td>
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<tr>
<td></td>
<td>—</td>
<td>1.0 × 10⁻⁵</td>
</tr>
<tr>
<td>Between doublets/ODFs 2 and 4</td>
<td>—</td>
<td>[1.46 to 3.25] × 10⁻⁵</td>
</tr>
<tr>
<td>Between doublets/ODFs 1 and 5–6</td>
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<td>—</td>
</tr>
<tr>
<td>Iteration interval (sec)</td>
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<td>— 0.005</td>
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<td>Drag coefficient (dyne cm⁻² sec)</td>
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<td>— 0.025</td>
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<tr>
<td>Passive stiffness (IE) (dyne cm⁻²)</td>
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<td>— 0.42 to 4.81 × 10⁻¹²</td>
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<td>Force per active dynein head (dyne)</td>
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<td>1.0 × 10⁻⁷</td>
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<td>Elastic constant per nexin link (Kₑ) (dyne/cm)²</td>
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<td>— 0.03</td>
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<td>Transfer coefficient P-R</td>
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<td>Resting probability of dynein bridge attachment (Base.P)</td>
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<td>For principal bend bridges</td>
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<td>For reverse bend bridges</td>
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<td>Adhesion scaling factor</td>
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<td>Dynein heads per segment</td>
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<td>8,000</td>
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<tr>
<td>In principal file</td>
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<td>400</td>
</tr>
<tr>
<td>In reverse file</td>
<td>— 130</td>
<td>400</td>
</tr>
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</table>

²Functional diameter is based on the spacing between doublets 2–4, and between doublets 1, 5–6. The simple cilium model used a single value for D. The bull sperm adaptation uses the inter-ODF spacing between elements 2 and 4 (or 7 and 9) for t-force calculation, and the full spacing between elements 1 and 5–6 was used for the bending torque calculation. Additionally, the range of inter-ODF spacing from the tip to the base of the flagellum is shown in brackets.

bValues in brackets are the range of stiffness from the tip to the base of the flagellum.

Based on a 100 nm nexin spacing.

From t-force to bridge attachment probability.

From adhesion force to bridge attachment probability.

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those in the ciliary simulation shown in Figure 4D. Since the cilium model uses 100 nm as the working diameter approximation, instead of the true functional diameter between doublets 5–6 and 1, the resulting torque values have been multiplied by 1.4 to provide a fair comparison. Figure 6 shows that torque generated at segment 2 in the bull sperm model peaks at 4.4 × 10⁻⁷ dyne cm, while in the simple cilium model a peak value of 2.0 × 10⁻⁶ dyne cm is developed at segment 2. The t-force profile of the flagellar beat is displayed in Figure 7. The t-force is the force acting between doublets (or in the case of mammalian sperm, between ODFs) to compress or distend the axoneme in the plane of beating. The magnitude of the t-force in simple cilia and flagella is already surprisingly large [Lindemann, 1994b; Lindemann and Kanous, 1995], raising the question of how the axoneme can maintain its integrity. In the case of bull sperm, the t-force is even larger, and the issue of how the axoneme remains intact becomes profound.

**DISCUSSION**

Mammalian sperm possess a number of interesting modifications in their flagellar ultrastructure. The most obvious of these features are the nine outer dense fibers (ODFs) paired to each of the nine outer microtubule doublets. These fibers are functionally attached along most of the principal piece to each corresponding doublet, and the basal anchoring mechanism of the axoneme is through these fibers rather than directly to the basal body [Fawcett, 1975; Lindemann and Gibbons, 1975]. Accordingly, the pattern of force transmission in these sperm is affected by this unique structural arrangement. Transmitting the forces generated by the dynein bridges through the ODFs increases the effective diameter of the flagellum. Increasing the effective diameter raises the active moment (torque) developed by a given number of dynein motors. It also amplifies the amount of sliding taking place as the flagellum bends.

Incorporating this principle of action into the earlier "geometric clutch" model of a simple axoneme successfully produces a bovine sperm-like flagellar beating pattern when a flagellum of the size and stiffness of a bull sperm is simulated. This is interesting from a second aspect. The greater sliding and larger bending moments which are generated by the ODF/doublet geometry have been increased just sufficiently to bend a structure that has been stiffened by the added structures of the ODFs.
Fig. 4. Comparative simulations to evaluate the effects of stiffness and the ODFs on the beat cycle. All of the simulations displayed utilized common values for bridge force \((8 \times 10^{-8} \text{ dyne})\), nexin elasticity \((0.03 \text{ dyne/cm})\), and \(P\) and \(R\) bridge attachment probabilities \((0.06\) and \(0.03\), respectively.) Each figure exhibits one complete cycle of beating, at least three cycles into the simulation run to insure adequate time for the beat to stabilize. The insets in \(A\) also display two additional beat cycles, the ones previous to (lower) and subsequent to (upper) the main figure. In \(A\), the bull sperm simulation is displayed substituting a single, uniform stiffness of \(2 \times 10^{-13} \text{ dyne cm}^2\) for the stiffer, tapered function used in the complete bull sperm model. The bull sperm simulation in \(B\) replaces the matrix of diameter values (derived from the incorporation of the varied ODF spacing) used in the complete model with a single, continuous working diameter of 100 nm. In \(C\), the simulation is that of the fully restored bull sperm model. The figures displayed in \(A\), \(B\), and \(C\) all have immobilized basal ends, for easier comparison. \(D\) and \(E\) consist of output from the earlier Geometric Clutch model [Lindemann, 1994b], employing the same bridge force, nexin elasticity, and \(P\) and \(R\) probability values as used in the bull sperm simulations in \(A–C\). This uniformity in values allows comparison of the behaviors of the 10 \(\mu\)m ciliary and 30 \(\mu\)m flagellar simulations of the previous report, utilizing standardized modelling parameters. A bull sperm simulation at the same settings, but with a free, pivoting base is displayed in \(F\). Matching \(D\) and \(E\) to \(C\) and \(F\) allows direct comparison of the individual effects of stiffness and ODFs on scaling up the original model to a 60 \(\mu\)m bull sperm flagellum. These modifications permit a much larger structure to generate a similar oscillation cycle while using the same basic axonemal parameters. The bars in \(D\), \(E\), and \(F\) are each 5 \(\mu\)m, to provide perspective of scale. Plotting intervals are as follows: \(A = 0.0045\) sec, \(B = 0.009\) sec, \(C = 0.009\) sec, \(D = 0.0012\) sec, \(E = 0.0009\) sec, and \(F = 0.009\) sec.

and the sheath. Nature has proportionately increased both the torque and stiffness of a mammalian sperm flagellum to allow it to be both larger and stronger, while utilizing the same central axoneme of a simple flagellum as the driving motor. The model demonstrates that while the same basic motor can be employed, the mammalian version is the flagellar equivalent of an eight cylinder engine compared to the simpler four cylinder design. This is accomplished by involving a longer stretch of axoneme in each bend formation cycle due to the extra stiffness of the flagellum. This, in turn, entails that a greater number of dynein arms are pulling together when generating each bend. It appears that the generation of greater force is achieved by a concomitant decrease in flagellar curvature effected by the presence of the stiffening properties of the sheath and ODFs.

While the model suggests that a bull sperm axoneme proportionately increases the torque development just sufficiently to bend a larger, stiffer flagellum, it does not tell us what advantage is achieved by having larger, stiffer sperm flagella. Perhaps a longer wavelength and slower beat facilitate motility in high viscosity media, like the mucus of the mammalian female reproductive tract. Large mammalian sperm (i.e., bull sperm) have been observed to move well under increased viscous loading [Rikmenspoel, 1984]. This ability to push hard against heavy, viscous loads is related to the flagellar capacity for optimized torque development. As demon-
Fig. 5. Numbers of dynein bridges contributing to bend formation in cilia and sperm flagella. The number of dynein bridges contributing to the bending moments at segment 2 (one segment from the base) is shown for one beat cycle of the bull sperm model (as shown in Fig. 4C). Directly comparable data from the simple cilium model (as shown in Fig. 4D) is co-plotted. For best direct comparison, both are shown for the condition where the base is immobilized. Note that development of a single bending episode in a bull sperm recruits the action of approximately ten times as many dynein bridges as that required by a 10 μm cilium.

Fig. 6. Comparison between the torque development of a bull sperm flagellum and that of a simple 10 μm cilium. The bending torque at segment 2 (one segment from the base) is displayed for both a simulated bull sperm flagellum and a simulated simple cilium. The simulations of the simple cilium and the bull sperm are the same as those shown in Figure 4C and D, and listed in Table I. However, since the simple cilium model used 100 nm as the functional diameter approximation, instead of the true spacing between doublets 5–6 and 1 (~140 nm), the cilium values have been multiplied by 1.4 to provide a fairer comparison. Together, the greater number of contributing dynein bridges and the greater effective diameter contributed by the outer dense fibers in bull sperm result in approximately a 30X differential in the bending torque between a bull sperm flagellum and a simple 10 μm cilium.

In an early study of bull sperm motility [Gray, 1958], it was recognized that the propulsive properties of bull sperm flagella increase progressively as one travels toward the distal end. This characteristic seems to stem from the greater stiffness of the mammalian sperm flagellum, particularly the extreme stiffness near the base of the flagellum. The motility of bovine sperm was likened to that of a fish, where the front end provides a fulcrum against which the tail can exert propulsive effort. In comparison, sea urchin sperm motility was equated to that of an eel, where the motion is nearly the same throughout. Gray [1958] proposed that each type of movement was dependent on length and flexibility. Phillips and Olson [1973] noted that the radius of flagellar bend curvature was inversely related to the size of the ODFs in many mammalian species. Another study of certain infertile human sperm [Serres et al., 1986] identified flagella with a very low amplitude of curvature in the first half of the flagellum. Upon electron microscopic investigation the only perceivable ultrastructural defects involved ODF anomalies. This also points to the possible role of ODFs in limiting flagellar curvature.

The role of the connecting piece as a basal anchor is supported by recent work of Woolley and Bozkurt [1995]. This study was conducted on Gallus domesticus (rooster) sperm, which are similar to mammalian sperm in that they have a basal connecting piece and ODFs [Nagano, 1962]. Investigators have shown that isolated flagella which are missing the basal connecting piece
Pivoting base t-force profiles

**Fig. 7.** The internal force transverse to the axoneme (t-force) developed in the bull sperm simulation. The t-force represents the force acting to squeeze doublets together (positive t-force) or spread doublets apart (negative t-force). The t-force at each of the 30 modelling segments of the bull sperm simulation is displayed at intervals of 0.012 sec (every 24 iterations) over one beat cycle. The modelling conditions used are given in Table I, the output of which is shown in Figure 3A. T-force was calculated using the same formulations given in Lindemann [1994b].

will not spontaneously reactivate with ATP. Furthermore, pinching the base of the flagellum to mechanically re-anchor the internal fibers does restore flagellar beating. These findings support a major premise of the current analysis; the connecting piece provides the necessary basal resistance to microtubular sliding. This anchoring function is necessary for bending torque generation, and it is a key element in normal dynein bridge switching. Both functions performed by the basal anchor are predicted as necessary for axonemal operation by the geometric clutch hypothesis [Lindemann and Kanous, 1995]. In simple flagella, the needed sliding resistance is provided by the basal body, while in mammalian and rooster sperm this opposition to sliding is provided by the connecting piece.

The sheath of mammalian sperm is another special feature with possibly unexplored functional significance. Analysis of the t-force acting in bull sperm (Fig. 7) reveals that the t-force in large sperm is proportionately increased over that of a smaller, simple flagellum. The peak t-force is negative, which in the convention of the geometric clutch model is that force acting to rip the flagellum apart (to split the axoneme in half). Could it be that the sheath is a secondary containment system provided to contain the distortions of the axoneme within a sub-critical level? In discussions with a colleague, Dr. Patricia Olds-Clarke, the author has been made aware of the similarity of the flagellar sheath to anatomical structures called retinacula. The sheath may in fact serve as a retinaculum to allow the tendon-like ODFs to curve around a bend without buckling away from the axoneme. In essence, it may help to restrain the outward thrust of the t-force. Detailed electron micrographs of rapidly fixed, swimming hamster sperm seem to reveal that this is exactly the case in the midpiece region [Woolley, 1977], where the ODFs appear to move away from the central axoneme and press against the restraining envelope of the mitochondrial sheath. The radially directed t-force is greatest in the basal portion of the flagellum. It may be significant that the ODF/outer doublet junctions observed in the principal piece are not typically present in the middle piece. This suggests that the ODFs may be free to move away from the central axonemal in the basal area of the flagellum (where the outward t-forces are very large), implicating the mitochondrial sheath as a containment structure in this part of the sperm flagellum. When bull sperm are subjected to a freeze-thaw method that removes the mitochondrial sheath, the addition of Mg-ATP causes the axoneme to split, extruding microtubules/ODFs from the unrestrained midpiece and (unlike reactivated cells) motility does not result [Lindemann et al., 1980; Kanous et al., 1993]. This observation is supportive of the retinaculum concept; however, proteolytic and glycolytic degradation may be contributing to the axonemal disintegration. In order to confirm the role of the mitochondrial sheath in maintaining the integrity of the axoneme, it will be necessary to demonstrate that splitting can occur when both proteolysis and glycolysis have been inhibited. If experimental support is obtained for a restraining function by the mitochondrial sheath, the model will be modified to test the consequences of such a structure on t-force and torque development in the basal region.

**CONCLUSION**

In conclusion, using the original geometric clutch model as a template, it can be demonstrated that the basic
Axonemal motor is capable of driving the motility of the larger mammalian sperm. The main distinctive structures of the mammalian sperm flagellum (the ODFs, the sheath, and the connecting piece) contribute to the functionality of the larger flagella in the following ways:

1. The ODFs and sheath provide stiffness that reduces the maximum flagellar curvature, distributing each bend over a longer span of the axoneme. This enables a greater number of dyneins to work together to develop greater torque.

2. The design of the connecting piece/axoneme junction requires that force be transmitted through the ODFs, thus substantially enlarging the working diameter to boost torque production. This larger working diameter also increases the interdoublet sliding and may also increase dynein cross-bridge turnover. Production of higher torque at the expense of more cycles of the motor is the equivalent of gearing down the operating mode. Speed is sacrificed for increased torque.

3. The higher torque production and additional stiffness are proportional, allowing the same basic molecular motor (dynein) to generate the force necessary to produce and propagate bends in the larger flagella.

4. Although it still speculative, the sheath may play a role in restraining the very large t-forces developed in large mammalian sperm axonemes (acting as a retinaculum).

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REFERENCES


Array of Values That Was Used to Simulate the Inter-ODF Spacing in a Bull Sperm*

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*At each modeling segment, a value was assigned for the inter-ODF spacing between elements 2 and 4. The spacing between elements 2 and 4, and that between elements 7 and 9 were found to be nearly identical, therefore the same matrix values were used for calculations on both the P and R bend sides of the axoneme. The second spacing value is the estimated center-to-center spacing between element 1 and elements 5-6. The 2-4 (7-9) spacing values were used to calculate the interdoublet sliding displacements. These sliding displacements were then used to find the stretch of the nexin links, an important component in determining the t-force that controls the engagement and disengagement of the dynein bridges as proposed earlier [Lindemann, 1994b]. In the bull sperm version of the model, it is assumed that force from the dynein bridges is ultimately conveyed to elements 1 and 5-6 (as shown in Fig. 2). Therefore, the active moment for bend production at each segment was calculated using the second array value multiplied by the force contributed by the active dynein molecules.