STARTING TRANSIENTS IN SEA URCHIN SPERM FLAGELLA

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ABSTRACT

Live sea urchin spermatozoa were rendered immotile by lowered pH; Triton-extracted spermatozoa were rendered immotile by either lowered pH or by deprivation of ATP. The spermatozoa began to beat after an increase in pH or as ATP was supplied, and the first bends were recorded on ciné film. Triton-extracted spermatozoa deprived of ATP retained a partially formed basal bend which could be either principal or reverse, and which resumed its development and propagation as ATP was supplied. Both live and tritonated flagella straightened at low pH. As the pH was increased, a series of principal bends formed near the base and propagated to the tip. Reverse bends began to develop as the pH continued to increase. The principal and reverse bends thus exhibited different sensitivities to pH, which suggests differences in the mechanisms that produce them. Straight flagella began to move by synchronous sliding all along the flagellum, thus forming principal bends. Flagella that contained a basal bend began to move by primarily metachronous sliding within that bend.

KEY WORDS flagella · spermatozoa · motility · pH · transients

Although some studies have been made of transient activities of flagella (e.g., references 10, 11, 16, 18, 23, and 27) and cilia (e.g., references 21, 29, and 30), most studies have concentrated on the steady-state beating of these organelles. In the study presented here, starting transients were observed. Quiescent sea urchin spermatozoa—both live and tritonated—were recorded on ciné film as they began to beat. The steady-state beating of tritonated flagella in solutions at lowered pH was also filmed.

Some of the results have been presented at meetings (13, 15). Related experiments have been reported by Gibbons (7) and Rikmenspoel (24).

MATERIALS AND METHODS

Spermatozoa were obtained by injecting the California sea urchins Lytechinus pictus and Strongylocentrotus purpuratus with 0.6 M KCl (31), and were then stored on ice. They exhibited some motility at first, but became quiescent within minutes. For experiments on live spermatozoa, samples were diluted ~1:5,000, as needed, in artificial seawater containing 470 mM NaCl, 10 mM KCl, 54 mM MgCl₂, 10 mM CaCl₂, 10 mM tris buffer, and usually containing 0.25% (wt/vol) bovine serum albumin, at pH 5.3, in which they briefly exhibited very asymmetric beating before becoming quiescent. Resumption of beating was then induced by introduction of the same solution, at pH 8.2, from the tip of a micropipette (10, 27). This technique was used to control the commencement of beating for filming. The initial bends induced under these conditions appeared very similar to those which formed when concentrated spermatozoa were diluted directly into seawater at pH 8.2.

Tritonated spermatozoa (5) were observed under two sets of conditions: (a) they were suspended in reactivating solution that contained 10⁻⁶ to 10⁻⁷ M free Ca²⁺ at pH 8.0 and lacked ATP, and were induced to beat by introduction via micropipette of the same solution containing 0.2 mM ATP, and (b) they were suspended in reactivating solution that contained 0.2 mM ATP, at pH...
Live spermatozoa were often rather straight and 7.3–7.5, in which they were quiescent, and were induced to beat by introduction via micropipette of the same solution at pH 8.0. Steady-state beating in solutions of intermediate pH was also observed.

Micropipette tips were broken to diameters of about 10–20 μm to provide reasonably uniform supplies of activating solutions along flagella. Pipettes were maneuvered with a Research Instruments model TVC 500 micromanipulator (Bailey Instruments Co., Inc., Saddle Brook, N. J.). A length of solution-filled polyethylene tubing was connected to the back of the pipette, and the flow rate was controlled by adjusting the height of the tubing.

A Zeiss WL microscope was used with a Zeiss X 40 water-immersion objective lens and a Zeiss “ultra” oil-immersion dark field-condenser (Carl Zeiss, Inc., New York). A Zeiss split-beam trinocular head was used with ×10 wide-field viewing oculars. One of the viewing oculars contained a reticle insert on which an image of the photographic field had been drawn, so that this field plus a large surrounding area could be viewed through the binocular eyepieces during filming. Results were recorded on 16-mm Kodak 4X negative film with a Locam model 51 camera (Redlake Corp., Photo Instruments Div., Santa Clara, Calif.), at between 20 and 200 frames per second. A ×4 ocular and an ×0.4 reflex adapter produced a magnification on the film of ×64. A Chadwick-Helmuth stroboscopic illuminator (Chadwick-Helmuth Co., Inc., Monrovia, Calif.) (1) was triggered by an independent oscillator during observation, and by amplified synchronizing pulses from the camera during filming. Experiments were generally performed at ~15°C.

A sperm suspension was placed between the objective lens and the slide and left for several minutes to allow some spermatozoa to settle to the slide. For the filming of live spermatozoa, the pipette was placed in or near the photographic field, and quiescent spermatozoa were introduced into the field by moving the slide just before starting the camera. Generally, the plane of beating was not parallel to the slide, and thus spermatozoa only occasionally remained in focus long enough to be filmed successfully. Tritonated spermatozoa, however, could be conveniently stopped and started by the introduction and withdrawal of the pipette, so usable tritonated spermatozoa could often be selected before filming was begun.

Measurements were made directly from the negatives on a Vanguard film analyzer, using an M-16C head, a C-11 case and an A-11 screen (Vanguard Instrument Corp., Melville, N. Y.). Curvature of bends was measured by overlaying circles of known size (12); angles were measured with the projector’s angle-measuring screen.

RESULTS

Live Spermatozoa

Live spermatozoa were often rather straight when quiescent, and the heads were often tilted slightly in the direction of the “reverse” bends (8), as shown in Figs. 1–4. Beating sometimes began with the formation of a sharp bend at the base, in a fashion which resembled the formation of new bends during steady-state beating (12, 14), as shown in Fig. 1. This type of flagellum resembled cilia: an “effective” stroke, which involved synchronous sliding of the microtubules and the formation of a basal bend, was followed, after a delay, by a “recovery” stroke in which that bend traveled to the tip. Usually, however, beating began with the formation of a deep bend along the basal 25–30% of a flagellum, as shown in Fig. 2. The length and radius decreased at first, in contrast to the development seen during steady-state beating. When the subtended angle reached ~1.6 rad the bend began to travel to the tip. It increased in radius and bend angle as it traveled and typically attained an angle of ~3.4 rad. It often required several seconds to develop before propagating, but typically traveled to the tip rather rapidly, and attained a speed of about one-third of that attained during steady-state beating. When normal beating developed, this initial bend could be seen to have been in the direction of the “principal” bends (8).

Normal beating ensued when a reverse bend formed at the base as the principal bend began to travel to the tip. This sometimes occurred as the first bend passed to the tip, as shown in Fig. 3. More commonly, however, several principal bends developed and traveled to the tip before the first reverse bend developed.

The development of the first bend near the base was often accompanied by the formation of a broad, more distal, curve in the opposite direction, as shown in Figs. 2–4. This curve usually subtended a smaller angle than the developing basal bend, and usually did not propagate, as shown in Figs. 2 and 3. However, it occasionally developed into a traveling bend, as shown in Fig. 4.

Tritonated Spermatozoa: ATP

Flagella can stop in rigor, with bends maintained along their length, when their supply of ATP diminishes rapidly (9). However, the supply of ATP was usually depleted slowly enough in these experiments so that flagella were straight or only gently curved along most of their length before beating was induced, as shown in Figs. 5 and 6. In media that lacked ATP, these sperma-

\[ \text{\textit{The Journal of Cell Biology} \cdot Volume 80, 1979} \]
Figure 1-3 Selected frames from films of live *L. pictus* spermatozoa that show the development of bends upon increase of pH. Numbers in lower right-hand corners are elapsed time (in milliseconds) from first frame. Bar of Fig. 3, 50 μm.
tozoa usually had a partially formed basal bend that typically subtended about 30–60% of the maximum angle that it would obtain after introduction of ATP. These flagella could be stopped and started repeatedly, and a flagellum could exhibit some arrests with the basal bend in the direction of principal bends and others in the direction of reverse bends. This bend increased in length, radius, and subtended angle and traveled toward the tip when ATP was supplied, as shown.
in Figs. 5 and 6. As this bend developed, the next bend began to form in the opposite direction, in a manner similar to that seen during steady-state beating (12). The angles of these two developing angles often, but not always, increased at the same rate, as shown in Figs. 5 and 6. The subsequent behavior of the new basal bend appeared to depend on the concentration of ATP: When the speed of propagation of the original bend was more than about 15–25 μm-s⁻¹—which is typical for beating of about 0.5–1.0 Hz and indicates ATP concentrations of about 0.005 mM (8)—the new basal bend continued to develop and propagate normally, so that a typical steady-state waveform was attained as the first bend reached the tip, as shown in Fig. 5. With lower ATP concentrations, the development of the new basal bend was delayed, and so it remained partially formed at the base as the original bend traveled to the tip, which resulted in an abnormally long straight region between them. In extreme cases, the first bend reached the tip before the next one completed its development and began to propagate, as shown in Fig. 6.

**Tritonated Spermatozoa: pH**

Tritonated spermatozoa of both *L. pictus* and *S. purpuratus* tended to beat asymmetrically at lowered pH's. However, the percentage of the spermatozoa of *L. pictus* that were motile decreased appreciably below pH 8.0, and only those of *S. purpuratus* were photographed during steady-state beating. No consistent differences were noted between the starting transients of these species.

When tritonated spermatozoa of *S. purpuratus* were suspended in reactivating solution adjusted to pH 7.5–7.7 (0.1–0.2 pH units above the highest pH at which they were quiescent), they beat very asymmetrically, as shown in Fig. 7. Bends began to form in both directions at the base. Those in one direction developed and propagated, although they attained a smaller final angle than at pH 8.0, while those in the other direction failed to develop and propagate. Observations on starting transients in live spermatozoa suggest that the propagating bends were principal bends, but tritonated flagella usually beat rather symmetrically at pH 8.0, and so their principal and reverse bends could not always be distinguished easily (5). The beat frequency was about one-third of that at pH 8.0.

Quiescent tritonated flagella at low pH could be reasonably straight, as shown in Fig. 8, or curved, as shown in Fig. 9. This curvature sometimes spread over most of a flagellum, but it was often confined to about one-third of the flagellum nearest the base, and typically subtended about 2.5 rad. When the pH was raised, straight flagella sometimes began to form a bend with a sharp basal angle, as shown in Fig. 8, although they...
FIGURES 8 AND 9 Selected frames from films of tritonated S. purpuratus spermatozoa that show the development of bends upon increase of pH. Numbers in lower right-hand corners are elapsed time (in milliseconds) from first frame. Same magnification as Fig. 3.

usually formed bends similar to the typical starting bends seen in live spermatozoa. When a bend was already present, it continued to develop as pH was increased, and traveled to the tip, as shown in Fig. 9. The angle of this bend generally increased, and typically attained a value of about 3.5 rad. The radius generally decreased, and there was usually a stage in which this bend included a sharp angle at the base. This initial bend often attained a speed of propagation similar to those of steady-state bends at pH 8.0. The results of increase of pH in live spermatozoa suggest that the initial bends were principal bends. A new bend often formed at the base as the initial bend began to travel to the tip, and a flagellum could exhibit a normal steady-state waveform when the first bend reached the tip, as shown in Fig. 9. They could also exhibit a series of very asymmetric cycles before normal beating ensued, as shown in Fig. 8. The duration of the asymmetric beating presumably depended upon the speed of increase of pH.

DISCUSSION

Echinoderm spermatozoa can be rendered quiescent with either CO₂ or low pH (6, 20); sea urchin spermatozoa are probably quiescent within the animal because of high CO₂. The difference between the pH's needed to render live and reactivated spermatozoa quiescent in this study suggests that an appreciable pH gradient can be maintained across the plasma membrane of a spermatozoon.

Live echinoderm spermatozoa beat somewhat asymmetrically (14, 17). The waveforms of reactivated echinoderm flagella, which are rather symmetric in media of low Ca²⁺ (5), can be rendered asymmetric with high Ca²⁺.

Gibbons (7) has reported asymmetric commencement of beating in live spermatozoa of the sea urchin Tripneustes gratilla, which stop and start in seawater containing 0.2 mM EDTA. They maintain principal bends at the base and tip when quiescent, and, during the first several cycles, exhibit asymmetric beating which resembles the steady-state beating seen in tritonated spermatozoa in media which contains a high concentration of Ca²⁺. Beating occurs only near the base at first. The region of beating expands to the tip during several cycles. The initial appearance of a principal bend resembles the pH-dependent behavior of spermatozoa seen in the present study.

Rikmenspoel (24) has published tracings from ciné sequences of two individual sea urchin sperm flagella in the process of spontaneously beginning to beat. The species are not indicated, and so direct comparisons cannot be made with other data. However, in one (his Fig. 11), beating appears to begin along the entire flagellum, although the compression of the most distal bend as
it approaches the tip suggests that the distal bends could be caused by resistance to sliding near the tip during the formation of a bend near the base, which develops and propagates in a manner similar to those of the live spermatozoa reported in the present study.

The steady asymmetric beating of tritonated spermatozoa in solutions at low pH—and the asymmetric commencement of beating of spermatozoa rendered quiescent by lowered pH—indicate a different between the sensitivities of principal and reverse bends to lowered pH. Thus, there appears to be a qualitative difference between the mechanisms of formation of principal and reverse bends. The production and propagation of only principal bends cannot result from the prevention of active sliding in one direction between adjacent doublets: The production of a bend at the base requires active sliding in one direction, and the propagation of that bend toward the tip requires active sliding in the opposite direction (12, 26); thus, active sliding in both directions is required for either principal or reverse bends. The asymmetry may result instead from a defect in the control of the direction of sliding, such as the failure of a curvature-feedback mechanism to cause a reversal of the sliding in one direction.

Some theoretical models have been developed that contain a series of identical oscillators arranged along their length; beating typically starts as shallow bends formed along the entire flagellum, and these bends grow in amplitude and begin to travel toward the tip (2, 19). This behavior was not seen under any of the conditions observed in this study; instead, beating generally began with the development of a basal bend.

The localization of the initial bend to the basal end of live flagella was not caused by localization of ATP to this region: The ability of the initial bend to travel at speeds greater than the diffusion rate of ATP (10) indicates the presence of ATP along the entire flagellum before that bend began to travel. In addition, live flagella that have been removed from the cell body at low pH can beat as the pH is increased (14), thus indicating that substantial ATP is stored within these quiescent flagella.

Although the formation of a single basal bend requires sliding along the entire flagellum, a unique distribution of ATPase activities along a flagellum cannot be inferred from the observed waveforms, and it is not clear whether the generation of the forces that produced a basal bend was localized within that bend or spread along the entire flagellum. However, a new bend that developed at the base was sometimes accompanied by a more distal curve in the opposite direction. This curve could have been passive if the active force had been localized to the base and if there had been resistance to sliding near the tip. The occurrence of this distal curve, in which the degree of sliding diminished near the tip instead of being constant all along the flagellum, suggests that the forces responsible for the development of the basal bends were not produced uniformly along a flagellum. Similarly, initial bends often cancelled one another as they developed in pairs in tritonated flagella that were controlled with ATP, which indicates that there was no distal sliding associated with their development, and suggests that the associated ATPase activity was localized within them. This behavior is in agreement with observations that bends can form in regions supplied locally with ATP (3, 10, 27), and that basal bends can cancel during steady-state beating (12).

The ability of newly formed distal bends to propagate is in agreement with the findings of Thurm (30), Lindemann and Rikmenspoel (18), Okuno and Hiramoto (23), and Shingyoji et al. (27), that bends produced with microneedles can propagate. The failure of basal bends to propagate along flagella in solutions containing less than ~0.005 mM ATP—while more distal, fully developed bends traveled to the tip—is consistent with the observations of Shingyoji et al. (27) that bends can propagate in 0.008 mM ATP, but require a higher ATP concentration at the base to form and begin traveling, although these authors did not indicate whether partially formed basal bends developed at the lower ATP concentration.

The ability of these spermatozoa to form initial bends very slowly is not surprising in view of their capacity to produce normal steady-state waveforms at very low beat frequencies (4). This suggests that viscous forces are not important in the control of flagellar waveforms, although, as Brokaw and Joslin (4) point out, these observations do not rule out the possibility that the ratio of internal to external viscosities—which is independent of frequency—is a control factor.

In straight quiescent flagella, initial bends typically began as curves of ~15 µm in length at the base. Active sliding can occur in straight flagella (28). This sliding may produce a curvature which is greatest near the base (25), although the exact
shape expected depends on the detailed assumptions of the model. Once a curve forms, shear forces in the curved basal region could become dominant (2). As the initial bend developed in the present study, the direction of microtubular sliding reversed, and it began to propagate, as occurs during steady-state beating (12). In quiescent flagella which contained a basal bend, the bend began to develop and propagate as beating commenced. Straight flagella thus exhibited synchronous microtubular sliding along their length, while flagella that contained a basal bend exhibited primarily metachronous sliding within that bend.

I thank Dr. C. J. Brokaw for valuable discussion. This work was supported by National Science Foundation grant BM573-06710-A01.

Received for publication 19 May 1978, and in revised form 21 August 1978.

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THE JOURNAL OF CELL BIOLOGY, VOLUME 80, 1979