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ROTATIONAL MOVEMENT OF A SPERMATOZOON AROUND ITS LONG AXIS

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Summary

The rotational movement of a spermatozoon around its longitudinal axis was investigated by two methods: by observing a spermatozoon attached vertically to a coverslip by the tip of its head, and by observing a spermatozoon freely swimming in a medium by means of 'double-focal microscopy', which yielded simultaneous images at two different focal planes.

Similar results were obtained by these two methods. Sea urchin, starfish, medaka, human, golden hamster and bull spermatozoa rolled in both clockwise and counterclockwise directions, although there was a large difference in the proportion of spermatozoa rolling in each direction in the different species. The majority of sea urchin and starfish spermatozoa rolled in a clockwise direction when an observer viewed the cell from its anterior end, whereas the majority of medaka, golden hamster, human and bull spermatozoa rolled in a counterclockwise direction relative to the same observer. Moreover, some spermatozoa occasionally changed their rotational direction.

These results suggest that the mechanism regulating the direction of rotation of the spermatozoa is lax. As rotational movement of a spermatozoon around its longitudinal axis is due to the three-dimensional component of the beat of the flagellum, the direction of the three-dimensional movement presumably changes as the spermatozoa swim.

Introduction

Most spermatozoa roll around their longitudinal axis as they swim freely (Gray, 1955, 1958; Bishop, 1958; Rikmenspoel, 1965; Phillips, 1972). This is very strong presumptive evidence that sperm flagellar movement is not confined to a single plane (Taylor, 1952; Gray, 1962; Chwang and Wu, 1971), although the unlikely

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possibility that the asymmetrical shape of the sperm head causes the rotation of the whole spermatozoon cannot be ruled out.

Several investigators have tried to determine the sense of rotation of a spermatozoon, using a variety of approaches, because information on the direction and the rate of rotation are fundamental to the determination of the three-dimensional geometry of flagellar movement (Bishop, 1958; Drake, 1974; Woolley, 1977; Yeung and Woolley, 1984). However, there is no agreement as to the direction of rotation reported in these studies (Woolley, 1979).

The geometry of the flagellar waveform is technically difficult to determine precisely, although several methods have been used to overcome this problem, including high-speed cinemicrography, video micrography, stroboscopic illumination, micromanipulative techniques, and so on (Woolley, 1979; Ishijima and Mohri, 1990).

In order to elicit detailed information on the shape of the flagellar waveform to aid our understanding of the basic control mechanisms of the flagellar movement, the following micromanipulative technique has been developed (Ishijima and Hiramoto, 1982; Ishijima and Mohri, 1985; Ishijima *et al.* 1986; Ishijima and Witman, 1987). A spermatozoon is held by its head with a sucking micropipette and its flagellar movement observed from various directions under the microscope while the spermatozoon is alive and beating. This method was very useful for studying whether flagellar movement occurred within a single plane or, if not, how far the waveform of the beating flagellum deviated from the major beating plane within the limit of resolution of the light microscope. However, it could not be used to ascertain the handedness or three-dimensional shape of the beating flagellum.

In the present study, rotational movement of a spermatozoon around its longitudinal axis was investigated by two methods in order to reconstruct the exact three-dimensional geometry of flagellar movement of various spermatozoa. The present studies will not only help to clarify both the existence and nature of the rotational movement of spermatozoa, but will also suggest ways for analyzing aspects of their three-dimensional movement.

Materials and methods

Sperm preparations

Bull sperm were kindly provided as a frozen straw of semen in liquid nitrogen by the Snow Brand Embryotransfer Laboratory (Tomakomai, Japan). For each experiment, a straw was thawed in warm (37 °C) water before use. To select motile spermatozoa from the semen by 'swimming-up' procedures (Lopata *et al.* 1976; Ishijima and Witman, 1991), the thawed semen was placed at the bottom of a short glass tube (15 mm×50 mm) and carefully covered with 3 ml of warm (37 °C) Tyrode's solution. After leaving the tube to stand for almost 10 min at room temperature, the sperm suspension in the upper 1.0–1.5 ml in each tube was collected and transferred into another tube.

Golden hamster spermatozoa were obtained from a cauda epididymis removed surgically from a mature male under ether anesthesia (Yanagimachi, 1982; Ishijima and Witman, 1991). A dense mass of the 'dry' sperm, which had oozed out when the cauda epididymis was punctured with a sharp needle, was placed at the bottom of a short glass tube (15 mm×50 mm). The dry sperm were covered with 3 ml of warm (37 °C) Tyrode's solution, and then actively motile golden hamster spermatozoa were selected by the swimming-up method outlined above.

Human semen was collected by masturbation from four healthy men after sexual abstinence for at least 4 days. The semen was washed three times in Tyrode's solution by centrifugation at 250 *g* for 10 min. Approximately 30 μl samples of the loosely packed sperm pellet were suspended in 3 ml of Tyrode's solution.

The spermatozoa of the medaka, *Oryzias latipes*, were obtained as follows (Yamamoto, 1961). The testis was isolated by dissecting the abdomen of an adult male from the opening of the rectum, after pithing the brain with the fine point of straight iris scissors, and then put into Ringer's solution for the medaka (7.5 g NaCl, 0.2 g KC1, 0.2 g $CaCl_2$ and 0.02 g $NaHCO_3$ per liter of deionized water, pH 7.3) in a plastic culture dish (35 mm×10 mm). The spermatozoa were liberated by teasing apart the testis in the Ringer's solution.

Concentrated spermatozoa of the sea urchin, *Hemicentrotus pulcherrimus*, and those of the starfish, *Asterina pectinifera*, were obtained by dissecting out the gonad and placing it in a plastic culture dish (35 mm×10 mm).

Observations and recording

The rotational movement of various spermatozoa was investigated both by observing a spermatozoon attached vertically to the coverslip at the tip of its head and by observing a spermatozoon swimming freely in a medium by means of double-focal microscopy (see below). Using a suitably pretreated coverslip (see below), sperm could be found that were stuck by the anterior surface of their head with the wave axes of the beating flagellum oriented nearly vertical to the coverslip surface. The anterior regions of the beating flagella of these spermatozoa were observed using a Nikon Optiphot microscope equipped with a phase-contrast condenser, objectives (plan 40× BM and plan 20× BM) and 10× eyepieces. In the case of the experiments on rotational movement of the head of bull spermatozoa, differential interference contrast (DIC) microscopy was used with objectives (plan 40× DIC and plan 20× DIC) and 10× eyepieces. For mammalian spermatozoa, the condenser was modified for a microscope stage warmer; the top lens was removed and home-made phase annuli corresponding to the phase plates of objectives were inserted. Images were recorded on video tape with a National video camera (WV-1300A, Matsushita Communication Industrial Co., Ltd, Yokohama, Japan), a video timer (VTG-33, For-A Corp., Tokyo) and a National time-lapse video tape recorder (NV-8030, Matsushita Electric Industrial Co., Ltd, Oosaka) which yielded 60 images per second. Images were displayed on a National TN-96 monitor (Matsushita Communication Industrial Co., Ltd). Illumination

was provided by stroboscopic flashes from a Chadwick-Helmuth model 100 (Chadwick-Helmuth Corp., El Monte, CA), the intensity of the flash being modulated by a number of neutral density filters placed between the condenser and the lamp. A heat-absorption filter affording some thermal protection was also placed in this position. Turning a rotating mechanical stage confirmed that the direction of rotation of a specimen was identical to that of the images formed by the video system and shown on the monitor.

Double-focal microscopy was used for medaka, bull and human spermatozoa. This method yielded images at two different focal planes at the same time (Hamaguchi *et al.* 1991). One video camera (National WV-1300A) equipped with a Nikon zoom lens was mounted on one of the binocular eyepiece tubes while another video camera (AVC-1550, Sony Corp., Tokyo) with a Nikon zoom lens was placed on the other binocular eyepiece tube; the second lens had a mechanical tube length different from that of the first. The difference in the mechanical tube length between the two lenses was adjusted so that the distance between two focal planes was approximately 60 % of the maximum displacement of beating flagella in medaka, bull and human spermatozoa. The output of the two video cameras was montaged using a National special effects generator (WJ-545, Matsushita Communication Industrial Co., Ltd) and then recorded on the video tape with a National time-lapse video tape recorder (NV-8030) after first being fed through a video timer. Images were displayed on a National WV-5360 monitor. The same system of illumination and filters as described for the other method was used for observation and recording. Rotating a wire model of a spermatozoon around its long axis confirmed that the rolling direction of a specimen was identical to that of images formed by the video system and appearing on the monitor.

The observation chamber was constructed by gluing three strips of glass onto a glass slide. The spermatozoa, prepared as described above, were diluted to a concentration of approximately 10^6 sperm ml^{-1} and the sperm suspension was transferred to the chamber. In the experiments in which we observed a spermatozoon attached to a coverslip by the tip of its head, the coverslips were precoated in different ways for the different species. The coverslips used for bull and human spermatozoa were precoated with 0.5 % agar dissolved in 0.9 % NaCl. For golden hamster spermatozoa, the coverslips were coated with 0.5 % agar dissolved in 0.9 % NaCl and then dried. 0.5 % agar dissolved in artificial sea water (Jamarin U, Jamarin Laboratory, Oosaka) was used for precoating coverslips in the case of sea urchin and starfish spermatozoa, and 0.5 % agar dissolved in the Ringer's solution was used in the case of medaka spermatozoa. The presence of agar on the coverslip did not change the rolling direction of spermatozoa.

Observations and recording were made at 37 °C for mammalian spermatozoa and at room temperature (23 °C) for medaka, sea urchin and starfish spermatozoa.

Data analysis

The rotational movement of each spermatozoon was recorded for intervals of approximately 15 s. For each spermatozoon, three parts of the video tape record

were randomly chosen for analysis. For detailed field-by-field analysis, images of the flagellum and head were traced from the video monitor onto transparent plastic sheets using a fine-point marker. The direction of sperm rotation around its longitudinal axis was determined from the direction of rotation of flagellar images. For analysis by double-focal microscopy, the beating plane was first determined by reconstructing a bending wave fitting the trace of images focused on the two different planes. The direction of sperm rotation was determined from the rotational direction of this beating plane. Rotation frequency of the beating plane or the head was calculated from the mean period required for one complete revolution of the beating plane or the head, respectively.

Results

Rotational movement of medaka, golden hamster, bull and human spermatozoa attached vertically to the coverslip

Most spermatozoa (58–100 %) of medaka, golden hamster, bull and human rolled in a counterclockwise direction around their longitudinal axes, when the cell was viewed from the anterior end, but the remainder of them rolled in a clockwise direction when they were attached vertically to a coverslip at the tip of the head (Table 1).

Bull spermatozoa

Bull spermatozoa fairly easily attached vertically to the surface of the coverslip by the anterior surface of their head and rolled around their longitudinal axis. The axis of rotation did not oscillate to any great extent, suggesting that flagellar waves of bull spermatozoa were fairly symmetrical on the two sides of the tail. When the anterior region of the beating flagellum of these spermatozoa was brought into focus, cross sections of beating flagella were recorded on the video tape as bright spots (Fig. 1). Judging the rolling direction of the spermatozoon from the direction in which the bright spots moved revealed that bull spermatozoa rolled in both directions; clockwise and counterclockwise relative to the observer, although counterclockwise rotation was predominant over clockwise rotation (Table 1). The average rotation frequency determined from the rolling flagellum was 9.78±4.35 Hz (Table 1).

Bull spermatozoa have broad, flat heads and it was possible to determine the direction and frequency of rotation of the sperm head by taking advantage of this morphological characteristic (Fig. 2). The sperm head rotated in the same direction as the tail. The rotation frequency of the head (9.25±6.09 Hz, N=12) was, moreover, little different from that of the tail. These results suggest that the head of a bull spermatozoon does not rotate freely but rolls together with the tail around its longitudinal axis.

Golden hamster spermatozoa

Few golden hamster spermatozoa attached vertically to the surface of the

Table 1. *Characteristics of rotational movement of various spermatozoa*

Species	Direction of sperm rotation: Counterclockwise (%)	Clockwise (%)	*N*	Rotation frequency (Hz)	*N*
Bull	73.3	26.7	30	9.78±4.35	19
	(57.1)	(42.9)	(84)	(9.08±1.75)	(49)
Golden hamster	76.2	23.8	21	1.48±0.69	8
Human	57.1	42.9	21	9.33±4.85	15
	(58.8)	(41.2)	(51)	(10.18±1.91)	(51)
Medaka *Oryzias latipes*	88.2	11.8	17	3.77±2.82	17
	(100)	(0)	(20)	(5.30±1.21)	(20)
Sea urchin *Hemicentrotus pulcherrimus*	5.0	95.0	20	2.55±0.98	16
Starfish *Asterina pectinifera*	30.4	69.6	23	0.50±0.57	15

Measurements were carried out at 37°C for mammalian spermatozoa and at room temperature (23°C) for medaka, sea urchin and starfish spermatozoa.

N, number of spermatozoa measured. The figures in parentheses are data measured by means of double-focal microscopy.

Direction of a spermatozoon rotation around its long axis was noted by an observer viewing the cell from its anterior end. Spermatozoa that rotated in both directions were included in both the clockwise and counterclockwise categories.

Values of rotation frequency are mean±S.D.

coverslip because of their hook-shaped heads. Thus, the axis of rotation tended to oscillate back and forth when a spermatozoon rotated around its longitudinal axis. In those spermatozoa whose axis of rotation did not oscillate too much, the direction of roll of most (76.2%) was counterclockwise, whereas the remainder (23.8%) rolled in a clockwise direction (Table 1). In this experiment, one spermatozoon was found to change its direction of roll from counterclockwise to clockwise, suggesting that each spermatozoon can occasionally change its direction of roll. The average rotation frequency of golden hamster spermatozoa was 1.48±0.69 Hz (Table 1). Observations of the rotational movement of the sperm head and tail reveal that the sperm head and tail rolled together (data not shown).

Human spermatozoa

A human spermatozoon attached vertically to the coverslip by its head rolled around its longitudinal axis with small and rapid oscillations. This oscillation of the rotational axis was probably because flagellar movement in human spermatozoa did not occur within the plane containing the head and proximal region of the flagellum (see Ishijima *et al.* 1986). 57.1% of human spermatozoa rotated in a

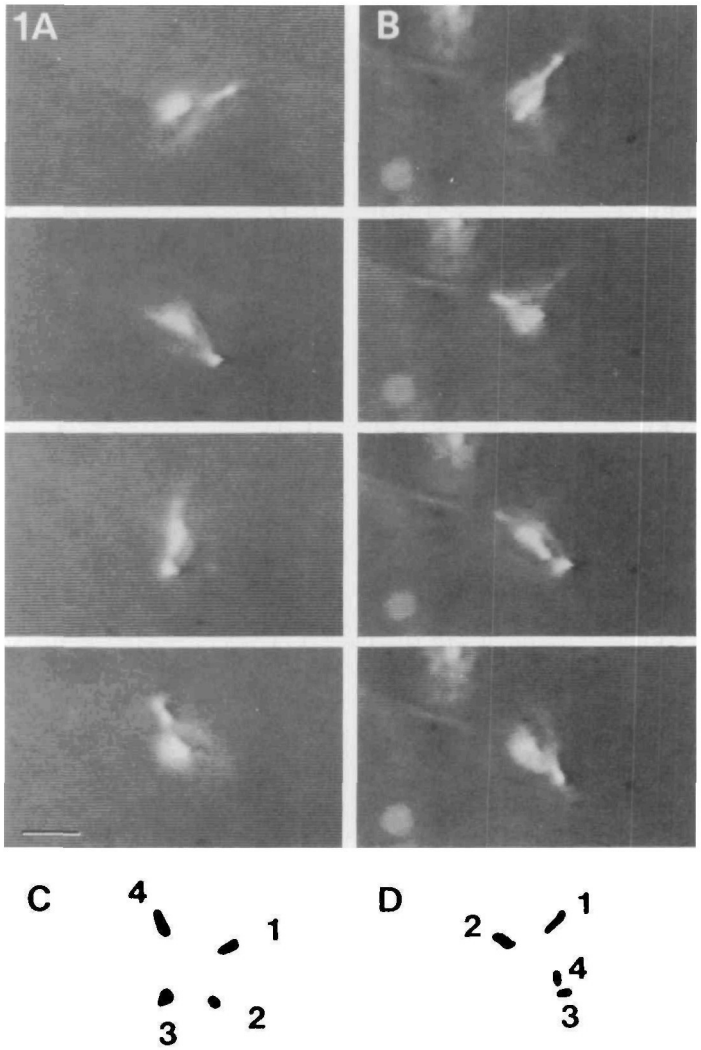

Fig. 1. Phase-contrast video micrographs of rotational movement of bull sperm flagellum observed from the sperm long axis. (A) Clockwise movement. (B) Counterclockwise movement. The time interval between successive images is 1/60 s. In C and D, tracings made from A and B, respectively, have been superimposed. Numbers indicate the positions of successive images. The scale bar represents 10 μm.

counterclockwise direction, the remainder in a clockwise direction. The average rotation frequency of human spermatozoa was 9.33±4.85 Hz, similar to that of bull spermatozoa.

Medaka spermatozoa

Medaka spermatozoa have nearly spherical heads with an average diameter of

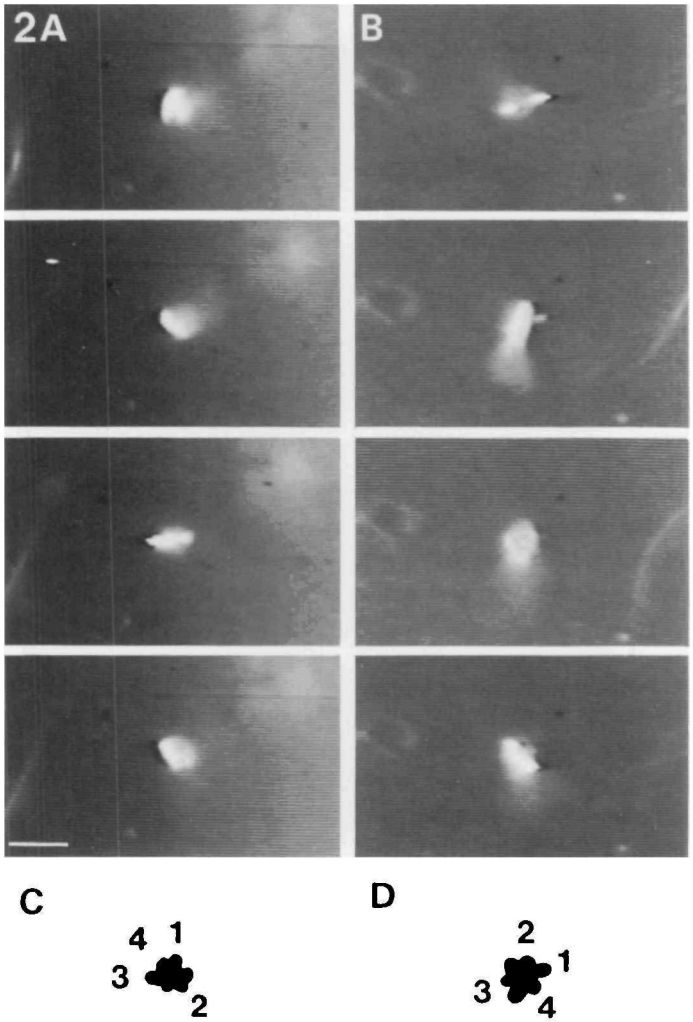

Fig. 2. Differential interference contrast video micrographs of rotational movement of bull sperm head. (A) Clockwise movement. (B) Counterclockwise movement. The time interval between successive images is 1/60 s. In C and D, tracings made from A and B, respectively, have been superimposed. Numbers indicate the positions of successive images. The scale bar represents 10 μm.

3.71±0.60 μm and tails of 30.9±1.60 μm (N=17) long. These spermatozoa moved forward at a rate of 86.6±20.1 $\mu m\,s^{-1}$ with a maximum displacement of beating flagella of 5.14±1.73 μm (N=16) near the coverslip at 23 °C. These values were much smaller than those of sea urchin spermatozoa, which are approximately the same size as medaka spermatozoa. When medaka spermatozoa attached vertically to the coverslip, most rolled counterclockwise (88.2 %), the remainder (11.8 %) undergoing clockwise rotation. Average rotation frequency of medaka spermatozoa was 3.77±2.82 Hz at 23 °C.

Rotational movement of sea urchin and starfish spermatozoa attached vertically to the coverslip

In contrast to medaka and mammalian spermatozoa, the majority of sea urchin and starfish spermatozoa rolled in a clockwise direction when the spermatozoa attached vertically to the surface of a coverslip by the tip of their head.

Sea urchin spermatozoa

Most sea urchin spermatozoa attached vertically to the surface of a coverslip by the tip of their head rolled around their longitudinal axis with little or no oscillation of the rolling axis. The beating plane of flagellar movement was observed as a rolling short segment because of the high beat frequency of flagellar waves compared to the rotation frequency. The rolling motion of this segment was sometimes interrupted by intermittent pauses and, moreover, changed direction of rotation from clockwise to counterclockwise. Judging the sense of rotation of spermatozoa from the direction of rotation of the short segment, 95 % of sea urchin spermatozoa rolled clockwise and the spermatozoa of only 5 % rolled counterclockwise. Average rotation frequency was 2.55±0.98 Hz.

Starfish spermatozoa

Most starfish spermatozoa did not attach vertically to a coverslip because of the almost spherical shape of the sperm head, so the rolling axis underwent a 'precession' movement, i.e. a slow, rotary motion of the rolling axis of a spinning spermatozoa, so that the axis of rotation described a cone and the poles of rotation described circles in the medium. The rolling movement of starfish spermatozoa (Fig. 3) was rather slow (average rotation frequency=0.50±0.57 Hz) compared to that of the other species studied (Table 1). The rolling direction of the majority of starfish spermatozoa (69.6 %) was clockwise, with 30.4 % rolling counterclockwise. One spermatozoon of 22 examined in this experiment rolled in a clockwise direction after first rolling counterclockwise.

Rotational movement of medaka, bull and human spermatozoa investigated by means of double-focal microscopy

Spermatozoa swimming freely in a medium were recorded by double-focal microscopy, which yielded different images of beating flagella on two different focal planes (Fig. 4). A set of these images enabled us to determine the direction of the beating plane of the sperm flagellum (see Fig. 4). Several successive sets of these images yielded the sense of rotation of the sperm flagellum.

The direction of sperm rotation around its long axis determined by this method was counterclockwise for most medaka, human and bull spermatozoa (Table 1). These results were similar to those obtained from observing the spermatozoa attached vertically to the coverslip, although there were some differences in the percentages rotating clockwise and counterclockwise (Table 1).

The rotation frequency determined by this method was almost identical to that obtained by the other method (see Table 1).

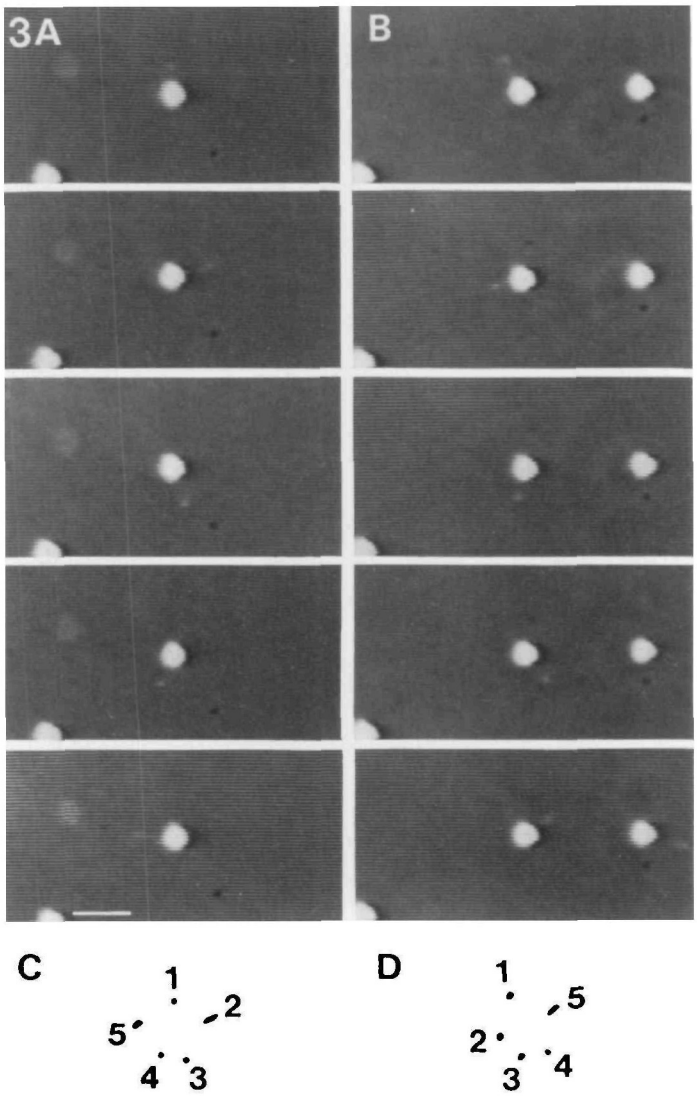

Fig. 3. Phase-contrast video micrographs of rotational movement of starfish sperm flagellum observed from the sperm long axis. (A) Clockwise movement. (B) Counterclockwise movement. The time interval between successive images is 0.4 s. In C and D, tracings made from A and B, respectively, have been superimposed. Numbers indicate the positions of successive images. The scale bar represents 10 μm.

Discussion

There are three important findings in the present study. (1) Spermatozoa of all species examined rolled both clockwise and counterclockwise. (2) The proportion of spermatozoa rotating counterclockwise to that rotating clockwise was different in the different species. (3) The sense of rotation of most sea urchin and starfish spermatozoa was opposite to that of most medaka and mammalian (bull, golden hamster and human) spermatozoa.

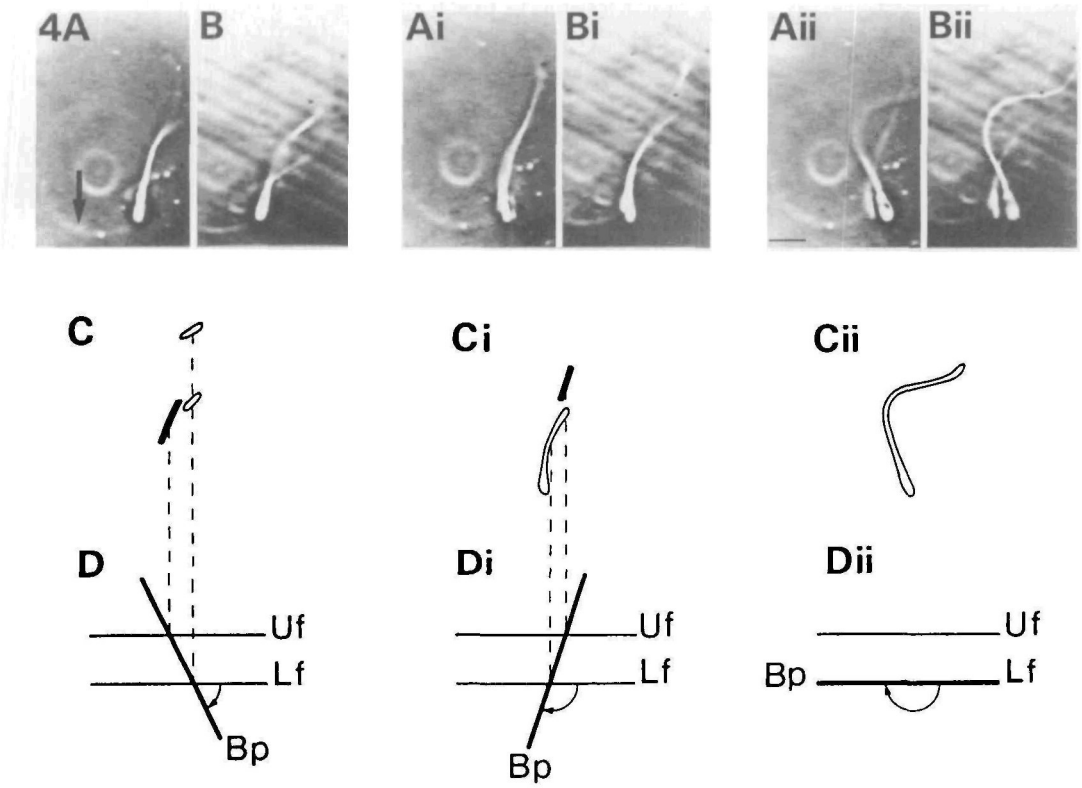

Fig. 4. Determination of the direction of roll of a bull spermatozoon from the images obtained by double-focal microscopy. A, Ai and Aii are micrographs of a bull spermatozoon focused on the upper focal plane and B, Bi and Bii are images focused on the lower focal plane. The spermatozoon is swimming in the direction of the arrow in A. The distance between the two focal planes is 4 μm. The time interval between successive images (A, Ai and Aii or B, Bi and Bii) is 1/60 s. The scale bar represents 10 μm. The superimposed tracings made from these micrographs are shown in C, Ci and Cii, where the filled areas represent the flagellar parts focused on the upper focal plane and the open traces are the counterparts on the lower focal plane. The major beating plane of a sperm flagellum is determined from these superimposed tracings as shown in D, Di and Dii, which are the side views; the image on the upper focal plane (Uf) and that on the lower focal plane (Lf) lie in the major beating plane (Bp). The direction of sperm rotation is determined from the direction of rotation of this major beating plane. The specimen in this figure rotates clockwise when viewed from the anterior end because the clockwise angle (arrow) from the focal plane increased with time.

Bidirectional rotation of spermatozoa

The observation that spermatozoa of all species examined could roll in both directions suggests that the mechanism controlling the direction of rotation of beating flagella is not fixed but, on the contrary, is easily changeable. This was confirmed by the observations that several golden hamster, sea urchin and starfish

spermatozoa changed their direction of roll from counterclockwise to clockwise or *vice versa*. Taking into account that the rotational movement of spermatozoa was due to the three-dimensional movement of their beating flagella (Taylor, 1952; Chwang and Wu, 1971; Woolley, 1979; and see below), the observation that sperm could rotate in both directions suggests that the three-dimensional geometry of flagellar movement of these spermatozoa changes while the spermatozoa are beating.

The results obtained in the present experiments explain earlier confusion over the rolling direction of spermatozoa. The observation by Drake (1974) that bull spermatozoa rolled in either a clockwise or a counterclockwise direction when viewed from in front is the same as that obtained in the present study, although that report did not contain any quantitative data. The suggestion that human spermatozoa roll counterclockwise (Linnet, 1979) agrees with a part of our results.

The significance of changing the direction of sperm rotation is unclear. However, when the direction of sperm rotation changes, sperm can quickly and effectively change their direction of movement. This kind of sperm behavior implies a tactical response, although no such response has yet been established in the species examined in the present experiments (see Miller, 1985).

Some cilia show an almost half-funnel-shaped movement (Sleigh, 1974), the effective stroke of the ciliary movement taking place in the vertical plane whilst in the recovery stroke the cilium swings out to the side relative to an observer looking down onto the cell surface. The direction of the recovery stroke in such three-dimensional ciliary movement is opposite in different species. Thus, a *Paramecium* cilium rotates counterclockwise during the recovery stroke, as viewed from the ciliary tip (Párducz, 1967; Machemer, 1972), while the lateral cilia of *Mytilus edulis* gill rotate clockwise (Aiello and Sleigh, 1972). The observation that cilia can beat in both directions during the recovery stroke may correspond to the finding that a sperm flagellum can change the direction of its bending waves.

Factors regulating the direction of sperm rotation

Even though spermatozoa rolled in both directions, the proportion of spermatozoa rolling counterclockwise to those rolling clockwise was not equal. Clockwise rotation was predominant to counterclockwise rotation in sea urchin and starfish spermatozoa, and *vice versa* in medaka, human, golden hamster and bull spermatozoa. Much more work is required to explain the difference in the proportion of counterclockwise to clockwise rotation. Some biochemical factors may be involved in regulating the ratio because the proportion of counterclockwise to clockwise rotation in sea urchin and starfish spermatozoa was opposite to that in the others. Calcium ion concentration is a possible candidate for such a regulating factor because intracellular calcium ion concentration in sea urchin spermatozoa is greater than $2\times10^{-6}\,\mathrm{mol\,l^{-1}}$ (Schackmann and Chock, 1986), whereas that in mammalian spermatozoa is less than $4\times10^{-7}\,\mathrm{mol\,l^{-1}}$: human, $1.46\times10^{-7}\,\mathrm{mol\,l^{-1}}$ (Irvine and Aitken, 1986); rabbit, $1.44\times10^{-7}\,\mathrm{mol\,l^{-1}}$ (Mahanes *et al.* 1986); ram, $1.93\times10^{-7}\,\mathrm{mol\,l^{-1}}$ (Simpson and White, 1988); ram,

4×10^{-7} mol l^{-1} (Babcock and Pfeiffer, 1987). Therefore, a relatively high concentration of intracellular calcium, more than 10^{-6} mol l^{-1} for example, could induce clockwise rotation whereas a relatively low concentration, less than 10^{-6} mol l^{-1} for example, could induce counterclockwise rotation. Our preliminary experiments (Ishijima and Hamaguchi, 1990) revealed that Ca^{2+} in the reactivation solution changed the direction of rotational movement of demembranated sea urchin spermatozoa. Cilia of *Paramecium* change the direction of the effective stroke according to the intracellular calcium ion concentration (Naitoh and Kaneko, 1972), and this type of regulating mechanism may also exist in flagella.

Three-dimensional geometry of sperm flagella and cilia

We cannot completely rule out the possibility that sperm rotation is caused by the asymmetrical shape of the sperm head, especially in a free-swimming spermatozoon (Phillips and Olson, 1975), but the more likely explanation is that it is caused by the three-dimensional movement of the flagellum (Gray, 1958; Chwang and Wu, 1971; Phillips and Olson, 1975, and see below). A knowledge of the direction of sperm rotation obtained from the present study therefore gives us the sense of the three-dimensional geometry of flagella beating; that is, a spermatozoon rotates in the direction opposite to that of the revolving motion of segments of the sperm tail (see Fig. 1 of Gray, 1962). Most medaka, bull, golden hamster and human spermatozoa beat with left-handed waves (i.e. with the same twist as a left-handed screw), whereas most sea urchin and starfish spermatozoa beat with right-handed waves (i.e. with the same twist as a right-handed screw). This agrees with previous observations of bending flagella of golden hamster spermatozoa (Woolley and Osborn, 1984). The three-dimensional geometry of the flagellar waves of some spermatozoa and the ciliary bends in each species are summarized in Table 2. The results imply that most cilia and flagella of freshwater animals beat with left-handed waves while those of marine animals beat with right-handed waves.

Influence of the sperm head on sperm rotation

In principle, the shape of the sperm head could be involved in promoting rotation of spermatozoa that swim freely (Phillips and Olson, 1975), just as a pinwheel revolves in the wind. Assuming that the rotation frequency of spermatozoa will increase as a result of the acceleration of rotation caused by the interaction between the asymmetrical sperm head and the stream of medium, it is possible to find some differences in beat frequency from the two methods used in this study. Double-focal microscopy measured the rotation frequency of spermatozoa swimming freely in a stream of medium, whereas the other method measured that of spermatozoa attached vertically to the coverslip, in which situation the sperm heads were rotating in little or no stream. But the observation that the rotation frequencies of spermatozoa obtained by the two methods were almost the same does not support this conjecture, at least for the spermatozoa examined in this study.

Table 2. *Three-dimensional geometry of flagellar waves of sperm and cilia in a variety of species*

Species	Three-dimensional geometry*	Minor semiaxis† / Major semiaxis	References
Human	Left-handed helicoid	0.2	Ishijima *et al.* (1986) Present study
Bull	Left-handed helicoid	0.3–0.5	Rikmenspoel (1965 Present study S. Ishijima, S. Oshio, T. Umeda and Y. Hamaguchi (unpublished data)
Golden hamster	Left-handed waves	0‡	Woolley and Osborn (1984) Ishijima and Mohri (1985) Present study
Medaka *Oryzias latipes*	Left-handed waves	0‡	Present study S. Ishijima, Y. Hamaguchi and T. Iwamatsu (unpublished data)
Eel *Anguilla anguilla*	Left-handed helix	1	Gibbons *et al.* (1985)
Sea urchin *Hemicentrotus pulcherrimus*	Right-handed waves	0‡	Hiramoto and Baba (1978) Present study
Starfish *Asterina pectinifera*	Right-handed waves	0‡	Hiramoto and Baba (1978) Present study
Horseshoe crab *Tachypleus gigas* *Tachypleus tridentatus*	Right-handed helix	1	Ishijima *et al.* (1988)
Mytilus edulis lateral cilia	Right-handed helicoid		Aiello and Sleigh (1972)
Paramecium multi-micronucleatum cilia	Left-handed helicoid		Machemer (1972)

* The term 'helix' describes a waveform with a cross section that is approximately circular, whereas the term 'helicoid' describes a waveform with an approximately elliptical cross section.

† The ratio of the lengths of the semiaxes of the elliptical cross section of the three-dimensional waves.

‡ The three-dimensional component (minor semiaxis) was not detected by ordinary light microscopic observation.

The present experiments clearly prove that the sperm head rotated together with the tail in bull and golden hamster spermatozoa, as previously reported in rodent spermatozoa (Phillips, 1972; Woolley, 1974), even though this was

presumed from the fact that the major beating plane, which was parallel to the major axis of the sperm head cross section in bull, golden hamster, human and ram, did not rotate when the sperm head was captured by a sucking micropipette (Ishijima and Mohri, 1985; Ishijima *et al.* 1986; Ishijima and Witman, 1987; S. Ishijima, S. Oshio, T. Umeda and Y. Hamaguchi, unpublished data).

Comparison of the two methods

Each method used in the present study has some advantages and disadvantages. The observation technique that examined the spermatozoon attached vertically to a coverslip is very useful for studying rotational movement of spermatozoa because it is easy to do and clearly establishes the rotation of the spermatozoa. But this method can be applied only to those spermatozoa with a more or less rounded tip to the sperm head. Double-focal microscopy can be employed for almost all spermatozoa, although it involves somewhat troublesome procedures to determine the direction of rotation of spermatozoa.

Although there is no agreement, as mentioned above, about the direction of rotation of spermatozoa, there are several reports presenting the values of rotational frequency of spermatozoa (see Table 3 of Ishijima *et al.* 1986, or Table I of Ishijima and Mohri, 1990, for details). There are no significant differences between these data and those obtained in the present study.

As shown in Table 2, we can now determine the three-dimensional geometry of flagellar movement of spermatozoa by combining the handedness of bending waves of sperm flagella with the information obtained by means of a micromanipulative technique developed by one of us. Moreover, the handedness of bending waves of sperm flagella and the rotation frequency obtained in this study are necessary for describing sperm behavior in a medium.

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