Flagellar Gyration and Midpiece Rotation during Extension of the Acrosomal Process of Thyone Sperm: How and Why This Occurs

Lewis G. Tilney and Shinya Inoué

Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104; and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Abstract. The midpiece of Thyone sperm contains a large mitochondrion and a centriolar pair. Associated with one of the pair, i.e., the basal body of the flagellum, are satellite structures which apparently anchor the flagellar axoneme to the mitochondrion and to the plasma membrane covering the midpiece. Immediately before and as the acrosomal process elongates, the flagellum and the midpiece begin to rotate at 1–2 rotations per second even though the head of the sperm, by being firmly attached on its lateral surfaces to the coverslip, does not rotate at all. This rotation is not observed in the absence of flagellar beating whose frequency is much greater than that of its gyration. To understand how the midpiece rotates relative to the sperm head, it is first necessary to realize that in Thyone sperm the flagellar axoneme projects at an acute angle to the principal axis of the sperm and is bent towards one side of this axis. Thus movement of the flagellum induces the sperm to tumble or yaw in solution. If the head is stuck, the midpiece will rotate because all that connects the sperm head to the midpiece is the plasma membrane, a liquid-like layer. A finger-like projection extends from the proximal centriole into an indentation in the basal end of the nucleus. In contrast to the asymmetry of the flagellum, this indentation is situated exactly on the principal axis of the sperm and, along with the finger-like projection, acts as a biological bearing to maintain the orderly rotation of the midpiece. The biological purpose of flagellar gyration during fertilization is discussed.

ELUCIDATING the steps in sperm–egg fusion (fertilization) has occupied the careers of countless investigators. This seemingly simple event actually involves a large number of discrete steps, many of which are now at least partially understood. What is interesting is that the exact mechanism can differ significantly in different species. The most commonly studied group of organisms is the echinoderms and among the group, the sea urchins hold the limelight, largely for historic reasons.

We have been studying the acrosomal reaction of living sperm of Thyone briareus, a sea cucumber, using high extinction video microscopy. During the course of these studies we noticed on our video sequences something which was not only remarkable but also inexplicable. Immediately before and as the acrosomal process elongates, the flagellum and the entire midpiece (which consists of its plasma membrane, a large mitochondrion, and a centriolar pair and associated satellite structures) begins to rotate even though the head of the sperm, by being firmly attached to the coverslip, does not rotate at all. The relative motion of the midpiece and the head proper is interesting on its own, but what is even more puzzling is that the rotary motion of the midpiece at 1–2 rotations per second is an order of magnitude slower than the rate of flagellar beat which, if Thyone sperm are similar to the sperm of sea urchins, is planar, not rotary (Gibbons and Gibbons, 1972).

In this paper we document the motion elicited in the midpiece, and from thin sections of fixed sperm, demonstrate how this type of motion could be generated with the complex equipment we find in the midpiece. In the discussion we will consider the biological function of this bizarre type of motion.

Materials and Methods

Thyone briareus were collected by the Marine Resources Department (Marine Biological Laboratory, Woods Hole, MA). To obtain sperm, the testes were removed and minced in sea water. The suspension was filtered through cheesecloth and the supernatant centrifuged at 1,000 g for 5 min to pellet the sperm. To examine the midpiece rotation, we recorded the antics of Thyone sperm using a Leitz 100×/1.3 NA Smith T differential interference contrast objective lens with the condenser oil immersed and used at an NA of 0.91. The video tape, recorded at 60 fields per second in the “1.2 hour mode” on a time lapse recorder (Sony TVO-9000), was played back in the “72 hour mode.” Each field was transformed during an interval free of noise onto a video laser disk recorder (Panasonic model TQ-2032 FBC high resolution monochrome optical disk recorder). Selected fields played back from the disk recorder were photographed off a monitor (Panasonic WV 5310) on 35-mm Plus-X film through a Ronchi grating. Details of the diffusion chamber, video apparatus etc., are found in Inoué and Tilney (1982) and Inoué (1986).

Since the sperm are thought to be immotile in the testes and to become

© The Rockefeller University Press, 0021-9525/87/03/407/9 $1.00
The Journal of Cell Biology, Volume 104, March 1987 407-415
Results

Observations on Living Sperm

Some of the sperm introduced into the perfusion chamber attach to the clean surfaces of the slide or coverslip. While the attachment is secure enough so that most sperm, once attached, are not dislodged by additional perfusion, attachment seems to occur mainly by the side of the head, leaving the midpiece and the tail free. Thus, the tail of the attached sperm can beat at its normal frequency. Furthermore, the acrosomal process can elongate without hindrance of a glass surface because it extends from the apical, not the lateral surface of its head.

Close examination of the midpiece reveals that it is asymmetric, a feature which allows us to document the rotation of this region. Although it is difficult to understand the basis for this asymmetry exclusively by light microscopy, by a combination of light microscopy and thin sections we now know that the asymmetry is due to the asymmetric shape of the mitochondrion and the location of the flagellar axoneme.
projecting basolaterally from the midpiece where the mitochondrion is either reduced in thickness or absent (see Figs. 1 and 3).

By playing the video tape back at various speeds, and by following the progressively changing location of the flagellum and the shape of the midpiece in successive frames of the video sequence, it is relatively easy to convince ourselves that the flagellum is gyrating and the midpiece is indeed rotating, rather than just swinging back and forth. A portion of such a sequence is illustrated in the top panel in Fig. 1 in which each frame is separated from its neighbor by 1/15 s. The discrete, bright spot on the midpiece is an optical section of the basal portion of the flagellum extending from the midpiece directly towards the viewer. The flagellum then curves around the sperm. These relationships are most easily understood by comparing the drawings in the lower panel of Fig. 1 with the appropriate video frames.

The flagellum beats so vigorously that, outside of the optical section at its base, only portions of it (see arrows) are visible in a single field of the video (1/60s), the rest appearing as a blur. On tape playback we see the whipping motion of the sperm tail, but definitive data on the gyration of the flagellum swinging completely around and around the sperm axis comes from sequential frame analysis of the movement of the base of the tail (the discrete, bright spot in a-d, i-k, and q-r in Fig. 1). The bright spot moves from right to left on successive frames in which the base of the tail is pointing towards the observer. Then it disappears for several frames until it reappears on the right side of the midpiece and the process repeats, again periodically (see composite top view, Fig. 1 i), at a frequency of approximately once every half second. The more distal part of the tail (arrows in e-i and l-p in Fig. 1) wraps partly around the midpiece as shown in the interpretive drawings (Fig. 1, a-y). Therefore the base of the tail that extends from the midpiece and the part that is presumably generating the thrust are pointing in different directions, thus producing a net torque which results in its gyration.

In the last six frames in the top panel of Fig. 1 (m-r), the acrosomal process is seen elongating out of the anterior end of the sperm.

From these reconstructions of the video sequence and others like it we have determined that the flagellum and midpiece invariably rotate in a clockwise direction as viewed from the sperm head towards the midpiece. The speed of midpiece rotation, which was measured in two sperm in detail (one is graphed in Fig. 2) and in several others where the asymmetry of the midpiece or swinging of the tail could be distinguished at least briefly, is 1-2 rotations per second.

In the video micrographs, the lipid droplet (labeled in Figs. 1 and 7) does not rotate with the midpiece but remains stationary. The droplet, which appears near the left margin of the midpiece (a-s) can be distinguished by its reversed shadowing (brighter to the right as in the acrosome owing to its lower refractive index; see Inoué and Tilney, 1982). We do not understand why the lipid droplet does not rotate with the rest of the midpiece; perhaps it is more firmly connected to the sperm head rather than to the midpiece.

Midpiece rotation has only been observed in sperm whose flagella are beating; however, not all sperm whose flagella are beating display midpiece rotation. In the latter case, both the midpiece and the head may be stuck to the glass surface.

Electron Microscopic Observations

The Flagellum, Its Basal Body, and Associated Centriole Are Located not on the Principal Axis of the Sperm, but to One Side. In thin sections through sperm which were fixed while still in the testis, we find that the flagellum invariably extends from the basolateral end of the midpiece (Fig. 3). An identical morphology is encountered in unreacted sperm which have been liberated from the testis by mincing the gonad. Not only is the basal body of the flagellum (often referred to as the distal centriole) located off to one side of the major axis of the sperm, but also the proximal centriole is situated off axis. A curved finger-like projection extends anteriorly from the centriole into an indentation in the basal end of the nucleus. The free end of this projection lies on a line that extends posteriorly from the center of the acrosomal vacuole, the cup of profilactin, and the nucleus (Fig. 3). In short, the free end of this projection is situated precisely along the principal axis of the sperm in contrast to the proximal centriole and basal body of the flagellum, which are located to one side of this axis.

The Basal Body and Its Associated Satellite Structures. In thin sections cut through the basal body, we find nine spoke-like satellites that extend radially from the nine triplets. Each satellite bifurcates at a dense node to connect to two dense bodies (Fig. 4) which in turn are connected to a periodically striated substance (Figs. 5 and 6). This striated substance is in intimate contact with the plasma membrane along one of its surfaces and with the outer mitochondrial membrane along another (Fig. 6). The net result of this complex organization around the basal body is that this organelle and its associated axoneme appear to be firmly connected to not only the plasma membrane at this point, but also to the mitochondrion. Thus, gyration of the flagellum would result in rotation of the entire midpiece.

The Relationship between the Proximal and Distal Centrioles. Unlike what one might predict from the literature on starfish (Sousa and Azevedo, 1983; Kuriyama and Kanatani, 1981) and sea urchin sperm (Longo and Anderson, 1969), in Thyone sperm the proximal and distal centrioles are not
Figure 3. Thin section of a Thyone sperm. The head (H) and midpiece (MP) region are indicated. Located within an invagination at the anterior of the nucleus (N) is the acrosomal vacuole (V) and the profilactin region (periacrosomal cup: A). The flagellum (F) extends from the basolateral surface of the sperm. The flagellar axoneme is connected to the distal centriole or basal body (B); associated with this is the proximal centriole (C). A curved, finger-like projection (P) extends from the proximal centriole towards the major axis of the sperm (shown by the dotted line), its unassociated end fitting into an indentation in the nuclear envelope. Bar, 1 μm.

Figures 4-6. (Fig. 4) Thin section cut through the basal body of the flagellum. Extending from each of the nine triplet microtubules are spokes, each of which bifurcates at a dense node (N) into two dense structures. These structures in turn contact the outer mitochondrial membrane and the plasma membrane. M indicates some of the cristae of the mitochondrion. Bar, 1 μm. (Fig. 5) Higher magnification of the basal body which shows the spokes in greater detail. Most interesting is that not only is each spoke striated, but also the nodes connect to a striated material (S) that is connected to both the plasma membrane and the mitochondrion (not visible in this figure, but in Fig. 6). Bar, 0.2 μm. (Fig. 6) Grazing, thin section cut parallel to the basal body but in front of it. In this section are two satellites, their nodes (N), and the striated material (S) that extends from the nodes. Of interest is that the striated material is in close contact with the plasma membrane and the surface of the mitochondrion (M). By comparing this micrograph with Figs. 4 and 5, and relating all this to the reconstructed drawing, Fig. 10, the reader can determine the orientation of the section. Bar, 1 μm.

Discussion

Two observations have convinced us that midpiece rotation is brought about by movement of the flagellum. First, midpiece rotation is only observed when the flagellum is also in motion, and second, in sperm the cytoplasm is so reduced in volume that there is not only no space for additional motile elements, but also none are found (see Tilney, 1985). If we accept that the flagellum provides the motile force for midpiece rotation which is seen when the sperm head is stuck...
to a slide or coverslip, one wonders how this occurs and why
the frequency of rotation is a small fraction of the frequency
of flagellar beat.

The key to understanding how the flagellum induces the
rotation of the midpiece is the observation that the basal body
of the flagellum is not located along the principal axis of the
sperm, but instead is situated off to one side and is bent at
its base so that it projects at an angle to the head axis. The
result of this is that when the flagellum beats, irrespective
of the type of motion, albeit by planar waves, by three-dimen-
sional waves, or by some combination, the sperm head would
not only be induced to move forward but at the same time
there would be a component to the motion that would tend
to make the sperm tumble or yaw. The frequency of the tum-
bling or yawing would bear no obvious relationship to the
frequency of flagellar motion because it would be dependent
on a variety of factors such as the angle of the flagellum rela-
tive to the principal axis and the resistance of the sperm head
to midpiece rotation.

When the sperm head is stuck to the slide, a portion of the
plasma membrane covering the midpiece would be induced
to move because it is attached to the gyrating flagellum by
the satellite structures. Yet another portion of the same mem-
brane, that covering the nucleus, would be stationary being
stuck to the slide. One might wonder where the shear zone
is located on the membrane and if the membrane is special-
ized in this region. Fortunately for us there is a biological
precedence for rotary motors. The most relevant case is the
devescovinid protozoa in which one part of the plasma mem-
brane, the head, rotates relative to a neighboring part, the
body (Tamm and Tamm, 1974). By electron microscopy
(Tamm and Tamm, 1974), conventional freeze fracture (Tamm
and Tamm, 1980), and analysis of the sterol composition of
the membrane at the point of rotation (Tamm and Tamm,
1983), it has been shown that this is not a unique membrane.
Rather shearing is a natural consequence of the fluid nature
of plasma membranes. Thus, different parts of the plasma
membrane of this same organism can rotate with respect to
other parts. A similar situation, albeit documented in much
less detail occurs during the acrosomal reaction of Limulus
sperm (Tilney, 1985). Thus, by analogy to these systems,
midpiece rotation with respect to the nucleated portion of
Thyone sperm could occur. Because the satellite structures
associated with the basal body morphologically are con-
ected to both the plasma membrane and the outer mitochon-
drial membrane, it seems likely that the entire midpiece
(with the possible exception of the lipid droplet) would rotate
as a unit relative to the nuclear half of the sperm, which is
stuck. Logically then the shear region would be situated on
the lateral surface of either the plasma membrane or the
nuclear envelopes to pinpoint the position of shear more ac-
curately.

If the plasma membrane is fluid, as we are lead to believe,
and if it is the major connector of the midpiece and the
nucleated half of the sperm, then one wonders why the mid-
piece always remains basal to the head. Why doesn't it creep
around as it moves relative to the head? The answer to this
question seems related to the finger-like projection that ex-
tends from the proximal centriole. Extending from the proximal centri-
ole is the finger-like projection which nestles into an indentation
in the nucleus. Notice that the tip of this projection is aligned on the
principle axis. It acts as a pivot around which the midpiece can ro-
tate by gyration of the flagellum. Notice that the flagellum extends
from the basolateral surface of the sperm and then wraps around
the midpiece. If the sperm head were stuck, motion of the flagellum
would induce a rotation of the midpiece.

Figures 7-9. (Fig. 7) Thin section cut perpendicular to the principle axis of the sperm through the basal end of the nucleus (N). Two nuclear pores (Np), the mitochondrion (M), and an eccentrically positioned lipid droplet (L) are seen in this section. Of interest to this report is the projection (P) that extends from the proximal centriole. This projection extends into an indentation in the nucleus. It is limited by the outer nuclear envelope. Bar, 1 μm. (Fig. 8) Thin section through a portion of the basal end of the nucleus (N). Extending into an indentation in the nucleus is the projection (P). Notice that it does not penetrate either the outer (O) or inner (I) nuclear envelopes. Fine strands connect these two nuclear envelopes at this point of indentation but are absent from the other parts of the nuclear envelopes. Bar, 0.2 μm. (Fig. 9) Thin section through a portion of the midpiece. Included in this section are the basal end of the nucleus (N) with its nuclear pores (Np), a portion of the mitochondrion (M), the proximal centriole (C) with its associated nuclear projection (P), the basal body of the flagellum (B), and a portion of one of its satellites (Sz). Of interest is that the projection is striated. Bar, 1 μm.
determine if midpiece rotation ever occurs in free swimming sperm. What is important is whether midpiece rotation occurs during fertilization. We do not yet know the answer to this question, but we hope our study will stimulate others to examine it.

Unlike the sea urchin (e.g., Arbacia or Strongylocentrotus), the jelly surrounding the sea cucumber (Thyone) and starfish (e.g., Asterias or Marthasterias) egg is so rigid that when a sperm makes contact, its forward motion is arrested. One of the first steps in the acrosomal reaction is to firmly attach the sperm to the surface of the jelly layer so that as the acrosomal process extends, the sperm proper is not translocated backwards away from the jelly layer. Adhesion is presumably accomplished by a “bindin-like” protein, formerly present in the acrosomal vacuole (see Vacquier and Moy, 1977). An additional way to keep the sperm attached to the egg is for the flagellum to maintain its motion so that it continuously pushes the sperm towards the egg. In fact, many investigators (e.g., Chambers, 1930; Dan, 1954) have stated that the flagella of fertilizing sperm continue to beat during the acrosomal reaction. Thus, during the steps which immediately precede fusion of sperm and eggs, the flagellum of the fertilizing sperm is beating maximally which is what one would have predicted. If the flagellum were to move with a rotary motion, then the sperm would be pressed continuously and consistently towards the surface of the egg, a situation that would be ideal. However, if the flagellum is beating with a planar wave as it does in sea urchin sperm (see Gibbons and Gibbons, 1972), then the sperm head might be dislodged on the jelly layer as it oscillates backward and forward in one axis. (To inhibit this dislodgement it is possible that the pattern of bend generation is different at the junction of the flagellum with the sperm head, but we lack evidence for this.)

Irrespective of the motion of the flagellum, albeit with a planar wave or a three-dimensional wave, bindin must fix the head of the sperm to the jelly sufficiently tightly so that the head will remain stationary. The midpiece, however, could rotate independently of the attached head and if so, convert the gyrating flagellar beat into a forward thrust, a thrust needed to combat the backward directed force induced by elongation of the acrosomal process. In short, it seems to us as if the midpiece of Thyone sperm is perfectly designed to function in fertilization.

Consistent with the above speculation are observations made on the eggs and sperm of different groups of echinoderms. Sea urchin and sand dollar sperm such as Arbacia (Longo and Anderson, 1969), Paracentrotus, Strongylocentrotus, and Echinarchaeus (Christen et al., 1982; Lee et al., 1983; Summers and Hylander, 1974) all have short acrosomal processes (<1 μm in length) and all have flagella whose basal bodies are situated on the principal axis. Furthermore, in all cases the flagella extend directly posteriorly, not posterolaterally as in Thyone. The jelly of the eggs of these organisms is soft so that the sperm can swim through it. On the other hand, sperm which produce long acrosomal processes, such as sea cucumbers (Colwin and Colwin, 1956; Tilney, 1985), brittle stars (Hylander and Summers, 1975), and starfish (Tilney, 1975, Sousa and Azavedo, 1985) have eccentric flagella and the jelly of the eggs of the same species is sufficiently hard so that the sperm cannot swim through it.

Thus, if the acrosomal process is long, e.g., more than a few micrometers, then the posteriorly directed force produced by the anterior extension of the acrosomal process must be compensated for by a force in the opposite direction which pushes the sperm towards the egg. This force is presumably produced by the flagellum which in all cases of sperm that form long acrosomal processes is eccentric in its location. Thus, a propeller type movement might be indicated. On the other hand, sperm with short acrosomal processes do not have to be concerned with a force directed posteriorly and accordingly do not need a propeller type movement.

Many investigators have noticed that sperm which have undergone the acrosomal reaction can be readily identified by the midpiece which tends to round up; concomitantly the flagellum reorients, now projecting laterally from the point of connection of the midpiece and the sperm head proper (Chambers, 1930; Dan, 1952; Tilney, 1975; Schroeder and Christen, 1982). Since sperm “taken directly from the testis are sluggish and frequently motionless, but become active when diluted in sea water” (Chambers, 1930), it is possible that the trigger of this activity in turn induces the motility by changing the position of the flagellar complex. It turns out that this is not correct. Dan (1954) could see no difference in the morphology of nonmotile and motile sperm and in her study the fine structure of the midpiece region in the sperm examined in pieces of fixed testis was identical to that of those fixed while suspended (presumably motile) in sea water. Furthermore, careful analysis of our video tapes show that the rounding up of the mitochondrion and the reorientation of the flagellum is a late event, occurring after the completion of extension of the acrosomal process. Thus, the eccentric location of the flagellar basal body, which we presume leads to the rotary motion of the midpiece is present in nonmotile, motile, and fertilizing sperm.

We would particularly like to express our thanks to Janna Knudson for photographing, printing, matching, and mounting the video frames illustrated in Fig. 1; to Bob Golder and Betti Goren for the art work; to Pat Connolly for cutting thin sections; and to Richard Christen for animated discussions during the early phases of this work.

This work was supported by grants HD 14474 (L. G. Tilney) and 2 R37 GM 3617 from the National Institutes of Health and grant DCB 8518672 from the National Science Foundation (S. Inoué).

Received for publication 24 July 1986, and in revised form 19 November 1986.

References

Chambers, R. 1930. The manner of sperm entry in the starfish egg. Biol. Bull. 58:344-369.

Christen, R., R. W. Schackman, and B. M. Shapiro. 1982. Elevation of intracellular pH activates respiration and motility of sperm of the sea urchin, Strongylocentrotus purpuratus. J. Biol. Chem. 257:14881-14887.

Colwin, L. H., and A. L. Colwin. 1956. The acrosome filaments and sperm entry in Thyone briareus and Asterias. Biol. Bull. (Woods Hole). 110:243-257.

Dan, J. C. 1952. Studies on the acrosome. I. Reaction to egg water and other stimuli. Biol. Bull. (Woods Hole). 103:44-66.

Dan, J. C. 1954. Studies on the acrosome. II. Acrosome reaction in starfish spermatozoa. Biol. Bull. 107:203-218.

Gibbons, B. H., and I. R. Gibbons. 1972. Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with Triton X-100. J. Cell Biol. 54:75-97.

Hylander, B. L., and R. G. Summers. 1975. An ultrastructural investigation of the spermatozoa of two ophiuroids, Ophiocoma echinata and Ophiocoma ventralis. Acrosomal morphology and reaction. Cell Tissue Res. 158:151-168.

Inoué, S. 1986. Video Microscopy. Plenum Publishing Corp., New York. 584 pp.
Inoué, S., and L. G. Tilney. 1982. The acrosomal reaction of Thyone sperm. I. Changes in the sperm head visualized by high resolution video microscopy. *J. Cell Biol.* 93:812–819.

Kuriyama, R., and H. Kanatani. 1981. The centriolar complex isolated from starfish spermatozoa. *J. Cell Sci.* 49:33–49.

Lee, H. C., C. Johnson, and D. Epel. 1983. Changes in internal pH associated with initiation of motility and acrosome reaction of sea urchin sperm. *Dev. Biol.* 95:31–45.

Longo, F. J., and E. Anderson. 1969. Sperm differentiation in the sea urchins Arbacia punctulata and Strongylocentrotus purpuratus. *J. Ultrastruct. Res.* 27:486–509.

Schroeder, T. E., and R. Christen. 1982. Polymerization of actin without acrosomal exocytosis in starfish sperm. Visualization with NBD-phallacidin. *Exp. Cell Res.* 140:363–371.

Sousa, M., and C. Azevedo. 1983. Fine structure of the spermatozoon of Marthasterias glacialis (Linnaeus) (Echinodermata; Asteroidea), with special reference to acrosomal morphology. *Int. J. Invertebr. Reprod.* 6:171–180.

Sousa, M., and C. Azevedo. 1985. Acrosomal reaction and early events at fertilization in Marthasterias glacialis (Echinodermata: Asteroidea) *Gamete Res.* 11:157–167.

Summers, R. G., and B. L. Hylander. 1974. An ultrastructural analysis of early fertilization in the sand dollar, Echinarachnius parma. *Cell Tissue Res.* 150:343–366.

Tamm, S. L., and S. Tamm. 1974. Direct evidence for fluid membranes. *Proc. Natl. Acad. Sci. USA.* 71:4589–4593.

Tamm, S. L., and S. Tamm. 1980. Membrane movements and fluidity during rotational motility of a termite flagellate. *J. Cell Biol.* 80:141–149.

Tamm, S. L., and S. Tamm. 1983. Distribution of sterol-specific complexes in a continually shearing region of plasma membrane and at procaryotic-eucaryotic cell junctions. *J. Cell Biol.* 97:1098–1106.

Tilney, L. G. 1975. The role of actin in non-muscle cell motility. In Molecules and Cell Movement. S. Inoué and R. E. Stephens, editors. Raven Press, Inc., New York. 339–388.

Tilney, L. G., and S. Inoué. 1982. The acrosomal reaction of Thyone sperm. II. The kinetics and possible mechanism of acrosomal process elongation. *J. Cell Biol.* 93:820–827.

Tilney, L. G. 1985. The acrosomal reaction. In The Biology of Fertilization, Vol. 2. C. Metz and A. Monroy, editors. Academic Press, Inc., New York. 157–213.

Vacquier, V. D., and G. W. Moy. 1977. Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. *Proc. Natl. Acad. Sci. USA.* 74:2456–2460.