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The Movements During Fertilization

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ABSTRACT

The movements during fertilization, the systems responsible for each motion, the effects of microtubule and microfilament inhibitors and the regulation of the associated motility are reviewed using the sea urchin system as a paradigm. Following the swimming of the sperm to the egg surface, mediated by the sliding of axonemal microtubules, polymerization of actin present in the periacrosomal cap of the sperm results in the elongation of the acrosomal process. The acrosomal process, extruded as a result of the formation of bundles of microfilaments, establishes the initial contact between the sperm and egg. The egg actively responds to the successful sperm by elongating adjacent microvilli to surround and engulf the successful sperm. These microvilli continue to enlarge, forming a bulbous structure, the fertilization cone, which is responsible for sperm incorporation. Sperm incorporation occurs in two stages, first the enlargement of the fertilization cone around the sperm and second the lateral displacement of the sperm from the site of fusion into the egg cytoplasm proper. Sperm incorporation requires the assembly of egg cortical microfilaments. The lateral displacement of the sperm along the egg cortex is terminated by the assembly of microtubules nucleated at the base of the sperm head, the position occupied by the sperm centrioles. Microtubule assembly continues off of the sperm centrioles to form at first a radial structure, the sperm aster. The growth and the formation of the sperm aster first displace the male pronucleus (sperm nucleus) from the egg cortex towards the egg center. When the microtubules from the sperm aster contact the surface of the female pronucleus (egg nucleus) these microtubules seem to undergo a selective disassembly, resulting in the pulling of the female pronucleus to the center of the sperm aster. Subsequently the now adjacent pronuclei are moved to the egg center by the continuing enlargement of the sperm aster. Pronuclear fusion (syngamy) typically occurs at the egg center after the sperm aster has disassembled. The first cell cycle is characterized by other cycles of assembly and disassembly of cytoskeletal components, including bursts in microvillar length and the microtubule-mediated formation of the interim apparatus or "streak." In this system, within an hour, the microtubules responsible for the formation of the mitotic apparatus form and regress as do the microfilaments comprising the contractile ring that effect cytokinesis. Fertilization represents a unique model in which to study cellular motility since the proper functioning of each gamete requires microtubule- and microfilament-mediated activities and since virtually every example of movement is manifested.

1. The Requirement for Movement During Fertilization

For fertilization to be successful several movements must occur. Sperm must be transported to a position near the egg surface, the sperm and egg must achieve the close contact required to effect membrane fusion and sperm must be drawn into the egg cytoplasm proper. Once within the egg cytoplasm the sperm and egg nuclei must locate one another and move together to establish a contact that will eventually result in the fusion of their nuclear envelopes. Following the successful fusion of the maternal and paternal genomes, subsequent events necessary to prepare the zygote for cell division and embryogenesis must

shortly ensue (see accompanying review by Dr. Belisle).

As a model for studying cellular motility, fertilization is unique for a variety of reasons. Perhaps foremost is polarity of each gamete's quest for survival; the nature of the sperm's motility is to propel it through the suspending fluid first to the proximity of and then into actual contact with the egg. Contrasting with this cellular migration are the egg's movements, which consist entirely of intracellular translocations. First the egg participates in sperm incorporation, i.e., the motion bringing the attached sperm from the egg exterior into its cytoplasm, and then the intracellular migrations of the sperm and egg nuclei that result in syngamy. Simultaneous with these motions of the sperm and egg, dramatic surface alterations involved with the establishment of the block to polyspermy and metabolic activation occur. With the completion of pronuclear fusion the now fertilized egg is prepared to begin to undergo cleavages and the morphogenetic movements required for proper development. Though fertilizaton is indeed unexcelled as a system for studying motion, few animal systems readily lend themselves to the sort of experimentation required for biochemical and microscopic analyses. Of the available systems in which *in vitro* fertilization is routinely obtainable, the echinoderm system is unrivaled. Closely mimicking the events occurring in mammals, this system permits the routine collection of hundreds of millions of perfectly synchronized eggs, which will fertilize at a near-perfected rate. The synchrony, yield and routine availability of this system, when coupled with the glass-like transparency of eggs of some species, has rendered sea urchin fertilization the system of choice for cellular, biochemical and molecular investigations for over a century. In this review the movements during echinoderm fertilization will be predominantly considered; readers interested in mammalian fertilization are directed to the recent reviews by Gwatkin[1] and Yanagimachi.[2]

2. Sperm Incorporation

The ultrastructural features of sperm incorporation have been documented by Longo and Anderson,[3] Schatten and Mazia,[4] Schatten and Schatten,[5] and Tilney and Jaffe.[6] In Figure 1a, the attachment of sperm to the egg shortly after insemination is depicted following the binding of the sperm to the egg surface by the apical region of the sperm head, the acrosome. As observed in Figure 1b, microvilli adjacent to the successful sperm elongate and cluster to form the anlage of the fertilization cone. The activity of the egg surface during sperm incorporation is particularly well documented in Figures 1c and d, in which the extracellular surface coats were removed to allow a direct view of the plasma membrane; here the engulfment of the sperm by the egg cortex and plasma membrane is clearly visible because the overlying layers have been removed.

Recent advances in video microscopy using differential interference contrast optics have permitted the recording of the movements during fertilization in the living.[7,8] In Figure 2 the entire sequence of fertilization is documented. Unlike electron microscopy, which requires the study of fixed, and therefore static, specimens, video tape recording permits a relatively high degree of resolution in the living state in which the sequence of fertilization is directly observed rather than compiled from a sequence of still micrographs. In Figure 2, the initial stages of sperm incorporation are observed as involving first the attachment of the sperm by the acrosomal process to the egg surface. Following a varying period of time, during which the sperm gyrates about its attachment site, the sperm stands erect on the egg surface and the motility of the sperm tail ceases. Moments later the elevation of the fertilization coat around the successful sperm occurs and the unsuccessful sperm attached to the egg surface are lifted from the plasma membrane by the elevation of this extracellular coat. The fertilization cone begins to form around

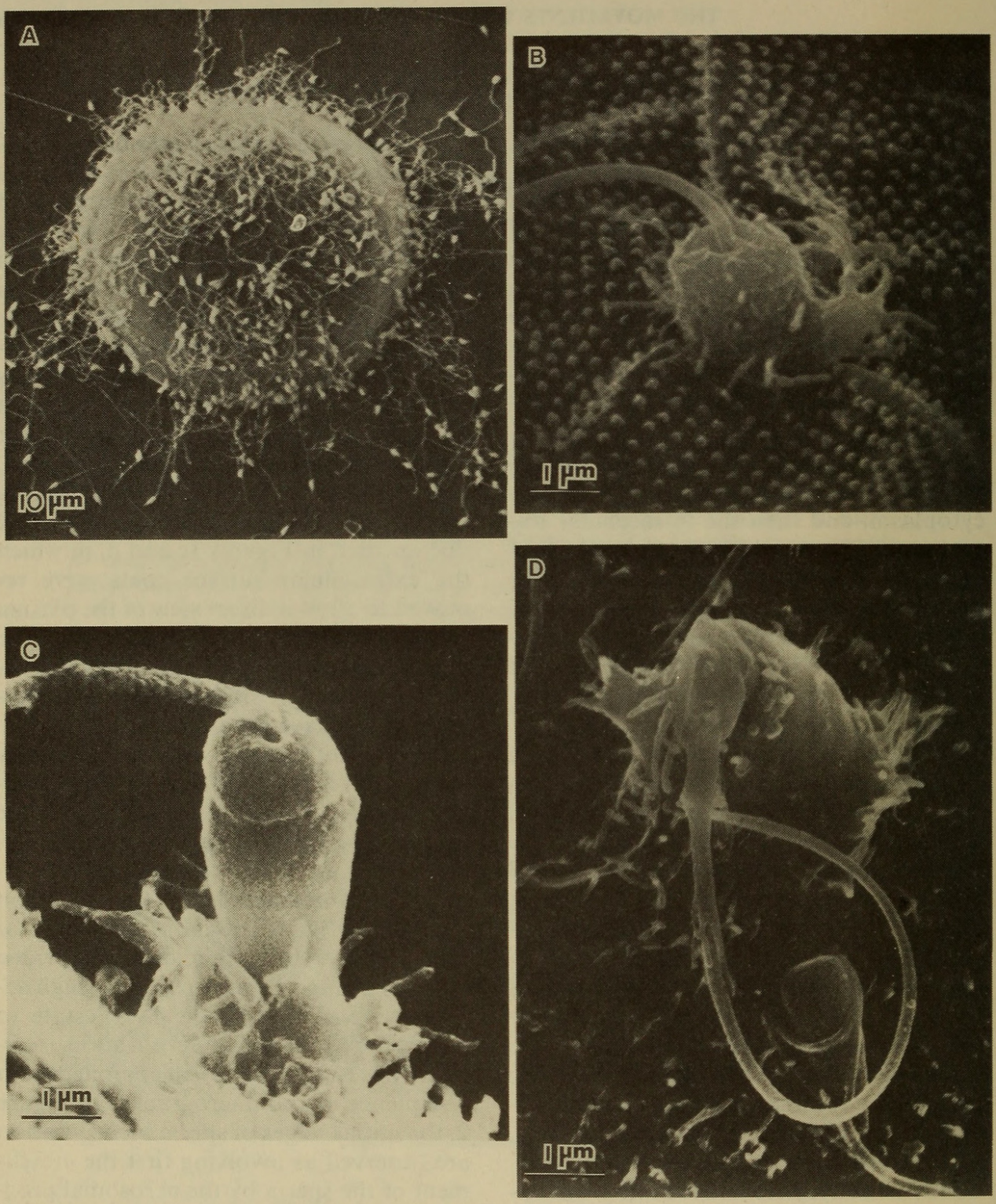
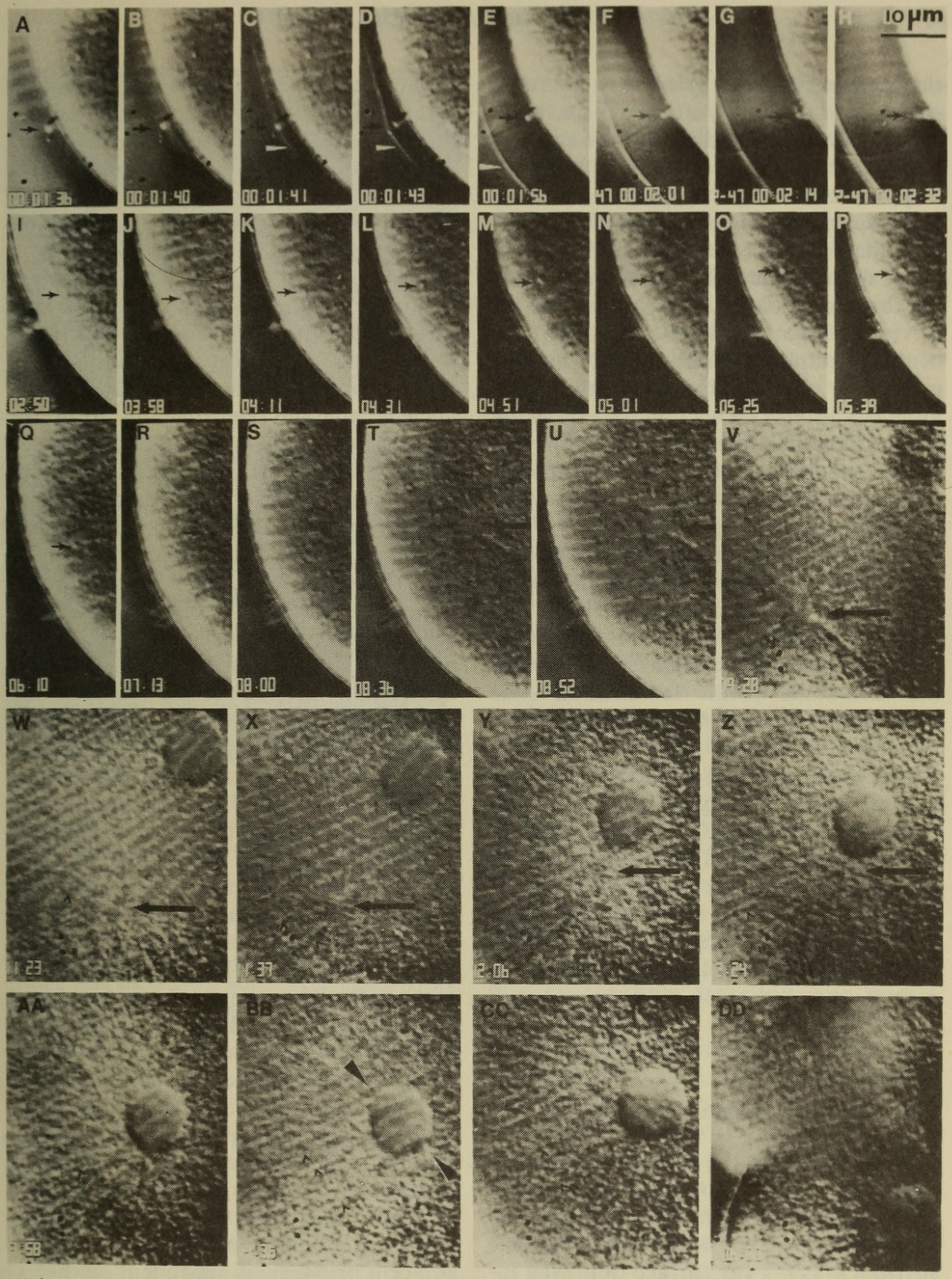


Fig. 1a. Insemination observed by scanning electron microscopy. An early stage of insemination of an egg glued to a polylysine-coated slide. Only the tops and sides of the egg are available for sperm binding. *Strongylocentrotus purpuratus*. Bar: 10 µm. Reprinted, with permission, from ref. #4. 1b. The egg membrane rises around the spermhead. Microvilli elongate around the spermatozoon as the membrane derived from the sperm appears slack and convoluted. *S. purpuratus*. Bar: 1 µm. Reprinted, with permission, from ref. #4. 1c. In these eggs, devoid of their vitelline layers, the activity of the egg surface in engulfing the sperm is clearly apparent. Microvilli have elongated, to 1.2 µm, to completely surround the successful sperm. These microvilli will continue to elongate to form the fertilization cone. *L. variegatus*. Bar: 1 µm. Reprinted, with permission, from Schatten and Schatten, 1980. 1d. The fertilization cone forms from these elongating microvilli, which surround the base of the fertilization cone, and which continue to engulf the sperm. Note the microvilli surrounding the sperm tail. *L. variegatus*. Bar: 1 µm. Reprinted, with permission, from ref. #5.

Fig. 2. Movements during Fertilization Studied in Living Eggs. Time-lapse video microscopy of fertilization with water immersion, differential interference contrast optics. Sperm-egg attachment occurs in A at 1:36 (min: sec). The sperm tail becomes immotile in B and a second later the fertilization coat (white arrow) elevates over the attached sperm (black arrow). The fertilization cone forms around and above the erect sperm in F–I. The



static sperm tail, which projects through the elevated fertilization coat, can be observed in E–H. The displacement of the sperm nucleus (male pronucleus) within the egg cytoplasm occurs in I–P; the sperm tail beats erratically at this stage. The sperm aster forms as the male pronucleus is moved centripetally (Q–U). In V, the field has been shifted to include the sperm aster (large black arrow) and the female pronucleus. Fibers radiating from the sperm aster are denoted by black v's in T–CC. The migration of the female pronucleus to the center of the sperm aster occurs in W–Z; the female pronucleus is distorted from a sphere to an ovoid during this migration. Pronuclear centration (AA–BB) occurs as the fibers of the sperm aster (black v's) continue to elongate. Small particles (black triangles) appear on the nuclear surface in BB; these particles may represent the centrioles since they are positioned along the presumptive axis for mitosis. Cleavage (DD) occurs parallel to the direction of pronuclear centration. *L. variegatus.* Bar: 10 μm. Reprinted, with permission. from ref. #8.

the perpendicularly oriented and static spermatozoon. Shortly afterward, the sperm rotates 90° to lie parallel with the egg cortex, and then begins to undergo a lateral displacement along the egg cortex from the site of sperm-egg fusion. Concomitant with this lateral displacement the erratic beating of the sperm tail is observed, perhaps causing this displacement. The momentary arrest in tail beating and the later resumption of this example of ciliary motility may be indicators of the changes in cytoplasmic ionic conditons (see section 6). Following the movement of the sperm along the egg cortex, the sperm is discharged into the egg cytoplasm proper with its mid-piece directed towards the egg center.

In summary then, the events during sperm incorporation following the swimming of the sperm to the egg surface and the contact by the exterior acrosomal process of the sperm with the egg involve first the formation of the fertilization cone around the erect and static sperm and then the rotation and displacement of the sperm along the egg cortex, which discharges it into the egg cytoplasm in a rotated position so that its centriole faces toward the egg center. This latter point is of importance when the significance of the centriole contributed by the sperm during the pronuclear migrations is considered and during later development (see section 3).

3. Pronuclear Migrations

In this section, the cytoplasmic migrations of the male pronucleus (sperm nucleus) and female pronucleus (egg nucleus) will be traced from the moment when the sperm leaves the egg surface following incorporation to that at which the pronuclei fuse. Syngamy completes the fertilization process. The terminology used throughout this chapter will refer to the unincorporated sperm nucleus as a sperm nucleus; the sperm nucleus within the egg cytoplasm will be referred to as the male pronucleus; the egg nucleus will be referred to as the female pronucleus. The transition from the genetically inactive sperm nucleus into the active metabolic state is reviewed in this monograph by Professor Poccia.

The documentation of the pronuclear migrations has been a difficult undertaking since most eggs are relatively opaque because of the presence of numerous yolk platelets and since the incorporated sperm nucleus migrates centripetally from the surface, where it is visible, into the egg center, where it is not. Modern sophistications in optics have increased the visibility of the pronuclei and their motile structure, and quite importantly, the nearly transparent egg of the Gulf coast sea urchin *Lytechinus variegatus* has contributed greatly in the living documentation. The pronuclear movements at fertilization involve the formation of the sperm aster, at the initial stages a radially symmetrical structure emanating from the sperm centrioles at the base of the rotated sperm mid-piece (see Figures 2 and 3a). The formation of the sperm aster moves the male pronucleus centripetally at a rate of 4.9 μm/min. Concomitant with this centrad motion the male pronucleus begins to undergo chromatin condensation (see accompanying review by Professor Poccia). When the microtubules of the sperm aster contact the surface of the female pronucleus the next of the three pronuclear migrations occurs, i.e. the migration of the female pronucleus (Figure 2). The movement of the female pronucleus to the center of the sperm aster is the swiftest and most dramatic of the pronuclear migrations, occurring at a rate of 14.6 μm/min, often traversing half the diameter of the egg. The final movement of the now adjacent pronuclei is dependent on the extension of the sperm astral microtubules, which push the pronuclei to the egg center. This final motion occurs at a rate of 2.6 μm/min. The fusion of the pronuclei typically occurs at the egg center shortly after

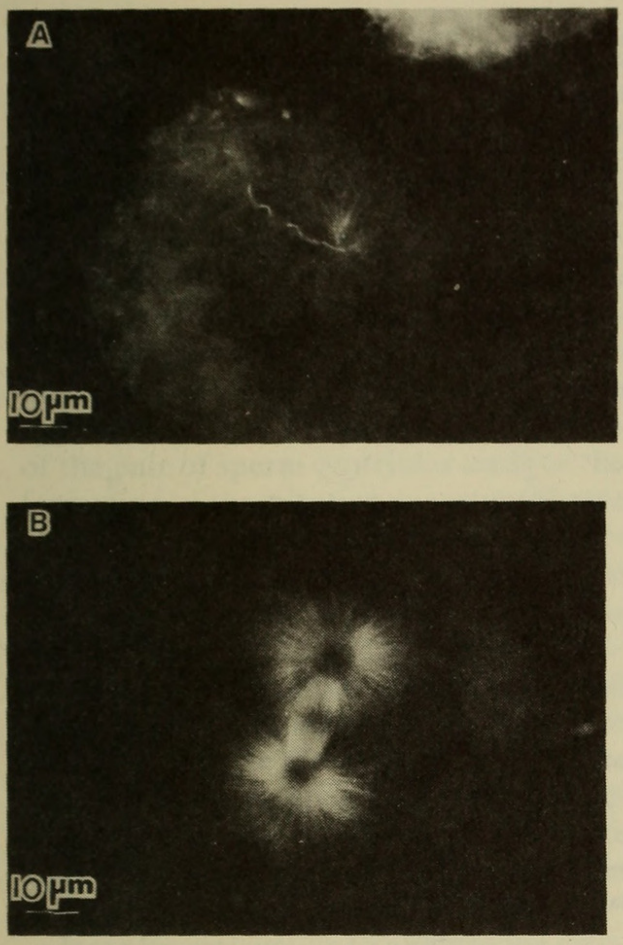


Fig. 3. Antitubulin Immunofluorescence Microscopy. A. Growth of sperm aster. The sperm aster is moved into the cytoplasm of the egg, accompanied by the elongation of astral microtubules. Sperm nucleus is visible as an area from which microtubules are excluded; all microtubules appear to be organized around the sperm midpiece. *A. punctulata.* Many of the fibers visible in these micrographs are of substantially lower intensity than the sperm axoneme, suggesting that microtubule bundles containing only a few microtubules are visible by immunofluorescence microscopy. Bars: 10 μm. B. Mitotic Apparatus. From unpublished work of Balczon and Schatten.

the sperm aster has reached its maximal size.

4. Effects of Motility Inhibitors

Fertilization is a superb model for studying the effects of selective inhibitors of motility. The beating of the sperm tail, which propels the sperm to the egg surface, is an example of the sliding of adjacent microtubules.[9] The extrusion of the sperm acrosomal process requires the assembly of microfilaments,[10] as does the formation of the fertilization cone required during sperm incorporation.[5,11-14] The centripetal migration of the male pronucleus and the later centration of the adjacent pronuclei[15] are systems of movement in which microtubules appear to push the pronuclei away from the surface. In this case the egg cortex, following the migration of the female pronucleus to the center of the sperm aster (Figure 3a), appears to require the pulling of microtubules; this is emphasized by the distortion of the typically spherical female pronucleus into an oblate spheroid during its migration (Figure 2). The use of motility inhibitors to study the mechanism responsible for fertilization has been investigated by a number of workers. Though the acrosome reaction appears insensitive to cytochalasin B,[16] eggs treated with microfilament inhibitors are unable to incorporate the spermatozoon even though sperm-induced egg activation occurs. In contrast, microtubule inhibitors have been utilized to demonstrate that syngamy requires microtubule assembly and that pronuclear fusion and the onset of DNA synthesis are independent processes.[17-21]

The effects of inhibitors of microfilament assembly, microfilament disassembly, microtubule assembly, and microtubule disassembly are summarized in Table 1. The inhibitors of microfilament assembly, cytochalasins B, D and E, prevent sperm incorporation if added prior to or simultaneously with insemination, but they have no effect on pronuclear migrations if added after incorporation. This pattern indicates that the functioning of the egg cortical microfilaments is necessary for the drawing of the sperm from the exterior into the egg cytoplasm but that these microfilaments play no role during the subsequent movements of the pronuclei. The inhibitor of microfilament disassembly phalloidin slows the rate of sperm incorpo-

Table 1—Summary of Effects of Motility Inhibitors

	Microfilament Inhibitors		Microtubule Inhibitors	
	(Assembly)	(Disassembly)	(Assembly)	(Disassembly)
	Cytochalasins	Phalloidin	Colcemid, griseofulvin, nocodazole, maytansine, vinblastine, et al.	Taxol, D_2O
Sperm-Egg Attachment and Fusion	+	+	+	+
Cortical Reaction, Fertilization Coat Elevation	+	−	+	+
Fertilization Cone Formation	−−	++	+	+
Lateral Displacement of Sperm during Incorporation	−−	−	++	+
Restructuring of Fertilized Egg Cortex	−−	+	+	+
Formation of Sperm Aster	+	+	−−	++
Migration of Female Pronucleus	+	+	−−	−−
Pronuclear Centration	+	+	−−	−−
Syngamy	+	+	+	−−
Formation of Streak	+	+	−−	−−
Mitosis	+	+	−−	−−
Cytokinesis	−−	−−	+	+

Legend: −− (event blocked); − (event retarded); + (normal event); ++ (event enhanced).

ration and results in a larger persistant fertilization cone; indications that an active dynamic equilibrium between assembly and disassembly of actin in microfilaments might well be occurring in the fertilization cone. Inhibitors of microtubule assembly, colchicine, colcemid, griseofulvin, maytansine, nocodazole, podophyllotoxin, and vinblastine, all permit sperm incorporation but prevent the subsequent migrations of the pronuclei by blocking the assembly of microtubules that form the sperm aster. This clearly indicates that whereas sperm incorporation does not involve the functioning of microtubules, these subsequent cytoplasmic movements of the pronuclei do. The inhibitor of microtubule disassembly taxol permits the initial formation of the sperm aster but blocks its disassembly with the result that the migration of the female pronucleus, perhaps moved by the disassembly of microtubules, is inhibited. Furthermore the sperm aster remains as an almost crystalline structure and in the absence of its disassembly the centrioles cannot separate and the mitotic apparatus cannot form. This then indicates that, in addition to the proper assembly of microtubules, their subsequent disassembly is also required for pronuclear fusion and subsequent development. In summary then almost any inhibitor of motility will block the normal repertoire of motility during fertilization; the proper union of the sperm and egg genomes requires an intricate and orderly assemblage of motile components, followed by their subsequent disassembly.

5. The Egg Cytoskeleton

The cytoskeleton arrays of the egg are as exceptional as are its other features. The unfertilized egg is the only higher cell completely devoid of any assembled micro-

tubules and microfilaments. Following insemination, cytoskeletal elements form *de novo*, another extraordinary event. In the normal case of fertilization, the sperm brings with it the trigger to assemble microfilaments of the fertilization cone and the microtubules of the sperm aster. These localized assemblages are converted into global events with the subsquent restructuring of the fertilized egg cortex and the microfilament-mediated extension of the egg microvilli. Alternately the separation of the pair of sperm centrioles leads to the formation of the mitotic apparatus (Figure 3b) and the subsequent cytoskeletal reorganizations during division, which result in the mathematical paradox of multiplication by division. In addition to the cytoskeletal rearrangements active during cell division, bursts in microvillar length[22] are noted during the first cell cycle as is the formation of a transient microtubule structure, the streak or interim apparatus. The significance of the streak and the bursts in microvillar elongation is not yet clear.

It is also instructive to analyze the cytoskeletal rearrangements during artificial activation. Clues concerning the natural regulation during fertilization can be easily inferred and furthermore, in the absence of a sperm, its direct contribution to the reorganization of the cytoskeleton following fertilization can be assessed. Microvilli of activated eggs elongate as shown by Mazia *et al.*[23] Microtubules also form in artificially activated eggs.[24,25] However, in the absence of a sperm centriole, these microtubules do not develop into the sperm aster but rather start as a subcortical disarray. These microtubules elongate to form a radial shell that moves the female pronucleus towards the egg center, and finally the shell coalesces at the egg center to form the apolar mitotic apparatus.[24] It appears then in the unfertilized egg that all the components to form the microfilaments and microtubules of the egg cytoskeleton exist. However, the seeds that normally result in the proper polarity and orientation of the cytoskeletal components and the trigger signalling the onset of polymerization are missing and must be either artificially induced or provided by the inseminating sperm.

6. Regulation of Motility During Fertilization

The program of activation, reviewed by Professor Nishioka in this monograph, has been compiled as a result of nearly a century of work. Though details of the scheme are still under active investigation the essential features are summarized as follows: following the acrosome reaction the sperm has greatly elevated levels of internal calcium and intracellular pH. It is at this stage that it fuses its membrane with the plasma membrane of the egg and thereby triggers the onset of development. It is presently thought that the sperm enters the egg effectively as a calcium bomb (estimates of the intracellular calcium concentration in the acrosome reacted sperm are over 10mM) and perhaps as a pH bomb. An alternative view is that the contribution of the sperm membrane to the egg membrane, with its presumed calcium channels serving as endogenous ionophores, permits an influx of sufficient external Ca^{++} to trigger activation. The sudden and localized increase in cytoplasmic calcium at the site of sperm-egg fusion is sufficient to trigger the explosive discharge of adjacent cortical granules. This initial discharge stimulates a propagating wave of released calcium from intracellular stores,[26] which is followed by the secretion of the cortical granules. This calcium transient is concluded within four minutes of sperm-egg fusion when the calcium is presumably resequestered. The initial release of calcium triggers a sodium: proton exchange, which then results in an increased cytoplasmic pH. This alkalinization of the egg cytoplasm appears to be the pervasive ionic trigger for signaling the egg that it is now fertilized.

For the study of the ionic regulation of the egg cytoplasm, two signals appear attractive: the intracellular calcium release triggered by the sperm, and/or the subsequent increase in intracellular pH. Four different sorts of experimental manipulations can be used to pose the question,[27] "Which is the dominant regulator for the formation and functioning of the egg cytoskeleton during fertilization, the intracellular calcium release and/or the rise in intracellular pH."

The first groups of experiments involve cases in which both intracellular pH and calcium fluxes occur: fertilization or activation with the divalent ionophore, A23187. In these cases the cytoskeleton forms and functions as indicated by the intracellular translocations of the pronuclei.

In the second case for studying the ionic manipulations during fertilization only the intracellular release of calcium is permitted. These are eggs in which either the $Na^+ : H^+$ exchange has been blocked by the removal of all extracellular Na^+ or sodium acetate at acidic pH's, which will diffuse and reduce the intracellular pH to the unfertilized values, is added. In this group of experiments, using either natural fertilization or artificial activation with the divalent ionophore A23187, no microfilament-mediated and no microtubule-mediated motion occurs and the cytoskeleton does not form. This indicates that the intracellular release of calcium alone is insufficient to permit the formation and functioning of the egg cytoskeleton.

The third group of experiments pertaining to this question involve cases in which intracellular pH elevation alone is induced using alkaline NH_4Cl in the absence of external Ca^{++}, procaine, or nicotine. In these cases of artificial activation microfilament-mediated events as indicated by microvillar elongation observed with the scanning electron microscope, microtubule-mediated events observed by antitubulin microscopy, and time-lapse video studies of female pronuclear centration indicate the formation and motility of the egg cytoskeleton. It appears that intracellular pH in the absence of an intracellular Ca^{++} release can result in cytoskeletal formation and activity.

The last group of experiments bearing on the ionic regulation of motility during fertilization are cases in which the intracellular Ca^{++} release is first triggered and then subsequently the rise in intracellular pH is permitted. The Ca^{++} release is triggered by the divalent ionophore A23187 or by sperm and when the intracellular elevation is blocked no motility is observed. However, when the intracellular pH is either permitted to rise or artificially elevated by diffusible weak bases, motion then ensues and, in the case of fertilization, development will occur but is delayed by the time span during which the intracellular pH rise was prevented.

To summarize these experiments, intracellular pH appears to be a primary regulator of motility during fertilization.[27] However, it also appears reasonable to conclude at this stage that secondary regulators and

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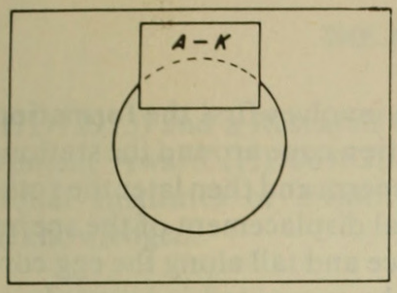
Fig. 4. The Movements During Fertilization. Sperm attach to the egg surface (A) and gyrate about their attachment sites (B) for varying times prior to fusion (C). Following a rapid cortical contraction radiating from the fusion site, the fertilization coat elevates (D–F). Sperm incorporation is characterized by the formation of the fertilization cone around the erect and stationary sperm; the sperm tail is immotile at this stage (D–F). The sperm glides along the egg cortex during penetration (G, H). The formation of the sperm aster moves the male pronucleus centripetally (I, J). The migration of the female pronucleus occurs when the fibers of the sperm aster interconnect the pronuclei (K, L). The adjacent pronuclei are moved to the egg center by the continuing elongation of the sperm aster (M); the centrioles may separate during this motion, and the sperm aster appears to have two focal points. Syngamy typically occurs at the egg center after the disassembly of the sperm aster (N). The streak forms around and distorts the zygote nucleus (O). The axis of the streak (O) is usually identical to the axis of the mitotic apparatus (Q). The streak is disassembled prior to the nuclear breakdown at prophase (P). Cleavage is perpendicular to the axis of the mitotic apparatus and is usually parallel to the egg radius passing through the sperm entry site (Q). Reprinted, with permission, from ref. #8.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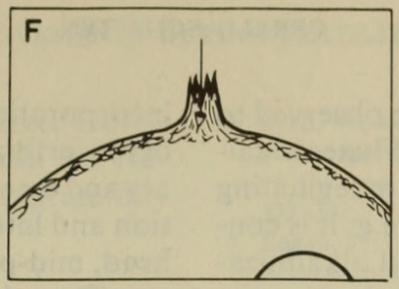
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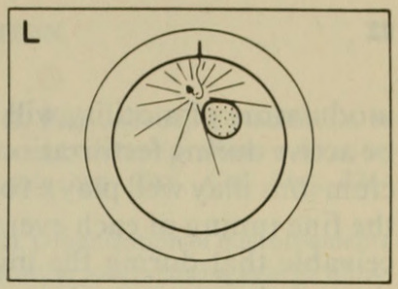
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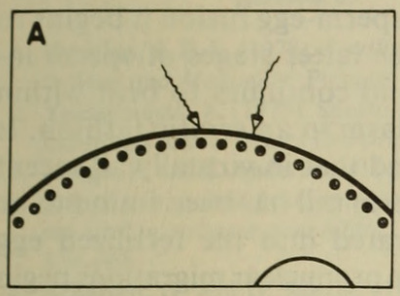
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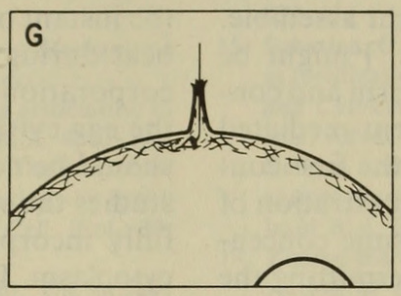
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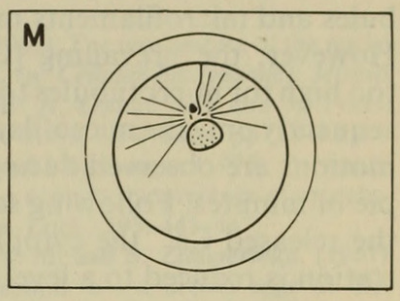
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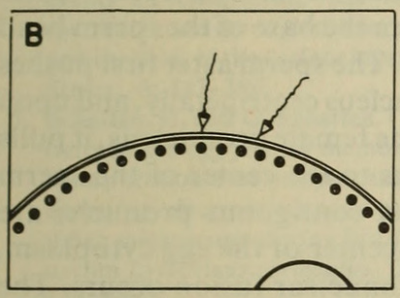
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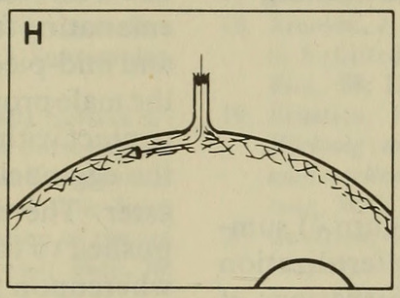
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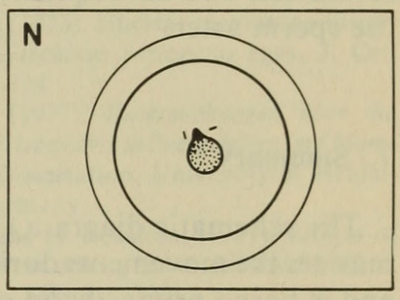
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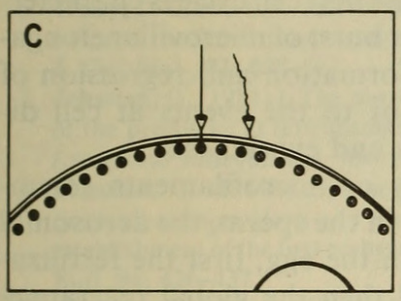
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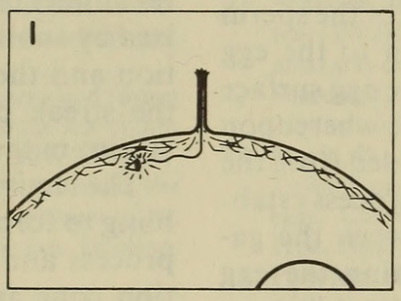
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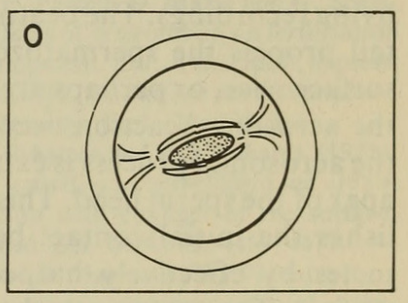
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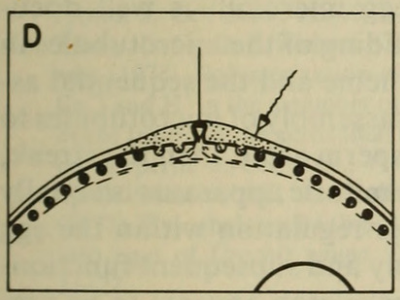
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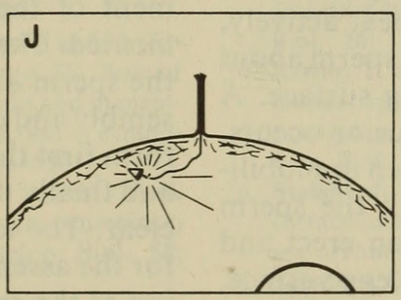
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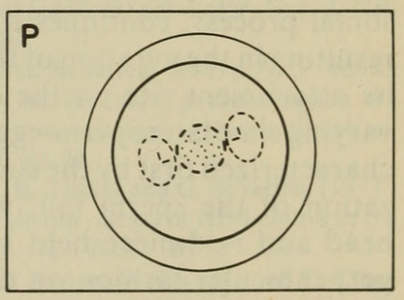
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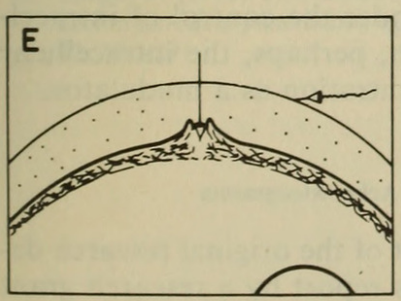
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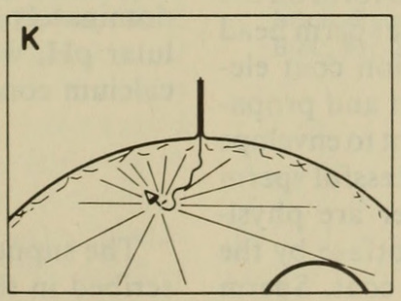
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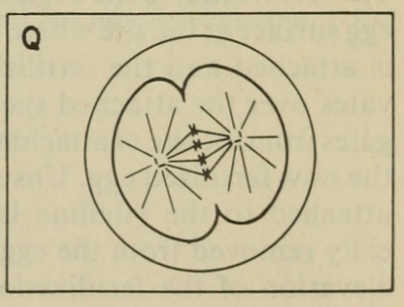
Q

modulators of motility will be observed to be active during fertilization. Fluxes in calcium ions may well play a role in regulating the fine tuning of each event, e.g. it is conceivable that during the initial alkalinization of the egg cytoplasm both microtubules and microfilaments might assemble. However, the prevailing [Ca^{++}] might be too high for microtubules to form and consequently only the microfilament-mediated motions are observed during the first couple of minutes. Following sequestration of the released Ca^{++} the cytoplasmic concentration is reduced to a level permitting the formation and subsequent functioning of the sperm aster.

7. Summary

The schematic diagram (Figure 4) summarizes the movements during fertilization and is based primarily on observations of living recordings. The beating of the sperm tail propels the spermatozoon to the egg surface near, or perhaps at, the egg surface the acrosome reaction occurs, whereupon the acrosomal process is extruded from the apex of the sperm head. This process establishes the initial contact between the gametes by effectively harpooning the egg surface. The sperm, attached by this acrosomal process, continues to beat actively, resulting in the gyration of the sperm about its attachment site on the egg surface. A varying time later sperm-egg fusion occurs, characterized first by the sudden immobilization of the sperm tail, with the sperm head and mid-piece held in an erect and perpendicular fashion on the egg surface. The fertilization cone begins to form on the egg surface at the site where the sperm head is attached and the fertilization coat elevates over the attached sperm and propagates from the site of attachment to envelope the now fertilized egg. Unsuccessful sperm attached to the vitelline layer are physically removed from the egg surface by the elevation of the fertilization coat. Sperm incorporation involves first the formation of the fertilization cone around the stationary and erect sperm and then later the rotation and lateral displacement of the sperm head, mid-piece and tail along the egg cortex. Though the sperm tail is immotile at the instant of sperm-egg fusion it begins to beat during the latter stages of sperm incorporation and continues to beat within the egg cytoplasm in an erratic fashion. It should be noted that in virtually all recent studies the sperm tail has been found to be fully incorporated into the fertilized egg cytoplasm. The pronuclear migrations begin with the formation of the sperm aster emanating from the base of the sperm head and mid-piece. The sperm aster first pushes the male pronucleus centripetally, and upon contact with the female pronucleus, it pulls the egg nucleus to the center of the sperm aster. The now contiguous pronuclei are pushed to the center of the egg cytoplasm, whereupon pronuclear fusion occurs. The remainder of the first cell cycle is characterized by another burst of microvillar elongation and the formation and regression of the streak prior to the events at cell division: mitosis and cytokinesis.

The activity of microfilaments assembling to form, in the sperm, the acrosomal process and, in the egg, first the fertilization cone and then the global rearrangement of the egg microvilli is well documented. The sliding of the microtubules in the sperm axoneme and the sequential assembly and disassembly of microtubules to form first the sperm aster, then the streak, and finally the mitotic apparatus is equally clear. The ionic regulation within the egg for the assembly and subsequent functioning of the cytoskeleton appears to be predominately under the control of intracellular pH, with, perhaps, the intracellular calcium concentration as a modulator.

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