PHYSIOLOGICAL REVIEWS
Vol. 79, No. 2, April 1999
Printed in U.S.A.

Ion Channels in Sperm Physiology

ALBERTO DARSZON, PEDRO LABARCA, TAKUYA NISHIGAKI, AND FELIPE ESPINOSA

Departamento de Genética y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Morelos, Mexico; and Centro de Estudios Científicos de Santiago and Facultad de Ciencias, Universidad de Chile, Santiago, Chile

Darszon, Alberto, Pedro Labarca, Takuya Nishigaki, and Felipe Espinosa. Ion Channels in Sperm Physiology. *Physiol. Rev.* 79: 481–510, 1999.—Fertilization is a matter of life or death. In animals of sexual reproduction, the appropriate communication between mature and competent male and female gametes determines the generation of a new individual. Ion channels are key elements in the dialogue between sperm, its environment, and the egg. Components from the outer layer of the egg induce ion permeability changes in sperm that regulate sperm motility, chemotaxis, and the acrosome reaction. Sperm are tiny differentiated terminal cells unable to synthesize protein and difficult to study electrophysiologically. Thus understanding how sperm ion channels participate in fertilization requires combining planar bilayer techniques, in vivo measurements of membrane potential, intracellular Ca^{2+} and intracellular pH using fluorescent probes, patch-clamp recordings, and molecular cloning and heterologous expression. Spermatogenic cells are larger than sperm and synthesize the ion channels that will end up in mature sperm. Correlating the presence and cellular distribution of various ion channels with their functional status at different stages of spermatogenesis is contributing to understand their participation in differentiation and in sperm physiology. The multi-faceted approach being used to unravel sperm ion channel function and regulation is yielding valuable information about the finely orchestrated events that lead to sperm activation, induction of the acrosome reaction, and in the end to the miracle of life.

I. INTRODUCTION

Fertilization is essential for sexual reproduction and for the generation of a new individual. This fundamental process requires communication between mature and competent male and female gametes so that they may fuse. Components from the external layers of the egg profoundly influence sperm physiology, priming it for fertili-

zation. Close to a century has gone by since the sperm-egg dialogue began to be studied (184), yet the detailed molecular mechanisms involved remain elusive.

Traveling toward the egg, spermatozoa undergo significant changes in their ionic milieu that modulate their functional state. In organisms of external fertilization (external fertilizers), i.e., sea urchins and teleost fishes, sperm develop the potential for motility only after leaving the testis. For instance, a sea urchin can deliver as many as 10^{10} sperm into the sea that, upon release, become active swimmers, powered by a microscopic flagellar engine. An amazing, still to be understood molecular orchestra tightly couples sperm behavior to chemical signals from the environment and the egg, guiding it through the waters in its most eventful journey. In spite of the wonderful physiological hardware they are endowed with, for most sperm the extenuating adventure will end nowhere. From the millions of sperm released by a male, only a few will find the egg to initiate the crucial event of fertilization (106, 288). A sea urchin sperm released as close as 1 cm away from an egg must swim around 50-fold its length to reach its target. How, then, do sperm manage to find the egg and fuse to it? There is still debate as to the role of sperm chemotaxis in nature. It is probably only effective at distances shorter than 0.2–0.5 mm (207).

In internal fertilizers such as reptiles, birds, and mammals, sperm develop the potential for motility as they pass through the epididymis (212). The behavior of sperm after being released from the gonads is a most dramatic example of continuous coupling between the cell machinery and the outer environment. The sperm is not a deterministic device oblivious to the external medium, tuned only to the chemical signals from the egg outer layer. It must avoid fusing with any other cells but the egg. The concentration of ions, pollutants, pH, temperature, and other physicochemical variables influence sperm behavior and metabolism. Importantly, signals from the egg modulate sperm physiology, inducing sperm to undergo a series of ordered changes in configuration that enable it to complete fertilization.

When sperm are spawned into the reproductive ground or ejaculated into the female reproductive tract, motility ensues. Activation is triggered by ionic or osmotic changes. These transduction events are likely to involve sperm ion channels (reviewed in Ref. 209). The fact that the sperm can accomplish a variety of configurational changes in a short time makes this tiny cell a most attractive model in cell physiology (68, 309).

In the early 1950s, J. C. Dan (60, 61), studying the entrance of sea urchin sperm into eggs by phase-contrast and electron microscopy, discovered the acrosome reaction (AR). It is now established that this exocytotic process is one of the fundamental steps for fertilization in many species, including mammals (68, 249, 288, 309). The AR synchronizes the exposure of membrane elements required for penetration of the egg coat and subsequent fusion with the egg plasma membrane. Her discovery brought to the attention of scientists that the spermatozoan, a tiny overlooked cell, needed to be studied to understand fertilization (142). Soon after, Dan (62) discovered that this reaction depended on the presence of Ca^{2+} in seawater. The dramatic influence of the external ion composition on motility and the AR strongly suggests that ion channels actively participate in these fundamental cell processes. Ion channels are essential elements in cell signaling (140, 148). Certain ion channel blockers and altered ionic conditions can inhibit sperm motility, sperm maturation, and the AR.

Sperm ion channels are the subject of this review. Although, at present, there is increasing and convincing evidence that ion channels are fundamental to sperm physiology, a full understanding of how these integral membrane proteins influence sperm physiology is needed. The ion channel mechanisms operating in sperm must be elucidated, not a trivial thing to do due to their small size. Another, not less involved, challenge is to unveil the mechanisms by which ion channels in the cell surface are regulated by the external environment and the intracellular metabolic machinery. Such a dialogue makes it possible for free-swimming sperm to operate as effective units in the course of a quite momentous event for eukaryotes.

In the past 10 years, ion channel mechanisms have been investigated in sperm using voltage- and ion-sensitive dyes, bilayer reconstitution, DNA recombinant techniques, cRNA expression in heterologous systems, immunocytochemistry, pharmacology, and, to a lesser extent, the patch clamp. Such studies have provided precious information about sperm ion channels as well as on some of the mechanisms that modulate them (reviewed in Ref. 68). Because of their incredible efficiency in catalyzing the flow of millions of ions per second through the nonconducting lipid bilayer, a few ion channels can cause changes in the configuration of a small cell, like the sperm, in milliseconds, a feat that cannot be achieved by any other known membrane transporter or metabolic device (140). Ion concentrations not only determine cell membrane potential through ion-selective channels in a classic Nernstian fashion, but permeant ions can control the extent of channel activity and therefore membrane potential and ion flow. In turn, membrane potential governs the rates and direction of ion transport in channels and exchangers; its fluctuations allow, for example, for local pH and Ca^{2+} concentration changes. Intracellular Ca^{2+} is key to flagellar motility and to the fusion of the acrosomal vesicle. Moreover, in sperm, membrane potential is known to modulate the activity of membrane-bound enzymes, causing changes in second messenger levels, which modulate sperm ion channels (22).

Sperm are tiny differentiated terminal cells unable to synthesize protein and difficult to study electrophysiologi-

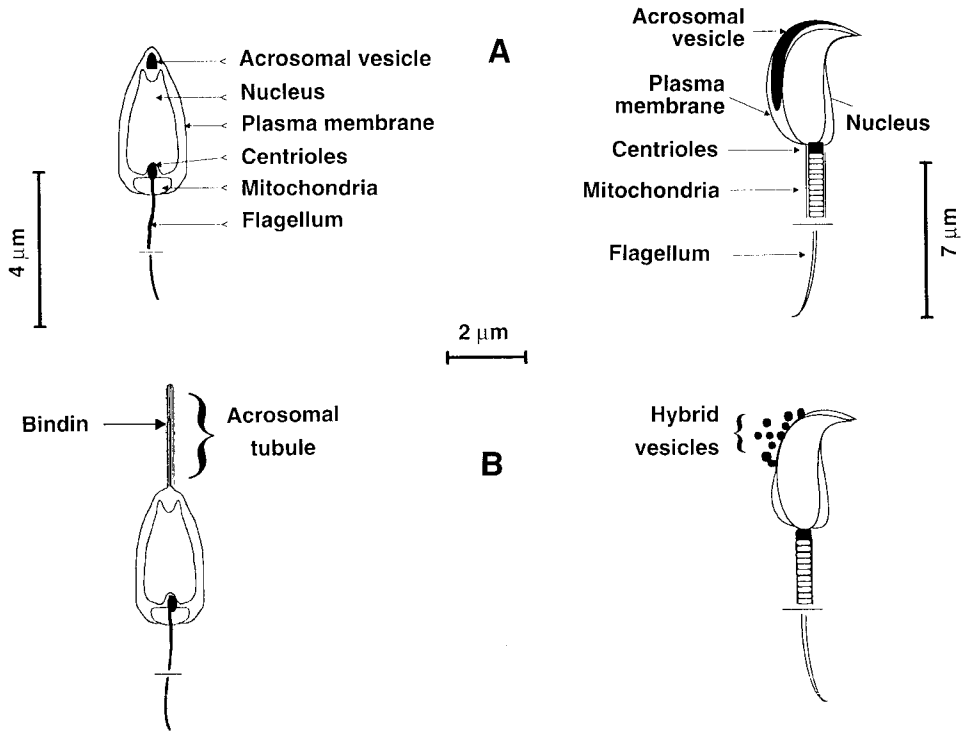

FIG. 1. Schematic diagram of a sea urchin (*left*) and mouse sperm (*right*) before (*A*) and after acrosome reaction (*B*). Sperm head contains all cell organelles except flagellum.

cally. Because of this, studying their ion channels has required combining experimental approaches. Because spermatogenic cells are larger than sperm and synthesize the ion channels that will end up in mature sperm, they are also being used to explore sperm ion channel function. Correlating the presence and cellular distribution of various ion channels with their functional status at different stages of spermatogenesis will allow a better understanding of their participation in differentiation and in sperm physiology. This review starts with the general characteristics of sperm, then the strategies used to learn about sperm ion channels are described. The properties and ways in which ion channels participate in sensing environmental changes and transducing signals from the egg are then discussed in detail. The authors apologize for leaving out some important contributions due to space limitations. There are several helpful reviews on general aspects of gamete interaction and function (68, 90, 106, 156, 245, 249, 309, 310).

A. General Characteristics of Spermatozoa

Most animal sperm display a similar general design (Fig. 1). They are quite small and are mainly composed of *1*) a head (2–5 μm in diameter), containing condensed packages of chromosomes in the nucleus, two centrioles, and in many species, the acrosome, a membranous structure lying over the nucleus in the anterior part of the sperm head. The nucleus occupies most of the head. *2*) The tail, of variable length depending on the species (10–100 μm), has the characteristic "9+2" complex of microtubules found in eukaryotic flagella and cilia. The mammalian flagellum has accessory fibers not seen in lower organisms. *3*) A few mitochondria power the tail movement at its base. They can be inside the sperm head as in sea urchins or spirally arranged in the midpiece of the tail as in mammals. The cytoplasmic volume of sperm is very small; the internal volume per sea urchin and human sperm has been estimated to be ~35 and 15 fl, respectively (155, 252). Spermatozoa are unable to synthesize proteins or nucleic acids. They are specialized cells committed to find, fuse, and deliver their genetic information to the egg.

II. STRATEGIES TO STUDY SPERM ION CHANNELS

A. Fluorescent Indicators

Fluorescent probes have been used to measure, in vivo, sperm intracellular Ca^{2+} ($[Ca^{2+}]_i$), intracellular pH (pH_i) (12, 94, 127, 128, 250, 327) and membrane potential (E_M) (10, 13, 114, 123, 251). Figure 2 illustrates how, in *Strongylocentrotus purpuratus* sea urchin sperm, the egg factor that triggers the AR (F), induces a 10- to 20-fold increase in $[Ca^{2+}]_i$, a 0.2–0.3 change in pH_i, and a E_M depolarization. All these ion

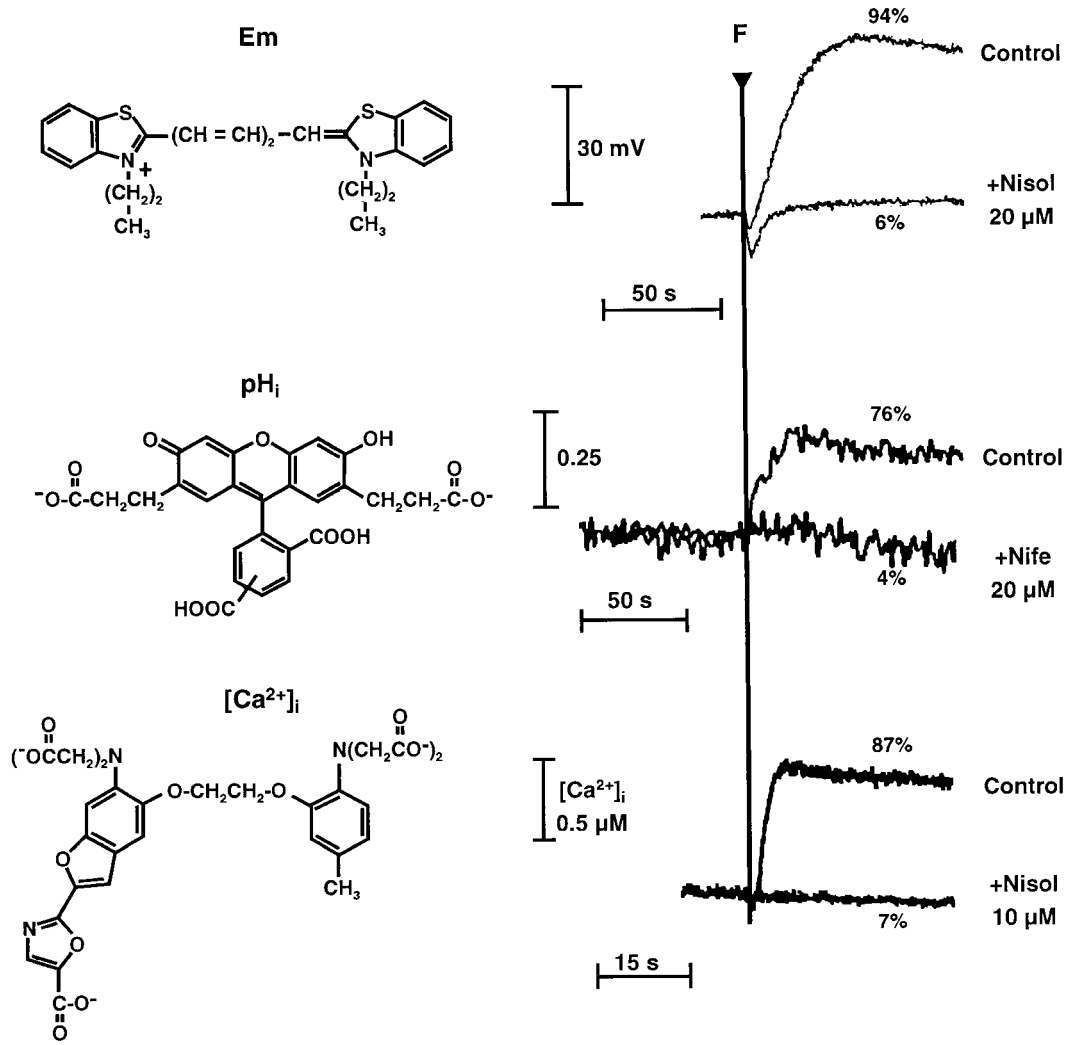

FIG. 2. Ca^{2+} channel blockers inhibit membrane potential (E_M), intracellular pH (pH_i), and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) changes induced by egg factor (F) that triggers acrosome reaction (AR) in *S. purpuratus* sperm. Black vertical line indicates addition of F. Increases in measured parameters correspond to upward deflections. Percent numbers above traces indicate AR determined by phase-contrast microscopy. Fluorescent probes used are shown on *left*: a cyanine dye $DisC_3$-(5) for E_M, BCECF for pH_i, and fura 2 for $[Ca^{2+}]_i$. Sperm were loaded overnight with permeant fura 2-AM or BCECF-AM dyes at 4°C in 0Ca artificial seawater, pH 7.0, for pH_i and $[Ca^{2+}]_i$ measurements, respectively. For E_M determinations, cells were preequilibrated with 500 nM $DisC_3$(5) for 2–3 min (see Ref. 67 for experimental details). Nifedipine (Nife) and nisoldipine (Nisol) were used.

permeability changes, and the AR, are blocked by dihydropyridines (123, 128, 129). Similar observations using the homologous AR-inducing component have been made in mammals and in many other sperm species (reviewed in Ref. 68).

B. Planar Bilayers

The availability of large quantities of sperm allows the isolation and characterization of plasma membrane fractions from the different regions of the cell. A mature sea urchin male can provide up to $\sim 5 \times 10^{10}$ sperm, whereas a mature mouse can provide $\sim 10^8$ sperm. The isolated sperm plasma membrane vesicles can be reassembled in various model systems to study sperm ion channels (Fig. 3, *A* and *B*; reviewed in Refs. 67, 68). The first single-channel recordings from sea urchin sperm were obtained in bilayers made at the tip of patch-clamp pipettes from monolayers generated from a mixture of lipid vesicles and isolated sea urchin sperm flagellar membranes (181). Thereafter, the fusion of isolated sperm plasma membranes from various species into black lipid membranes (BLM) revealed the presence of several types of ion channels (Table 1).

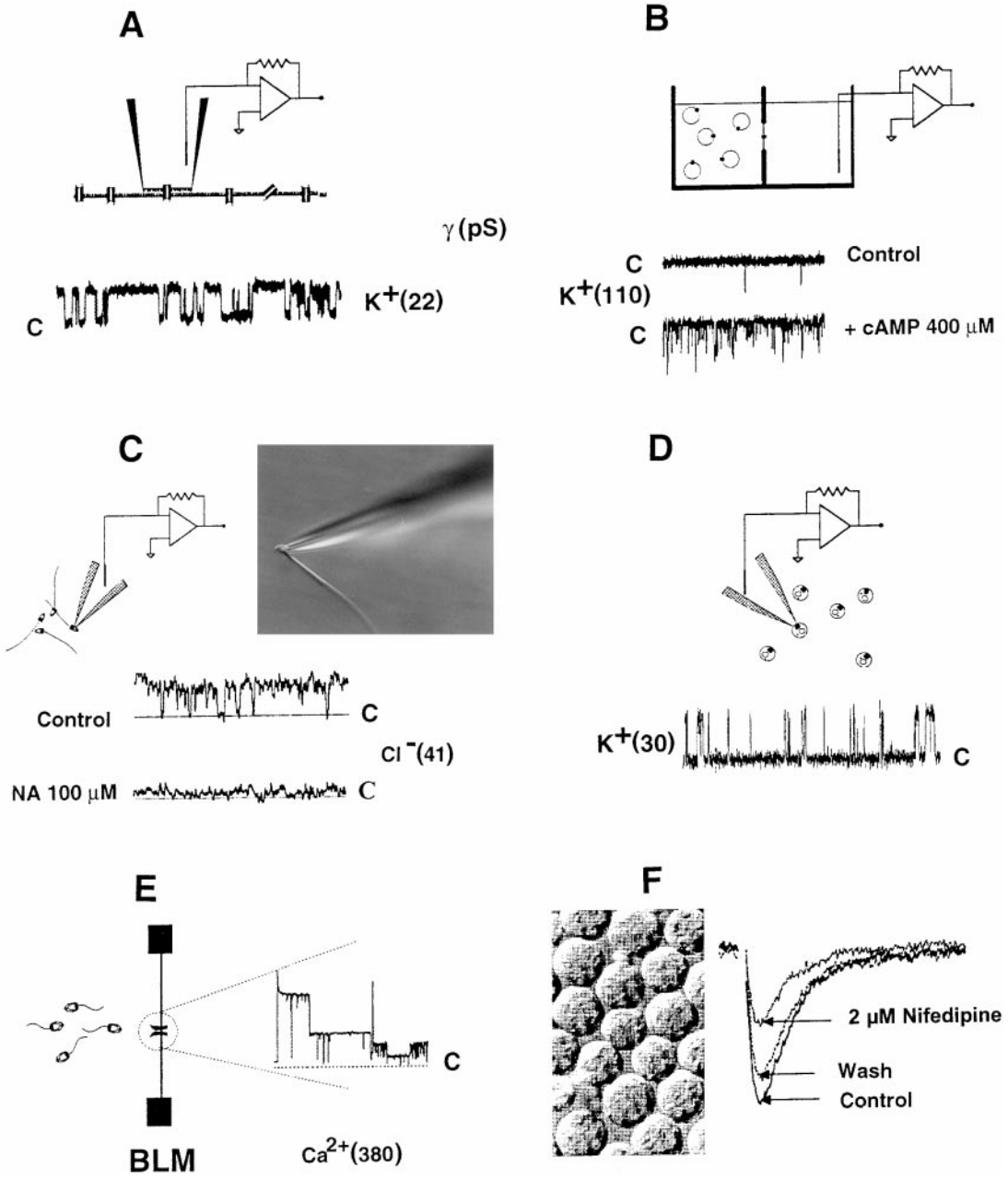

FIG. 3. Electrophysiological strategies to study ion channels in sperm and spermatogenic cells. An example of a channel detected with each technique is illustrated, indicating main ion transported and single-channel conductance (γ) in pS (see Table 1 for ionic conditions). *A*: bilayers at tip of a patch-clamp pipette. C, closed channel. *B*: black lipid membranes (BLM) with fused sperm plasma membranes. *C*: mouse sperm on-cell patch-clamp recordings of a niflumic acid (NA)-sensitive Cl^- channel. (From Sánchez and A. Darszon, unpublished data). *D*: one-cell patch-clamp recordings in osmotically swollen sea urchin sperm. *E*: direct ion channel transfer from sperm to BLM. *F*: photograph of purified mouse pachytene spermatocytes. [From Bellvé et al. (20).] *Right* shows T-type Ca^{2+} currents recorded from mouse pachytene spermatocytes are reversibly blocked by nifedipine.

C. Transfer of Ion Channels to Planar Bilayers Directly From Spermatozoa

An alternative to circumvent the sperm size limitation is the transfer of ion channels from live sperm to BLM (21) (Fig. 3*E*). The probability of ion channel transfer is at least doubled by the AR, both in sea urchin and in mouse sperm. Cell-cell interactions, such as sperm-egg fusion, can be explored using this new strategy (21).

D. Patch Clamp

Although the planar bilayer experiments described above have yielded important information regarding the

TABLE 1. *Ion channel classes in sperm cells*

Source/Channel Property	Method	Type of Channel	Gating Properties	γ, pS	Blockers	Cloned?	Reference No.
Sea urchin/single-channel current	Planar bilayer reconstitution	K^+ selective	?	22, 46[a]	TEA	−	181
	Patch clamp	K^+ selective	?	60, 172[b]	?	−	130
	Patch clamp	K^+ selective	Activated by Speract	2–5[c]	?	−	13
	Planar bilayer reconstitution; heterologous expression	K^+ selective	Upward modulated by cAMP; gated by voltage	103[d]	TEA, Ba^{2+}	− +	118, 163
Mouse/single-channel current	Heterologous expression	K^+ selective	Gated by voltage; pH sensitive	106[e]	?	+	256
Rat/single-channel current	Planar bilayer reconstitution	K^+ selective	?	24[f]	?	−	40
	Planar bilayer reconstitution	Na^+ selective	?	109[f]	?	−	40
Sea urchin/single-channel current	Planar bilayer reconstitution	Cation selective	?	82[g]	?	−	181
Mouse/single-channel current	Planar bilayer reconstitution	Cation selective	?	103[h]	?	−	164
	Patch clamp	Cation selective	?	23, 318[i]	?	−	85
Bovine/macroscopic current	Heterologous expression	Cation selective	Gated by cGMP		?	+	312
Human/single-channel current	Planar bilayer reconstitution	Cation selective	?	130,[j] 29[k], 65[k]	?	−	324 40
Sea urchin/single-channel current	Planar bilayer reconstitution	Ca^{2+} selective	Gated by voltage	172[l]	Mg^{2+}, Co^{2+}, Cd^{2+}	−	21, 183
Boar/single-channel current	Planar bilayer reconstitution	Ca^{2+} selective (L type?)	Weak voltage dependence	16[m](Nitrendipine, (R22)-BAY K 8644,		− −	283
Mouse/single-channel current	Planar bilayer reconstitution	Ca^{2+} selective	Gated by voltage	381[n]	Co^{2+}, ruthenium red	−	21
Mouse/macroscopic current and monitored on spermatogenic cells	Patch clamp	Ca^{2+} selective T-type channel	Gated by voltage		Nifedipine, amiloride, pimozide, Ni^{2+}	−	7, 182, 246
Sea urchin/single-channel current	Planar bilayer reconstitution	Cl^- selective	?	148[o]	DIDS	−	208
Mouse/single-channel current	Patch clamp	Cl^- selective	Gated by voltage	41[p]	Niflumic acid, (F^-?)	−	85
Mouse, rat/single-channel current	Planar bilayer reconstitution	Cl^- selective	?	83[q] 15[f]	?	−	164 40

Ionic conditions (in mM) in which single-channel conductance (γ) was obtained are as follows: [a]symmetrical, 100 KCl; [b]cell excised, 486 NaCl in pipette/10 KCl in bath; [c]cell attached, 7.5 or 25 potassium gluconate in pipette; [d]symmetrical, 100 KCl; [e]symmetrical, 213 KCl; [f]100 KCl *cis*/200 NaCl, 300 mM KCl *trans;* [g]symmetrical, 100 KCl; [h]600 KCl *cis*/100 NaCl *trans;* [i]23-pS channel, cell-excised patch, 145 NaCl in bath/120 CsCl in pipette; 318-pS channel, cell-excised patch, 145 NaCl in bath/90 KCl in pipette; [j]symmetrical 100 NaCl; [k]200 KCl *cis*/100 KCl *trans;* [l]symmetrical, 50 $CaCl_2$, 25 $BaCl_2$, 125 KCl *cis*/25 $BaCl_2$ *trans;* [m]100 $BaCl_2$, 50 NaCl *cis*/50 NaCl *trans;* [n]200 KCl *cis*/25 $BaCl_2$ *trans*, 600 KCl *cis*/100 NaCl *trans;* [o]symmetrical, 100 KCl; [p]cell-attached, 120 NaCl, 30 NaF in pipette; [q]600 KCl *cis*/100 KCl *trans*.

properties of sperm ion channels, it is desirable to directly record them in the cell. The development by Neher and Sackman of the patch-clamp technique in the 1970s, which revolutionized cell physiology, did not have a crucial impact in the sperm front. The reason for this is easy to understand: because they are tiny and have a complex morphology, sperm are tough nuts to crack, even for the patch clamp. Despite this, single channels were recorded directly from sea urchin sperm heads using the patch-clamp technique. Single-channel transitions were documented (Table 1), one of which was K^+ selective (130). Recently, it was possible also to obtain patch-clamp recordings from the head of mouse sperm (85).

To overcome the sperm size limitation, sea urchin sperm have been swollen in diluted seawater. Swollen sperm are spherical (~4 μm diameter) and can be patch clamped (13; Fig. 3*D*). They can regulate their E_M, pH_i, and $[Ca^{2+}]_i$. Swelling *S. purpuratus* sperm improved the success rate of sealing from 1% in nonswollen cells to >20%. Patch-clamp experiments revealed

the presence of a K^+-selective channel. Swollen sea urchin sperm open new possibilities to directly study ion channel regulation (13).

E. Molecular Cloning and Heterologous Expression

Many physiologically relevant ion channels have been sequenced (140). Now, testicular libraries are being tested with probes designed for specific channels and receptors. It is interesting that members of the olfactory receptor gene family have been found in spermatogenic cells and in mature mammalian spermatozoa (296). With the consideration of the involvement of cyclic nucleotides in the physiology of sperm (reviewed in Ref. 68), it is not surprising that the first sperm channel to be cloned using a bovine testis library was a cyclic nucleotide-gated (CNG) cation channel (312, see sect. III*D1*). This important contribution was followed by the cloning of a somewhat atypical pH- and voltage-dependent K^+ channel (256) and a sea urchin sperm cAMP-modulated mildly K^+-selective channel (118). This channel, named SPIH, together with a similar hyperpolarization-activated cation channel found in spontaneously active neurons and heart (189) are the first cyclic nucleotide-modulated K^+-selective channels cloned in animal cells.

F. Spermatogenic Cells

In addition to being very small and difficult to study electrophysiologically, spermatozoa are differentiated terminal cells unable to make proteins. Thus gene expression and protein assembly have to be studied in the progenitor spermatogenic cells. Spermatogenesis is a striking process where spermatogonia divide producing spermatocytes which undergo meiosis and yield spermatids that differentiate and mature into spermatozoa (11, 20). Pachytene spermatocytes, and round and condensing spermatids, are at the later stages of differentiation and are translationally active and much larger than sperm, therefore easier to patch clamp (7, 8, 131, 182, 246; see Fig. 3).

Back in 1984 when Hagiwara and Kawa (131) reported the first whole cell recordings from spermatogenic cells, only a couple of electrophysiological studies (intracellular recording) had attempted to investigate sperm cells (186, 195). Hagiwara and Kawa (131) concentrated their attention on late primary spermatocytes and early spermatids which, in adult rats (>80 days old), are most abundant (~80% of dissociated cells). Dissociated, late primary spermatocytes are 16–18 μm in diameter and exhibit a large nucleus with condensed chromatin. Dissociated early condensing spermatids are smaller in diameter (11–14 μm) and display a short flagellum as well as a developing acrosomal vesicle on the nucleus. Transient inward Ca^{2+} currents whose density increased during spermatogenesis, from spermatogonia to early spermatids, were described. These cells also displayed slowly developing voltage-dependent K^+ outward currents blocked by tetraethylammonium ion (TEA^+), and insensitive to external Ca^{2+}, that significantly decreased during spermatogenesis. These observations suggest that distinct expression of ion channels during spermatogenesis may influence differentiation (131). In addition, Hagiwara and Kawa's paper (131) indicated " . . . genes for ionic channels and receptors, which have been considered to be characteristic of excitable tissues, can be expressed and function at early stages of embryogenesis." This prediction has been proven to be correct (182, 256). Furthermore, some of these channels end up in mature sperm, determining their physiological properties.

The resting E_M of rat spermatids has been estimated using a E_M-sensitive dye in suspension (−22 mV; Ref. 236) and in single cells (−57 mV; Ref. 223). These cells regulate their pH_i by means of a V-type H^+-ATPase, a HCO_3^- entry pathway, a Na^+/HCO_3^--dependent transport system, and a putative H^+-conductive pathway. Apparently, rat spermatids do not have base extruder transport systems. Their pH_i regulation seems tuned to manage acid challenges (223).

Regulation of $[Ca^{2+}]_i$ is likely to be important for spermatogenesis and is critical for sperm maturation, capacitation, and AR. Because of this, the genotypic and phenotypic expression of voltage-dependent Ca^{2+} channels (VDCC) (7, 182, 246) as well as the role of Ca^{2+} internal stores in determining $[Ca^{2+}]_i$ are being studied in spermatogenic cells (247, 285). The functional findings relevant for sperm are discussed in section V*C*.

Messenger RNA for the three inositol 1,4,5-triphosphate receptor (IP_3R) subtypes (I, II, and III) were detected in spermatogonia as well as in all subsequent stages of spermatogenesis (285). Antibodies raised against mammalian IP_3R revealed distinct distribution patterns of the mature receptor during sperm differentiation. At early stages, IP_3R are homogeneously distributed throughout the cytoplasm, and as differentiation proceeds, they become selectively localized to the Golgi complex. Consistent with this distribution pattern, spermatogonia undergo a large intracellular Ca^{2+} release in response to Ca^{2+}-ATPase inhibitor thapsigargin, whereas smaller responses were detected in late spermatocytes and spermatids (285).

The cytoplasmic distribution of IP_3R and the larger Ca^{2+} release responses found in spermatogonia suggest that IP_3R could be involved in cell proliferation at this stage. This notion received support from experiments with a spermatogenic derived cell line showing that depletion of intracellular Ca^{2+} pools after thapsigargin treatment dramatically inhibits cell division. On the other hand, incubation with an antisense oligonucleotide used

to arrest the synthesis of functional type I IP_3R completely inhibited proliferation (285).

The three known genes (I, II, and III) encoding for ryanodine receptor proteins (RyR) are expressed at all stages of spermatogenesis. However, specific antibodies raised against each of the RyR subtypes indicate that only types I and III are present in spermatogenic cells. In contrast to IP_3R, which undergo a dramatic subcellular redistribution, RyR remain homogeneously scattered in the cytoplasm at all stages of differentiation. In mature sperm, only type III RyR was detected immunocytochemically. Functional responses to caffeine and ryanodine were completely absent in spermatogenic cells and in mature sperm. Thus IP_3R may participate more significantly in spermatogenesis, particularly during cell proliferation, than RyR (285).

III. ION CHANNELS AND SPERM ACTIVATION

A. Sea Urchin

Spermatozoa cannot swim in the sea urchin male gonads because the high CO_2 tension in semen maintains pH_i acid (~7.2) with respect to seawater (150). Dynein, the ATPase that drives the flagella, is inactive below pH 7.3, repressing motility and respiration (42, 174). Spawning decreases the CO_2 concentration surrounding sperm as well as induces H^+ release, a pH_i increase to ~7.4, and dynein activation. Production of ADP activates mitochondrial respiration 50-fold and initiates motility (42, 150, 220). A phosphocreatine shuttle allows the energy produced in the mitochondria to reach the flagella (284).

The activation of motility depends on the concentration of external Na^+ ($[Na^+]_o$), external K^+ ($[K^+]_o$), and pH_i (27, 42, 43, 150, 174). Sea urchin sperm possess a Na^+/H^+ exchange activity in the flagella (169, 170) that has been studied in flagellar vesicles. This Na^+/H^+ exchange is unusual in that it is amiloride insensitive and Mg^{2+} and voltage dependent (169, 170, 171, 173). By keeping intracellular Na^+ ($[Na^+]_i$) low, the Na^+-K^+-ATPase contributes to pH_i regulation (117). Zinc also modulates pH_i (45).

1. K^+ channels

The sea urchin sperm resting E_M (−36 to −56 mV) is influenced by $[K^+]_o$ (114, 251). Sperm activation is inhibited when $[K^+]_o$ is 100 mM in seawater. These results suggest the presence of K^+ channels in the plasma membrane of these cells. Two of the three types of cation-selective single-channel transitions identified in tip-dip formed bilayers were blocked by TEA^+, indicating they were due to K^+ channels (Table 1) (181). Single channels were also recorded directly from sea urchin sperm heads using the patch-clamp technique (Table 1), one of which was K^+ selective (130). Because $[K^+]_o$ is higher in semen than in seawater (44), spawning could hyperpolarize sperm. The hyperpolarization could stimulate the voltage-dependent Na^+/H^+ exchange and contribute to the pH_i rise that accompanies sperm activation. It has been shown that the sea urchin adenylyl cyclase (AC) is modulated by voltage (22). A cAMP increase may activate a cAMP-dependent protein kinase (PKA), which phosphorylates axonemal proteins contributing to sperm motility (106, 209).

B. Salmonid Fish

It has been known since 1938 that millimolar $[K^+]_o$ in the seminal tract is primarily responsible for keeping trout sperm inactive (255). Morisawa and Suzuki (214) further investigated this phenomena and showed that salmonid fish sperm motility can be initiated in K^+-free medium, and not in K^+-supplemented medium, which is similar to the seminal fluid. This group also showed that cAMP increases and reaches a plateau seconds after suspending trout sperm in K^+-free medium (211). This cAMP elevation is required for motility initiation (213), which involves the cAMP-dependent phosphorylation of a 15-kDa axonemal protein (210).

Although $[K^+]_o$ and cAMP were known to influence motility, their relationship was unknown. Potassium channel blockers like, TEA^+, nonyltriethylammonium$^+$, Ba^{2+}, and Cs^+, inhibited sperm motility initiation (278). Potassium was shown to contribute to the resting E_M of trout sperm (115); therefore, a membrane hyperpolarization caused by sperm suspension in low $[K^+]_o$ could be the first step in this signal cascade. Divalent cations, including Ca^{2+}, Mg^{2+}, and Sr^{2+}, can initiate trout sperm motility even in K^+-supplemented medium (278). Boitano and Omoto (31) proposed that divalent cations can mask the surface potential of trout sperm membrane, leading to a hyperpolarization. They demonstrated that a hyperpolarization induced with Cs^+ and valinomycin in K^+-supplemented medium could initiate motility. These results indicated that K^+ efflux through sperm plasma membrane K^+ channels would lead to a hyperpolarization under physiological conditions (31). Recently, K^+ efflux from salmonid sperm was measured upon initiation of sperm motility in K^+-free medium (277).

Calcium is also thought to be important for initiating salmonid sperm motility. The Ca^{2+} channel blockers verapamil (278) and desmethoxyverapamil (53) inhibit sperm motility initiation in K^+-free media. In this medium, sperm AC activity is higher in the presence than in the absence of external Ca^{2+} ($[Ca^{2+}]_o$) (211). Uptake of $^{45}Ca^{2+}$ is accelerated in trout sperm incubated in K^+-free medium (277). The Ca^{2+}-sensitive fluorescent probes

have indicated $[Ca^{2+}]_i$ increases in single sperm (277) and in sperm populations (32, 53) upon initiation of motility. A transient $[Ca^{2+}]_i$ elevation was detected in Ca^{2+}-free medium after providing hyperpolarization by addition of Mg^{2+} (32). The contribution of $[Ca^{2+}]_o$ and internal Ca^{2+} stores to the $[Ca^{2+}]_i$ increase that occurs when motility is initiated still remains to be established. The river water into which sperm are spawned contains 0.3–0.4 mM Ca^{2+}, enough to contribute to Ca^{2+} influx through specific sperm plasma membrane Ca^{2+} channels under physiological conditions. In salmonid sperm, there is no evidence that pH_i increases accompany sperm motility initiation (31).

C. Teleosts and Amphibians

An osmolarity change is a key factor for sperm motility initiation among many species of teleosts and amphibians. In marine teleosts, such as puffer fish, spermatozoa start swimming upon incubating them in a hypertonic medium regardless of the presence of electrolytes (214). On the contrary, in freshwater teleosts, such as goldfish and zebrafish, and amphibians, such as newt, sperm start swimming when treated with hypotonic solutions (136, 214). The conditions described above correspond to the physiological environments found by these animals when spawned. Unlike salmonid fish, K^+ efflux-dependent hyperpolarization is not required to initiate sperm motility in these species. Instead, changes in intracellular ion concentration caused by swelling or shrinkage, according to external osmolarity, appear to regulate sperm motility in marine and freshwater teleosts (276). In addition, high pH_i seems preferable for the initiation of sperm motility, whereas it is not clear whether sperm pH_i increases or decreases upon hypertonic treatment in marine teleosts (221, 276). The initiation of sperm motility in marine teleosts appears to also involve an increase in $[Ca^{2+}]_i$ derived from intracellular Ca^{2+} stores (221).

D. Mammals

The ionic environment encountered by spermatozoa in its journey through the epididymis undergoes significant changes. In the caput, $[Na^+]_o$ is higher than 100 mM and decreases to <50 mM in the cauda (149), whereas $[K^+]_o$ rises from ~20 to ~40 mM in these two regions. The mouse sperm membrane resting potential is determined mainly by K^+ (84, 326), whose internal concentration has been estimated to be ~120 mM both in bull (12) and in human (185). Increasing $[K^+]_o$ can thus depolarize the cell and open voltage-dependent Ca^{2+} channels (14, 21, 55, 92), possibly triggering premature AR. Nonetheless, the decrease in $[Na^+]_o$, which acidifies pH_i (327), and the low Ca^{2+} concentration in epididymal fluids (149), would compensate the tendency to open Ca^{2+} channels, preventing spontaneous AR. As discussed below, an alkaline pH_i is necessary for capacitation and AR (10, 327).

Sperm must undergo capacitation, and thereafter the AR, to fertilize the egg. The most significant changes experienced by sperm during capacitation are reorganization (in composition and topology) of sperm surface antigens, changes in plasma membrane permeability, increases in intracellular second messengers (cAMP, IP_3, diacylglycerol), and increased phosphorylation of a set of proteins by different kinases (16, 270, 300, 313).

During sperm maturation, $[Ca^{2+}]_i$ progressively rises in some species (15, 69), leading to hyperactivated motility (313) and spontaneous AR (319). Seminal plasma factors and other factors present in the female fluids in vivo or added to the capacitating media (165, 319) can regulate $[Ca^{2+}]_i$ and sperm capacitation (30, 222). For instance, caltrin, a seminal plasma protein, inhibits sperm $^{45}Ca^{2+}$ uptake (46, 243). It has been reported that heparin, which is required for bovine sperm in vitro capacitation, regulates $[Ca^{2+}]_i$ by modulating voltage-dependent Ca^{2+} channels possibly binding to specific plasma membrane receptors (37, 52, 227). A minimum of 90 μM $[Ca^{2+}]_o$ is required for mouse sperm capacitation (101), but it may differ for human sperm (78). The role of internal Ca^{2+} stores in capacitation is not yet understood. Calreticulin, a Ca^{2+}-binding protein (217), and the IP_3R (285, 303) have been detected in the acrosome of several mammalian species, indicating that Ca^{2+} may be stored and released from this organelle. Compounds that favor Ca^{2+} release from internal stores, like thapsigargin, appear to accelerate this process (204).

The lipid content of membranes may modulate their fluidity and ion channel activity (18, 41, 190). Bovine serum albumin scavenging of cholesterol during capacitation (121) is thought to change the membrane fluidity and the permeability to Ca^{2+} and HCO_3^- (301). Protein phosphorylation during mouse (299, 301), bovine (103), and human sperm capacitation (16) are modulated by $[Ca^{2+}]_o$ as well as $[HCO_3^-]_o$. Hyperactivation and phosphorylation of several proteins by tyrosine kinase require HCO_3^- in the capacitating media. These effects may be due to cAMP increases mediated by AC (29, 261, 299, 301). The increase in cAMP activates PKA, which in turn stimulates tyrosine kinases, which finally phosphorylate a set of proteins important for capacitation (300).

The influence of pH_i on maturation and capacitation is an open question (313). Changes in extracellular pH (pH_o) linearly affect sperm pH_i (116, 132, 327). In mouse sperm, pH_i increases during capacitation mainly through a Na^+-, Cl^--, and HCO_3^--dependent mechanism (327). This pH_i increase also occurs in bovine (291, 302) and human sperm, where it has been related to sperm cholesterol content (57). Bovine sperm exposed to PKA inhibitors undergo the normal pH_i changes during capacitation

(103), implying that internal alkalinization precedes PKA activation or that both processes are independent. The second option seems more likely (at least in mouse sperm), since alkaline pH_o, which should elevate pH_i, does not substitute for HCO_3^- depletion, indicating that HCO_3^- itself stimulates AC and not through a pH_i increase (301). As mentioned above, considering that pH_i may influence sperm Ca^{2+} permeability (14, 68), an acidic pH_i may contribute to maintain E_M (38) and to maintain $[Ca^{2+}]_i$ low, thus preventing untimely AR.

Capacitation in bovine and mouse sperm is accompanied by K^+ permeability increases that hyperpolarize the cells from around −30 to −60 mV (326). This hyperpolarization could stimulate AC if it is similar to the sea urchin sperm AC (22). Increases in cAMP would activate PKA, leading to protein phosphorylation. The hyperpolarization would also affect voltage-dependent channels, especially the T-type Ca^{2+} channels likely to be present in sperm (see sect. v) (182). A subtle balance must persist between conditions that promote premature AR and those that counterbalance the environmental changes so that only the tightest and fittest sperm survive the excursion through the epididymis and the female reproductive tract to achieve fertilization.

1. Cyclic nucleotide-gated channels

The presence of CNG channels in mammalian sperm was suspected since cAMP and Ca^{2+} levels are important modulators of motility (also capacitation and the AR). Indeed, the first sperm channel to be cloned using a bovine testis library was a CNG cation channel (312). The CNG channels are heteroligomeric complexes made from at least two subunits (α and β). The α-subunit displays the channel activity, whereas β alone is not functionally active. However, coexpression of α- and β-subunits yields channel species with different properties when compared with homoligomeric channels (reviewed in Ref. 152). The α-subunit from bovine testis was cloned first (312) and shows 78% amino acid sequence homology to CNG channels in chicken photoreceptors. It contains the cyclic nucleotide binding site, pore sequence, transmembrane segments, and S4-voltage sensor motif characteristic of the CNG channel family. When expressed in *Xenopus* oocytes, its single-channel conductance is 20 pS (Table 1). The channel selects poorly between Na^+ and K^+, is blocked by Mg^{2+}, and exhibits permeability to Ca^{2+}. Guanosine 3′,5′-cyclic monophosphate [dissociation constant (K_d) = 8.3 μM, Hill coefficient = 2.6] is far more effective in activating the bovine testis channel than cAMP (K_d = 1,700 μM, Hill coefficient = 1.5). Small cGMP-induced currents associated with single-channel transitions of <10 pS were detected in vesicles thought to be sperm cytoplasmic droplets. Inside-out patches from human and bovine sperm responded to cGMP with similar small currents (312).

Very recently, one short and several long less abundant transcripts of CNG channels β-subunits were identified in bovine testis (315). Immunodetection showed that the α-subunit is present along the entire sperm flagellum, whereas the short β-subunit is only found in the principal piece of the flagellum. These sperm CNG channels permeate Ca^{2+} and are more sensitive to cGMP than to cAMP. If various types of CNG channels have different permeability to Ca^{2+} and are distinctly localized in the flagellum, as indicated by the dissimilar localization of the α- and β-subunits, then Ca^{2+} microdomains may exist. This could be the basis for flagellar bending control (315).

IV. SPERM ION CHANNEL REGULATION BY DIFFUSIBLE EGG COMPONENTS

External fertilizers undergo an immense dilution upon spawning. Gamete encounter demands information about their whereabouts. Although sperm in internal fertilizers have a determined trajectory through the female reproductive tract, gamete interaction also requires signals that prepare them for fertilization and promote preferential interactions of the egg with the fittest sperm. Some of these signals stimulate vectorial sperm movement toward the egg (chemotaxis) and/or enhance their motility and metabolism (chemokinesis). It has been reported that secretions from the egg or from the female reproductive organs may cause chemotaxis and/or chemokinesis in plant and animal sperm (206, 209).

A. Sea Urchin

The metabolic state and motility of sperm are altered, species specifically (with restrictions), by small peptides (~10–14 amino acids) contained in the jelly surrounding the egg. Possibly, these peptides may also facilitate AR, acting in concert with the main egg jelly inductor of this process (263, 318; but see Ref. 294).

Speract, a decapeptide (Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly) isolated from *S. purpuratus* and *Hemicentrotus pulcherrimus* egg jelly, stimulates at picomolar concentrations and pH_o 6.6, sperm phospholipid metabolism, respiration, and motility (134, 275). In normal seawater, this peptide induces complex plasma membrane permeability changes in sea urchin spermatozoa. Speract and resact (Cys-Val-Thr-Gly-Ala-Pro-Gly-Cys-Val-Gly-Gly-Gly-Arg-Leu), a similar peptide from *Arbacia punctulata* eggs (275), stimulate sperm uptake of $^{22}Na^+$ and $^{45}Ca^{2+}$, and H^+ and K^+ release, at nanomolar concentrations (133, 173, 235). As a result of these permeability changes, $[Ca^{2+}]_i$ and pH_i increase (250). Furthermore, these peptides elevate the cGMP and cAMP levels (106, 157).

Speract analogs cross-link to a 77-kDa transmembrane peptide in *S. purpuratus* sperm (64) that has been cloned (63, 65). The speract-receptor complex transiently activates the sperm membrane guanylyl cyclase (GC) (106). Resact (nM) directly stimulates this GC in *A. punctulata* (264). The sea urchin resact receptor was the first sequenced member of a family of GC that are major regulators of cell physiology (107, 266).

Speract (nM) induces a K^+-dependent hyperpolarization in *S. purpuratus* sperm flagella and flagellar plasma membrane vesicles (106, 173). Starting at picomolar concentrations, this peptide causes a TEA^+-insensitive, K^+ permeability increase in swollen sea urchin sperm that is mediated by K^+-selective channels, as shown in patch-clamp experiments (13, see Fig. 3*D*). The speract-induced hyperpolarization activates a Na^+/H^+ exchange in swollen (13, 49, 237) and nonswollen sperm, whose stoichiometry was estimated to be 1:1 from measurements of $^{22}Na^+$ influx and pH_i using BCECF (169, 170, 250). Although this exchange is electroneutral, it is stimulated by the speract-induced hyperpolarization (169). It would be important to confirm the stoichiometry of this exchange using methods that have equal time resolution. The speract-induced pH_i increase dephosphorylates GC and reduces its activity (25, 233, 274, 306–308); it stimulates AC, which is pH_i (49, 50), E_M (22), and Ca^{2+} sensitive (106). The cGMP decrease may lower K^+ permeability and repolarize sperm (49). Lee (172) suggested the participation of G proteins in the speract-induced hyperpolarization, since guanosine 5′-*O*-(3-thiotriphosphate) stimulates it in flagellar vesicles. Although sea urchin sperm contain G_i (24, 158), G_s, and low-molecular-weight G proteins (39, 58), their role in sea urchin sperm physiology remains to be established.

In swollen (13, 49, 237) and nonswollen sperm (22, 162), speract (>100 pM) induces a Ca^{2+}-dependent depolarization after the hyperpolarization and transiently increases $[Ca^{2+}]_i$, possibly opening a Ca^{2+}-permeable channel. Such a channel appears to be regulated by cAMP and allows Mn^{2+} through (50). It has not been ruled out that, as in photoreceptors and mouse sperm, cGMP could also upregulate a cation-selective channel permeable to Ca^{2+} (152, 312, 315). A Na^+/Ca^{2+} exchanger probably contributes to the speract-induced $[Ca^{2+}]_i$ increase and to $[Ca^{2+}]_i$ regulation (250).

Sperm from *A. punctulata* are attracted by nanomolar resact, changing their swimming pattern from a circular to a straighter trajectory; $[Ca^{2+}]_o$ is required for the response. Only in this species has chemotaxis been demonstrated (305). In *S. purpuratus* sperm, the simultaneous addition of 50 nM speract and 100 μM IBMX, a phosphodiesterase inhibitor, produces asymmetric flagellar movements (51). These results have been used to derive an interesting model to explain how sperm may detect an increasing egg peptide gradient over a broad concentration range (51; see Ref. 68 for discussion). The drawback of this work is that the simultaneous addition of IBMX and speract is not a physiological condition, and it induces AR (250). A working model for the action mechanism of speract that incorporates the available information is presented in Figure 4.

The transmembrane topographical homology between the somatic AC and various ion channels and transporters led to the proposal, not yet demonstrated, that this protein might have a dual life, converting ATP to cAMP and operating as an ion channel (160). An AC not modulated by G proteins, and stimulated by hyperpolarization, was described in *Paramecium* (257). The sea urchin sperm AC is modulated by pH_i and $[Ca^{2+}]_i$ (49, 50, 106) and appears to be insensitive to G proteins (106, 139). Sea urchin sperm hyperpolarization stimulates this AC independently of $[Ca^{2+}]_i$ and pH_i (22). Because sperm hyperpolarization is induced by egg outer envelope components, such as speract (reviewed in Ref. 68), E_M activation of AC could modulate sperm motility, chemotaxis, and AR. It will be interesting to explore if mammalian and fish sperm AC are also voltage dependent. A few somatic cell AC have been shown to be regulated by E_M (234).

1. Cyclic nucleotide-gated channels

It is likely that two cAMP-regulated ion channels with distinct selectivity and pharmacology may contribute to the depolarization triggered by nanomolar speract in sperm: a Ca^{2+} channel (13, 50, 66) and a poorly selective K^+ channel that was detected in planar lipid bilayers (163). In bilayers exposed to symmetrical 100 mM KCl, this latter channel has a single-channel conductance of 103 pS. Its open probability is low and weakly voltage dependent, increasing at negative potentials. Addition of cAMP to the *cis*-side increases the open probability of the channel in a dose-dependent and reversible fashion (Fig. 3*B*). The channel does not allow Ca^{2+} through, is blocked in a voltage-dependent fashion by millimolar Ba^{2+} or TEA in the *trans*-side, and displays a low P_K/P_{Na} of ~5, indicating a sizable permeability to Na^+ (163). Because seawater contains ~0.5 M Na^+, and in sea urchin sperm cells, $[K^+]_i$ = 0.18 M, the reversal potential of this cAMP-modulated channel in the sea is close to −10 mV, 30 mV more positive than the sperm resting potential. Thus its opening in seawater would depolarize sperm. The cAMP dependence, selectivity, and pharmacological profile of this channel suggest it participates in the Na^+-dependent speract-induced repolarization in sea urchin sperm (163). The E_M studies in *S. purpuratus* sperm are consistent with this proposal (162, 237).

A cAMP-regulated K^+ channel has been cloned from sea urchin testis and functionally expressed in HEK 293 cells (118). The cDNA encodes a 767-amino acid polypeptide (molecular mass ~88 kDa) named SPIH with significant sequence similarity to CNG and ether-a-gogo (EAG/

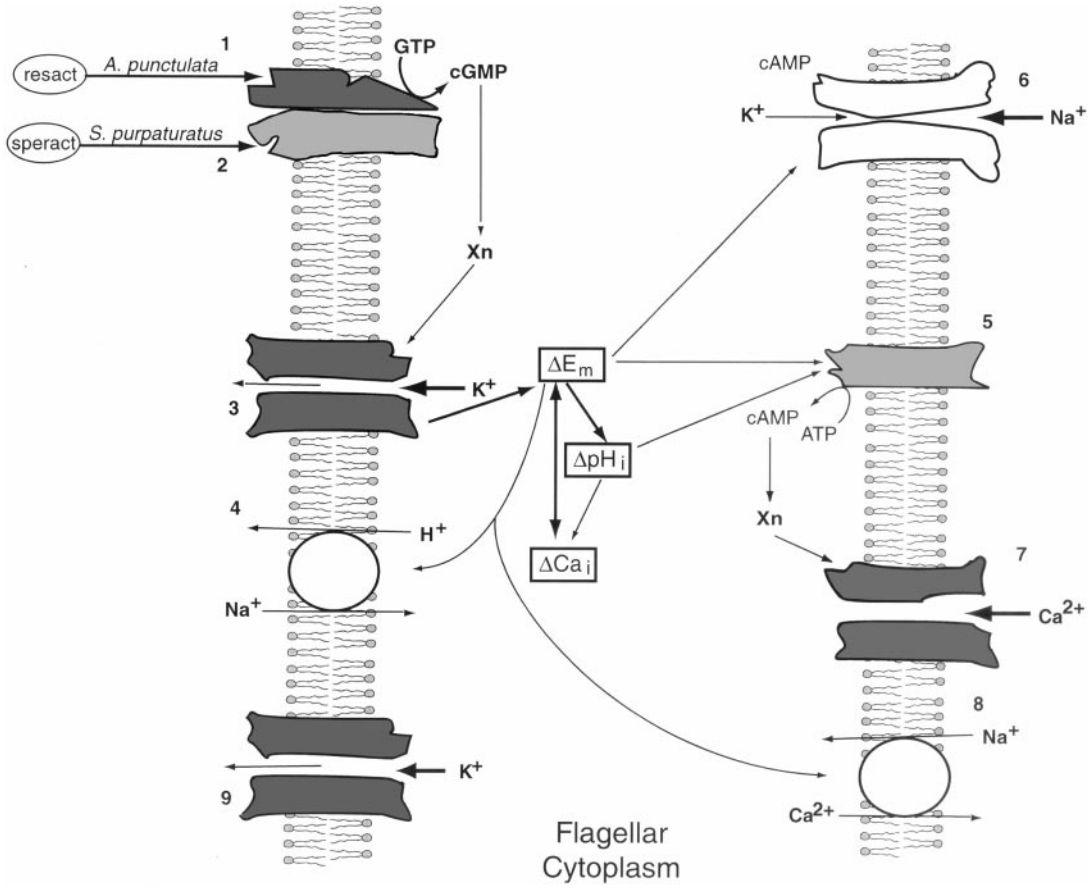

FIG. 4. Regulation model of main ion transport systems involved in sea urchin sperm responses to small egg peptides. Resact directly activates *A. punctulata* sperm flagellar guanylyl cyclase (GC; *1*), whereas in *S. purpuratus* sperm, speract indirectly activates GC after binding to its receptor (*2*). Two different receptors to speractlike peptides have been detected (323). After a momentary concentration increase, cGMP directly or after Xn steps, opens a K^+ channel (*3*) which transiently hyperpolarizes sperm. This hyperpolarization enhances Na^+/H^+ exchange (*4*), stimulates adenylyl cyclase (AC; *5*), which is also sensitive to pH_i, and activates a cation channel (*6*), studied in bilayers and recently cloned, that is directly regulated by cAMP. Because of its poor K^+/Na^+ selectivity, this channel depolarizes sperm. Depolarization and increase in cAMP concentration may stimulate an ill-defined Ca^{2+} channel (*7*). Combined changes in pH_i, $[Na^+]_i$, and E_M could lead to reverse Na^+/Ca^{2+} exchange (*8*). GC (*1*), some kinases, phosphatases, and phosphodiesterases may be pH_i sensitive.

HERG) channels. This similarity is particularly notorious in transmembrane segments S3 and S5, in segment S4 or voltage-sensing region, as well as in the pore and cyclic nucleotide binding domains. On the other hand, the voltage sensor of SPIH is peculiar in that it exhibits a long string of regularly spaced positively charged amino acids, interrupted by a serine residue located right at the center of the motif. Other K^+ channels, like Shaker and EAG/HERG, display an arginine at this position (152). The pore region of SPIH has the GYG triplet characteristic of K^+ channel pores, but in addition, it exhibits two positively charged residues and lacks the threonine cluster which, in Shaker channels, determines K^+ selectivity (76, 137). The cyclic nucleotide binding region of SPIH is similar to other cyclic nucleotide binding proteins (152). In addition, SPIH contains sites for potential phosphorylation by PKA, cGMP-dependent protein kinase, Ca^{2+}-dependent protein kinase (PKC), and tyrosine kinase. Functional SPIH channels exist in a phosphorylated form when expressed in HEK 293 cells (118). They resemble voltage-gated I_f K^+ currents from pacemaker sinoatrial node myocytes. These channels are gated by hyperpolarization and are upwardly modulated by cAMP and phosphorylation (1). This channel has also been cloned recently and is present in heart and brain (189, 248). Thus a family of channels activated by hyperpolarization and cAMP exists that are important in determining the resting E_M, depolarizing cells, and limiting their hyperpolarization currents (72, 118, 166, 189, 248).

Whole cell and excised patch-clamp recordings of SPIH channels expressed in HEK 293 cells showed that these channels are closed at voltages more positive than 10 mV and opened by membrane hyperpolarization (118). A voltage jump from a holding potential of 10 mV to more

negative voltages induces an early current ("instant current"), which then increases in sigmoidal fashion, reaches a peak in 20–100 ms, and relaxes nearly exponentially to a lower steady-state value. This complex behavior suggests that the channel displays involved kinetic properties, including more than one activation process and inactivation. Steady-state activation of SPIH channels, assessed from instant currents, were well described by a Boltzmann function with $V_{1/2} = -26$ mV and apparent gating charge = 3.5, in the whole cell configuration. Steady-state activation curves shift to the left by as much as 30 mV in excised patches, where it was found that cAMP ($K_{0.5} = 0.74$ μM), but not cGMP, directly and reversibly upwardly modulates the SPIH channel in a hyperbolic ratio. Adenosine 3′,5′-cyclic monophosphate acts by increasing channel open probability and stabilizing the peak currents. Permeability ratios indicated that the selectivity sequence of SPIH is as follows: $K^+ > Rb^+ > Na^+ > Li^+ > Cs^+$, with $P_K/P_{Na} = 4$ (118), similar to that of cAMP-modulated channels from sperm flagellar membranes reconstituted in planar lipid bilayers (163). The shape of the current versus voltage relation derived from tail currents measurements was found to depend on $[K^+]_o$. When $[K^+]_o$ is high (20 mM), a fairly linear current-voltage relation is observed. In the absence of $[K^+]_o$, but in the presence of Na^+, inward currents are abolished, indicating that $[K^+]_o$ governs ion conduction through SPIH channels. Antibodies directed against the COOH terminus of SPIH stained almost exclusively the sperm flagellum. Western blots of purified flagellar and head sperm indicated also that SPIH is preferentially in the flagellar membrane. The SPIH channels are unique among known K^+ channels, since they can be regulated by a myriad of mechanisms that include voltage, cAMP, phosphorylation, as well as $[K^+]_o$. Further work will be necessary to define, in a precise way, how this intriguing K^+ channel participates in sea urchin sperm physiology.

B. Ascidian

Ciona spermatozoa are immotile even after ejaculation. They become motile and are attracted to eggs under the influence of an egg factor called sperm-activating and attracting factor (SAAF) (205, 322). The SAAF is a proteolysis resistant, dialyzable, small molecule. It activates cAMP synthesis and sperm motility only in the presence of $[Ca^{2+}]_o$ (321). The SAAF increases K^+ sperm permeability and fails to activate *Ciona* sperm in high-K^+ seawater. A voltage-dependent K^+ channel blocker, mast cell-degranulating peptide, depresses the SAAF-induced hyperpolarization and inhibits sperm activation. Thus K^+ channels seem essential for the SAAF-induced sperm activation in *Ciona* (147).

A K^+ channel-mediated hyperpolarization is likely to be the initial step for sperm motility initiation in salmonid fish and ascidians. The possibility that sperm adenylyl cyclase is regulated by E_M, first demonstrated in sea urchin sperm (22), could explain the link between E_M, cAMP, and motility in many species.

C. Mammals

Long-range gamete communication may also be important in mammals, even though after being delivered to the female reproductive tract spermatozoa follow an arranged pathway toward the egg. Storage in the caudal isthmus of the oviduct after ejaculation reduces the motility of a significant fraction of sperm from various mammalian species (144). Minutes after ovulation, sperm abandon their storage sites to reach the ampullary region (88). These results suggest that eggs or follicle cells may release factors that activate motility and guide sperm toward the ovulated egg. Because the sperm-to-egg ratio is low (1:1 to 1:10) at the fertilization site, these factors could enhance productive encounters among the fittest gametes (309, 319).

Follicular factors have been reported to attract human spermatozoa in vivo (232, 298). Recently, it was shown that only a small fraction of human sperm (2–12%) undergoes chemoattraction by follicular factors. It appears that sperm acquire their chemotactic responsiveness as they become capacitated, a state proposed to be transient. Thus sperm chemotaxis to follicular factors in vivo may selectively recruit capacitated sperm for egg fertilization (47).

V. SPERM ION CHANNELS AND THE ACROSOME REACTION

All sperm species possessing an acrosome must undergo the AR to fertilize the egg. This exocytotic reaction enables sperm to penetrate the outer envelope of the egg and to recognize and fuse with the egg plasma membrane (319). This fundamental sperm process is triggered by components from the egg's outer layers and is modulated by factors from the female reproductive tract in internal fertilizers.

A. Sea Urchin

The AR is triggered when sperm encounter the jelly layer surrounding the egg (60, 282). The egg jelly component that triggers the AR is a fucose sulfate polymer (FSP) (3, 109, 259, 294). This reaction encompasses acrosomal vesicle exocytosis (60, 272), exposure of material necessary for sperm-egg binding (119, 293), and extension of the acrosomal tubule with its surrounding membrane des-

tined to fuse with the egg (see Fig. 1) (288). Recently, homologs of two proteins that form part of a complex involved in the fusion of plasma and vesicle membranes during exocytosis have been identified. These are syntaxin, an intracellular protein integral to the plasma membrane, and vesicle-associated membrane protein (VAMP; synaptobrevin), a protein associated with secretory vesicle membrane (23). Immunoprecipitation indicates that sea urchin sperm syntaxin and VAMP are associated with a complex. During acrosomal exocytosis, syntaxin and VAMP are shed with the vesicles that result from multiple fusions of the plasma membrane over the acrosome and the acrosomal membrane. These observations suggest that syntaxin and VAMP participate in the AR (258).

External Ca^{2+} and Na^+ are required for the AR under physiological conditions (48, 62, 254). Seconds after FSP binds to sperm, Na^+ and Ca^{2+} influx as well as H^+ and K^+ efflux are activated (106, 108, 249, 253, 254). These ion fluxes result in changes in E_M (114, 123, 252) and increases in $[Ca^{2+}]_i$ (127, 128, 286) and pH_i (128, 174). Furthermore, FSP elevates cAMP concentration (108) and inositol 1,4,5-trisphosphate (IP_3) (75) and stimulates protein kinase A (110, 112) and phospholipase D (74). Adenylyl cyclase activation leads to cAMP concentration increases (108), which can occur in isolated heads, when triggered by A-23187 or nigericin. Even though the cAMP rise depends on Ca^{2+} uptake (311), it was reported to precede the AR (105). How the FSP-induced permeability changes and the second messenger levels are related is still an open question.

The receptor for egg jelly (REJ) that triggers the AR was cloned (215). Monoclonal antibodies (MAb) to REJ bind to a narrow collar of plasma membrane over the acrosome and to the entire flagellum (287). Some of them cause large increases in $[Ca^{2+}]_i$ (286, 295) and induce the AR (215). These MAb can also activate sperm AC (295). Receptor for egg jelly is 1,459 amino acids. Greater than 900 amino acids are related to only one protein, which is polycystin. Polycystin is the protein mutated in polycystic kidney disease-1, the most frequent human genetic disease. The role of normal polycystin is unknown. REJ has been postulated to participate in ion permeability regulation (215).

In *L. pictus* sperm, FSP induces a transient hyperpolarization that is followed by a depolarization. The hyperpolarization is K^+ dependent, which suggests it is mediated by K^+ channels (123). The AR and the increases in Ca^{2+} uptake (253) and pH_i (128) associated with this reaction are blocked by rising $[K^+]_o$ from 10 to 40 mM. The pH_i increase observed during the AR is Na^+ dependent (128, 174). These results taken together suggest that FSP increases pH_i, at least in part, by activating a Na^+/H^+ exchange stimulated by a hyperpolarization (124). It is not known if the E_M-sensitive Na^+/H^+ exchange induced by speract participates in the pH_i increase that occurs during the AR. Neither the mechanism nor the stoichiometry of this apparent Na^+/H^+ exchange associated to the AR is known.

Some Ca^{2+} and K^+ channel blockers inhibit Ca^{2+} uptake and the AR (108, 113, 153, 253). These observations emphasize the crucial participation of ion channels in triggering the sperm AR. As indicated earlier, several sperm channels have been detected in planar bilayers and patch-clamp recordings, some of which are sensitive to blockers, which inhibit the AR (see Fig. 3 and Table 1). In addition, ionophores such as A-23187, a Ca^{2+}/H^+ exchanger (48), and nigericin, a Na^+ or K^+/H^+ exchanger (253), that artificially alter the sperm plasma membrane permeability induce the AR in the absence of the physiological ligand.

1. Ca^{2+} channels

$[Ca^{2+}]_i$ determinations using fluorescent Ca^{2+}-sensitive dyes have revealed the participation of two different Ca^{2+} channels in the sea urchin sperm AR (127, 128, 249). Binding of FSP, the factor that triggers AR, to its receptor opens a Ca^{2+}-selective channel that inactivates and is blocked by verapamil and dihydropyridines. Five seconds later, a second channel opens that is insensitive to the later blockers, does not inactivate, and is permeable to Mn^{2+}. Inhibition of the egg jelly-induced pH_i increase associated with the AR with high $[K^+]_o$, TEA^+, or in the absence of $[Na^+]_o$ prevents the opening of the second channel and the AR. Under these conditions, a transient rise in $[Ca^{2+}]_i$ remains, due to the opening of the first channel. However, the two Ca^{2+} channels are somehow linked, since blocking the first channel inhibits the second (127, 128). The pH_i change associated with the AR is Ca^{2+} dependent (129); therefore, the opening of the first channel could allow Ca^{2+} in so that pH_i can increase, and open the second channel, which is regulated by pH_i. Other possible links between the two channels could be Ca^{2+}-induced Ca^{2+} release or the emptying of internal stores (26) and proteolysis (86). Both channels are required apparently for development of a normal AR (Darszon and González-Martínez, unpublished data).

The fusion of isolated *S. purpuratus* sperm plasma membranes into BLM revealed the presence of a voltage-dependent high-conductance channel (183). The single main-conducting state of the high-conductance channel displays rare closing events at voltages more positive than −25 mV and tends to close through several subconductance states of lesser conductance at more negative potentials. As in some other Ca^{2+} channels (19), the main state conductance size sequence is $Ba^{2+} > Sr^{2+} > Ca^{2+}$. However, the channel discriminates poorly between divalent and monovalent cations, $P_{Ca}/P_{Na} = 5.9$, and is permeable to Mg^{2+} when added to the *cis*-side (the side of membrane addition) ($P_{Ca}/P_{Mg} = 2.8$). In contrast, addition

of Mg^{2+} to the *trans*-side blocks the channel in a voltage-independent manner. Both Cd^{2+} and Co^{2+} block the channel at millimolar concentrations and also inhibit the AR and the Ca^{2+} uptake associated with it. This channel is basically insensitive to verapamil and nisoldipine. Although the channel is detected fusing purified flagellar membranes to BLM, possible contamination from acrosomal membranes does not allow its definitive localization (183).

Although the high-conductance Ca^{2+} channel and the second type of Ca^{2+} channel that participates in the AR share some properties (183), it remains to be established if they are the same channel. The characteristics of the high-conductance Ca^{2+} channel are closer to those disclosed by the RyR (26) than to VDCC (267). There is extensive homology in the pore region of the RyR and IP_3 receptor, and both channels are sensitive to Ca^{2+} and pH (26). It is necessary to explore if any of the agonists of these channels regulate the second Ca^{2+} channel that participates in the AR; alternatively, it could be a store-operated Ca^{2+} channel (SOC) (226).

Inositol 1,4,5-trisphosphate accumulates during the AR and could modulate Ca^{2+} influx (75). An IP_3 binding component obtained by affinity chromatography from *S. purpuratus* sperm extracts has similar characteristics as the IP_3 receptor from other sources (325). It displays pH-dependent high-affinity for $InsP_3$ (dissociation constant = 200 nM), specificity (IC_{50} >5 mM for inositol 1-monophosphate, inositol 1,4-bisphosphate, and inositol 1,3,4,5-tetrakisphosphate, and 75% binding inhibition by 10 mg/ml heparin sodium. It is interesting that a plasma membrane component in the sperm head was recognized by an antibody against the COOH terminal of the type I IP_3 receptor of somatic cells. Although less intensely, this antibody also recognized a flagellar component. Consistent with these findings, the antibody detected a 240-kDa band from isolated head plasma membranes and weakly in flagellar membranes. The presence of IP_3 receptors in the sperm plasma membrane, although somewhat controversial, has been described in other systems (59, 154). This receptor may link IP_3 increases to Ca^{2+} permeability changes during the AR. However, IP_3 regulation of the large-conductance Ca^{2+} channels, sensitive to Co^{2+} and ruthenium red, found in mouse and sea urchin sperm plasma membranes, monitored in planar lipid bilayers, awaits experimental demonstration (325).

2. Cl^- channels

The stilbene disulfonate DIDS that inhibits anion channels and transporters blocks the sea urchin sperm AR (208). A DIDS-sensitive anion channel was identified fusing sperm plasma membranes into BLM (Table 1). This channel was enriched from detergent-solubilized sperm plasma membranes using a wheat germ agglutinin-Sepharose column. The anion selectivity sequence found was $NO_3^- > CNS^- > Br^- > Cl^-$. The channel has a high open probability at the holding potentials tested and often displays substates. This channel could be involved in the AR, influencing the sperm resting E_M, or being modulated during the reaction (208).

3. AR inactivation

Acrosome reaction inactivation (ARI) turns sperm irreversibly refractory to egg jelly and may involve ion channels. This process is triggered by the egg jelly when $[Ca^{2+}]_o$ is lowered from 10 mM in seawater to 2 mM and is associated with a transient $[Ca^{2+}]_i$ increase. However, a rise in $[Ca^{2+}]_i$ alone is not sufficient to induce ARI, since artificially increasing $[Ca^{2+}]_i$ with an ionophore or rising pH_o does not trigger ARI. In contrast to the AR that strictly requires Ca^{2+}, ARI can be triggered almost equally well when Ca^{2+} is replaced by Sr^{2+}. On the other hand, although Mn^{2+} does not affect AR, it inhibits ARI. Thus the mechanisms involved in ARI differ from those leading to AR. High pH_o can trigger AR in previously inactivated sperm by opening the same Ca^{2+} channels activated by the egg jelly. Thus ARI requires egg jelly receptor activation and originates from uncoupling of the egg jelly receptor from Ca^{2+} channels, and also from the mechanism that elevates pH_i during AR (129).

4. Unsolved puzzles

There are still many intriguing and fundamental questions about the sea urchin sperm AR. How is ion transport finely choreographed by the egg jelly receptor? It is unfortunate that no functional clues emerged from the sequence of REJ. Until now, there is no evidence for the participation of G proteins in the AR, although they are present in sperm. Is the Na^+/H^+ exchange that occurs during the AR the same as the one involved in the speract response? Probably not because the first is Ca^{2+} dependent (129, 249), and the other is not (250). How are the H^+ and Na^+ movements coupled during the AR? Why are they voltage dependent? Why does the AR require a pH_i increase: to open the high conductance pH-sensitive Ca^{2+} channel, to activate a poorly selective K^+ channel modulated by pH of the type recently cloned from mouse testis or to stimulate proteases (86, 193), AC, some kinase or phosphatase? Do second messengers like cAMP and IP_3 modulate the permeability changes that occur during AR? Is the second Ca^{2+} channel that opens during AR a capacitative channel? The sea urchin sperm AC could be a coincidence detector involved in AR, since it is modulated by $[Ca^{2+}]_i$, pH_i, and E_M (22). Figure 5 illustrates briefly the main events and possible mechanisms that encompass the sea urchin sperm AR.

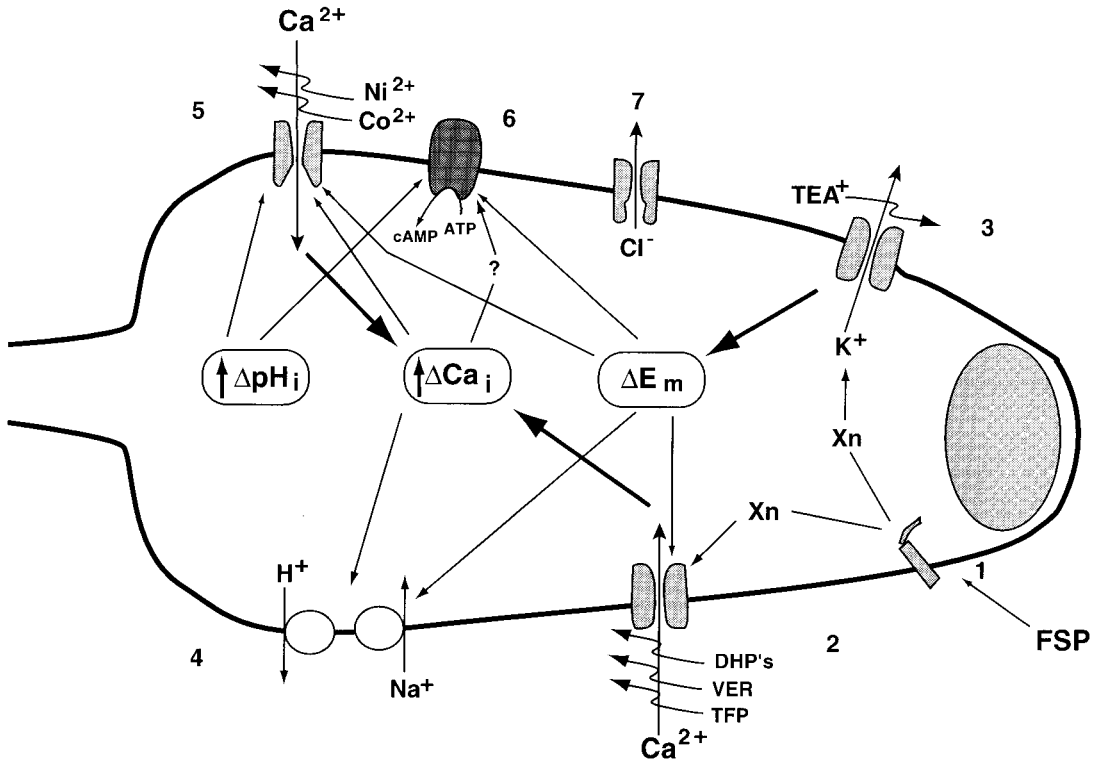

FIG. 5. Simplified working model of sea urchin sperm acrosome reaction. By unknown mechanisms, binding of egg factor [fucose sulfate polymer (FSP)] to sperm receptor, REJ (*1*), transiently opens Ca^{2+} channels (*2*), and possibly K^+ channels (*3*) that hyperpolarize *L. pictus* sperm. Ca^{2+} channel initiates $[Ca^{2+}]_i$ elevation, which is blocked by dihydropyridines (DHP), verapamil (VER), and trifluoperazine (TFP). Hyperpolarization stimulates a voltage- and Ca^{2+}-dependent Na^+/H^+ exchange (*4*), raising pH_i. These changes open a second pH_i-dependent Ca^{2+} channel (*5*) that keeps $[Ca^{2+}]_i$ elevated, further depolarizing sperm, and leading to AR. Because inositol 1,4,5-trisphosphate (IP_3) increases during AR, second channel could be a store-operated Ca^{2+} channel. FSP induced hyperpolarization and increases in $[Ca^{2+}]_i$ and pH_i activate sperm adenylyl cyclase (*6*), mainly found in flagella, but also present in head. cAMP increases could regulate various channels. DIDS-sensitive Cl^- channels (*7*) may participate in setting resting E_M or otherwise. Role of second messengers and intracellular Ca^{2+} stores in AR deserves further study.

B. Starfish

Starfish spermatozoa undergo the AR upon encountering the jelly coat. They stop swimming immediately after extruding a long acrosomal process (10–25 μm). Starfish sperm do not have to swim through the egg jelly; their long acrosomal tubule reaches the egg plasma membrane. Three egg jelly components are involved in AR induction (142): *1*) AR-inducing substance (ARIS), a high-molecular-weight ($>10^7$) sulfated glycoprotein whose higher order structure seems important for molecular recognition (159); *2*) co-ARIS, a sulfated steroidal saponin that is not species specific and whose biological activity depends on the sulfate moiety and steroid side chain; and *3*) asterosaps, tetratriacontapeptides containing an intramolecular disulfide bond (Cys-8—Cys-32) essential for function (219). An NH_2-terminal partial sequence of the 13-kDa sperm chemoattractant from the starfish *Pycnopodia helianthoides* shows high homology with asterosap (207), suggesting asterosap is a potent chemoattractant. Both co-ARIS and asterosap are diffusible components in the egg jelly.

The starfish egg jelly causes Ca^{2+} influx, modulates cAMP concentration, increases pH_i in a Na^+-dependent fashion (141, 143, 191, 192, 289), and leads to sperm histone degradation (4, 5). Alone, ARIS can induce the AR only in high-pH or high-Ca^{2+} seawater (145, 146, 191).

In normal seawater, ARIS and co-ARIS together are necessary to increase $[Ca^{2+}]_i$ and induce AR and thereafter decrease pH_i (191, 192). In contrast to sea urchins, the starfish sperm AR does not appear to require a pH_i increase. Acrosome reaction-inducing substance plus asterosap can also induce AR under physiological conditions. Antiasterosap rabbit IgG neutralizes the ability of egg jelly to induce AR; thus, in seawater, asterosap is important for this reaction.

Studies using a fluorophore- and radioisotope-labeled ARIS demonstrated the presence of species-specific receptors in the head of starfish sperm (292). Colloidal gold-tagged ligands confirmed these results (187). Similar

experiments revealed the presence of an asterosap receptor in the sperm flagella. Asterosap derivatives photoaffinity labeled a 130-kDa flagellar sperm membrane protein, probably GC (218).

It has been suggested that maitotoxin induces the AR in starfish *Asterina pectinifera* sperm by activating Ca^{2+} channels. This marine toxin stimulates Ca^{2+} channels in other cells (6). The toxin-induced response depends on $[Ca^{2+}]_o$ and is inhibited by verapamil. On the other hand, increasing K^+ to 30 mM KCl in seawater inhibits the toxin-induced AR. This result indicates the possible participation of K^+ channels in the starfish AR.

C. Mammals

The main physiological inducer of the mammalian sperm AR is the zona pellucida (ZP). Three sulfated glycoproteins (ZP1, ZP2, and ZP3) principally constitute ZP. ZP3 (~83 kDa) exhibits most of the sperm binding and AR-inducing activity (reviewed in Refs. 196, 310). Both protein and carbohydrate regions of ZP3 appear to be involved in its AR-inducing activity (91). The sperm binding sites of ZP3 are likely to be *O*-linked oligosaccharides located in the COOH-terminal half of the polypeptide (240; reviewed in Ref. 310). *N*-linked oligosaccharides of porcine zona have been suggested to participate in sperm binding (161).

Several candidates have been postulated as primary receptors for ZP3, e.g., a 56-kDa protein (sp56) (34), a 95-kDa tyrosine kinase (177, 196), β1–4 galactosyltransferase (265), trypsinlike proteins (30), and spermadhesins (37, 104, 135; for review, see Ref. 196). However, the physiological relevance of many of these candidates is under active debate (100, 151, 178, 188). Multiple concerted and cooperative interactions between ZP3 and various surface components of sperm, possibly involving receptor aggregation and phosphorylation, may be required to achieve AR.

Extracellular Ca^{2+} concentration is required for ZP-induced AR in mature sperm (319). Essential to this process is the elevation of pH_i and $[Ca^{2+}]_i$ (93, 94). Zona pellucida triggers $[Ca^{2+}]_i$ increases that precede exocytosis in single sperm loaded with fluorescent ion indicators (89, 94, 271). Several G proteins, such as G_i and G_z, are present in mammalian sperm (120). In mouse sperm, ZP activates G_{i-1} and G_{i-2} (304). Pertussis toxin (PTX), a specific inactivator of the G_i class of heterotrimeric G proteins, inhibits the ZP-induced AR and many of the ion fluxes associated with it in mouse, bovine, and human sperm (80, 81, 94, 175). Recently, it was shown that the PTX-sensitive step in the ZP-induced AR is the pH_i increase (10). Determining which plasma membrane proteins interact with activated G_i will help understand how pH_i is regulated during AR. GalTase-R has been shown to interact with G_i; its overexpression in transgenic mice makes sperm hypersensitive to ZP3 (122). However, galactosyltransferase-null sperm, which do not seem to undergo AR or bind to ZP3 oligosaccharides, still bind to the ZP and fertilize. Other sperm membrane components must participate to achieve fertilization (188).

Multiple results suggest the involvement of VDCC in the mammalian sperm AR (10, 68, 95, 224). Elevation of $[K^+]_o$ depolarizes bull (90), ram (14), and human sperm (35, 185, 238) and induces $[Ca^{2+}]_i$ increases sensitive, in some species, to dihydropyridines, benzothiazepine, and phenylalkylamine, which depend on $[Ca^{2+}]_o$ and pH_o (10, 92). The AR can be induced under these conditions in mouse, bull, and ram and is blocked by the above-mentioned Ca^{2+} channel antagonists and by inorganic divalent cations such as Co^{2+} and Ni^{2+}. These blockers also inhibit the ZP-induced AR in mouse and bull sperm (92). Moderately high-affinity binding sites for PN-200–110 (dissociation constant ~0.4 μM), an L-type VDCC antagonist, are present in both species (92). In human sperm, micromolar dihydropyridines block the AR induced by neoglycoproteins bearing mannose residues and by a K^+-induced depolarization at pH_o of 8.5 (35). Thus VDCC play a key role during mammalian sperm AR. Early on, these VDCC were identified as L-type channels on the grounds of the micromolar sensitivity to dihydropyridines of the mammalian sperm AR and the $[Ca^{2+}]_i$ increase associated with it. Nevertheless, now it is known that such dihydropyridine concentrations also block T-type Ca^{2+} channels (2, 9, 180).

1. Ca^{2+} channels

In tip-dip bilayers formed from liposomes containing boar sperm plasma membrane, two Ca^{2+} channels (Table 1) were monitored, one of which was blocked by nitrendipine and La^{3+} (55). Fusion of plasma membranes from cauda epididymal or ejaculated boar sperm into BLM revealed the activity of a nonselective cation channel. The channel allowed monovalent and divalent cations through, was not voltage dependent, and was blocked by high concentrations of verapamil, nitrendipine, and ruthenium red (54). With the use of the same strategy, an interesting 10-pS Ca^{2+} channel from boar sperm membranes (Table 1) was characterized by Tiwari-Woodruff and Cox (283). This channel selects poorly between monovalent and divalent cations (P_{Ca}/P_{Na} = 3.4; P_{Ba}/P_{Na} = 1) and is blocked by nitrendipine (mean affinity constant = 0.5 μM) but activated by the agonist (*S*)-BAY K 8644 (mean affinity constant = 0.3 μM). The channel does not display the voltage dependence characteristics of T- or L-type Ca^{2+} channels in planar bilayers; this could be due to the lipid composition or the membrane isolation procedure, where some component is lost. Future work

with this channel should explore its mode of regulation and possible participation in the AR.

The relevance of Ca^{2+} channels in sperm physiology motivated the study of their genotypic and phenotypic expression in the late differentiation stages of mouse spermatogenesis. Oligonucleotide probes to α_1-subunits A, B, C, D, and E, which contain the pore and the voltage sensor of the various voltage-dependent Ca^{2+} channels (L, N, T?, P/Q, and R), detected the presence of all these α_1-subunits in mouse testicular mRNA. However, mainly α_{1E}- and to a much lesser extent α_{1A}-transcripts were found in pachytene spermatocytes and in round and condensed spermatids (182). Only low-voltage-activated, rapidly inactivating Ca^{2+} currents, with properties similar to T-type Ca^{2+} currents described in other cell types, were seen in whole cell patch-clamp recordings from primary spermatocytes at the pachytene stage of spermatogenesis (7, 182, 246). It is interesting that pachytene spermatocytes have the lowest resting $[Ca^{2+}]_i$ (~50 nM) among the population of germ cells. This value gradually elevates in subsequent stages of germ cell differentiation (247) and so do α_{1E}-transcripts (C. Serrano and A. Liévano, unpublished data). Calcium currents start activating at about −60 mV and reach a maximum between −20 and −30 mV. Stronger depolarizations did not activate a second Ca^{2+} current component. The mean peak Ca^{2+} current density ranges from 6 to 11 $\mu A/cm^2$ in external solutions containing 10 mM Ca^{2+}. These Ca^{2+} currents display voltage-dependent inactivation (half-inactivation at −60 mV) and have also been observed in round spermatids (7, 8, 246). T-type Ca^{2+} currents are sensitive to micromolar nifedipine, Ni^{2+}, amiloride, and pimozide (see Fig. 3*F*). Because the mouse sperm AR and the uptake of Ca^{2+} that triggers it are also inhibited by these blockers (7, 92), at similar concentrations, it is very likely that a T-type Ca^{2+} channel is involved in inducing this reaction (7, 182, 246). The above-mentioned results are also consistent with antifertility effects reported for dihydropyridines in human males (138).

Other than pH, which does not have profound effects (247), little is known about the mechanisms regulating T-type Ca^{2+} channels in spermatogenic cells, or in the mature sperm. Further work is required to study these mechanisms, since they may influence spermatogenesis and sperm physiology during the early stages of mammalian fertilization. It has been reported that in dissociated mouse pachytene spermatocytes and round spermatids, the T-type Ca^{2+} currents are facilitated after strong depolarizations or high-frequency stimulation (8).

The molecular identity of T-type channels in spermatogenic cells remains to be defined. Soong et al. (268) showed that a rat α_{1E}-clone expressed in *Xenopus* oocytes yielded Ca^{2+} channels exhibiting functional properties compatible with those of low-voltage-activated Ca^{2+} channels. To the contrary, α_{1E}-clones from other species were reported to form exclusively high-voltage-activated Ca^{2+} channels (83). On the other hand, antisense oligonucleotides against rat brain α_{1E} were found to decrease T-type Ca^{2+} currents in one system (229) and R-type Ca^{2+} currents in another (230).

In apparent settlement of the issue, Pérez-Reyes et al. (228) recently cloned from rat brain a neuronal Ca^{2+} channel and called it α_{1G}. Expression of α_{1G} in *Xenopus* oocytes yielded channels whose properties defined it, neatly, as a T-type Ca^{2+} channel, indicating that α_{1G} represented the first member of a putative family of low-voltage-activated T-type Ca^{2+} channels. The channel α_{1G} is present also in mouse and humans, where it mapped to chromosome 17q22. These findings question the notion that α_{1E} might contribute to the formation of T-type Ca^{2+} channels in spermatogenic cells (182). However, Meir and Dolphin (197) have demonstrated that expression of α_{1B}, α_{1E}, or α_{1C} in COS-7, a cell line devoid of endogenous Ca^{2+} channel subunits or Ca^{2+} channels, can yield low-conductance, low-voltage-activated Ca^{2+} channels whose voltage dependence and kinetics of activation and inactivation makes them undistinguishable from native T-type Ca^{2+} channels.

A 2,169-base clone was isolated by RT-PCR from rat testis mRNA whose sequence is closely related to α_{1C} found in rat cardiac muscle (126). This is not unexpected, as shown by Liévano et al. (182), considering various cellular types are found in testis and only a probe specific for this subunit was used. Antibodies against skeletal L-type cardiac α_{1S} were used, without peptide or protein controls, as the sole proof that the cardiac α_{1C} is present in mature sperm. An 84-base difference with the rat cardiac muscle α_{1C} was detected and attributed to splicing and alternate exon usage. Goodwin et al. (125) indicated that this change could alter dihydropyridine affinity and activation kinetics that would explain the discrepancies between AR properties and L-type Ca^{2+} channels. Their more recent studies have indicated a second difference in the sequence of their testis α_{1C}-clone, which encodes for another putative dihydropyridine binding site. In situ RT-PCR in rat testis frozen sections using primers specific to this site revealed PCR products associated with all stages of spermatogenesis. Although it is most likely that other α_1-subunits are present in mature sperm (7, 182, 246) and it is not known if their clone is functional, they have concluded that the relevant VDCC for the AR is an L-type channel (125).

In view of all these findings, it becomes necessary to determine if α_{1G} or α_{1H} (56), another α_1-subunit that codes for T-type Ca^{2+} currents in human heart, is present in the later stages of spermatogenesis. Experiments are needed to decipher which α_1-subunit codes for the T-type Ca^{2+} currents of spermatogenic cells that appear to be crucial for the mouse sperm AR.

It is unclear if T-type Ca^{2+} channels can be opened by a depolarization at the resting potential of capacitated

sperm (−55 mV; Ref. 326), since they are probably inactivated. Acrosome reaction cannot be triggered by depolarization with K^+ in ram, mouse, and bull sperm unless external or internal pH is raised (10, 92). A transient hyperpolarization could be needed, as proposed in sea urchin sperm (124), to remove Ca^{2+} channel inactivation and then open the T-type Ca^{2+} channel (182). Depending on the equilibrium potential for Cl^-, anion channels could hyperpolarize sperm; alternatively, a K^+ channel could open.

Once VDCC are ready to open, is a ZP-induced sperm depolarization required, or could a transient hyperpolarization that would return the sperm potential to its resting value (approximately −55 mV) be enough to initiate T-type Ca^{2+} channel opening? Although K^+ channels are present in sperm, so far, ZP3 has not been shown to cause a transient hyperpolarization. Zona pellucida or ZP3 has been reported to induce a 30-mV depolarization in bovine or mouse sperm. However, this depolarization seems too slow to activate T-type Ca^{2+} channels (10). For the time being, two candidates may be considered to accomplish a ZP3-induced depolarization: *1*) mSlo3, if its voltage dependence is shifted to more negative potentials in capacitated sperm and the pH_i increase can open it, and *2*) a homolog of sea urchin sperm SPIH, if present in mature sperm, and if the ZP3-induced increase in cAMP is fast enough (309).

2. K^+ and cation-selective channels

The experiments inducing AR by depolarizing with K^+ at high pH imply the presence of K^+ channels in the sperm plasma membrane. Indeed, K^+-selective and TEA^+-sensitive channels have been observed in spermatogenic cells (131) and in bilayers containing rat sperm plasma membranes (40). Little is known about the regulation of K^+ channels in spermatogenic cells and in sperm.

Planar bilayer (40, 54, 55, 164) and patch-clamp studies (85) have revealed the presence of poorly selective cationic channels in mammalian sperm, which could depolarize sperm to open VDCC and trigger AR (Fig. 3 and Table 1). However, the modes of regulation of these channels are unknown.

The recently cloned mSlo3 K^+ channel found in mouse spermatogenic cells could contribute to depolarize mature sperm (256). This channel, named Slo3, exhibits extensive sequence similarity to Slo1, the large-conductance K^+ channel activated by Ca^{2+} and voltage. In contrast to Slo1, Slo3 is refractory to Ca^{2+} but is activated by depolarization and basic pH. At +80 mV, pH 7, the channel's open probability is <1%, whereas at pH 8.0, it increases by as much as 100-fold. Furthermore, Slo3 is poorly selectivity for K^+ over Na^+, as revealed by a $P_K/P_{Na} = 5$, compared with a $P_K/P_{Na} = 50$ in Slo1. Slo3 channel opening requires a somehow extreme depolarization, due to its quite positive half-activation voltage (+70 mV) as well as its shallow voltage dependence (16 mV/*e*-fold). Because it displays voltage and pH sensitivity, Slo3 differs from other cloned channels exhibiting only pH dependence (77, 273). Northern blot analysis demonstrated that Slo3 message is expressed prominently in mouse and human testis but is absent from brain, muscle, lung, kidney, and heart. In situ hybridization revealed that mSlo3 message is present in the seminiferous tubules, signals being more abundant over maturing spermatocytes and in the later stages of spermatogenesis. Because sperm basically lack translational activity, it is reasonable to speculate that Slo3 might be present and functional in the mature sperm, translating changes in H^+ concentration into changes in sperm cell E_M. Antibodies against Slo3 will be important to establish its presence in mature sperm. Moreover, finding specific blockers for Slo3 will be helpful in assessing its role in sperm function.

If Slo3 is functionally present in mouse sperm, could it open in response to the ZP-induced pH_i increase? Considering its P_K/P_{Na} ~5 and pH dependence (256), it could depolarize sperm contributing to activate VDCC, possibly T-type Ca^{2+} channels, and trigger AR (7, 182, 246). However, unless its voltage dependence is shifted to more negative potentials in sperm, compared with *Xenopus laevis* oocytes, this would be difficult, since it requires a large depolarization to open (256). In addition, although the ZP-induced sperm pH_i increase is inhibited by PTX, the depolarization is not. This result questions the role of Slo3 in this sperm E_M change (10). The molecular mechanisms involved in the ZP-induced opening of VDCC are still ill defined.

3. Other Ca^{2+}-permeable channels

As in sea urchin sperm (128), more than one type of Ca^{2+} channel has been proposed to participate in the ZP-induced mammalian sperm AR (89). T-type Ca^{2+} channels activate transiently (7, 246); therefore, they cannot sustain $[Ca^{2+}]_i$ elevated, as it occurs during the AR. A high-conductance, voltage-dependent poorly Ca^{2+}-selective channel ($P_{Ca}/P_{Na} = 4$), similar to the one described in sea urchin sperm membranes, has been detected directly transferring ion channels from mouse sperm to BLM in planar bilayers. Possibly this channel could be responsible for the sustained Ca^{2+} influx, since at certain potentials it remains open. This channel must be important considering its presence in diverse species and its sensitivity to Co^{2+} and ruthenium red which block AR (21, 164).

In many cells (e.g., Ref. 320) including sea urchin (128) and mammalian sperm (10), an interrelationship between pH_i and $[Ca^{2+}]_i$ has been established. Recently, it was shown that controlled intracellular alkalinization with NH_4Cl pulses results in important $[Ca^{2+}]_i$ increases

in pachytene spermatocytes, round and condensing spermatids, and testicular sperm (247). After an initial decrease in $[Ca^{2+}]_i$ in response to alkalinization, $[Ca^{2+}]_i$ increases along several seconds. The $[Ca^{2+}]_i$ increase is abolished by Ni^{2+} but is refractory up to 20 μM nifedipine and to antagonists of Ca^{2+} release from internal stores. The pH-induced increases in $[Ca^{2+}]_i$ are reversible and, moreover, their magnitude becomes larger in successive alkalinization episodes, revealing the occurrence of facilitation. The fact that nifedipine, a blocker of T-type Ca^{2+} channels in spermatogenic cells, has no effect on alkalinization-dependent $[Ca^{2+}]_i$ increases discards, in principle, the participation of T-type Ca^{2+} channels. On the other hand, the direct contribution of intracellular stores to the alkalinization-induced $[Ca^{2+}]_i$ increase in spermatogenic cells is minor. The alkalinization-induced $[Ca^{2+}]_i$ increases grow with maturation and are the largest in testicular sperm. These results suggest that this pH-dependent Ca^{2+} permeability pathway could operate in mature sperm (247).

Although Ca^{2+} release from internal stores does not contribute significantly to the alkalinization-induced $[Ca^{2+}]_i$ increases, these stores could contribute indirectly through the modulation of SOC (226). These channels may be present in the plasma membrane of sperm. In fact, Ca^{2+} uptake is stimulated in spermatogenic cells by compounds known to release Ca^{2+} from internal stores such as thapsigargin and cyclopiazonic acid (247). The pH-dependent Ca^{2+} influx pathway is permeable to Sr^{2+}, Ba^{2+}, and Mn^{2+}. These findings indicate that spermatogenic cells, and probably mature sperm, can undergo important $[Ca^{2+}]_i$ changes in response to increases in pH_i. Although probably a SOC-type channel, the mechanism leading to alkalinization-induced elevations in $[Ca^{2+}]_i$ in spermatogenic cells and testicular sperm remains to be investigated further. If present in mature sperm, this novel Ca^{2+} permeation pathway could be responsible, at least in part, for the dihydropyridine-insensitive Ca^{2+} influx that occurs during the ZP-induced AR (247). It is interesting that transcripts from a transient receptor potential homolog have been found in bovine spermatocytes (316).

Consistent with the observations just described, thapsigargin triggers AR in mouse and human sperm (199, 303). The response depends on $[Ca^{2+}]_o$; thus cross-talk between internal and external Ca^{2+} pathways occurs. Inositol 1,4,5-trisphosphate receptors have been selectively immunolocalized to the acrosomal cap of mature nonreacted mammalian sperm (303) and may also be present after AR in their plasma membrane (285). Furthermore, Walensky and Snyder (303) observed IP_3-induced release of $^{45}Ca^{2+}$ from the acrosome that was prevented by thapsigargin. These results led them to propose that IP_3-regulated Ca^{2+} release from the acrosome participates in the induction of the AR (303).

H-89, a PKA inhibitor, decreases IP_3-induced Ca^{2+} efflux from isolated acrosomes, suggesting that PKA may regulate the IP_3 receptor (36, 269). The ZP-induced AR increases pH_i, and alkaline pH_i favors Ca^{2+} release through IP_3 receptors, adding to the possible modulation pathways of $[Ca^{2+}]_i$ rise in sperm (26). Future experiments will determine the nature of the cross-talk between internal (acrosomal) and plasma membrane Ca^{2+}-permeant pathways, like IP_3 receptors or SOC, in the ZP-induced AR (see Fig. 6).

4. AR agonists distinct from ZP

The AR can be induced by other agonists like progesterone (15, 28, 201, 281), GABA (239, 262, 317), glycine (202), epidermal growth factor (167), ATP (96, 97), hyposmotic shock (241), and platelet-activating factor (260). Adenosine 5′-triphosphate induces a Na^+-dependent depolarization through a P_2 purinergic poorly selective cation channel, independently of $[Ca^{2+}]_o$ (97). Calcium-independent secretory exocytosis triggered by ATP (179) or Ca^{2+}-dependent exocytosis triggered by osmotic changes have been described (71, 87). Do these "alternative" pathways to achieve AR have a physiological role? Some of these transduction systems could be vestiges from previous differentiation stages. Others, like progesterone, may potentiate the ZP-induced AR (239); enhance capacitation (17, 70); promote sperm hyperactivation, a motility state important for fertilization; and/or induce chemotaxis (297).

Progesterone significantly increases $[Ca^{2+}]_i$ and produces AR in human sperm in a $[Ca^{2+}]_o$-dependent fashion (15, 28, 281). This process has been reported to involve Cl^- efflux (200, 244, 290). Progesterone elevates $[Ca^{2+}]_i$, rapidly reaching a long-lasting plateau. Conflicting results have been reported about the effects of tyrosine kinase inhibitors on these $[Ca^{2+}]_i$ changes (33, 203). Various proteins ranging from 20 to 220 kDa are phosphorylated during ZP- or progesterone-induced AR (16, 224, 280); some could be ion channels. Pertussis toxin does not inhibit the progesterone-induced $[Ca^{2+}]_i$ rise and AR, implying a different signaling path from the one triggered by ZP (98, 216, 279).

The sensitivity to dihydropyridines of the progesterone-induced human sperm AR is in dispute, and so is the participation of VDCC in this process (95, 198, 224, 225). Progesterone also triggers a depolarization (98). Two channels have been implicated in it: a cationic poorly selective channel that allows Na^+ in (98, 99) and a $GABA_A$ receptor proposed to mediate Cl^- efflux (200, 244, 290). Additionally, there is controversy regarding the Na^+ dependence of the progesterone-induced increase in $[Ca^{2+}]_i$ and AR in human sperm. In the absence of $[Na^+]_o$, Foresta et al. (98) reported that the progesterone-induced $[Ca^{2+}]_i$ increase is enhanced and there is AR at 60 and 180 min, while Garcia and Meizel (111) do not see stimulation of the progesterone-induced Ca^{2+} signal nor AR, but at 5

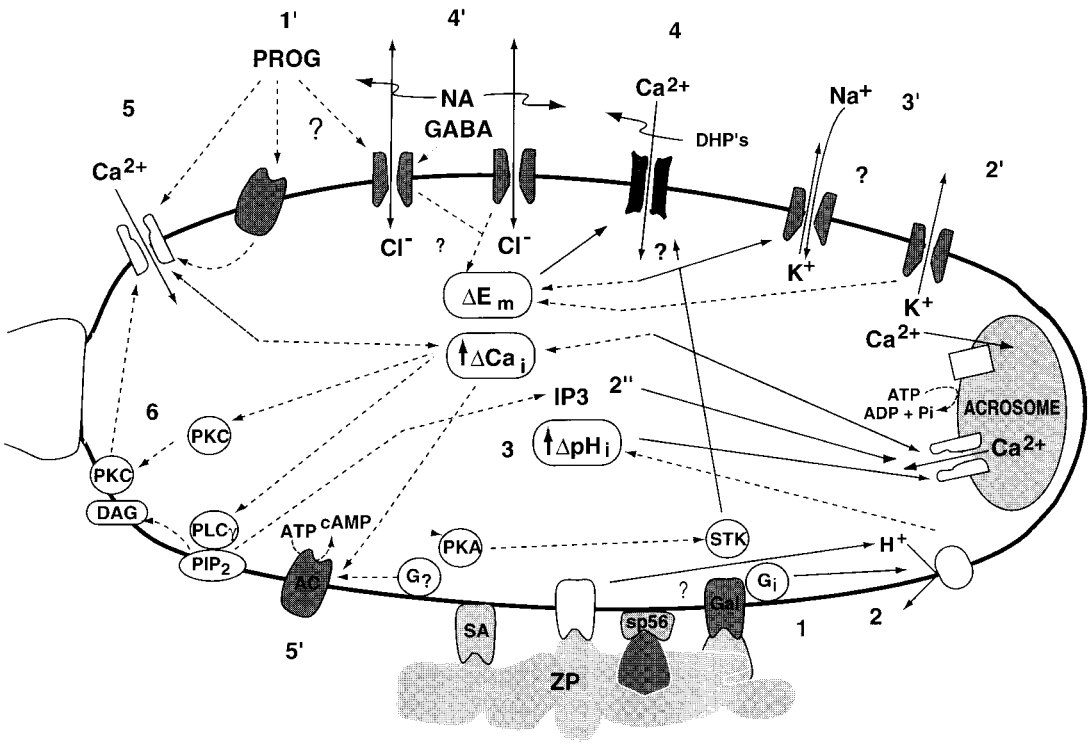

FIG. 6. Schematic model of mammalian sperm ion channel participation in acrosome reaction (*1*). Zona pellucida (ZP) 3 binds to sperm receptors and possibly aggregates them initiating AR. Three receptor candidates, of several possible, are illustrated: spermadhesin (SA), sp56, and GalTase (Gal). AR could be modulated or primed by progesterone (Prog) and GABA (*1′*), whose receptors are displayed on *top*. Stimulated ZP receptors could then activate G_i proteins sensitive to pertussis toxin (PTX) that directly or indirectly stimulate a H^+ transporter (*2*) increasing pH_i (*3*). Opening of a K^+-selective channel (*2′*) could hyperpolarize sperm, removing inactivation from T-type Ca^{2+} channels. Removal of inactivation is at least partially accomplished by hyperpolarization that occurs during capacitation. If ZP3 can induce a transient hyperpolarization (not measured yet), repolarization could open T-type Ca^{2+} channels (*4*). Alternatively, a nonselective cationic channel (*3′*) depolarizes sperm so that T-type Ca^{2+} channels may open. This channel could be either mSlo3, if its voltage dependence is modified in sperm, or a homolog of sea urchin sperm SPIH. Receptor activation by ZP3 may also lead to IP_3 production (*2′′*), which together with increase in pH_i and/or $[Ca^{2+}]_i$ would release Ca^{2+} from acrosome and activate a store-operated channel (SOC) channel in plasma membrane (*5*). SOC channel would explain sustained increase in $[Ca^{2+}]_i$ that occurs during AR. IP_3 receptor is present in acrosome. Progesterone can also open cation channels that depolarize sperm. Cl^- fluxes via $GABA_A$-type receptor or other niflumic acid (NA)-sensitive Cl^- channels (*4′*) may modulate E_M. Adenylyl cyclase (AC) regulation by $[Ca^{2+}]_i$ and possibly G proteins (G?) and E_M (*5′*) may also participate in AR. Increases in cAMP and other second messengers (*6*) may affect plasma membrane and acrosome ion channels, directly or through cAMP, protein kinase (PKA), tyrosine kinases, and/or protein kinase C (PKC), influencing course of AR. $[Ca^{2+}]_i$ and pH_i increases modulate phospholipases, proteases, kinases, and phosphatases, which regulate fusion machinery to achieve AR. DAG, diacylglycerol; PIP_2, phosphatidylinositol 4,5-bisphosphate.

min. These discrepancies could be due to different conditions used to measure $[Ca^{2+}]_i$ and the times for AR determination.

Progesterone metabolites enhance the interaction of GABA with the GABA receptor, a multisubunit protein containing a Cl^- channel (231). The GABA receptor was immunodetected in boar and ram sperm (82). Progestins may trigger human sperm responses by interacting with a $GABA_A$ receptor-like/Cl^- channel complex (317).

The neuronal $GABA_A$ receptor has consensus sequence sites for phosphorylation (194). Possibly the 50-kDa α-subunit of the $GABA_A$ receptor, immunocytochemically detected to the equatorial sperm head segment, could be phosphorylated in tyrosine, in response to progesterone (280, 317).

Glycine receptors have also been immunodetected in porcine sperm and reported to mediate glycine and zona-induced AR (202). Antagonist studies indicate that a glycine-like receptor participates in the AR induced by this neurotransmitter and by ZP in porcine and human sperm, while the GABA-like receptor is involved in the AR triggered by progesterone (198). These results suggest that different Cl^- channels are required to induce AR by different ligands.

5. *Cl^- channels*

The first single-channel recordings made in cell-attached and excised patches from mouse sperm revealed anion channels sensitive to micromolar niflumic acid

(IC_{50} = 11 μM) (Fig. 3*C*, Table 1) (85). Niflumic acid has been used to block Ca^{2+}-dependent Cl^- channels (314). Importantly, niflumic acid was an effective inhibitor of the AR induced by GABA, progesterone, and ZP. Inhibition of AR induced by GABA and ZP required lower niflumic acid concentrations (IC_{50} = 1 and 7 μM, respectively) than AR induced by progesterone (IC_{50} = 84 μM), suggesting that anion channels activated by GABA and ZP might be different from those activated by progesterone or, alternatively, that progesterone acts on other surface receptors in addition to Cl^- channels. The results suggest that anion-selective channels are important actors in the sperm-egg dialogue. Voltage-gated Cl^- currents, blocked by niflumic acid (IC_{50} = 100 μM), were also recorded in mouse pachytene spermatocytes (85). Anion channels have been detected in BLM containing mouse sperm plasma membranes (Table 1, Fig. 3) (164).

6. Second messengers and phosphorylation

Acrosome reaction induced by ZP, progesterone, and nonphysiological agents like the Ca^{2+} ionophore A-23187 is accompanied by phospholipid and cAMP metabolic changes (90, 102, 156, 225, 239, 281). Activity of PKC and PKA may be influenced by these pathways, resulting in phosphorylation changes of several proteins during the AR (73, 203). Antibodies have detected PKC-α and PKC-βII in the equatorial segment of human sperm heads (242) and $G_{q/11}\alpha$ and phospholipase C-β1 in the anterior mouse acrosomal region (303). Biologically active phorbol diesters and diacylglycerols influence the cell distribution of PKC and the time course of the ZP-induced AR (79, 176, 239). Furthermore, PKC translocation from cytosol to the plasma membrane depends on $[Ca^{2+}]_o$ (168). Activation of Ca^{2+} uptake by progesterone and ZP is sensitive to PKC and PKA inhibitors in plasma membrane vesicles and in isolated acrosomes from bovine sperm (36, 269), and to PKC inhibitors in human sperm (99). Agonists for these kinases, especially when combined, appear to circumvent the $[Ca^{2+}]_o$ requirement of the AR (73, 203). These findings suggest that $[Ca^{2+}]_i$ rises may activate these kinases during intermediate steps of the physiologically relevant AR. Artificial stimulation of the kinases overcomes the $[Ca^{2+}]_o$ requirements for the final stages, where membrane fusion occurs. Alternatively, Ca^{2+} from intracellular stores could be liberated during kinase stimulation, bypassing the need for $[Ca^{2+}]_o$ uptake. Future experiments will determine if the physiologically relevant AR involves cross-talk between internal (acrosomal) and plasma membrane Ca^{2+}-permeant pathways, like IP_3 receptors or SOC (see Fig. 6). Unraveling the mammalian sperm AR still requires an understanding of the delicately organized participation of several sperm receptors in the regulation of ionic fluxes involving G proteins, E_M, and second messengers (see Fig. 6 for a working hypothesis).

VI. CONCLUDING REMARKS

Ion channels play a cardinal role in the dialogue between gametes and thus in the generation of a new individual in many species. Several new avenues are being pursued that have great potential to contribute to our knowledge of sperm physiology and fertilization. Interweaving strategies of molecular biology and electrophysiology in spermatogenic cells, together with ion channel incorporation directly from sperm or using purified proteins, may yield information as to how ion channels are regulated and participate in spermatogenesis, sperm maturation, the AR, and during fertilization. The long-awaited crystal structure of an ion channel (76) sets a new better-defined stage to think about possible regulation mechanisms and generates many new interesting questions relating structure-function relationships.

We thank Otilia Zapata, José Luis de la Vega, and Yolanda Sandoval for technical help as well as Carmen Beltrán, Claudia Treviño, Celia Santi, Jesús García-Soto, and Marco González for discussions and help with the manuscript. A. Darszon expresses special gratitude to Arturo Liévano who has been a source of inspiration to the group in many significant ways.

This work was supported by grants from Consejo Nacional de Ciencia y Tecnología, Dirección General de Asuntos del Personal Académico, the Howard Hughes Medical Institute (to A. Darszon), and the International Centre for Genetic Engineering and Biotechnology (to P. Labarca and A. Darszon). P. Labarca is a Howard Hughes International Fellow and holds a Cátedra Presidencial from Chile.

REFERENCES

1. ACCILI, E. A., G. REDAELLI, AND D. DI FRANCESCO. Differential control of the hyperpolarization-activated currents by cAMP gating and phosphatase inhibition in rabbit sino-atrial node myocytes. *J. Physiol. (Lond.)* 500: 643–651, 1997.
2. AKAIKE, N., P. G. KOSTYUK, AND Y. V. OSIPCHUK. Dihydropyridine-sensitive low-threshold calcium channels in isolated rat hypothlamic neurones. *J. Physiol. (Lond.)* 412: 181–195, 1989.
3. ALVES, A.-P., B. MULLOY, J. A. DINIZ, AND P. A. S. MOURAO. Sulfated polysaccharides from the egg jelly layer are species-specific inducers of acrosomal reaction in sperms of sea urchins. *J. Biol. Chem.* 272: 6965–6971, 1997.
4. AMANO, T., Y. OKITA, AND M. HOSHI. Treatment of starfish sperm with egg jelly induces the degradation of histones. *Dev. Growth Differ.* 34: 99–106, 1992.
5. AMANO, T., Y. OKITA, T. OKINAGA, T. MATSUI, I. NISHIYAMA, AND M. HOSHI. Egg jelly components responsible for histone degradation and acrosome reaction in the starfish, *Asterina pectinifera*. *Biochem. Biophys. Res. Commun.* 187: 274–278, 1992.
6. AMANO, T., Y. OKITA, T. YASUMOTO, AND M. HOSHI. Maitotoxin induces acrosome reaction and histone degradation of starfish *Asterina pectinifera* sperm. *Zool. Sci.* 10: 307–312, 1993.
7. ARNOULT, C., R. A. CARDULLO, J. R. LEMOS, AND H. M. FLORMAN. Activation of mouse sperm T-type Ca^{2+}channels by adhesion to the egg zona pellucida. *Proc. Natl. Acad. Sci. USA* 93: 13004–13009, 1996.
8. ARNOULT, C., J. R. LEMOS, AND H. M. FLORMAN. Voltage-dependent modulation of T-type calcium channel by protein tyrosine phosphorylation. *EMBO J.* 16: 1593–1599, 1997.
9. ARNOULT, C., M. VILLAZ, AND H. M. FLORMAN. Pharmacological

properties of T-type Ca^{2+} current of mouse spermatogenic cells. *Mol. Pharmacol.* 53: 1104–1111, 1998.
10. ARNOULT, C., Y. ZENG, AND H. FLORMAN. ZP3-dependent activation of sperm cation channels regulates acrosomal secretion during mammalian fertilization. *J. Cell Biol.* 134: 637–645, 1996.
11. AUSTIN, C. R., AND R. V. SHORT. *Germ Cells and Fertilization.* Cambridge, UK: Cambridge Univ. Press, 1972.
12. BABCOCK, D. F. Examination of the intracellular ionic environment and of ionophore action by null point measurements employing the fluorescein chromophore. *J. Biol. Chem.* 258: 6380–6389, 1983.
13. BABCOCK, D. F., M. M. BOSMA, D. E. BATTAGLIA, AND A. DARSZON. Early persistent activation of sperm K^+ channels by the egg peptide speract. *Proc. Natl. Acad. Sci. USA* 89: 6001–6005, 1992.
14. BABCOCK, D. F., AND D. R. PFEIFFER. Independent elevation of cytosolic [Ca^{2+}] and pH of mammalian sperm by voltage-dependent and pH-sensitive mechanisms. *J. Biol. Chem.* 262: 15041–15047, 1987.
15. BALDI, E., R. CASANO, C. FALSETTI, C. KRAUSZ, M. MAGGI, AND G. FORTI. Intracellular calcium accumulation and responsiveness to progesterone in capacitating human spermatozoa. *J. Androl.* 12: 323–330, 1991.
16. BALDI, E., M. LUCONI, L. BONACCORSI, C. KRAUSZ, AND G. FORTI. Human sperm activation during capacitation and acrosome reaction: role of calcium, protein phosphorylation and lipid remodelling pathways. *Front. Biosci.* 1: 189–205, 1996.
17. BARBONI, E., M. MATTIOLI, AND E. SEREN. Influence of progesterone on boar sperm capacitation. *J. Endocrinol.* 144: 13–18, 1995.
18. BASTIAANSE, E. M. L., K. M. HOLD, AND A. VAN-DER-LAARSE. The effect of membrane cholesterol content on ion transport processes in plasma membranes. *Cardiovasc. Res.* 33: 272–283, 1997.
19. BEAN, B. P. Classes of calcium channels in vertebrate membranes. *Annu. Rev. Physiol.* 51: 367–389, 1989.
20. BELLVÉ, A. R., C. F. MILLETTE, Y. M. BHATNAGAR, AND D. A. O'BRIEN. Dissociation of the mouse testis and characterization of isolated spermatogenic cells. *J. Histochem. Cytochem.* 25: 480–494, 1977.
21. BELTRÁN, C., A. DARSZON, P. LABARCA, AND A. LIÉVANO. A high-conductance voltage-dependent multistate Ca^{2+} channel found in sea urchin and mouse spermatozoa. *FEBS Lett.* 338: 23–26, 1994.
22. BELTRÁN, C., O. ZAPATA, AND A. DARSZON. Membrane potential regulates sea urchin sperm adenylylcyclase. *Biochemistry* 35: 7591–7598, 1996.
23. BENNETT, M. K., N. CALAKOS, AND R. H. SCHELLER. Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. *Science* 257: 255–259, 1992.
24. BENTLEY, J. K., D. L. GARBERS, S. E. DOMINO, T. D. NOLAN, AND C. VAN DOP. Spermatozoa contain a guanine nucleotide binding protein ADP ribosylated by pertussis toxin. *Biochem. Biophys. Res. Commun.* 138: 728–734, 1986.
25. BENTLEY, J. K., D. J. TUBB, AND D. L. GARBERS. Receptor-mediated activation of spermatozoan guanylate cyclase. *J. Biol. Chem.* 261: 14859–14862, 1986.
26. BERRIDGE, M. J. Inositol trisphosphate and calcium signalling. *Nature* 361: 315–325, 1993.
27. BIBRING, T., J. BAXANDALL, AND C. C. HARTER. Sodium-dependent pH regulation in active sea urchin sperm. *Dev. Biol.* 101: 425–435, 1984.
28. BLACKMORE, P. F., S. J. BEEBE, D. R. DANFORTH, AND N. ALEXANDER. Progesterone an 17α-hydroxyprogesterone. Novel stimulators of calcium influx in human sperm. *J. Biol. Chem.* 265: 1376–1380, 1990.
29. BOATMAN, D. E., AND R. S. ROBBINS. Bicarbonate: carbon-dioxide regulation of sperm capacitation, hyperactivated motility and acrosome reactions. *Biol. Reprod.* 44: 806–813, 1991.
30. BOETTGER-TONG, H., D. J. AARON, B. E. BIEGLER, B. GEORGE, AND G. R. POIRIER. Binding of a murine proteinase inhibitor to the acrosome region of the human sperm head. *Mol. Reprod. Dev.* 36: 346–353, 1993.
31. BOITANO, S., AND C. K. OMOTO. Membrane hyperpolarization activates trout sperm without an increase in intracellular pH. *J. Cell Sci.* 98: 343–349, 1991.
32. BOITANO, S., AND C. K. OMOTO. Trout sperm swimming patterns and role of intracellular Ca^{2+}. *Cell Motil. Cytoskeleton* 21: 74–82, 1992.
33. BONACCORSI, L., M. LUCONI, G. FORTI, AND E. BALDI. Tyrosine kinase inhibition reduces the plateau phase of the calcium increase in response to progesterone in human sperm. *FEBS Lett.* 364: 83–86, 1995.
34. BOOKBINDER, L. H., A. CHENG, AND J. D. BLEIL. Tissue- and species-specific expression of sp56, a mouse sperm fertilization protein. *Science* 269: 86–89, 1995.
35. BRANDELLI, A., P. V. MIRANDA, AND J. G. TEZÓN. Voltage-dependent calcium channels and G_i regulatory protein mediate the human sperm acrosomal exocytosis induced by *N*-acetylglucosaminyl/mannosyl neoglycoproteins. *J. Androl.* 17: 522–529, 1996.
36. BREITBART, H., AND B. SPUNGIN. The biochemistry of the acrosome reaction. *Mol. Hum. Reprod.* 3: 195–202, 1997.
37. CALVETE, J. J., K. MANN, L. SANZ, M. RAIDA, AND E. TÖPFER-PETERSEN. The primary structure of BSP-30K, a major lipid-, gelatin-, and heparin-binding glycoprotein of bovine seminal plasma. *FEBS Lett.* 399: 147–152, 1996.
38. CALZADA, L., A. BERNAL, AND E. LOUSTAUNAU. Effect of steroid hormones and capacitation on membrane potential of human spermatozoa. *Arch. Androl.* 21: 121–128, 1988.
39. CASTELLANO, L. E., G. MARTíNEZ-CADENA, J. LÓPEZ-GODINEZ, A. OBREGON, AND J. GARCíA-SOTO. Subcellular localization of the GTP-binding protein Rho in the sea urchin sperm. *Eur. J. Cell Biol.* 74: 329–335, 1997.
40. CHAN, H. C., T. S. ZHOU, W. O. FU, W. P. WANG, L. SHI, AND P. Y. D. WONG. Cation and anion channels in rat and human spermatozoa. *Biochim. Biophys. Acta* 1323: 117–129, 1997.
41. CHANG, H. M., R. REITSTETTER, R. P. MASON, AND R. GRUENER. Attenuation of channel kinetics and conductance by cholesterol: an interpretation using structural stress as a unifying concept. *J. Membr. Biol.* 143: 51–63, 1995.
42. CHRISTEN, R. W., R. W. SCHACKMANN, AND B. M. SHAPIRO. Elevation of intracellular pH activates respiration and motility of sperm of the sea urchin *Strongylocentrotus purpuratus*. *J. Biol. Chem.* 257: 14881–14890, 1982.
43. CHRISTEN, R. W., R. W. SCHACKMANN, AND B. M. SHAPIRO. Interaction between sperm and sea urchin egg jelly. *Dev. Biol.* 98: 1–14, 1983.
44. CHRISTEN, R. W., R. W. SCHACKMANN, AND B. M. SHAPIRO. Ionic regulation of sea urchin sperm motility, metabolism and fertilizing capacity. *J. Physiol. (Lond.)* 379: 347–365, 1986.
45. CLAPPER, D. L., J. A. DAVIES, P. J. LAMOTHE, C. PATTON, AND D. EPEL. Involvement of zinc in the regulation of pH_i, motility, and the acrosome reaction. *J. Cell Biol.* 100: 1817–1824, 1985.
46. CLARK, E. N., M. E. CORRON, AND H. M. FLORMAN. Caltrin, the calcium transport regulatory peptide of spermatozoa, modulates acrosome exocytosis in the response to the egg's zona pellucida. *J. Biol. Chem.* 268: 5309–5316, 1993.
47. COHEN-DAYAG, A., I. TUR-KASPA, J. DOR, S. MASHIASH, AND M. EISENBACH. Sperm capacitation in humans is transient and correlates with chemotactic responsiveness to follicular factors. *Proc. Natl. Acad. Sci. USA* 92: 11039–11043, 1995.
48. COLLINS, F., AND D. EPEL. The role of calcium ions in the acrosome reaction of sea urchin sperm. Regulation of exocytosis. *Exp. Cell Res.* 106: 211–222, 1977.
49. COOK, S. P., AND D. F. BABCOCK. Selective modulation by cGMP of the K^+ channel activated by speract. *J. Biol. Chem.* 268: 22402–22407, 1993.
50. COOK, S. P., AND D. F. BABCOCK. Activation of Ca^{2+} permeability by cAMP is coordinated through the pH_i increase induced by speract. *J. Biol. Chem.* 268: 22408–224013, 1993.
51. COOK, S. P., C. J. BROKAW, C. H. MULLER, AND D. F. BABCOCK. Sperm chemotaxis: egg peptides control cytosolic calcium to regulate flagellar responses. *Dev. Biol.* 165: 10–19, 1994.
52. CÓRDOBA, M., T. A. SANTA-COLOMA, N. B. BEORLEGUI, AND M. T. BECONI. Intracellular calcium variation in heparin-capacitated bovine sperm. *Biochem. Mol. Biol. Int.* 41: 725–733, 1997.
53. COSSON, M. P., R. BILLARD, AND L. LETELLIER. Rise of internal Ca^{2+} accompanies the initiation of trout sperm motility. *Cell Motil. Cytoskeleton* 14: 424–434, 1989.

54. COX, T., P. CAMPBELL, AND R. N. PETERSON. Ion channels in boar sperm plasma membranes: characterization of a cation selective channel. *Mol. Reprod. Dev.* 30: 135–147, 1991.
55. COX, T., AND R. N. PETERSON. Identification of calcium conducting channels in isolated boar sperm plasma membranes. *Biochem. Biophys. Res. Commun.* 161: 162–168, 1989.
56. CRIBBS, L. L., J. H. LEE, J. YANG, J. SATIN, Y. ZHANG, A. DAUD, J. BARCLAY, M. P. WILLIAMSON, M. FOX, M. REES, AND E. PEREZ-REYES. Cloning and characterization of alpha1H from human heart, a member of the T-type Ca^{2+} channel gene family. *Circ. Res.* 83: 103–109, 1998.
57. CROSS, N. L., AND P. RAZY-FAULKNER. Control of human sperm intracellular pH by cholesterol and its relationship to the response of the acrosome to progesterone. *Biol. Reprod.* 56: 1169–1174, 1997.
58. CUELLAR-MATA, P., G. MARTÍNEZ-CADENA, L. E. CASTELLANO, G. ALDANA-VELOZ, G. NOVOA-MARTÍNEZ, I. VARGAS, A. DARSZON, AND J. GARCíA-SOTO. Multiple GTP-binding proteins in sea urchin sperm: evidence for G_s and small G-proteins. *Dev. Growth Differ.* 37: 173–181, 1995.
59. CUNNINGHAM, A. M., D. K. RYUGO, A. H. SHARP, R. R. REED, S. H. SNYDER, AND G. V. RONNETT. Neuronal inositol 1,4,5-trisphosphate receptor localized to the plasma membrane of olfactory cilia. *Neuroscience* 57: 339–352, 1993.
60. DAN, J. C. Studies on the acrosome. I. Reaction to egg-water and other stimuli. *Biol. Bull.* 103: 54–66, 1952.
61. DAN, J. C. Sperm entrance in echinoderms, observed with the phase contrast microscope. *Biol. Bull.* 99: 399–3411, 1954.
62. DAN, J. C. Studies on the acrosome. III. Effect of Ca^{2+} deficiency. *Biol. Bull.* 107: 335–349, 1954.
63. DANGOTT, L. J. Isolation and characterization of a cDNA clone for a speract receptor homologue in the sea urchin *Arbacia punctulata* (Abstract). *FASEB J.* 5: A1227, 1991.
64. DANGOTT, L. J., AND D. L. GARBERS. Identification and partial characterization of the receptor for speract. *J. Biol. Chem.* 259: 13712–13716, 1984.
65. DANGOTT, L. J., J. E. JORDAN, R. A. BELLET, AND D. L. GARBERS. Cloning of the mRNA of protein that crosslinks to the egg peptide speract. *Proc. Natl. Acad. Sci. USA* 86: 2128–2132, 1989.
66. DARSZON, A., M. BOSMA, D. E. BATTAGLIA, AND D. F. BABCOCK. Volume expanded sperm: models for study of early responses required for fertilization (Abstract). *J. Cell Biol.* 111: 115a, 1990.
67. DARSZON, A., P. LABARCA, C. BELTRÁN, J. GARCÍA-SOTO, AND A. LIÉVANO. Sea urchin sperm: an ion channel reconstitution study case. *Methods* 6: 37–50, 1994.
68. DARSZON, A., A. LIÉVANO, AND C. BELTRÁN. Ion channels: key elements in gamete signaling. In: *Current Topics in Develomental Biology*, edited by R. A. Pedersen and G. P. Schatten. San Diego, CA: Academic, 1996, vol. 34, p. 117–167.
69. DASGUPTA, S., C. L. MILLS, AND L. R. FRASER. Ca^{2+}-related changes in the capacitation state of human spermatozoa assessed by a chlortetracycline fluorescence assay. *J. Reprod. Fertil.* 99: 135–143, 1993.
70. DASGUPTA, S., C. O'TOOLE, C. L. MILLS, AND L. R. FRASER. Effect of pentoxifylline and progesterone on human sperm capacitation and acrosomal exocytosis. *Hum. Reprod.* 9: 2103–2109, 1994.
71. DAY, R. N. AND P. M. HINKLE. Osmotic regulation of prolactin secretion. Possible role of chloride. *J. Biol. Chem.* 263: 15915–15921, 1988.
72. DI FRANCESCO, D. Pacemaker mechanisms in cardiac tissue. *Annu. Rev. Physiol.* 55: 455–472, 1993.
73. DOHERTY, C. M., S. M. TARCHALA, E. RADWANSKA, AND C. J. DEJONGE. Characterization of two second messenger pathways and their interactions in eliciting the human sperm acrosome reaction. *J. Androl.* 16: 36–46, 1995.
74. DOMINO, S. E., S. B. BOCCKINO, AND D. L. GARBERS. Activation of phospholipase D by the fucose-sulfate glycoconjugate that induces an acrosome reaction in spermatozoa. *J. Biol. Chem.* 264: 9412–9419, 1989.
75. DOMINO, S. E., AND D. L. GARBERS. The fucose-sulfate glycoconjugate that induces an acrosome reaction in spermatozoa stimulates inositol 1,4,5-trisphosphate accumulation. *J. Biol. Chem.* 263: 690–695, 1988.
76. DOYLE, D. A., J. MORAIS CABRAL, R. A. PFUETZNER, A. KUO, J. M. GULBIS, S. L. COHEN, B. T. CHAIT, AND R. MCKINNON. The structure of the potassium channel: molecular basis of K^+ conduction. *Science* 280: 69–77, 1998.
77. DUPRAT, F., F. LESAGE, M. FINK, R. REYES, C. HEURTEAUX, AND M. LAZDUNSKI. TASK, a human background K^+ channel to sense external pH variations near physiological pH. *EMBO J.* 16: 5464–5471, 1997.
78. EMILIOZZI, C., AND P. FENICHEL. Protein tyrosine phosphorylation is associated with capacitation of human sperm in vitro but is not sufficient for its completion. *Biol. Reprod.* 56: 674–679, 1997.
79. ENDO, Y., S. KOMATSU, M. HIRAI, S. SUZUKI, AND N. SHIMIZU. Protein kinase C activity and protein phosphorylation in mouse sperm. *Nippon Sanka Fujinka Gakkai Zasshi* 43: 109–116, 1991.
80. ENDO, Y., M. A. LEE, AND G. S. KOPF. Evidence for the role of a guanine nucleotide binding regulatory protein in the zona pellucida-induced mouse sperm acrosome reaction. *Dev. Biol.* 119: 210–216, 1987.
81. ENDO, Y., M. A. LEE, AND G. S. KOPF. Characterization of an islet activating protein-sensitive site in mouse sperm that is involved in the zona pellucida-induced acrosome reaction. *Dev. Biol.* 129: 12–24, 1988.
82. ERDO, S. L., AND L. WEKERLE. $GABA_A$ type binding sites on membranes of spermatozoa. *Life Sci.* 47: 1147–1151, 1990.
83. ERTEL, S. I., AND E. ERTEL. Low-voltage-activated T-type calcium channels. *Trends Pharmacol. Sci.* 18: 37–42, 1997.
84. ESPINOSA, F., AND A. DARSZON. Mouse sperm membrane potential: changes induced by Ca^{2+}. *FEBS Lett.* 372: 119–125, 1995.
85. ESPINOSA, F., J. L. DE LA VEGA-BELTRÁN, I. LÓPEZ-GONZÁLEZ, R. DELGADO, P. LABARCA, AND A. DARSZON. Mouse sperm patch-clamp recordings reveal single Cl^- channels sensitive to niflumic acid, a blocker of the sperm acrosome reaction. *FEBS Lett.* 426: 47–51, 1998.
86. FARACH, H. A., JR., D. I. MUNDY, W. J. STRITTMATTER, AND W. J. LENNARZ. Evidence for the involvement of metalloendoproteases in the acrosome reaction in sea urchin sperm. *J. Biol. Chem.* 262: 5483–5487, 1987.
87. FISCHER, R., F. SCHLIESS, AND D. HAUSSINGER. Characterization of the hypo-osmolarity-induced Ca^{2+} response in cultured rat astyrocytes. *Glia* 20: 51–58, 1997.
88. FLECHON, J. E., AND R. H. F. HUNTER. Distribution of spermatozoa in the utero-tubal junction and isthmus of pigs, and their relationship with the luminal epithelium after mating: a scanning electron microscope study. *Tissue Cell* 13: 127–139, 1981.
89. FLORMAN, H. M. Sequential focal and global elevations of sperm intracellular Ca^{2+} are initiated by the zona pellucida during acrosomal exocytosis. *Dev. Biol.* 165: 152–164, 1994.
90. FLORMAN, H. M., AND D. F. BABCOCK. Progress toward understanding the molecular basis of capacitation. In: *Elements of Mammalian Fertilization. I. Basic Concepts*, edited by P. M. Wasssarman. Boca Raton, FL: CRC, 1991, p. 105–132.
91. FLORMAN, H. M., K. B. BECHTOL, AND P. M. WASSARMAN. Enzymatic dissection of the functions of the mouse egg's receptor for sperm. *Dev. Biol.* 106: 243–245, 1984.
92. FLORMAN, H. M., M. E. CORRON, T. D.-H. KIM, AND D. F. BABCOCK. Activation of voltage-dependent calcium channels of mammalian sperm is required for zona pellucida-induced acrosomal exocytosis. *Dev. Biol.* 152: 304–314, 1992.
93. FLORMAN, H. M., AND N. L. FIRST. The regulation of acrosomal exocytosis. II. The zona pellucida-induced acrosome reaction of bovine spermatozoa is controlled by extrinsic positive regulatory elements. *Dev. Biol.* 128: 464–473, 1988.
94. FLORMAN, H. M., R. M. TOMBES, N. L. FIRST, AND D. F. BABCOCK. An adhesion associated agonist from the zona pellucida activates G protein-promoted elevations of internal Ca^{2+} and pH that mediate mammalian sperm acrosomal exocytosis. *Dev. Biol.* 135: 133–146, 1989.
95. FORESTA, C., AND M. ROSSATO. Calcium influx pathways in human spermatozoa. *Mol. Hum. Reprod.* 3: 1–4, 1997.
96. FORESTA, C., M. ROSSATO, AND F. DI VIRGILIO. Extracelular ATP is a trigger for the acrosome reaction in human spermatozoa. *J. Biol. Chem.* 267: 19443–19447, 1992.
97. FORESTA, C., M. ROSSATO, P. CHIOZZI, AND F. DI VIRGILIO.

Mechanism of human sperm activation by extracellular ATP. *Am. J. Physiol.* 270 (Cell Physiol. 39): C1709–C1714, 1996.
98. FORESTA, C., M. ROSSATO, AND F. DI VIRGILIO. Ion fluxes through progesterone-activated channel of the sperm plasma membrane. *Biochem. J.* 294: 279–283, 1993.
99. FORESTA, C., M. ROSSATO, AND F. DI VIRGILIO. Differential modulation by protein kinase C of progesterone-activated responses in human sperm. *Biochem. Biophys. Res. Commun.* 206: 408–413, 1995.
100. FOSTER, J. A., B. B. FRIDAY, M. T. MAULIT, C. BLOBEL, V. P. WINFREY, G. E. OLSON, K. S. KIM, AND G. L. GERTON. AM67, a secretory component of the guinea pig sperm acrosomal matrix, is related to mouse sperm protein sp56 and the complement component 4-binding proteins. *J. Biol. Chem.* 272: 12714–12722, 1997.
101. FRASER, L. R. Minimum and maximum extracellular Ca^{2+} requirements during mouse sperm capacitation and fertilization in vitro. *J. Reprod. Fertil.* 81: 77–89, 1987.
102. FRASER, L. R., AND N. J. MONKS. Cyclic nucleotides and mammalian sperm capacitation. *J. Reprod. Fertil.* 42, *Suppl.*: 9–21, 1990.
103. GALANTINO-HOMMER, H. L., P. E. VISCONTI, AND G. S. KOPF. Regulation of protein tyrosine phosphorylation during bovine sperm capacitation by a cyclic adenosine 3′,5′-monophosphate-dependent pathway. *Biol. Reprod.* 56: 707–719, 1997.
104. GAO, Z., AND D. L. GARBERS. Species diversity in the structure of zonadhesin, a sperm-specific membrane protein containing multiple cell adhesion molecule-like domains. *J. Biol. Chem.* 273: 3415–3421, 1998.
105. GARBERS, D. L. The elevation in cyclic AMP concentrations in flagella-less sea urchin sperm heads. *J. Biol. Chem.* 256: 620–624, 1981.
106. GARBERS, D. L. Molecular basis of fertilization. *Annu. Rev. Biochem.* 58: 719–742, 1989.
107. GARBERS, D. L. Guanylyl cyclase receptors and their endocrine, paracrine, and autocrine ligands. *Cell* 71: 1–4, 1992.
108. GARBERS, D. L., AND G. S. KOPF. The regulation of spermatozoa by calcium and cyclic nucleotides. *Adv. Cyclic Nucleotide Res.* 13: 251–306, 1980.
109. GARBERS, D. L., G. S. KOPF, D. J. TUBB, AND G. OLSON. The elevation of sperm cyclic AMP concentrations by a fucose-sulfate rich complex associated with eggs. I. Structural characterization. *Biol. Reprod.* 29: 1211–1220, 1983.
110. GARBERS, D. L., D. J. TUBB, AND G. S. KOPF. Regulation of sea urchin sperm cyclic AMP-dependent protein kinases by an egg associated factor. *Biol. Reprod.* 22: 526–532, 1980.
111. GARCIA, M. A., AND S. MEIZEL. Importance of sodium ion to the progesterone-initiated acrosome reaction in human sperm. *Mol. Reprod. Dev.* 45: 513–520, 1996.
112. GARCíA-SOTO, J., L. M. ARAIZA, M. BARRIOS, A. DARSZON, AND J. P. LUNA-ARIAS. Endogenous activity of cyclic nucleotide-dependent protein kinase in plasma membranes isolated from *Strongylocentrotus purpuratus* sea urchin sperm. *Biochem. Biophys. Res. Commun.* 180: 1436–1445, 1991.
113. GARCíA-SOTO, J., AND A. DARSZON. High pH-induced acrosome reaction and Ca^{2+} uptake in sea urchin sperm suspended in Na^{+}-free seawater. *Dev. Biol.* 110: 338–345, 1985.
114. GARCÍA-SOTO, J., M. GONZÁLEZ-MARTÍNEZ, L. DE DELA-TORRE, AND A. DARSZON. Internal pH can regulate Ca^{2+} uptake and the acrosome reaction in sea urchin sperm. *Dev. Biol.* 120: 112–120, 1987.
115. GATTI, J. L., R. BILLIARD, AND R. CHRISTEN. Ionic regulation of the plasma membrane potential of rainbow trout (*Salmo gairdneri*) spermatozoa: role in the initiation of sperm motility. *J. Cell. Physiol.* 143: 546–554, 1990.
116. GATTI, J. L., C. CHEVRIER, M. PAQUIGNON, AND J. L. DRACHEUX. External ionic conditions, internal pH and motility of ram and boar spermatozoa. *J. Reprod. Fertil.* 98: 439–449, 1993.
117. GATTI, J. L., AND R. CHRISTEN. Regulation of internal pH of sea urchin sperm. *J. Biol. Chem.* 260: 7599–7602, 1985.
118. GAUSS, R., R. SEIFERT, AND U. B. KAUPP. Molecular identification of a hyperpolarization-activated channel in sea urchin sperm. *Nature* 393: 583–587, 1998.
119. GLABE, C. G., AND W. LENNARZ. Species-specific sperm adhesion in sea urchins: a quantitative investigation of binding mediated egg agglutination. *J. Cell Biol.* 83: 595–604, 1979.
120. GLASSNER, M., J. JONES, I. KLIGMAN, M. J. WOOLKALIS, G. L. GERTON, AND G. S. KOPF. Immunocytochemical and biochemical characterization of guanine nucleotide-binding regulatory proteins in mammalian spermatozoa. *Dev. Biol.* 146: 438–450, 1991.
121. GO, K. J., AND D. P. WOLF. Albumin-mediated changes in sperm sterol content during capacitation. *Biol. Reprod.* 32: 145–153, 1985.
122. GONG, X., D. H. DUBOIS, D. J. MILLER, AND B. D. SHUR. Activation of a G protein complex aggregation of β-1,4-galactosyltransferase on the surface of sperm. *Science* 269: 1718–1721, 1995.
123. GONZÁLEZ-MARTÍNEZ, M., AND A. DARSZON. A fast transient hyperpolarization occurs during the sea urchin sperm acrosome reaction induced by egg jelly. *FEBS Lett.* 218: 247–250, 1987.
124. GONZÁLEZ-MARTÍNEZ, M. T., A. GUERRERO, E. MORALES, L. DE DE LATORRE, AND A. DARSZON. A depolarization can trigger Ca^{2+} uptake and the acrosome reaction when preceded by a hyperpolarization in *L. pictus* sea urchin sperm. *Dev. Biol.* 150: 193–202, 1992.
125. GOODWIN, L. O., N. B. LEEDS, I. HURLEY, G. W. COOPER, R. G. PERGOLIZZI, AND S. BENOFF. Alternative splicing of exons in the alpha1 subunit of the rat L-type voltage-dependent cacium channel generates germ line-specific dihydropyridine binding sites. *Mol. Hum. Reprod.* 4: 215–226, 1998.
126. GOODWIN, L. O., N. B. LEEDS, I. HURLEY, F. S. MANDEL, R. G. PERGOLIZZI, AND S. BENOFF. Isolation and characterization of the primary strucure of testis-specific L-type calcium channel: implications for contraception. *Mol. Hum. Reprod.* 3: 255–268, 1997.
127. GUERRERO, A., AND A. DARSZON. Egg jelly triggers a calcium influx which inactivates and is inhibited by calmodulin antagonists in the sea urchin sperm. *Biochim. Biophys. Acta* 980: 109–116, 1989.
128. GUERRERO, A., AND A. DARSZON. Evidence for the activation of two different Ca^{2+} channels during the egg jelly-induced acrosome reaction of sea urchin sperm. *J. Biol. Chem.* 264: 19593–19599, 1989.
129. GUERRERO, A., L. GARCÍA, O. ZAPATA, E. RODRÍGUEZ, AND A. DARSZON. Acrosome reaction inactivation in sea urchin sperm. *Biochim. Biophys. Acta* 1401: 329–338, 1998.
130. GUERRERO, A., J. A. SÁNCHEZ, AND A. DARSZON. Single-channel activity in sea urchin sperm revealed by the patch-clamp technique. *FEBS Lett.* 220: 295–298, 1987.
131. HAGIWARA, N., AND K. KAWA. Calcium and potassium currents in spermatogenic cells dissociated from rat seminiferous tubules. *J. Physiol. (Lond.)* 356: 135–149, 1984.
132. HAMAMAH, S., E. MAGNOUX, D. ROYERE, C. BARTHELEMY, J. L. DACHEUX, AND J. L. GATTI. Internal pH of human spermatozoa: effect of ions, human follicular fluid and progesterone. *Mol. Hum. Reprod.* 2: 219–224, 1996.
133. HANSBROUGH, J. R., AND D. L. GARBERS. Sodium dependent activation of sea urchin spermatozoa by speract and monensin. *J. Biol. Chem.* 256: 2235–2241, 1981.
134. HANSBROUGH, J. R., G. S. KOPF, AND D. L. GARBERS. The stimulation of sperm metabolism by a factor associated with eggs and by 8-bromo-guanosine 3′,5′-monophosphate. *Biochim. Biophys. Acta* 630: 82–91, 1980.
135. HARDY, D. M., AND D. L. GARBERS. A sperm membrane protein that binds in a species-specific manner to the egg extracellular matrix is homologous to von Willebrand factor. *J. Biol. Chem.* 270: 26025–26028, 1995.
136. HARDY, M. P., AND J. N. DENT. Regulation of motility in sperm of the red-spotted newt. *J. Exp. Zool.* 240: 385–396, 1986.
137. HEGINBOTHAM, L., T. ABRAMSON, AND R. MACKINNON. Mutations in the K^{+} channel signature sequence. *Biophys. J.* 66: 1061–1067, 1994.
138. HERSHLAG, A., G. W. COOPER, AND S. BENOFF. Pregnancy following discontinuation of a calcium channel blocker in the male partner. *Hum. Reprod.* 10: 599–606, 1995.
139. HILDEBRANDT, J. D., J. CODINA, J. S. TASH, H. J. KIRCHICK, L. LIPSCHULTZ, R. D. SEKURA, AND L. BIRNBAUMER. The membrane-bound spermatozoal adenylyl cyclase system does not share coupling characteristics with somatic cell adenylyl cyclases. *Endocrinology* 116: 1357–1366, 1985.

140. HILLE, B. *Ionic Channels of Excitable Membranes* (2nd ed.). Sunderland, MA: Sinauer, 1992.
141. HOSHI, M., T. AMANO, Y. OKITA, T. OKINAGA, AND T. MATSUI. Egg signals for triggering the acrosome reaction in starfish spermatozoa. *J. Reprod. Fertil.* 42, *Suppl.*: 23–31, 1990.
142. HOSHI, M., T. NISHIGAKI, A. USHIYAMA, T. OKINAGA, K. CHIBA, AND M. MATSUMOTO. Egg-jelly signal molecules for triggering the acrosome reaction in starfish spermatozoa. *Int. J. Dev. Biol.* 38: 167–174, 1994.
143. HOSHI, M., T. M. OKINAGA, K. KONTANI, T. ARAKI, AND K. CHIBA. Acrosome reaction-inducing glycoconjugate in the jelly coat of star fish eggs. In: *Comparative Spermatology 20 Years After*, edited by B. Baccetti. New York: Raven, 1991, p. 175–180.
144. HUNTER, R. H. F., AND R. NICHOL. A preovulatory temperature gradient between the isthmus and the ampulla of pig oviducts during the phase of sperm storage. *J. Reprod. Fertil.* 77: 599–606, 1986.
145. IKADAI, H., AND M. HOSHI. Biochemical studies on the acrosome reaction of the starfish, *Asterias amurensis*. I. Factors participating in the acrosome reaction. *Dev. Growth Differ.* 23: 73–80, 1981.
146. IKADAI, H., AND M. HOSHI. Biochemical studies on the acrosome reaction of starfish, *Asterias amurensis*. II. Purification and characterization of the acrosome reaction-inducing substance. *Dev. Growth Differ.* 23: 81–88, 1981.
147. IZUMI, H., T. MARIAN, K. INABA, Y. OKA, AND M. MORISAWA. Hyperpolarization of sperm plasma membrane mediated by K^+ efflux induces an increase in cAMP and initiation of sperm motility in the ascidian, *Ciona intestinalis* and *C. savignyi* (Abstract). *Zygote* 6: 5133, 1998.
148. JAN, Y. N., AND L. Y. JAN. Cloned potassium channels from eukaryotes and prokaryotes. *Annu. Rev. Neurosci.* 20: 91–123, 1997.
149. JENKINS, A. D., C. P. LECHENE, AND S. S. HOWARD. Concentrations of seven elements in the intraluminal fluids of the rat seminiferous tubules, rat testis, and epididymis. *Biol. Reprod.* 23: 981–987, 1980.
150. JOHNSON, C. H., D. L. CLAPPER, M. M. WINKLER, H. C. LEE, AND D. EPEL. A volatile inhibitor immobilizes sea urchin sperm in semen by depressing intracellular pH. *Dev. Biol.* 98: 493–501, 1983.
151. KALAB, P., P. VISCONTI, P. LECLERC, AND G. S. KOPF. p95, the major phosphotyrosine-containing protein in mouse spermatozoa, is a hexokinase with unique properties. *J. Biol. Chem.* 269: 3810–3817, 1994.
152. KAUPP, B. Family of cyclic nucleotide gated ion channels. *Curr. Opin. Neurobiol.* 5: 434–442, 1995.
153. KAZAZOGLOU, T., R. W. SCHACKMANN, M. FOSSETT, AND B. M. SHAPIRO. Calcium channel antagonists inhibit the acrosome reaction and bind to plasma membranes of sea urchin sperm. *Proc. Natl. Acad. Sci. USA* 82: 1460–1464, 1985.
154. KHAN, A. A., J. P. STEINER, AND S. H. SNYDER. Plasma membrane inositol 1,4,5-trisphosphate receptor of lymphocytes: selective enrichment in sialic acid and unique binding specificity. *Proc. Natl. Acad. Sci. USA* 89: 2849–2853, 1992.
155. KLEINHANS, F. W., V. S. TRAVIS, J. Y. DU, P. M. VILLENS, K. E. COLVIN AND J. K. CRITSER. Measurement of human sperm intracellular water volume by electron-spin resonance. *J. Androl.* 13: 498–506, 1992.
156. KOPF, G. S., AND G. L. GERTON. The mammalian sperm acrosome and the acrosome reaction. In: *Elements of Mammalian Fertilization. I. Basic Concepts*, edited by P. M. Wassarman. Boca Raton, FL: CRC, 1990, p. 153–203.
157. KOPF, G. S., D. J. TUBB, AND D. L. GARBERS. Activation of sperm respiration by a low molecular weight egg factor and by 8-bromoguanosine 3′,5′-monophosphate. *J. Biol. Chem.* 254: 8554–8560, 1979.
158. KOPF, G. S., M. J. WOOLKALIS, AND G. L. GERTON. Evidence for a guanine nucleotide-binding regulatory protein in invertebrate and mammalian sperm. Identification by islet activating protein-catalyzed ADP-ribosylation and immunochemical methods. *J. Biol. Chem.* 261: 7327–7331, 1986.
159. KOYOTA, S., K. M. SWARNA WIMALASIRI, AND M. HOSHI. Structure of the main saccharide chain in the acrosome reaction-inducing substance of the starfish, *Asterias amurensis*. *J. Biol. Chem.* 272: 10372–10376, 1997.
160. KRUPINSKI, J., F. COUSSEN, H. A. BAKALYAR, W. J. TANG, P. G. FEINSTEIN, K. ORTH, C. SLAUGHTER, R. R. REED, AND A. G. GUILMAN. Adenylyl cyclase amino acid sequence: possible channel- or transporter-like structure. *Science* 244: 1558–1564, 1989.
161. KUDO, K., N. YONEZAWA, T. KATSUMATA, H. AOKI, AND M. NAKANO. Localization of carbohydrate chains of pig sperm ligand in the glycoprotein ZPB of egg zona pellucida. *Eur. J. Biochem.* 252: 492–499, 1998.
162. LABARCA, P., C. SANTI, O. ZAPATA, C. BELTRÁN, A. LIÉVANO, Y. SANDOVAL, AND A. DARSZON. Possible participation of a cAMP regulated K^+ channel from the sea urchin sperm in the speract response. In: *From Ion Channels to Cell-to-Cell Conversations*, edited by R. Latorre. New York: Plenum, 1997.
163. LABARCA, P., C. SANTI, O. ZAPATA, E. MORALES, C. BELTRÁN, A. LIÉVANO AND A. DARSZON. A cAMP regulated K^+-selective channel from the sea urchin sperm plasma membrane. *Dev. Biol.* 174: 271–280, 1996.
164. LABARCA, P., O. ZAPATA, C. BELTRÁN, AND A. DARSZON. Ion channels from the mouse sperm plasma membrane in planar lipid bilayers. *Zygote* 3: 199–206, 1995.
165. LAKOSKI, K. A., C. P. CARRON, C. L. CABOT, AND P. M. SALING. Epididymal maturation and the acrosome reaction in mouse sperm: response to zona pellucida develops coincident with modification of M42 antigen. *Biol. Reprod.* 38: 221–233, 1988.
166. LATORRE, R., J. BACIGALUPO, R. DELGADO, AND P. LABARCA. Four cases of direct ion channel gating by cyclic nucleotides. *J. Bioenerg. Biomembr.* 23: 577–597, 1991.
167. LAX, Y., S. RUBINSTEIN, AND H. BREITBART. Epidermal growth factor induces acrosomal exocytosis in bovine sperm. *FEBS Lett.* 339: 234–238, 1994.
168. LAX, Y., S. RUBINSTEIN, AND H. BREITBART. Subcellular distribution of PKCα and β1 in bovine spermatozoa and their regulation by calcium and phorbol esters. *Biol. Reprod.* 56: 454–459, 1997.
169. LEE, H. C. Sodium and proton transport in flagella isolated from sea urchin spermatozoa. *J. Biol. Chem.* 259: 4957–4963, 1984.
170. LEE, H. C. A membrane potential-sensitive Na^+-H^+ exchange system in flagella isolated from sea urchin spermatozoa. *J. Biol. Chem.* 259: 15315–15319, 1984.
171. LEE, H. C. The voltage-sensitive Na^+/H^+ exchange in spermatozoa flagella membrane vesicles studied with an entrapped pH probe sea urchin. *J. Biol. Chem.* 260: 10794–10799, 1985.
172. LEE, H. C. Internal GTP stimulates the speract receptor mediated voltage changes in sea urchin spermatozoa membrane vesicles. *Dev. Biol.* 126: 91–97, 1988.
173. LEE, H. C., AND D. L. GARBERS. Modulation of the voltage sensitive Na^+/H^+ exchange in sea urchin spermatozoa through membrane potential changes induced by the egg peptide speract. *J. Biol. Chem.* 261: 16026–16032, 1986.
174. LEE, H. C., C. JOHNSON, AND D. EPEL. Changes in internal pH associated with the initiation of motility and acrosome reaction of sea urchin sperm. *Dev. Biol.* 95: 31–45, 1983.
175. LEE, M. A., J. H. CHECK, AND G. S. KOPF. A guanine nucleotide-binding regulatory protein in human sperm mediates acrosomal exocytosis induced by the human zona pellucida. *Mol. Reprod. Dev.* 31: 78–86, 1992.
176. LEE, M. A., G. S. KOPF, AND B. T. STOREY. Effects of phorbol esters and diacyglycerol on the mouse sperm acrosome reaction induced by the zona pellucida. *Biol. Reprod.* 36: 617–627, 1987.
177. LEYTON, L., P. LEGUEN, D. BUNCH, AND P. M. SALING. Regulation of mouse gamete interaction by a sperm tyrosine kinase. *Proc. Natl. Acad. Sci. USA* 89: 11692–11695, 1992.
178. LEYTON, L., C. TOMES, AND P. SALING. LL95 monoclonal antibody mimics functional effects of ZP3 on mouse sperm: evidence that the antigen recognized is not hexokinase. *Mol. Reprod. Dev.* 42: 347–358, 1995.
179. LI, G., D. MILANI, M. J. DUNNE, W. F. PRALONG, J. M. THELER, O. H. PETERSEN, AND C. B. WOLLHEIM. Extracellular ATP causes Ca^{2+}-dependent and -independent insulin secretion in RINm5F cells. *J. Biol. Chem.* 266: 3449–3457, 1991.
180. LIÉVANO, A., A. BOLDEN, AND R. HORN. Calcium channels in excitable cells: divergent genotypic and phenotypic expression of α1-subunits. *Am. J. Physiol.* 267 (*Cell Physiol.* 36): C411—C424, 1994.

181. LIÉVANO, A., J. SÁNCHEZ, AND A. DARSZON. Single channel activity of bilayers derived from sea urchin sperm plasma membranes at the tip of a patch-clamp electrode. *Dev. Biol.* 112: 235–257, 1985.
182. LIÉVANO, A., C. M. SANTI, C. J. SERRANO, C. L. TREVIÑO, A. R. BELLVÉ, A. HERNÁNDEZ-CRUZ, AND A. DARSZON. T-type Ca^{2+} channels and α_{1E} expression in spermatogenic cells, and their possible relevance to the sperm acrosome reaction. *FEBS Lett.* 388: 150–154, 1996.
183. LIÉVANO, A., E. C. VEGA SAENZ DE MIERA, AND A. DARSZON. Ca^{2+} channels from the sea urchin sperm plasma membrane. *J. Gen. Physiol.* 95: 273–296, 1990.
184. LILLIE, F. R. *Problems of Fertilization.* Chicago, IL: Univ. of Chicago Press, 1919.
185. LINARES-HERNANDEZ, L., A. M. GUZMAN-GRENFELL, J. J. HICKS-GOMEZ, AND M. GONZALEZ-MARTINEZ. Voltage dependent calcium influx in human sperm assessed by simultaneous optical detection of intracellular calcium and membrane potential. *Biochim. Biophys. Acta* 1372: 1–12, 1998.
186. LINDEMANN, C., AND R. RIKMENSPOEL. Intracellular potentials in bull spermatozoa. *J. Physiol. (Lond.)* 219: 127–138, 1971.
187. LONGO, F. J., A. USHIYAMA, K. CHIBA, AND M. HOSHI. Ultrastructural localization of acrosome reaction-inducing substance (ARIS) on sperm of the starfish *Asterias amurensis*. *Mol. Reprod. Dev.* 41: 91–99, 1995.
188. LU, Q., AND B. D. SHUR. Sperm from beta 1,4-galactosyltransferase-null mice are refractory to ZP3-induced acrosome reactions and penetrate the zona pellucida poorly. *Development* 124: 4121–4131, 1997.
189. LUDWIG, A., X. ZONG, M. JEGLITSCH, F. HOFMANN, AND M. BIEL. A family of hyperpolarization-activated mammalian cation channels. *Nature* 393: 587–591, 1998.
190. LUNDBAEK, J. A., P. BIRN, J. GIRSHMAN, A. J. HANSEN, AND O. S. ANDERSEN. Membrane stiffness and channel function. *Biochemistry* 35: 3825–3830, 1996.
191. MATSUI, T., I. NISHIYAMA, A. HINO, AND M. HOSHI. Induction of the acrosome reaction in starfish. *Dev. Growth Differ.* 28: 339–348, 1986.
192. MATSUI, T., I. NISHIYAMA, A. HINO, AND M. HOSHI. Intracellular pH changes of starfish sperm upon the acrosome reaction. *Dev. Growth Differ.* 28: 359–368, 1986.
193. MATSUMARA, K., AND K. AKETA. Proteasome (multicatalytic protease) of sea urchin sperm and its possible participation in the acrosome reaction. *Mol. Reprod. Dev.* 29: 189–199, 1990.
194. McDONALD, R. L., AND R. W. OLSEN. $GABA_A$ receptor channels. *Annu. Rev. Neurosci.* 17: 596–602, 1994.
195. McGRADY, A. V., AND L. NELSON. Cationic influences on sperm biopotentials. *Exp. Cell Res.* 73: 192–196, 1972.
196. McLESKEY, S. B., C. DOWDS, R. CARBALLADA, R. R. WHITE, AND P. M. SALING. Molecules involved in mammalian sperm-egg interaction. *Int. Rev. Cytol.* 177: 57–113, 1998.
197. MEIR, A., AND C. DOLPHIN. Known calcium channel α1 subunits can form low threshold small conductance channels with similarities to native T-type channels. *Neuron* 20: 341–351, 1998.
198. MEIZEL, S. Amino acid neurotransmitter receptor/chloride channels of mammalian sperm and the acrosome reaction. *Biol. Reprod.* 56: 569–574, 1997.
199. MEIZEL, S., AND K. O. TURNER. Initiation of the human sperm acrosome reaction by thapsigargin. *J. Exp. Zool.* 267: 350–355, 1993.
200. MEIZEL, S., AND K. O. TURNER. Chloride efflux during the progesterone-initiated human sperm acrosome reaction is inhibited by lavendustin A, a tyrosine kinase inhibitor. *J. Androl.* 17: 327–330, 1996.
201. MEIZEL, S., K. O. TURNER, AND R. NUCCITELLI. Progesterone triggers a wave of increased free calcium during the human sperm acrosome reaction. *Dev. Biol.* 182: 67–75, 1997.
202. MELENDREZ, C., AND S. MEIZEL. Studies of porcine and human sperm suggesting a role for a sperm glycine receptor/Cl^- channel in the zona pellucida-initiated acrosome reaction. *Biol. Reprod.* 53: 676–683, 1995.
203. MENDOZA, C., A. SOLER, AND J. TESARIK. Nongenomic steroid action: independent tartgeting of a plamsa membrane calcium channel and a tyrosine kinase. *Biochem. Biophys. Res. Commun.* 210: 518–523, 1995.
204. MENDOZA, C., AND J. TESARIK. A plasma-membrane progesterone receptor in human sperm is switched on by increasing intracellular free calcium. *FEBS Lett.* 330: 57–60, 1993.
205. MILLER, R. L. Sperm chemotaxis in ascidians. *Am. Zool.* 22: 827–840, 1982.
206. MILLER, R. L. Sperm chemo-orientation in the metazoa. In: *Biology of Fertilization. Biology of the Sperm*, edited by C. B. Metz and A. Monroy. New York: Academic, 1985, vol. 2, p. 275–337.
207. MILLER, R. L., AND R. VOGT. An N-terminal partial sequence of the 13 kDa *Picnopodia Helianthoides* sperm chemoattractant "star-trak" possesses sperm-attracting activity. *J. Exp. Biol.* 199: 311–318, 1996.
208. MORALES, E., L. DE DE LA TORRE, G. MOY, V. D. VACQUIER, AND A. DARSZON. Anion channels in the sea urchin sperm plasma membrane. *Mol. Reprod. Dev.* 36: 174–182, 1993.
209. MORISAWA, M. Cell signaling mechanisms for sperm motiity. *Zool. Sci.* 11: 647–662, 1994.
210. MORISAWA, M., AND H. HAYASHI. Phosphorylation of a 15 K axonemal protein is the trigger initiating trout sperm motility. *Biomed. Res.* 6: 181–184, 1985.
211. MORISAWA, M., AND K. ISHIDA. Short-term changes in levels of cyclic AMP, adenylate cyclase, and phosphodiesterase during the initiation of sperm motility in rainbow trout. *J. Exp. Zool.* 242: 199–204, 1987.
212. MORISAWA, M., AND S. MORISAWA. Acquisition and initiation of sperm motility. In: *Controls of Sperm Motility: Biological and Clinical Aspects*, edited by C. Gagnon. Boca Raton, FL: CRC, 1994, p. 137–151.
213. MORISAWA, M., AND M. OKUNO. Cyclic AMP induces maturation of trout sperm axoneme to initiate motility. *Nature* 295: 703–704, 1982.
214. MORISAWA, M., AND K. SUZUKI. Osmolarity and potassium ion: their roles in initiation of sperm motility in teleosts. *Science* 210: 1145–1147, 1980.
215. MOY, G. W., L. M. MENDOZA, J. R. SCHULZ, W. J. SWANSON, C. G. GLABE, AND V. D. VACQUIER. The sea urchin sperm receptor for egg jelly is a modular protein with extensive homology to the human polycystic kidney disease protein, PKD1. *J. Cell Biol.* 133: 809–817, 1996.
216. MURASE, T., AND E. R. S. ROLDAN. Progesterone and the zona pellucida activate different transducing pathways in the sequence of events leading to diacylglycerol generation during mouse sperm acrosome reaction. *Biochem. J.* 320: 1017–1023, 1996.
217. NAKAMURA, M., M. MORIYA, T. BABA, Y. MICHIKAWA, T. YAMANOBE, K. ARAI, S. OKINAGA, AND T. KOBAYASHI. An endoplasmic reticulum protein, calreticulin, is transported into the acrosome of rat sperm. *Exp. Cell Res.* 205: 101–110, 1993.
218. NISHIGAKI, T., K. CHIBA, AND M. HOSHI. Identification and characterization of receptors for starfish sperm-activating peptides (asterosaps). *Zool. Sci.* 13, *Suppl*: 71, 1996.
219. NISHIGAKI, T., K. CHIBA, W. MIKI, AND M. HOSHI. Structure and function of asterosaps, sperm-activating peptides from the jelly coat of starfish eggs. *Zygote* 4: 237–245, 1996.
220. NISHIOKA, D., AND N. CROSS. The role of external sodium in sea urchin fertilization. In: *Cell Reproduction*, edited by E. R. Dirksen, D. Prescott, and C. F. Fox. New York: Academic, 1978, p. 403–413.
221. ODA, S., AND M. MORISAWA. Rises of intracellular Ca^{2+} and pH mediate the initiation of sperm motility by hyperosmolality in marine teleosts. *Cell Motil. Cytoskeleton* 25: 171–178, 1993.
222. OKAMURA, N., S. ONOE, K. KAWAKURA, Y. TAJIMA, AND Y. SUGITA. Effects of a membrane-bound trypsin-like proteinase and seminal proteinase inhibitors on the bicarbonate-sensitive adenylate cyclase in porcine sperm plasma membranes. *Biochim. Biophys. Acta* 1035: 83–89, 1990.
223. OSSES, N., F. PANCETTI, D. J. BENOS, AND J. G. REYES. Intracellular pH regulation in rat round spermatids. *Biol. Cell* 89: 273–283, 1997.
224. O'TOOLE, C. M. B., E. R. S. ROLDAN, AND L. R. FRASER. Role for Ca^{2+} channels in the signal transduction pathway leading to acrosomal exocytosis in human spermatozoa. *Mol. Reprod. Dev.* 45: 204–211, 1996.
225. O'TOOLE, C. M. B., E. R. S. ROLDÁN, P. HAMPTON, AND L. R.

FRASER. A role for diacylglycerol in human sperm acrosomal exocytosis. *Mol. Hum. Reprod.* 2: 317–326, 1996.
226. PAREKH, A. B., AND R. PENNER. Store depletion and calcium influx. *Physiol. Rev.* 77: 901–930, 1997.
227. PARRISH, J. J., J. L. SUSKO-PARRISH, AND N. L. FIRST. Capacitation of bovine sperm by heparin: inhibitory effect of glucose and role of intracellular pH. *Biol. Reprod.* 41: 683–699, 1989.
228. PÉREZ-REYES, E., L. L. CRIBBS, A. DAUD, A. E. LACERDA, J. BARCLAY, M. P. WILLIAMSON, M. FOX, M. REES, AND J.-H. LEE. Molecular characterization of a neuronal low-voltage-activated T-type calcium channel. *Nature* 391: 896–900, 1998.
229. PIEDRAS-RENTERÍA, E. S., C. CHEN, AND P. BEST. Antisense oligonucleotides against rat brain α1E DNA and its atrial homologue decrease T-type calcium current in atrial myocytes. *Proc. Natl. Acad. Sci. USA* 94: 14936–14941, 1997.
230. PIEDRAS-RENTERÍA, E. S., AND R. W. TSIEN. Antisense oligonucleotides against alpha1E reduce R-type calcium currents in cerebellar granule cells. *Proc. Natl. Acad. Sci. USA* 95: 7760–7765, 1998.
231. RABOW, L. E., S. J. RUSSEK, AND D. H. FARB. From ion currents to genomic analysis: recent advances in $GABA_A$ receptor research. *Synapse* 21: 189–274, 1995.
232. RALT, D., M. GOLDENBERG, P. FETTEROLF, D. THOMPSON, J. DOR, S. MASHIACH, D. L. GARBERS, AND M. EISENBACH. Sperm attraction to a follicular factor(s) correlates with human egg fertilizability. *Proc. Natl. Acad. Sci. USA* 88: 2840–2844, 1991.
233. RAMARAO, C. S., AND D. L. GARBERS. Receptor-mediated regulation of guanylate cyclase activity in spermatozoa. *J. Biol. Chem.* 260: 8390–8396, 1985.
234. REDDY, R., D. SMITH, G. WAYMAN, Z. WU, E. C. VILLACRES, AND D. R. STORM. Voltage-sensitive adenylyl cyclase activity in cultured neurons. A calcium-independent phenomenon. *J. Biol. Chem.* 270: 14340–14346, 1995.
235. REPASKE, D. R., AND D. L. GARBERS. A hydrogen ion flux mediates stimulation of respiratory activity by speract in sea urchin spermatozoa. *J. Biol. Chem.* 258: 6025–6029, 1983.
236. REYES, J. G., J. BACIGALUPO, R. ARAYA, AND D. J. BENOS. Ion dependence of resting membrane potential of rat spermatids. *J. Reprod. Fertil.* 102: 313–319, 1994.
237. REYNAUD, E., L. DE DE LATORRE, O. ZAPATA, A. LIÉVANO, AND A. DARSZON. Ionic bases of the membrane potential and intracellular pH changes induced by speract in swollen sea urchin sperm. *FEBS Lett.* 329: 210–214, 1993.
238. ROBLERO, L., A. GUADARRAMA, M. E. ORTIZ, E. FERNÁNDEZ, AND F. ZEGERS-HOCHSCHILD. High potassium concentration improves the rate of acrosome reaction in human spermatozoa. *Fertil. Steril.* 49: 676–679, 1988.
239. ROLDÁN, E. R. S., T. MURASE, AND Q. SHI. Exocytosis in spermatozoa in response to progesterone and zona pellucida. *Science* 266: 1578–1581, 1994.
240. ROSIERE, T. K., AND P. M. WASSARMAN. Identification of a region of mouse zona pellucida glycoprotein mZP3 that possesses sperm receptor activity. *Dev. Biol.* 154: 309–317, 1992.
241. ROSSATO, M., F. DI VIRGILIO, AND C. FORESTA. Involvement of osmo-sensitive calcium influx in human sperm activation. *Mol. Hum. Reprod.* 2: 903–909, 1996.
242. ROTEM, R., G. F. PAZ, Z. T. HOMONNAI, M. KALINA, J. LAX, H. BREITBART, AND Z. NAOR. Ca^{2+}-independent induction of acrosome reaction by protein kinase C in human sperm. *Endocrinology* 131: 2235–2243, 1992.
243. RUFO, G. A., J. P. SINGH, D. F. BABCOCK, AND H. A. LARDY. Purification and characterization of a calcium transport inhibitor protein from bovine seminal plasma. *J. Biol. Chem.* 257: 4627–4632, 1982.
244. SABEUR, K., D. P. EDWARDS, AND S. MEIZEL. Human sperm plasma membrane progesterone receptor(s) and the acrosome reaction. *Biol. Reprod.* 54: 993–1001, 1996.
245. SALING, P. M. Mammalian sperm interaction with extracellular matrices of the egg. *Oxf. Rev. Reprod. Biol.* 11: 339–388, 1989.
246. SANTI, C. M., A. DARSZON, AND A. HERNÁNDEZ-CRUZ. A dihydropyridine-sensitive T-type Ca^{2+} current is the main Ca^{2+} current carrier in mouse primary spermatocytes. *Am. J. Physiol.* 271 (*Cell Physiol.* 40): C1583—C1593, 1996.
247. SANTI, C. M., T. SANTOS, A. HERNÁNDEZ, AND A. DARSZON. Properties of a novel pH-dependent Ca^{2+} permeation pathway present in male germ cells with possible roles in spermatogenesis and mouse sperm function. *J. Gen. Physiol.* 112: 33–53, 1998.
248. SANTORO, B., D. T. LIU, H. YAO, D. BARTSCH, E. R. KANDEL, S. A. SIEGELBAUM, AND G. R. TIBBS. Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. *Cell* 95: 717–729, 1998.
249. SCHACKMANN, R. W. Ionic regulation of the sea urchin sperm acrosome reaction and stimulation by egg-derived peptides. In: *The Cell Biology of Fertilization*, edited by H. Schatte and G. Schatten. San Diego, CA: Academic, 1989, p. 3–28.
250. SCHACKMANN, R. W., AND P. B. CHOCK. Alteration of intracellular $[Ca^{2+}]$ in sea urchin sperm by the egg peptide speract. *J. Biol. Chem.* 261: 8719–8728, 1986.
251. SCHACKMANN, R. W., R. CHRISTEN, AND B. M. SHAPIRO. Membrane potential depolarization and increased intracellular pH accompany the acrosome reactions of sea urchin sperm. *Proc. Natl. Acad. Sci. USA* 78: 6066–6070, 1981.
252. SCHACKMANN, R. W., R. CHRISTEN, AND B. M. SHAPIRO. Measurement of plasma membrane and mitochondrial membrane potentials in sea urchin sperm. *J. Biol. Chem.* 259: 13914–13922, 1984.
253. SCHACKMANN, R. W., E. M. EDDY, AND B. M. SHAPIRO. The acrosome reaction of *Strongylocentrotus purpuratus* sperm: ion requirements and movements. *Dev. Biol.* 65: 483–495, 1978.
254. SCHACKMANN, R. W., AND B. M. SHAPIRO. A partial sequence of ionic changes associated with the acrosome reaction of *Strongylocentrotus purpuratus*. *Dev. Biol.* 81: 145–154, 1981.
255. SCHLENK, W. J., AND H. KAHMANN. Die Chemische Zusammensetzung des Spermaliquors und ihre physiologische Bedeutung. *Bioch. Zeit.* 295: 283–301, 1938.
256. SCHREIBER, M., A. WEI, A. YUAN, J. GAUT, M. SAITO, AND L. SALKOFF. Slo3, a novel pH-sensitive K^+ channel from mammalian spermatocytes. *J. Biol. Chem.* 273: 3509–3516, 1998.
257. SCHULTZ, J. E., S. KLUMPP, R. BENZ, W. J. CH. SCHÜRHOFF-GOETERS, AND A. SCHMID. Regulation of adenylyl cyclase from *Paramecium* by an intrinsic potassium conductance. *Science* 255: 600–603, 1992.
258. SCHULZ, J. R., G. M. WESSEL, AND V. D. VACQUIER. The exocytosis regulatory proteins syntaxin and VAMP are shed from sea urchin during the acrosome reaction. *Dev. Biol.* 191: 80–87, 1997.
259. SEGALL, G. K., AND W. J. LENNARZ. Chemical characterization of the component of the jelly coat from sea urchin eggs responsible for induction of the acrosome reaction. *Dev. Biol.* 71: 33–48, 1979.
260. SENGOKU, K., K. TAMATE, N. TAKUMA, Y. TAKAOKA, T. YOSHIDA, K. NISHIWAKI, AND M. ISHIKAWA. Involvement of protein kinases in platelet activating factor-induced acrosome reaction of human spermatozoa. *Mol. Hum. Reprod.* 2: 401–404, 1996.
261. SHI, Q. X., AND E. R. S. ROLDÁN. Evidence that a $GABA_A$-like receptor is involved in progesterone-induced acrosomal exocytosis in mouse spermatozoa. *Biol. Reprod.* 52: 373–381, 1995.
262. SHI, Q. X., Y. YUAN, AND E. R. S. ROLDÁN. γ-Aminobutiric acid (GABA) induces the acrosome reaction in human spermatozoa. *Mol. Hum. Reprod.* 3: 677–683, 1997.
263. SHIMIZU, T., H. KINOH, M. YAMAGUCHI, AND N. SUZUKI. Purification and characterization of the egg jelly macromolecules, sialoglycoprotein and fucose sulfate glycoconjugate, of the sea urchin *Hemicentrotus pulcherrimus*. *Dev. Growth Differ.* 32: 473–487, 1990.
264. SHIMOMURA, H. L., L. J. DANGOTT, AND D. L. GARBERS. Covalent coupling of a resact analog to guanylate cyclase. *J. Biol. Chem.* 261: 15778–15782, 1986.
265. SHUR, B. D. Glycosyltransferases as cell adhesion molecules. *Curr. Opin. Cell Biol.* 5: 854–863, 1993.
266. SINGH, S., D. G. LOWE, D. S. THORPE, H. RODRIGUEZ, W. J. KUANG, L. J. DANGOTT, M. CHINKERS, D. V. GOEDDEL, AND D. L. GARBERS. Membrane guanylate cyclase is a cell-surface receptor with homology to protein kinase. *Nature* 334: 708–712, 1988.
267. SNUTCH, T. P., AND P. B. REINER. Ca^{2+} channels: diversity of form and function. *Curr. Opin. Cell Biol.* 2: 247–253, 1992.
268. SOONG, T. W., A. STEA, A. HODSON, S. J. DUBEL, S. VINCENT, AND T. P. SNUTCH. Structure and funtional expression of a member of the low voltage-activated calcium channel family. *Science* 260: 1133–1136, 1993.

269. SPUNGIN, B., AND H. BREITBART. Calcium mobilization and influx during sperm exocytosis. *J. Cell Sci.* 109: 1947–1955, 1996.
270. STOREY, B. T. Interactions between gametes leading to fertilization: the sperm's eye view. *Reprod. Fertil. Dev.* 7: 927–942, 1995.
271. STOREY, B. T., C. L. HOURANI, AND J. B. KIM. A transient rise in intracellular Ca^{2+} is a precursor reaction to the zona pellucida-induced acrosome reaction in mouse sperm and is blocked by the induced acrosome reaction inhibitor 3-quinuclidinyl benzilate. *Mol. Reprod. Dev.* 32: 41–50, 1992.
272. SUMMERS, R. G., AND B. L. HYLANDER. Species-specific acrosome reaction and primary gamete binding in echinoids. *Exp. Cell Res.* 96: 63–68, 1975.
273. SUZUKI, M., K. TAKAHASHI, M. IKEDA, H. HAYAKAWA, A. OGAWA, Y. KAWAGUCHI, AND O. SAKAI. Cloning of a pH-sensitive K^+ channel possessing two transmembrane segments. *Nature* 367: 642–645, 1994.
274. SUZUKI, N., H. SHIMOMURA, E. W. RADANY, C. S. RAMARAO, G. E. WARD, J. K. BENTLEY, AND D. L. GARBERS. A peptide associated with eggs causes a mobility shift in a major plasma membrane protein of spermatozoa. *J. Biol. Chem.* 259: 14874–14879, 1984.
275. SUZUKI, N., AND K. YOSHINO. The relationship between amino acid sequences of sperm-activating peptides and the taxonomy of equinoids. *Comp. Biochem. Physiol. B Biochem.* 102: 679–690, 1992.
276. TAKAI, H., AND M. MORISAWA. Change in intracellular K^+ concentration caused by external osmolality change regulates sperm motility of marine and freshwater teleosts. *J. Cell Sci.* 108: 1175–1181, 1995.
277. TANIMOTO, S., Y. KUDO, T. NAKAZAWA, AND M. MORISAWA. Implication that potassium flux and increase in intracellular calcium are necessary for the initiation of sperm motility in salmonid fishes. *Mol. Reprod. Dev.* 39: 409–414, 1994.
278. TANIMOTO, S., AND M. MORISAWA. Roles for potassium and calcium channels in the initiation of sperm motility in rainbow trout. *Dev. Growth Differ.* 30: 117–124, 1988.
279. TESARIK, J., A. CARRERAS, AND C. MENDOZA. Differential sensitivity of progesterone- and zona pellucida-induced acrosome reactions to pertussis toxin. *Mol. Reprod. Dev.* 34: 183–189, 1993.
280. TESARIK, J., J. MOOS, AND C. MENDOZA. Stimulation of a protein tyrosine phosphorylation by progesterone receptor on the cell surface of human sperm. *Endocrinology* 133: 328–335, 1993.
281. THOMAS, P., AND S. MEIZEL. Phosphatidylinositol 4,5-bisphosphate hydrolysis in human sperm stimulated with follicular fluid or progesterone is dependent upon Ca^{2+} influx. *Biochem. J.* 264: 539–546, 1989.
282. TILNEY, L. G. The acrosomal reaction. In: *Biology of Fertilization*, edited by C. B. Metz and A. Monroy. Orlando, FL: Academic, 1985, vol. 2, p. 157–213.
283. TIWARI-WOODRUFF, S. T., AND T. C. COX. Boar sperm plasma membrane Ca^{2+}-selective channels in planar lipid bilayers. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C1284—C1294, 1995.
284. TOMBES, R. M., AND B. M. SHAPIRO. Metabolite channeling: a phosphorylcreatine shuttle to mediate high energy phosphate transport between sperm mitochondria and tail. *Cell* 41: 325–334, 1985.
285. TREVIÑO, C. L., C. M. SANTI, C. BELTRÁN, A. HERNÁNDEZ-CRUZ, A. DARSZON, AND H. LOMELI. Localization of IP_3 and ryanodine receptors during mouse spermatogenesis: possible functional implications. *Zygote* 6: 159–172, 1998.
286. TRIMMER, J. S., R. W. SCHACKMANN, AND V. D. VACQUIER. Monoclonal antibodies increase intracellular Ca^{2+} in sea urchin spermatozoa. *Proc. Natl. Acad. Sci. USA* 83: 9055–9059, 1986.
287. TRIMMER, J. S., I. S. TROWBRIDGE, AND V. D. VACQUIER. Monoclonal antibody to a membrane glycoprotein inhibits the acrosome reaction and associated Ca^{2+} and H^+ fluxes of sea urchin sperm. *Cell* 40: 697–703, 1985.
288. TRIMMER, J. S., AND V. D. VACQUIER. Activation of sea urchin gametes. *Annu. Rev. Cell Biol.* 2: 1–26, 1986.
289. TUBB, D. J., G. S. KOPF, AND D. L. GARBERS. Starfish and horseshoe crab egg factors cause elevations of cyclic nucleotide concentrations in spermatozoa from starfish and horseshoe crabs. *J. Reprod. Fertil.* 56: 539–542, 1979.
290. TURNER, K. O., AND S. MEIZEL. Progesterone-mediated efflux of cytosolic chloride during the human sperm acrosome reaction. *Biochem. Biophys. Res. Commun.* 213: 774–780, 1995.
291. UGUZ, C., W. L. VREDENBURGH, AND J. J. PARRISH. Heparin-induced capacitation but not intracellular alkalinization of bovine sperm is inhibited by RP-adenosine-3′,5′-cyclic monophosphorothioate. *Biol. Reprod.* 51: 1031–1039, 1994.
292. USHIYAMA, A., T. ARAKI, K. CHIBA, AND M. HOSHI. Specific binding of acrosome-reaction-inducing substance to the head of starfish spermatozoa. *Zygote* 1: 121–127, 1993.
293. VACQUIER, V. D., AND G. W. MOY. Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. *Proc. Natl. Acad. Sci. USA* 74: 2456–2460, 1977.
294. VACQUIER, V. D., AND G. W. MOY. The fucose sulfate polymer of egg jelly binds to sperm REJ and is the inducer of the sea urchin sperm acrosome reaction. *Dev. Biol.* 192: 125–135, 1997.
295. VACQUIER, V. D., G. W. MOY, J. S. TRIMMER, Y. EBINA, AND D. C. PORTER. Monoclonal antibodies to a membrane glycoprotein induces the phosphorylation of histone H1 in sea urchin spermatozoa. *J. Cell Biol.* 107: 2021–2027, 1988.
296. VANDERHAEGHEN, P., S. SCHURMANS, G. VASSART, AND M. PARMENTIER. Olfactory receptors are displayed on dog mature sperm cells. *J. Cell Biol.* 123: 1441–1452, 1993.
297. VILLANUEVA-DÍAZ, C., J. ARIAS-MARTÍNEZ, L. BERMEJO-MARTÍNEZ, AND F. VADILLO-ORTEGA. Progesterone induces human sperm chemotaxis. *Fertil. Steril.* 64: 1183–1188, 1995.
298. VILLANUEVA-DÍAZ, C., F. VADILLO-ORTEGA, A. KABLY-AMBE, M. DÍAZ-PÉREZ, AND S. K. KRIVITZKY. Evidence that human follicular fluid contains a chemoattractant for spermatozoa. *Fertil. Steril.* 54: 1180–1182, 1990.
299. VISCONTI, P. E., J. L. BAILEY, G. D. MOORE, D. PAN, P. OLDS-CLARKE, AND G. S. KOPF. Capacitation of mouse spermatozoa. I. Correlation between the capacitation state and protein tyrosine phosphorylation. *Development* 121: 1129–1137, 1995.
300. VISCONTI, P. E., AND G. S. KOPF. Regulation of protein phosphorylation during sperm capacitation. *Biol. Reprod.* 59: 1–6, 1998.
301. VISCONTI, P. E., G. D. MOORE, J. L. BAILEY, P. LECLER, S. A. CONNORS, D. PAN, P. OLDS-CLARKEC, AND G. S. KOPF. Capacitation of mouse spermatozoa. II. Protein tyrosine phosphorylation and capacitation are regulated by a cAMP-dependent pathway. *Development* 121: 1139–1150, 1995.
302. VREDENBURGH-WILBERG, W. L., AND J. PARRISH. Intracellular pH of bovine sperm increases during capactitation. *Mol. Reprod. Dev.* 40: 490–502, 1995.
303. WALENSKY, L. D., AND S. H. SNYDER. Inositol 1,4,5-trisphosphate receptors selectively localized to the acrosomes of mammalian sperm. *J. Cell Biol.* 130: 857–869, 1995.
304. WARD, C. R., B. T. STOREY, AND G. S. KOPF. Selective activation of G_{i1} and G_{i2} in mouse sperm by the zona pellucida, the egg's extracellular matrix. *J. Biol. Chem.* 269: 13254–13258, 1994.
305. WARD, G. E., C. J. BROKAW, D. L. GARBERS, AND V. D. VACQUIER. Chemotaxis of *Arbacia punctulata* spermatozoa to resact, a peptide from the egg jelly layer. *J. Cell Biol.* 101: 2324–2329, 1985.
306. WARD, G. E., D. L. GARBERS, AND V. D. VACQUIER. Effects of extracellular egg factors on sperm guanylate cyclase. *Science* 227: 768–770, 1985.
307. WARD, G. E., G. W. MOY, AND V. D. VACQUIER. Phosphorylation of membrane-bound guanylate cyclase of sea urchin spermatozoa. *J. Cell Biol.* 103: 95–101, 1986.
308. WARD, G. E., AND V. D. VACQUIER. Dephosphorylation of a major sperm membrane protein is induced by egg jelly during sea urchin fertilization. *Proc. Natl. Acad. Sci. USA* 30: 5578–5582, 1983.
309. WARD, G. R., AND G. KOPF. Molecular events mediating sperm activation. *Dev. Biol.* 158: 9–34, 1993.
310. WASSARMAN, P. M., AND E. S. LITSCHER. Sperm-egg recognition mechanisms in mammals. *Curr. Top. Dev. Biol.* 30: 1–19, 1995.
311. WATKINS, H. D., G. S. KOPF, AND D. L. GARBERS. Activation of sperm adenylate cyclase by factors associated with eggs. *Biol. Reprod.* 19: 890–894, 1978.
312. WEYAND, I., M. GODDE, S. FRINGS, J. WELNER, F. MULLER, W. ALTENHOFEN, H. HATT, AND B. KAUPP. Cloning and functional expression of a cyclic-nucleotide-gated channel from mammalian sperm. *Nature* 368: 859–863, 1994.

313. WHITE, D. R., AND R. J. AITKEN. Relationship between calcium, cyclic AMP, ATP and intracellular pH and the capacity of hamster spermatozoa to express hyperactivated motility. *Gamete Res.* 22: 163–177, 1989.
314. WHITE, M., AND M. AYLWIN. Niflumic and flufenamic acids are potent reversible blockers of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. *Mol. Pharmacol.* 37: 720–724, 1990.
315. WIESNER, B., J. WEINER, R. MIDDENDORFF, V. HAGEN, U. B. KAUPP, AND I. WEYAND. Cyclic nucleotide-gated channels on the flagellum control Ca^{2+} entry into sperm. *J. Cell Biol.* 142: 473–484, 1998.
316. WISSENBACH, U., G. SCHROTH, S. PHILIPP, AND V. FLOCKERZI. Structure and mRNA expression of a bovine trp homologue related to mammalian trp2 transcripts. *FEBS Lett.* 429: 61–66, 1998.
317. WISTROM, C. A., AND S. MEIZEL. Evidence suggesting involvement of a unique human steroid receptor/Cl^- channel complex in the progesterone-initiated acrosome reaction. *Dev. Biol.* 159: 679–690, 1993.
318. YAMAGUCHI, M., T. NIWA, M. KURITA, AND N. SUZUKI. The participation of speract in the acrosome reaction of *Hemicentrotus pulcherrimus*. *Dev. Growth Differ.* 30: 159–167, 1987.
319. YANAGIMACHI, R. Mammalian fertilization. In: *The Physiology of Reproduction*, edited by E. Knobil and J. D. Neil. New York: Raven, 1994, p. 189–317.
320. YODOZAWA, S., T. SPEAKE, AND A. ELLIOTT. Intracellular alkalinization mobilizes calcium from agonist-sensitive pools in rat lachrymal acinar cells. *J. Physiol (Lond.)* 499: 601–611, 1997.
321. YOSHIDA, M., K. INABA, K. ISHIDA, AND M. MORISAWA. Calcium and cyclic AMP mediate sperm activation, but Ca^{2+} alone contributes sperm chemotaxis in ascidian, *Ciona savignyi*. *Dev. Growth Differ.* 36: 589–595, 1994.
322. YOSHIDA, M., K. INABA, AND M. MORISAWA. Sperm chemotaxis during the process of fertilization in the ascidians *Ciona savignyi* and *Ciona intestinalis*. *Dev. Biol.* 157: 497–506, 1993.
323. YOSHINO, K., AND N. SUZUKI. Two classes of receptor specific for sperm-activating peptide III in sand-dollar spermatozoa. *Eur. J. Biochem.* 206: 887–893, 1992.
324. YOUNG, G. P., S. S. KOIDE, M. GOLDSTEIN, AND J. D. E. YOUNG. Isolation and partial purification of an anion channel protein from human sperm membranes. *Arch. Biochem. Biophys.* 262: 193–225, 1988.
325. ZAPATA, O., J. RALSTON, C. BELTRÁN, J. B. PARYS, J. L. CHEN, F. J. LONGO, AND A. DARSZON. IP_3 receptors in sea urchin sperm. *Zygote* 5: 355–364, 1998.
326. ZENG, Y., E. N. CLARK, AND H. M. FLORMAN. Sperm membrane potential: hyperpolarization during capacitation regulates zona pellucida-dependent acrosomal secretion. *Dev. Biol.* 171: 554–563, 1995.
327. ZENG, Y., J. A. OBERDORF, AND H. M. FLORMAN. pH regulation in mouse sperm: identification of Na^+-, Cl^--, and HCO_3^--dependent and arylaminobenzoate-dependent regulatory mechanisms and characterization of their roles in sperm capacitation. *Dev. Biol.* 173: 510–520, 1996.