Regulating the acrosome reaction

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ABSTRACT The acrosome reaction is a secretory event that must be completed by the sperm of many animal species prior to fusion with eggs. In mammals, exocytosis is triggered by ZP3, a glycoprotein component of the egg pellucida, following gamete contact. ZP3 promotes a sustained influx of Ca\(^{2+}\) into sperm that is necessary for the acrosome reaction. Here, we discuss the mechanism by which ZP3 generates Ca\(^{2+}\) entry, as well as the upstream events leading to this influx and downstream processes that couple it with exocytosis.

KEY WORDS: acrosome reaction, signal transduction calcium channel, TRPC channel, PI3, kinase

Introduction

The sperm of many animals, including those of all mammals, contain a single secretory vesicle, or acrosome, in the anterior portion of the head. The contents of this vesicle are released during the early stages of sperm-egg interaction through an exocytotic process known as the acrosome reaction.

Exocytosis represents a key control point in the process of fertilization. Sperm must complete the acrosome reaction in order both to penetrate the egg zona pellucida and to fuse with the egg plasma membrane. However, if exocytosis is completed prematurely then sperm cannot enter the cumulus oophorus surrounding the egg (Cummins and Yanagimachi, 1986). Thus, the acrosome reaction functions as a behavioral switch, converting sperm into a state in which they are competent to interact with eggs. Moreover, it is an irreversible step and thus differs from other modes of regulation that operate within the female reproductive tract. Thus, the initiation of acrosome reactions must be strictly regulated and coordinated spatially and temporally with the availability of eggs.

This discussion will consider the control of the acrosome reaction by the egg zona pellucida, and will retain a narrow focus on the role of sperm intracellular Ca\(^{2+}\) (Ca\(^{2+}\)_i) activity in this process, on the mechanism by which egg-derived agonists regulate that cation, and on the downstream targets of agonist-induced Ca\(^{2+}\) entry. As such, this line of investigation follows from suggestions and thoughts in an influential and still relevant review by Garbers and Kopf (Garbers and Kopf, 1980). The signal transduction mechanisms that link zona pellucida stimulation to sperm exocytosis are complex, involving pathways in addition to Ca\(^{2+}\). Readers interested in more general information concerning the mammalian acrosome reaction or on events in non-mammalian species may wish to consult other sources (Yanagimachi, 1994; Florman and Ducibella, 2006; Darszon et al., 2006; Roldan and Shi, 2007).

Capacitation as a regulator of the acrosome reaction

Mammalian sperm are released by the male in a functionally restricted state and must complete a process of priming, or capacitation, within the female reproductive tract before acquiring the ability to fertilize eggs. Capacitation is associated with complex changes in the biochemical, physiological and cellular properties of the sperm. As a result of this process, sperm develop novel patterns of motility, including hyperactivation and oriented motility towards chemoattractants from the female reproductive tract, and also acquire the ability to undergo a zona pellucida-evoked acrosome reaction. A number of changes in the intracellular ionic environment of sperm occur during capacitation. These and other events associated with capacitation have been extensively reviewed recently (Florman and Ducibella, 2006; Eisenbach and Giojalas, 2006; Gadella and Visconti, 2006) and the present discussion of capacitation is not intended to be comprehensive. Rather, it will focus on alterations in sperm membrane potential that may regulate the ability of sperm to undergo acrosome reactions.

Sperm membrane potential hyperpolarizes during capacitation. Fluorescent probes report population-averaged values that are in the range of -25 to -40 mV for uncapacitated cells and that rise to ~-60 mV following incubation under capacitating conditions (Zeng et al., 1995; Arnoult et al., 1999; Munoz-Garay et al., 2001).

Abbreviations used in this paper: CNG, cyclic nucleotide gated channel; TRPC, transient receptor potential channel; ZP zona pellucida.
Single cell measurements revealed that sperm sort into two subpopulations during incubation: one group exhibits weak hyperpolarization to -45 mV and a second group shifts to ~30 mV. Only those sperm in the strongly hyperpolarizing group (~80 mV) were capacitated, as assessed both by chlortetracycline fluorescence and by the ability to undergo a zona pellucida-induced acrosome reaction (Arnoult et al., 1999). This hyperpolarization is associated with an enhanced contribution of $K^+$ permeability to membrane potential (Zeng et al., 1995), which may result from an increased $K^+$ channel activity or the closing of other conductance pathways. In this regard, a number of $K^+$ channels are present in sperm, although specific channels have not yet been linked to hyperpolarization (Darszon et al., 2006; Navarro et al., 2007). There is, additionally, evidence for a decreased $Na^+$ conductance that may contribute to hyperpolarization. Finally, ion transport pathways such as the Na$^+$/HCO$_3^-$ cotransporter and the cystic fibrosis transmembrane regulator participate in the regulation of membrane potential during capacitation (Demarco et al., 2003; Xu et al., 2007; Hernandez-Gonzalez et al., 2007).

It has been suggested that hyperpolarization regulates the ability of sperm to undergo zona pellucida-evoked acrosome reactions (Arnoult et al., 1999). Zonae pellucidae trigger a transient Ca$^{2+}$ entry through low voltage-activated channels (see below). These channels exhibit voltage-dependent inactivation and, at the prevailing membrane potential of uncapacitated sperm, dwell in an inactivated state from which openings are rare. Hyperpolarization to ~80 mV, such as occurs during capacitation, shifts those channels into a closed state, from which opening and current flow can occur following a depolarization (Perez-Reyes, 2003). According to this suggestion, the relatively depolarized membrane potential of uncapacitated sperm prevents premature acrosome reactions by holding low voltage-activated channels in a reluctant state, while hyperpolarization primes sperm, permitting a response respond to depolarizing signals by acrosome reacting (Arnoult et al., 1999).

**Acrosome reactions**

**Triggers**

This discussion focuses on the activation of sperm following contact with the zona pellucida or interaction with soluble extracts of the zona pellucida. The evidence for an acrosome reaction-inducing agonist activity in the zona pellucida has been reviewed frequently (Wassarman et al., 2001; Florman and Duchibella, 2006) and can be summarized as follows. Most of the sperm in the ampulla of the oviduct that are not associated with the egg/cumulus complex have intact acrosomes (Bryan, 1974; Cummins and Yanagimachi, 1982; Suarez et al., 1983) while sperm observed moving through the cumulus matrix either have intact acrosomes or may show initial signs of exocytosis (Cummins and Yanagimachi, 1982; Yanagimachi, 1994). In addition, sperm appear to make initial contact with the zona pellucida in vivo prior to initiation of acrosome reactions (Crozet, 1984; Crozet and Dumont, 1984) and can initiate and complete the acrosome reaction following zona pellucida contact in vitro (Gwatkin, 1976; Gwatkin et al., 1976; Saling et al., 1979; Saling and Storey, 1979). These and other observations suggest that the zona pellucida is the site of the acrosome reaction during the fertilization process.

Agonist activity was demonstrated in soluble extracts of the zona pellucida (Florman et al., 1982; Bleil and Wassarman, 1983). Mouse ZP3 retained agonist activity following purification whereas the other zona pellucida proteins, ZP1 and ZP2, did not (Bleil and Wassarman, 1983). Other observations that support the assignment of agonist activity to ZP3 include its presence at the site of fertilization, the direct binding of this protein to sperm (Thaler and Cardullo, 1996) and the loss of agonist activity following fertilization (Bleil and Wassarman, 1983). In addition, the effects of pharmacological inhibitors on the acrosome reaction induced by purified ZP3 and by soluble extracts of the zona pellucida recapitulate the response of sperm undergoing the acrosome reaction on structurally intact zonae pellucidae (Arnoult et al., 1999), suggesting that the same signal transduction mechanisms are activated in all cases. It should be noted that zonae pellucidae of many mammalian species are constructed from four proteins, however in mouse ZP4 has become a pseudogene (Conner et al., 2005). The question of whether ZP3 also functions independently as the agonist in zonae pellucidae containing all four components is, at present, unresolved.

A number of pharmacological agents and biological fluids will also induce acrosome reactions, yet are of uncertain biological significance. One informative example is that of progesterone, which is released by cumulus cells and so may be encountered by sperm in the vicinity of eggs at the time of fertilization. High concentrations (micromolar) of progesterone promote acrosome reactions (Osman et al., 1989) and potentiate the agonist effects of zona pellucida in vitro (Roldan et al., 1994). Yet, at lower concentrations (picomolar to nanomolar), or when presented as a concentration gradient to simulate sperm approach to the egg, the effects of progesterone are on the modulation of flagellar motility and chemotaxis rather than upon acrosome reactions (Harper et al., 2004; Harper and Publicover, 2005; Teves et al., 2006). The question of a physiological role of progesterone in control of acrosome reactions remains unresolved. Similarly, agonist activity is present in follicular fluid and released with eggs during ovulation, but it is uncertain how such factors would be captured by the oviduct following ovulation or be retained in the vicinity of the egg within the oviduct. The remainder of this discussion will focus on the mechanism of action of ZP3.

**From ZP3 stimulation to a Ca$^{2+}$ response**

Sperm interaction with the zona pellucida triggers a number of initial responses which are required in order to produce the sustained Ca$^{2+}$ influx that leads to the acrosome reaction (see below). An elevation of intracellular pH$_i$ is reported using fluorescent indicator dyes (Florman et al., 1989; Rockwell and Storey, 2000) and may be driven by a G protein-dependent pathway (Arnoult et al., 1996b). The heterotrimeric GTP-binding proteins, G$_{i1}$ and G$_{i2}$, are activated in sperm by the zona pellucida (Endo et al., 1988; Ward et al., 1994a; Ward et al., 1994b) and the zona pellucida-dependent intracellular alkalization does not occur when G protein stimulation is inhibited by treatment with pertussis toxin (Florman et al., 1989; Arnoult et al., 1996b). Transient alkalization, by treatment with a permeant weak base, bypasses the pertussis toxin block in ZP3 signaling and allows both Ca$^{2+}$ influx and acrosome reactions (Arnoult et al., 1996b). This suggests elevation of pH$_i$ is the key step in signal transduction that is blocked by inhibition of ZP3-activated G proteins in sperm. A specific role of pH$_i$ in ZP3 action has not been assigned, but may,
speculatively, include an enhancement of Ca\(^{2+}\) signals (Babcock and Pfeiffer, 1987; Arnoult \textit{et al.}, 1996b).

Zonae pellucidae also activate sperm phospholipase C (PLC) (Roldan \textit{et al.}, 1994; Roldan and Shi, 2007). Following targeted deletions of the \textit{plcd4} gene in mice, sperm cannot generate a sustained Ca\(^{2+}\) influx or acrosome reaction in response to zona pellucida stimulation (Fukami \textit{et al.}, 2001; Fukami \textit{et al.}, 2003). Other PLC isozymes are also present in the sperm head (Walensky and Snyder, 1995) and may contribute to ZP3 responses. PLC hydrolyzes phosphatidylinositol-1,4,5-bisphosphate (PIP2) to produce two products: inositol-1,4,5-trisphosphate (IP3), which gates IP3 receptors present in the acrosomal membrane (Walensky and Snyder, 1995); and diacylglycerol. Plcd4 may be regulated by Ca\(^{2+}\), by PIP2 and by a high molecular weight G protein, while other isozymes are controlled by G proteins (Rebecchi and Pentylala, 2000; Rhee, 2001; Fukami, 2002), but the mechanism by which ZP3 regulates PLC activity has not been defined.

Finally, zonae pellucidae trigger a transient elevation of Ca\(^{2+}\). Ca\(^{2+}\) increases from resting levels in the nanomolar range to micromolar values within 50 msec and then, in the continuous presence of ZP3, relaxes to basal levels during the next 200 msec (Arnoult \textit{et al.}, 1999). This activation time course and effects of pharmacological antagonists suggest that this transient Ca\(^{2+}\) entry is carried by a low voltage-activated Ca\(^{2+}\) channel, most likely a member of the Ca\(_{v}3\) family (Arnoult \textit{et al.}, 1996a; Arnoult \textit{et al.}, 1999). All three members of the Ca\(_{v}3\) family are present in sperm, with Ca\(_{v}3.1\) and 3.2 detected in the sperm head and so plausibly localized for a role in the acrosome reaction (Trevino \textit{et al.}, 2004; Jimenez-Gonzalez \textit{et al.}, 2005; Florman and Ducibella, 2006; Publicover \textit{et al.}, 2007; Escoffier \textit{et al.}, 2007). However, the disruption of the genes that encode either Ca\(_{v}3.1\) or 3.2 channels does not affect fertility and the concerted disruption of several Ca\(_{v}\) genes has not been reported (Chen \textit{et al.}, 2003; Stamboulian \textit{et al.}, 2004; Escoffier \textit{et al.}, 2007). Thus, the identity of this ZP3-activated channel is not yet resolved.

Also unresolved is the mechanism by which ZP3 activates low voltage activated currents. Voltage-dependent activation of these channels has been reviewed extensively (Perez-Reyes, 2003). ZP3 produces a membrane potential depolarization that is sufficiently strong to activate T channels, but this response is slow and might be expected to trigger voltage-dependent inactivation before there is significant current flow (Arnoult \textit{et al.}, 1996b). Alternative mechanisms of Ca\(_{v}\) channel activation have been described (Perez-Reyes, 2003), and these channels in sperm may also be regulated by either tyrosine phosphorylation (Arnoult \textit{et al.}, 1997) or by calmodulin (Lopez-Gonzalez \textit{et al.}, 2001).

These upstream elements of ZP3 signal transduction cannot complement each other and appear to function as parallel, essential processes rather than in series. As a result, it has been suggested that these early steps are organized as a bifurcated pathway, with separate limbs consisting of transient Ca\(^{2+}\) entry and of G protein and pH responses. Both limbs must operate for effective transmission. These signals are integrated at the level of the sustained Ca\(^{2+}\) entry (Arnoult \textit{et al.}, 1996b; Florman and Ducibella, 2006).

\textbf{Sculpting a sustained Ca\(^{2+}\) elevation}

A role of Ca\(^{2+}\) in the zona pellucida-evoked acrosome was anticipated based on observations that the frequency of agonist-independent acrosome reactions (the so-called “spontaneous” acrosome reaction) was strictly dependent on the presence of extracellular Ca\(^{2+}\) (Yanagimachi and Usui, 1974) and the understanding of the general role of that cation in exocytosis (Katz and Miledi, 1967; Douglas, 1968; Neher \textit{et al.}, 1987). Although it was understood by the early 1980s that an acrosome reaction-inducing agonist activity was present in the zona pellucida (Florman and Storey, 1982) and that ZP3 accounted for this activity (Beil and Wassarman, 1983), the heterogeneity of sperm populations and other technical difficulties (reviewed by Florman and Ducibella, 2006; Gadella and Visconti, 2006) prevented direct examination of this question until the introduction, in the mid-1980s, of ion-selective indicator dyes that could be loaded into cells passively (Gryniewicz \textit{et al.}, 1985) and of image analysis techniques that permitted events in single cells to be resolved.

In considering Ca\(^{2+}\) triggering of acrosome reactions, it should be recalled that Ca\(^{2+}\) also controls other behavioral responses in sperm, including capacitation (Fraser \textit{et al.}, 1995; Visconti \textit{et al.}, 1995), hyperactivated flagellar motility (Kamiya and Witman, 1984; Suarez \textit{et al.}, 1993; Suarez and Ho, 2003; Marquez and Suarez, 2004) and, possibly, chemotaxis (Spehr \textit{et al.}, 2003; Fukuda \textit{et al.}, 2004). It is necessary to regulate these cellular functions separately and several mechanisms are expected to contribute to that signaling isolation. First, sperm have a number of Ca\(^{2+}\)-conducting channels and transport proteins. These include both high- and, as discussed previously, low-voltage activated channels (Ca\(_{v}1\)-3), members of the TRPC family of Ca\(^{2+}\)-conducting cation channels, cyclic nucleotide-gated (CNG) channels, and the Catsper channels in the plasma membrane, as well as IP3 receptors and, possibly, ryanodine receptors on intracellular membranes (reviewed by Felix, 2005; Jimenez-Gonzalez \textit{et al.}, 2006; Publicover \textit{et al.}, 2007). Differences in the mechanisms of activation and inactivation of these channels provide a means of selective activation. Second, the unique biophysical properties of various channels, taken together with their spatial distribution in sperm (Walensky and Snyder, 1995; Westenbrook and Babcock, 1999; Wennemuth \textit{et al.}, 2000; Ren \textit{et al.}, 2001; Quill \textit{et al.}, 2001; Florman and Ducibella, 2006), facilitates the coupling of specific influx pathways with cellular responses. Finally, behavioral responses are encoded in the spatiotemporal pattern of ionic responses, thereby permitting individual processes to be driven separately. Ca\(^{2+}\) oscillations drive flagellar bending patterns while capacitation is associated with linear, low-slope increases (Florman and Ducibella, 2006; Publicover \textit{et al.}, 2007). In contrast, the signature response of to stimulation with ZP3 or with unfractonated extracts of the zona pellucida is a sustained elevation of Ca\(^{2+}\), as shown in studies in bovine (Florman \textit{et al.}, 1989), mouse (Arnoult \textit{et al.}, 1996a; Fukami \textit{et al.}, 2003), and hamster models (Shirakawa and Miyazaki, 1999). Fluorescent indicators report that Ca\(^{2+}\) levels in capacitated sperm rise from basal levels of 100-150 nM to a peak of 400-600 nM, which is maintained or relaxes slowly in the continuous presence of agonist (Florman \textit{et al.}, 1989; Florman, 1994; O’Toole \textit{et al.}, 2000; Fukami \textit{et al.}, 2003; Florman and Ducibella, 2006). This sustained rise precedes exocytosis and agents that block this Ca\(^{2+}\) response also inhibit the acrosome reaction (reviewed by Florman and Ducibella, 2006).

It was initially suggested, based on inhibitor studies, that sustained entry of Ca\(^{2+}\) into sperm during ZP3 stimulation was
due to the activation of an L-type (Ca\textsubscript{1,1}) high voltage-activated Ca\textsuperscript{2+} channel (Florman et al., 1992). However, it was not understood at that time that sperm also had low voltage-activated, Ca\textsubscript{3} channels; that transient Ca\textsuperscript{2+} entry through those Ca\textsubscript{3} channels was an essential pre-requisite for the sustained phase of response, such that inhibitors of Ca\textsubscript{3} block downstream processes, including the sustained Ca\textsuperscript{2+} rise (reviewed by Florman and Ducibella, 2006), or that there was considerable overlap in the pharmacology of Ca\textsubscript{1} and Ca\textsubscript{3} channel classes (Perez-Reyes, 2003). In addition, it was shown that the sustained influx pathway had a pharmacological profile that differed from that of Ca\textsubscript{3} channel (O’Toole et al., 2000). Thus, neither Ca\textsubscript{1} or Ca\textsubscript{3} channels account for the sustained phase of influx.

More recently, attention has focused on the role of TRPC (canonical transient receptor potential) channels in ZP3 signaling. The seven members of the TRPC family form Ca\textsuperscript{2+}-conducting channels that are widely expressed in animal cells and contribute to PLC-dependent Ca\textsuperscript{2+} entry (Montell, 2005; Putney, 2007). Several TRPC channels are expressed in sperm, with TRPC1, C2 and C5 are located in the anterior head where sperm interact with the zona pellucida and where acrosome reaction signaling occurs (Trevino et al., 2001; Jungnickel et al., 2001; Castellano et al., 2003; Sutton et al., 2004; Stamboulian et al., 2005). A substantial fraction of the ZP3-evoked Ca\textsuperscript{2+} influx into mouse sperm is conducted by TRPC2, as a function-blocking antibody against the second extracellular loop of that channel inhibits both 80-85% of the sustained Ca\textsuperscript{2+} response and also blocks the acrosome reaction (Jungnickel et al., 2001). ZP3 may activate TRPC2 through a store-operated mechanism (O’Toole et al., 2000; Jungnickel et al., 2001), although interaction with IP3 receptors on the adjacent acrosomal membrane is also plausible (Kiselyov et al., 1998). In addition, a component of the ZP3-induced Ca\textsuperscript{2+} entry cannot be inhibited by the TRPC2 function-blocking antibody (Jungnickel et al., 2001), suggesting that other channels may also contribute to this influx. This is also consistent with the fertility of mice following the disruption of the trpc2\textsuperscript{gene}, and of humans, where TRPC2 is a pseudogene (Stowers et al., 2002; Leypold et al., 2002; Montell, 2005). It is tempting to speculate that other channels in the anterior head, including other TRPC channels (see above) as well as additional channel families (reviewed by Florman and Ducibella, 2006), account for this Ca\textsuperscript{2+} entry and fertility when TRPC2 is absent.

**From Ca\textsuperscript{2+} entry to exocytosis**

An acute rise in Ca\textsuperscript{2+} triggers the release of the acrosomal granule, pointing to a role of Ca\textsuperscript{2+}-binding proteins in the downstream events the follow Ca\textsuperscript{2+} influx. Such downstream targets are expected to be localized within the spatial domains of that Ca\textsuperscript{2+} elevation. The recognition that TRPC channels mediate a major component of the sustained Ca\textsuperscript{2+} entry that is triggered by ZP3 resulted in an effort to identify Ca\textsuperscript{2+} binding proteins associated with these channels.

This lead to the identification of enkurin, a multidomain protein containing a proline-rich N-terminal region with predicted SH3 and WW ligand motifs and a C-terminal domain that binds TRPC2. It also binds TRPC1 and C5 (but not TRPC3), although these interaction sites have not yet been mapped (Sutton et al., 2004). This binding specificity corresponds to the functional and sequence classes of TRPC channels—(Nilius et al., 2007), suggesting that enkurin binds the TRPC1/4/5 and TRPC2 classes, but not the TRPC3/6/7 class. Enkurin also contains an IQ motif that binds Ca\textsuperscript{2+}-calmodulin, but not apocalmodulin, and therefore associates a Ca\textsuperscript{2+} sensor with the inner face of a Ca\textsuperscript{2+}-conducting ion channel (Sutton et al., 2004). Enkurin has the anticipated sequence characteristics of an adapter protein that binds SH3 and WW domain cargo to ZP3-activated TRPC channels, thereby assembling a signaling module at the site of ZP3 action.

One such cargo protein appears to be 1-phosphatidylinositol-3-kinase (PI3K). The SH3 domain of p85, the regulatory subunit of PI3K, binds to the sequence PKKPAVP (residues 83-89 of mouse enkurin) (Sutton et al., 2004). A role of PI3K in ZP3 signal transduction was unanticipated, yet several lines of evidence indicate that phosphatidylinositol signaling pathways are activated during the initiation of the acrosome reaction (Fig. 1; Jungnickel et al., 2007).

An increase in the levels of phosphatidylinositol-3,4,5-triphosphate (PIP3), one product of PI3K catalytic activity, can be detected within 30 sec of ZP3 treatment, whereas the production of other PI3K products, such as phosphatidylinositol-3-phosphate, could not be detected. The mechanism of PI3 kinase activation is unresolved. It is tempting to speculate that Ca\textsuperscript{2+} entry through TRPC channels permits enkurin/Ca\textsuperscript{2+}-calmodulin to associate with activated

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**Fig. 1. Model of the late events of the mouse sperm acrosome reaction.** Enkurin is a scaffold protein that binds PI3 kinase to sperm transient receptor potential channels (TRPC). Sperm contact with the zona pellucida results in ZP3 activation of TRPC channels, leading to both Ca\textsuperscript{2+} entry and to activation of PI3 kinase. The resulting D3-phosphorylation of (PIP2) (phosphatidylinositol-4,5-bisphosphate) leads to the local accumulation of PIP3 (phosphatidylinositol-3,4,5-triphosphate). PIP3 provides docking sites for proteins with appropriate lipid binding domains and results in the activation of the serine/theonine protein kinases, Akt (Protein Kinase B) and PKC\textsubscript{\gamma}. These protein kinases mediate the downstream stages of sperm exocytosis. See text for details.
channels, and thereby translocates PI3 kinase to the plasma membrane.
- Both the ZP3-dependent accumulation of PIP3 and the induction of acrosome reactions were inhibited by two different PI3K antagonists, wortmannin and LY294002.
- PI3K subunits and PTEN, the phosphoinositide D3-phosphatase, are both present in the acrosomal crescent of mouse sperm, thus co-localizing with both TRPC2 (and also with TRPC1 and C5) and with enkurein.
- Inhibitors of phosphoinositide D3-phosphatases, when used in the absence of ZP3, lead to PIP3 accumulation and to acrosome reactions, with both effects blocked by PI3K antagonists. Yet, the effects of phosphatase inhibitors and zona pellucida agonists on acrosome reactions were not additive, pointing to a common mechanism (Jungnickel et al., 2007).

Two inferences can be drawn from these observations. First, the effects of phosphatase inhibitors point to the constitutive operation of a phosphoinositide phosphorylation/dephosphorylation cycle in capacitated sperm. Cycling provides a means for rapid, finely-controlled enzymatic response (Newsholme and Start, 1973). Second, elevations of PIP3 are necessary and sufficient for the induction of the acrosome reaction.

PI3P is typically a trace component of the membranes of unstimulated cells and is produced locally at the site of PI3 kinase activation. Proteins containing specific PI3P binding domains, including a PH or PX domain, are then recruited to the membrane to permit the local assembly of a number of signal transduction modules (Cantley, 2002; Newton, 2003). In this regard, two protein kinases, Akt (protein kinase B, PKB) and the atypical protein kinase C, PKCζ, that are effectors of PI3P (Cantley, 2002; Newton, 2003; Fayard et al., 2005) have been shown to act downstream of PI3P during ZP3 signaling in sperm (Jungnickel et al., 2007). These Ser/Thr kinases are located in the acrosomal crescent region of the sperm head and inhibitors block the ZP3-induced acrosome reaction. Additionally, in the case of PKB/Akt there is direct evidence for activation by the zona pellucida (Jungnickel et al., 2007). A major activation mechanism for PKB/Akt is through the sequential phosphorylation of Thr-308 and of Ser-473, with PDK1 (the phosphoinositide-dependent protein kinase-1) acting as the Thr-308 kinase (Chan et al., 1999). Using phosphorylation state-specific antibodies, it was found that soluble extracts of the zona pellucida produced a 2-fold increase in Thr-308 phosphorylation in sperm and that this effect was blocked by the PI3 kinase inhibitor, wortmannin (Jungnickel et al., 2007).

A plausible activation mechanism then is the stimulation of PI3 kinase by the zona pellucida leads to local accumulation of PI3P, and then to the recruitment of PKD1 to that site as a result of the association of its PH domain with PI3P (Alessi and Cohen, 1998; Lizcano and Alessi, 2002). Localization at the membrane permits PKD1 access to substrate proteins. Among such substrates are the AGC superfamily of protein kinases, including both PKB/Akt and PKCζ (Newton, 2003). Thus, a common mechanism can account for the activation of both of these protein kinases by ZP3. In this regard, PKD1 is present in sperm, in both the acrosomal crescent and in the flagellum (Jungnickel et al., 2007).

PI3 kinase pathways, including PKB/Akt and PKCζ, have previously been linked to exocytosis in somatic systems (Fig. 1), either through the control of vesicle trafficking or by interaction with SNARE proteins and other elements of the secretory machinery (Cousin et al., 2003; Czech, 2003; Viard et al., 2004; Bezzerides et al., 2004; Bose et al., 2004; Hodgkinson et al., 2005a; Hodgkinson et al., 2005b; Evans et al., 2006). One focus of future work will be the identification of kinase substrates in sperm, and their role in the evoked acrosome reaction.

Closing comments

Understanding of the mechanisms by which the zona pellucida regulates the acrosome reaction is central to models of fertilization, and also shapes developing strategies for the control of fertility. Efforts were made in this narrative to outline our present knowledge of ZP3 signaling, specifically with regard to the regulation of Ca2⁺, and to downstream effectors of that ionic response. The stages of this signaling mechanism include: 1) early events, such as G protein activation, intracellular alkalinization, and transient Ca2⁺ entry; 2) these lead to a sustained influx of Ca2⁺, a major component of which is carried by TRPC channels in mouse sperm; and 3) downstream events, including the activation of phosphoinositide-dependent protein kinases. It is speculated that these downstream processes are linked to Ca2⁺ entry by enkurein, a TRPC and PI3 kinase-binding protein.

More generally, this discussion has focused on one aspect of the control of sperm behavior by the egg. As such, the pioneering work of David Garbers in this general area is still recognized and appreciated. His studies on chemosensory response mechanisms in echinoderm sperm and also on mammalian sperm function (Garbers and Kopf, 1980; Garbers, 1989) have informed much of the thinking in these areas.

References


CANTLEY LC (2002). The phosphoinositide 3-kinase pathway. Science, 296: 1655-


JUNGNICKEL MK, SUTTON KA, WANG Y and FLORMAN HM (2007). Phosphoi-
nositide-dependent pathways in mouse sperm are regulated by egg ZP3 and drive the acrosome reaction. *Dev. Biol.*, 304: 116-126.


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M Hoshi, T Nishigaki, A Ushiyama, T Okinaga, K Chiba and M Matsumoto
*Int. J. Dev. Biol.* (1994) 38: 167-174

**Failure of differentiation of the nuclear-perinuclear skeletal complex in the round-headed human spermatozoa.**

D Escalier
*Int. J. Dev. Biol.* (1990) 34: 287-297