New Research Horizon review

Mathematical Modeling of Calcium Signaling in Sperm

Mathematical modeling of calcium signaling during sperm hyperactivation

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ABSTRACT

Mammalian sperm must hyperactivate in order to fertilize oocytes. Hyperactivation is characterized by highly asymmetrical flagellar bending. It serves to move sperm out of the oviductal reservoir and to penetrate viscoelastic fluids, such as the cumulus matrix. It is absolutely required for sperm penetration of the oocyte zona pellucida. In order for sperm to hyperactivate, cytoplasmic Ca\(^{2+}\) levels in the flagellum must increase. The major mechanism for providing Ca\(^{2+}\) to the flagellum, at least in mice, are CatSper channels in the plasma membrane of the principal piece of the flagellum, because sperm from CatSper null males are unable to hyperactivate. There is some evidence for the existence of other types of Ca\(^{2+}\) channels in sperm, but their roles in hyperactivation have not been clearly established. Another Ca\(^{2+}\) source for hyperactivation is the store in the redundant nuclear envelope of sperm. To stabilize levels of cytoplasmic Ca\(^{2+}\), sperm contain Ca\(^{2+}\) ATPase and exchangers. The interactions between channels, Ca\(^{2+}\)-ATPases, and exchangers are poorly understood; however, mathematical modeling can help to elucidate how they work together to produce the patterns of changes in Ca\(^{2+}\) levels that have been observed in sperm. Mathematical models can reveal interesting and unexpected relationships, suggesting experiments to be performed in the laboratory. Mathematical analysis of Ca\(^{2+}\) dynamics has been used to develop a model for Ca\(^{2+}\) clearance and for CatSper-mediated Ca\(^{2+}\) dynamics. Models may also be used to understand how Ca\(^{2+}\) patterns produce flagellar bending patterns of sperm in fluids of low and high viscosity and elasticity.

Keywords: Spermatozoa, sperm motility, mathematical model, CatSper, Ca\(^{2+}\) signaling,
Introduction: Definition of hyperactivation

Hyperactivation is a type of motility that is characterized by a highly asymmetrical and high amplitude flagellar beat pattern (Yanagimachi, 1994; Ishijima et al., 2006) which gives rise to a whip-like movement of the flagellum that can produce circular, figure eight, or zigzag swimming trajectories. Initiation and maintenance of hyperactivated motility is associated with an increase in Ca^{2+} concentration in the flagellum (Suarez et al., 1993; Ho and Suarez, 2001b; Ho et al., 2002; Carlson et al., 2005). Hyperactivation can be induced in sperm with the use of Ca^{2+} ionophores A23187 or ionomycin, which facilitate Ca^{2+} transport across the plasma membrane (Suarez et al., 1987; Suarez et al., 1992; Marquez and Suarez, 2007; Xia et al., 2007). Pharmacological agents such as caffeine (Ho and Suarez, 2001b), procaine (Marquez and Suarez, 2004), thimerosal (Ho and Suarez, 2001b; Marquez et al., 2007), and thapsigargin (Ho and Suarez, 2001b, 2003) have all been shown to induce asymmetrical beating resembling physiological hyperactivation by increasing intracellular Ca^{2+}, by allowing increased Ca^{2+} transport across membranes, or by causing release of Ca^{2+} from stores. Sperm that have been demembranated by detergent and reactivated by adding exogenous ATP in a special “intracellular” medium, display the symmetrical beat pattern characteristic of activated motility in approximately 50 nM Ca^{2+}, but switch to asymmetrical beating characteristic of hyperactivation when Ca^{2+} is increased to about 400 nM (Ho et al., 2002). Therefore, it is believed that Ca^{2+} is the primary second messenger that triggers hyperactivated motility.

In this review, we first discuss the movement of sperm through the female reproductive tract in order to introduce the roles played by hyperactivation in bringing
about fertilization. Next, we review mechanisms of modulating Ca$^{2+}$ levels in sperm and the molecular targets of Ca$^{2+}$. Finally, we will describe how mathematical modeling can help us to understand how Ca$^{2+}$ distributes in sperm to trigger and support hyperactivation.

Hyperactivation in the context of movement of sperm through the mammalian female reproductive tract

The propulsion of sperm via flagellar bending is vital for the sperm to move through the female reproductive tract. When sperm are moved out of the epididymis during ejaculation and come into contact with secretions of the male accessory glands, soluble adenylyl cyclase (SACY) is activated and raises the intracellular level of cAMP (Chen et al., 2000; Carlson et al., 2007). This stimulates a cascade of protein phosphorylation to activate sperm motility (Tash and Means, 1982, 1983; San Agustin and Witman, 1994). Activated motility is characterized by symmetrical and low amplitude flagellar beating that produces linear swimming trajectories (Katz and Yanagimachi, 1994; reviewed by Turner, 2003).

In humans, sperm are inseminated into the anterior vagina and quickly enter the cervix. There is evidence that sperm pass through the cervix by entering mucus filled microchannels in the periphery of the cervical canal, which lead them to the uterine cavity (Mattner, 1968; Mullins and Saacke, 1989; Suarez, 2010).

The uterus produces coordinated waves of myometrial smooth muscle contractions during the late follicular phase in humans and estrus in nonprimates, which may serve to wash sperm toward the uterotubal junction (reviewed in Suarez and Pacey, 2006).
In order to pass through the uterotubal junction into the oviduct, sperm must not only possess normal motility (Smith et al., 1988; Shalgi et al., 1992), but also specific surface proteins on their heads (Yanamaguchi et al., 2009). The role of these proteins in enabling sperm to swim through the junction is not understood, although it has been proposed that the proteins serve to adhere sperm lightly to the epithelial lining of the junction, to enable them to move against a gentle fluid outflow from the oviduct (Suarez, 2010).

Most sperm that enter the oviduct soon bind to the mucosal epithelium, which serves to hold them in a storage reservoir (DeMott and Suarez, 1992; Suarez and Pacey, 2006). Binding involves specific interactions between proteins on sperm and receptors on the oviductal epithelium. The interaction of sperm with the oviductal epithelium serves to maintain their ability to fertilize while they are in storage (Pollard et al. 1991; Kervancioglu et al., 1994; Lloyd et al. 2008).

As the time of ovulation approaches, sperm are gradually released from the reservoir. There is evidence that release is brought about by loss of the oviduct-binding proteins (Gwathmey et al., 2003, 2006; Ignotz et al., 2007), as well as hyperactivation of sperm motility (DeMott and Suarez, 1992; Ho et al., 2009). It is likely that the gradual loss of the binding proteins reduces the binding strength, enabling the deep flagellar bends produced by hyperactivated sperm to tear sperm off of the epithelium. Both hyperactivation and shedding of binding proteins have been associated with sperm capacitation, which is defined as a series of physiological and molecular changes that impart on the sperm the ability to fertilize the oocyte (Visconti, 2009).

When sperm move beyond the oviductal reservoir, they continue to use
hyperactivated motility. Within the oviduct, hyperactivated motility is observed in sperm near the site of fertilization (Katz and Yanagimachi, 1980; Suarez and Osman, 1987). Hyperactivation has been shown to enable sperm to swim effectively through highly viscoelastic fluid, such as oviductal mucus and the matrix of the cumulus oophorus (Suarez et al., 1991; Suarez and Dai, 1992). When sperm finally reach the oocyte within the cumulus oophorus, they require hyperactivation in order to penetrate its zona pellucida (Stauss et al., 1995; Quill et al., 2001; Ren et al., 2001).

As the sperm nears the oocyte, chemical cues from the latter and/or its surroundings may modify the flagellar beat pattern in order to guide the sperm to the oocyte via a process of chemotaxis (Eisenbach, 2007; Chang and Suarez, 2010). The relationship of chemotaxis to hyperactivation is not well understood, but since sperm begin to hyperactivate in the lower oviduct, far from the site of fertilization, it has been proposed that chemotactic factors modulate the flagellar beat of hyperactivated sperm to re-direct them toward the oocyte (Chang and Suarez, 2010). There is evidence that Ca\textsuperscript{2+} signaling plays a role in the chemotactic response (Spehr et al., 2003, 2004) in addition to its role in triggering hyperactivation. Progesterone has been implicated as a chemoattractant for human sperm (Teves et al., 2006), although, at micromolar levels, it can also induce acrosome reactions (Osman et al., 1989; Baldi et al., 1998; Bedu-Addo et al., 2007).

Much remains to be learned about regulation of sperm flagellar beat patterns and their role in promoting the movement of sperm through the female reproductive tract and to the oocyte. Nevertheless, it is clear that Ca\textsuperscript{2+} signaling plays a vital role in the regulatory processes.
Ca\(^{2+}\) signaling modulates sperm motility

The flagellar bend amplitude and the asymmetry of the flagellar beat of mammalian sperm waveforms have been found to be highly dependent on the intracellular Ca\(^{2+}\) concentration, and thus Ca\(^{2+}\) plays a key role in hyperactivating sperm. The Ca\(^{2+}\) that triggers hyperactivation \textit{in vivo} may originate from the opening of Ca\(^{2+}\) channels in the plasma membrane or release of Ca\(^{2+}\) from intracellular stores, or both, acting together (Jimenez-Gonzalez \textit{et al.}, 2006; Suarez, 2008). Cytoplasmic Ca\(^{2+}\) levels also depend on Ca\(^{2+}\) clearance mechanisms that transfer Ca\(^{2+}\) out of the cell or into mitochondria or Ca\(^{2+}\) storage organelles. The next few sections will deal with the mechanisms of increasing cytoplasmic Ca\(^{2+}\) levels in order to trigger and maintain hyperactivated motility.

Plasma membrane Ca\(^{2+}\) channels

CatSper channels, located on the plasma membrane of the principal piece (Figure 1; Ren \textit{et al.}, 2001; Quill \textit{et al.}, 2003; Xia \textit{et al.}, 2007), are the primary source of Ca\(^{2+}\) flux into the cytosol for hyperactivation (Kirichok \textit{et al.}, 2006). CatSper channels are pH and voltage dependent Ca\(^{2+}\) permeable ion channels that are absolutely required for hyperactivated motility and are only found on sperm (Ren \textit{et al.}, 2001; Quill \textit{et al.}, 2003; Carlson \textit{et al.}, 2003 and 2005; Qi \textit{et al.}, 2007; Xia \textit{et al.}, 2007). CatSper channels are necessary for sperm to ascend beyond the oviductal reservoir (Ho \textit{et al.}, 2009) and are required for sperm penetration of the zona pellucida of the oocyte (Ren \textit{et al.}, 2001; Quill \textit{et al.}, 2003). The CatSper proteins that form the ion channel contain six transmembrane segments, with a voltage-sensor S4 domain and a pore region that is Ca\(^{2+}\) selective (Ren \textit{et al.}, 2001; Qi \textit{et al.}, 2007). Four CatSper proteins have been identified (CatSper-1 (Ren
et al., 2001), -2 (Quill et al., 2003), -3 and -4 (Lobley et al., 2003; Jin et al., 2007) that
together form the subunits of the Ca\textsuperscript{2+} channel. In addition to the subunits that directly
form the channel, other subunits associate with the channel complex, such as CatSper\(\beta\)
(Liu et al., 2007;) and CatSper\(\gamma\) (Wang et al., 2009), and are thought to regulate channel
activity.

Sperm from CatSper null mice cannot hyperactivate and suffer a general decline in
motility over time (Qi et al., 2007; Quill et al., 2003). Even the initial motility of CatSper
null sperm is abnormal, which may be the result of abnormally low resting levels of Ca\textsuperscript{2+}
(Marquez et al., 2007). The flagellar bend amplitudes of CatSper null sperm are low, but
can be raised to normal pre-hyperactivated levels with the application of thimerosal,
which triggers release of Ca\textsuperscript{2+} from stores (Marquez et al., 2007). Also, reducing Ca\textsuperscript{2+}
levels in wild type sperm by loading sperm with the cell permeant Ca\textsuperscript{2+} chelator BAPTA-
AM produces abnormal motility resembling that of CatSper null sperm. Therefore
CatSper channels may be involved in providing sufficient Ca\textsuperscript{2+} for activated, as well as
hyperactivated, motility (Marquez et al., 2007).

A pH-sensitive efflux of K\textsuperscript{+} has been identified and is thought to aid in the
hyperpolarization of the membrane, which would maximize Ca\textsuperscript{2+} entry through CatSper
channels. The channel responsible for the K\textsuperscript{+} efflux has been identified as KSper, a
sperm-specific channel, which co-localizes with CatSper in the principal piece of the
sperm flagellum (Navarro et al., 2007).

In addition to CatSper channels, a variety of Ca\textsuperscript{2+} channels have been identified in
mammalian sperm, mostly by channel-specific antibodies. Various voltage-operated Ca\textsuperscript{2+}
channels (VOCCs), have been detected in mouse and human sperm (Figure 1; Wiesner et
VOCCs are a family of transmembrane, channel forming proteins that are compromised of an α1 (pore-forming) subunit and several other auxiliary subunits (Catterall, 2000; Ertel et al., 2000; Jimenez-Gonzalez et al., 2006). Through immunostaining and pharmacological analyses, low voltage activated (T-type), and high voltage activated (L-, R-, P/Q, and N-type) VOCCs have been identified in sperm (Arnoult et al., 1996; Benoff, 1998; Wennemuth et al., 2000; Felix, 2005).

Capacitative Ca$^{2+}$ entry via transient receptor potential (TRPC) channels may influence sperm motility and play a role in resequestration of Ca$^{2+}$ into stores. TRPC channels are made of six transmembrane units and studies have identified four types in sperm, TRPC-1, 3, 4, and 6 (Trevino et al., 2001; Castellano et al., 2003). TRPC channels have been localized to the principal piece of human sperm flagellum and the head (Figure 1; Castellano et al., 2003; Jimenez-Gonzalez et al., 2006).

Cyclic nucleotide gated (CNG) Ca$^{2+}$ channels have been identified on the flagella of bull sperm using antibodies (Figure 1; Wiesner et al., 1998). When bull or mouse sperm were treated with cGMP or cAMP via cell-permeant analogs, intracellular Ca$^{2+}$ increased (Wiesner et al., 1998; Xia et al., 2007); however, the role played by CNG Ca$^{2+}$ channels in hyperactivation is difficult to determine, because mouse sperm that lack CatSper channels do not respond to cyclic nucleotides. These channels may not be required for hyperactivation or they may interact somehow with CatSper channels.
Ca\textsuperscript{2+} stores and their relevant pumps and receptors

The redundant nuclear envelope (RNE), which is located at the base of the flagellum (Figure 1) contains a Ca\textsuperscript{2+} store (Ho and Suarez, 2001b, 2003). This store is likely to be an inositol 1,4,5-trisphosphate (IP\textsubscript{3})-gated store, because the IP\textsubscript{3} receptor has been localized by antibodies to a portion of the RNE in bull sperm, as well calreticulin, which is the Ca\textsuperscript{2+} binding protein in reticular stores (Ho and Suarez, 2001b, 2003). Thapsigargin, a Ca\textsuperscript{2+} ATPase inhibitor that depletes intracellular Ca\textsuperscript{2+} stores, induces an increase in intracellular Ca\textsuperscript{2+} great enough to switch on hyperactivation, even in the absence of available external Ca\textsuperscript{2+} (Ho and Suarez, 2003). The increase in intracellular Ca\textsuperscript{2+} that occurs during hyperactivation has been proposed to derive from both the opening of CatSper plasma membrane channels and the release of Ca\textsuperscript{2+} from the RNE store via IP\textsubscript{3}-gated channels (Ho and Suarez, 2001b, 2003). At this time, however, it is not known whether Ca\textsuperscript{2+} release from the RNE normally plays a role in hyperactivation or if it only serves to modulate hyperactivated motility. Recent observations indicate that release from the store actually produces a reversed pattern of hyperactivation (Marquez \textit{et al.}, 2007; Chang and Suarez, 2010; Chang and Suarez, 2011). In mouse sperm, the direction of the dominant, high-amplitude bend of flagellum can be determined, because the hook of the head of mouse sperm can serve as a reference point. The dominant flagellar bend of mouse sperm hyperactivated during capacitation, or by pharmacological activation of CatSper channels using procaine or 4-aminopyridine, was primarily oriented in the same direction as the hook of the sperm head. In contrast, the dominant bend in sperm hyperactivated by thimerosal, which triggers release of Ca\textsuperscript{2+} from internal stores, was oriented in the opposite direction of the hook in the head (see videos published in...
Chang and Suarez, 2011

http://www.biolreprod.org/content/early/2011/03/08/biolreprod.110.089789/suppl/DC1

It was proposed that release of Ca\(^{2+}\) from the RNE store serves to correct the course of hyperactivated sperm to re-orient sperm toward the oocyte and thus it may act as a mechanism of chemotaxis (Chang and Suarez, 2011). This phenomenon requires further investigation.

The acrosome also acts as an IP\(_3\)-gated Ca\(^{2+}\) store in sperm, which is thought to contribute to raising cytoplasmic Ca\(^{2+}\) to support the acrosome reaction (Walensky and Snyder, 1995; Herrick et al., 2005; Lawson et al., 2007). Sperm of CatSper-1 null mice can undergo the acrosome reaction even though they do not hyperactivate under capacitating conditions (Ren et al., 2001; Quill et al., 2003; Xia et al., 2007). Stimulation of the IP\(_3\) receptor with thimerosal can trigger hyperactivation (Ho and Suarez, 2001a), or the acrosome reaction (Herrick et al., 2005), depending upon experimental conditions.

The sarcoplasmic endoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA), which pumps Ca\(^{2+}\) from the cytoplasm into intracellular stores, has been immunolocalized to the midpiece and acrosomal region of human sperm (Lawson et al., 2007). In other cell types, SERCA activity is highly sensitive to the inhibitor thapsigargin (Thastrup et al., 1990). Inhibition generally produces a rise in cytoplasmic Ca\(^{2+}\) by releasing it from endoplasmic stores. Thapsigargin has been shown to stimulate hyperactivation in bull sperm (Ho and Suarez, 2001) and the acrosome reaction in human sperm (Meizel and Turner, 1993; O’Toole et al., 2000); however, the doses required to produce the effects are much higher than those required for other cell types (Harper et al., 2005), indicating that the response may be nonspecific or that the drug has poor penetration into the
intracellular stores of sperm.

The secretory pathway Ca\(^{2+}\) ATPase (SPCA) could serve to refill sperm intracellular stores instead of, or along with, SERCA. SPCA has been immunolocalized to the posterior head or neck and the midpiece of human sperm (Figure 1), where it could affect RNE stores (Harper et al., 2005; Bedu-Addo et al., 2008; Costello et al., 2009). The SPCA pump in other cell types has been observed to transport Ca\(^{2+}\) with an affinity similar to that of SERCA pumps (Wuytack et al., 2002).

**Ca\(^{2+}\) clearance mechanisms**

Sperm utilize Ca\(^{2+}\) clearance mechanisms to control intracellular Ca\(^{2+}\) levels (Wennemuth et al., 2003; reviewed in Jimenez-Gonzalez et al., 2006). Even though there exists a constant leak of Ca\(^{2+}\) from the extracellular fluid (which contain mM levels of Ca\(^{2+}\)) through the plasma membrane into the cytosol, activated sperm are still able to maintain a resting level of Ca\(^{2+}\) that ranges from about 40 nM in hamster and bull sperm (Suarez et al., 1993; Ho et al., 2002) to 100-250 nM in mouse sperm (Wennemuth et al., 2003). The different levels reported may be due to species or experimental differences. For example, the measurements of Ca\(^{2+}\) levels in hamster and bull sperm were recorded at the specific body temperature of each species, whereas those of mouse sperm were performed at room temperature. In comparison with other cells types, the resting levels of astrocytes were reported to be 87 nM (Parpura and Hayden, 2000), while those of cultured skeletal myotubes were reported to be 116 +/- 7 nM (Eltit et al., 2010).

The primary mechanism of Ca\(^{2+}\) clearance in sperm, at least in the mouse, is via the plasma membrane Ca\(^{2+}\)-ATPase (PMCA). The PMCA pumps use ATP to export a
cytoplasmic Ca\(^{2+}\) ion out in exchange for one or two extracellular protons brought into the cytosol. Antibody labeling of PMCA shows a patchy distribution along the principal piece of the mouse sperm tail that tapers off distally toward the tip, similar to the distribution of CatSper channel subunits (Figure 1; Wennemuth et al., 2003; Okunade et al., 2004). In addition, some faint antibody labeling has been detected on the midpiece and head (Okunade et al., 2004). When the PMCA activity was depressed by raising the pH of the medium, other mechanisms cleared sperm cytoplasmic Ca\(^{2+}\), but only to an intermediate level (Wennemuth et al., 2003). The major isoform of PMCA expressed in mouse sperm is PMCA-4. PMCA-4 null mouse sperm quickly developed severely impaired motility when incubated under capacitating conditions that eventually produced hyperactivation in wild type sperm (Okunade et al., 2004; Schuh et al., 2004).

The \(\text{Na}^+ / \text{Ca}^{2+}\) exchanger (NCX) is another mechanism of Ca\(^{2+}\) clearance in sperm, which can remove Ca\(^{2+}\) out of the cell or into an intracellular store. The NCX has been localized to the postacrosomal region and flagellar midpiece in human sperm (Krasznai et al., 2006; Jimenez-Gonzalez et al., 2006), which differs from the distribution of PMCA-4 in mouse sperm (Figure 1). When the intracellular Ca\(^{2+}\) concentration is high, the exchanger will export a Ca\(^{2+}\) ion out of the cytosol in exchange for the influx of three \(\text{Na}^+\) ions. The NCX can reverse direction when sperm membranes are depolarized or when \(\text{Na}^+\) is removed from the medium (Wennemuth et al., 2003). The exchanger has been estimated to operate at about 1/3 the rate of PMCA in mouse sperm (Wennemuth et al., 2003).

Mitochondria can act as Ca\(^{2+}\) buffers and remove Ca\(^{2+}\) from the cytosol (Nicholls and Chalmers, 2004). In other cell types, the mitochondrial Ca\(^{2+}\) uniporter (MCU) brings
Ca\(^{2+}\) into energized mitochondria when intracellular Ca\(^{2+}\) is above 500 nM (Gunter et al., 2000), as it may do in hyperactivated sperm. The mitochondria at the base of the flagellum lie very close to the RNE Ca\(^{2+}\) store and, in bull sperm, electron micrographs revealed that these mitochondria are uncoiled from the tightly-wound mitochondrial helix in the flagellar midpiece, such that they associate closely with the RNE (Ho and Suarez, 2001). Such a close association between the two types of organelles strongly suggests that they interact. On the other hand, treatment of bull sperm with CGP-37157 to inhibit the Na\(^+\)/Ca\(^{2+}\) exchanger did not prevent procaine induction of hyperactivation (Ho and Suarez, 2003). Also, in human sperm, application of the mitochondrial uncoupler 2,4-dinitrophenol had no effect on progesterone induction of Ca\(^{2+}\) oscillations (Harper et al., 2004). It was found that treatment with protonophore m-chlorophenylhydrazone (CCCP) to depolarize the inner mitochondrial membrane and prevent Ca\(^{2+}\) uptake by the MCU has only a minor effect on Ca\(^{2+}\) clearance in mouse sperm (Wennemuth et al., 2003). Thus, there is little evidence for a specific role of mitochondria in regulating Ca\(^{2+}\) levels in sperm flagella.

**Where and how is Ca\(^{2+}\) acting to modify the waveform?**

The exact mechanism and signaling pathway by which Ca\(^{2+}\) acts to trigger hyperactivated motility, transitioning from symmetrical to asymmetrical flagellar beating, is not known. In the flagellar axoneme, the hydrolysis of ATP on the dynein arms can be converted into force, which causes the microtubules to slide past one another. Sliding is converted to bending by the restraining influence of other structures in the axoneme (Lindemann and Lesich, 2010). The demembranated sperm model has helped us to
understand where Ca$^{2+}$ is acting to modify the waveform. When reactivated, bull sperm show symmetrical flagellar beating at approximately 50 nM Ca$^{2+}$ and asymmetrical, hyperactivated beating at about 400 nM Ca$^{2+}$ (Ho et al., 2002). Similar results have been seen in sea urchin sperm (Brokaw, 1979) and in rat sperm (Lindemann and Goltz, 1988). These observations indicate that the Ca$^{2+}$ is acting directly at the axoneme, rather than at the plasma membrane or other cellular compartments. Further, in reactivated demembranated sea urchin sperm, it was demonstrated that Ca$^{2+}$ regulates the activity of a specific group of dynein arms (Bannai et al., 2000). Ni$^{2+}$ and Cd$^{2+}$ have been shown to block a specific subset of dyneins, presumably by binding to Ca$^{2+}$ receptors (Kanous et al., 1993; Lindemann et al., 1995) and thereby indicating that there are receptors with different binding specificities for divalent cations.

Ca$^{2+}$ can modify flagellar bending in several different ways. Mechanical effects such as changing the spoke length, nexin length, or elasticity of the nexins could be induced by Ca$^{2+}$ to modify the waveform (Lindemann and Kanous, 1995). One immediate target for Ca$^{2+}$ is calmodulin (CaM) (Means et al., 1982; Weinmann et al., 1986; Bendahmane et al., 2001), which has been localized to the principal piece of the flagellum (Schlingmann et al., 2007). When CaM was extracted from demembranated bull sperm, motility could not be reactivated unless exogenous CaM was added back (Ignitz and Suarez, 2005).

Binding of Ca$^{2+}$ to CaM could activate Ca$^{2+}$/CaM-dependent kinases (Marin-Briggiler et al., 2005), which could phosphorylate an axonemal protein, resulting in a modification of the waveform (Suarez, 2008). CaM kinases have been identified in the flagella of bovine (Ignotz and Suarez, 2005) and human (Marin-Briggiler et al., 2005).
sperm. Peptide inhibitors of CaMKII, as well as a membrane permeant inhibitor of CaMKII inhibited asymmetrical flagellar bending of intact and demembranated bull sperm (Ignotz and Suarez, 2005). There is evidence of additional Ca$^{2+}$-binding proteins in sperm flagella, such as calcium-binding tyrosine phosphorylation regulated protein (CABYR) in mammals (Naaby-Hansen et al., 2002) that could also play a role in modulating flagellar bending patterns.

**Mathematical models of Ca$^{2+}$ dynamics**

Hyperactivation is known to be Ca$^{2+}$ dependent and vital for fertilization, but how hyperactivation is triggered *in vivo* is still unknown. Experiments have provided insight into the signaling pathway, but there is not a detailed understanding of how each of the channels, stores, and clearance mechanisms are acting together to lead to hyperactivation. Along with experiments, mathematical models can be used to investigate nonlinear interactions in the Ca$^{2+}$ signaling pathway, and provide a systematic approach to examine the implications of hypothesized mechanisms of Ca$^{2+}$ signaling in sperm.

Since Ca$^{2+}$ is a universal signaling molecule, mathematical models of Ca$^{2+}$ dynamics have been developed for many cell types including endocrine cells of the pituitary gland (Blum et al., 2000), airway epithelial cells (Sneyd et al., 1995), pancreatic and parotid acinar cells (Sneyd et al., 2003), kidney epithelial cells (Roose et al., 2006), vertebrate olfactory receptor neurons (Dougherty et al., 2005), airway and pulmonary arteriole smooth muscle (Wang et al., 2008), and the Xenopus oocyte (Atri et al., 1993). Mathematical models that are based upon ordinary differential equations track the spatially averaged Ca$^{2+}$ concentration in the sperm cell, and do not account for the
location of channels and internal stores. Partial differential equation models are more
detailed and track both the temporal and spatial evolution of Ca^{2+} in a cell or group of
cells. The partial differential equation model presented in Olson et al. (2010) examines
fluxes of Ca^{2+} due to channels and stores at the appropriate spatial location. In addition,
spatial models may include the diffusion of ions along the length of the flagellum.
Moreover, the diffusivity may also be spatially varying due to the microtubules, fibers,
mitochondria, acrosome, nucleus, and Jensen’s ring. Olson et al. (2010) use a 1-
dimensional model, that represents the flagellum as a line, and assume a constant
diffusion coefficient as a first approximation. Buffering of ions such as Ca^{2+} can play a
large role in changing the free Ca^{2+}. Initial modeling efforts assume fast buffering,
corresponding to effective fluxes and only account for a free Ca^{2+} concentration. It is
noted that as models are extended from 1-dimensional to higher dimensions, special
considerations will need to be taken to model the flagellum as a tapering cylinder, and to
accurately account for many of the structures within the flagellum.

In a mathematical model, the Ca^{2+} dynamics are determined by the specific
extracellular signals (e.g. membrane depolarizations, neurotransmitters, shear stresses),
key second messengers, the Ca^{2+} channels and clearance mechanisms, and the
intracellular Ca^{2+} stores. Naturally, the components of the model are based upon
experimental findings. However, even if some of the parameters (i.e. rate constants) are
not known, overall dynamics can be measured for different ranges of parameter values,
and the sensitivity of model results to changes in these parameters can be quantified. The
model provides a platform to perform computational experiments that measure the effect
of perturbing the system. These simulations could, for instance, correspond to
pharmacologically inhibiting a channel or changing the extracellular Ca\textsuperscript{2+} concentration. The model can then be used to understand the relative importance of each mechanism, test hypotheses, and gain insight into the underlying physiology or cellular biology. Mathematical models can reveal interesting and unexpected relationships, suggesting experiments to be performed in the laboratory.

**Ca\textsuperscript{2+} clearance model for mouse sperm**

Wennemuth *et al.* (2003) performed a number of experiments on mouse sperm, blocking Ca\textsuperscript{2+} channels and pumps, to understand the relative importance of specific Ca\textsuperscript{2+} clearance mechanisms of sperm. The fundamental question was, how do sperm maintain Ca\textsuperscript{2+} levels and remove excess Ca\textsuperscript{2+}? Through carefully designed experiments, they were able to understand the relative importance of each Ca\textsuperscript{2+} clearance mechanism after a depolarizing stimulus. The results of these experiments were used to set up a differential equation for the rate of change of Ca\textsuperscript{2+} concentration in the cytosol of the sperm. The Ca\textsuperscript{2+} fluxes accounted for were: (1) a constant leak flux into the cytosol, (2) PMCA pumping Ca\textsuperscript{2+} out of the cytosol, (3) MCU sequestering Ca\textsuperscript{2+}, (4) a sodium-calcium (NCX) exchanger, and (5) Ca\textsuperscript{2+} influx due to membrane depolarization. The system of ordinary differential equation was solved numerically to determine the spatially averaged Ca\textsuperscript{2+} concentration in the sperm cell. These concentrations were compared to experimental data of spatially averaged Ca\textsuperscript{2+} concentrations determined using fluorescent Ca\textsuperscript{2+} indicators. Many qualitative features of the experimentally observed results were captured in the simulations. It was determined through experiments and simulations that the PMCA is the primary mechanism of Ca\textsuperscript{2+} clearance in mouse sperm after...
depolarization, with PMCA removing Ca\(^{2+}\) at a rate 3 times greater than the MCU and NCX. As discussed by Wennemuth et al. (2003), each of these fluxes is localized to a specific region, which is not taken into account in this model. The model also does not consider physiological and biochemical differences between activated and hyperactivated sperm.

**CatSper mediated Ca\(^{2+}\) dynamics model**

In experiments by Xia et al. (2007), the relative intracellular Ca\(^{2+}\) concentration was recorded in mouse sperm after the application of a cell permeant cAMP analog. The intracellular Ca\(^{2+}\) first started to increase in the principal piece, the location of the CatSper channels, and was followed by increases in Ca\(^{2+}\) along the length of the flagellum, with a sustained increase in the head region. After the initial increase in Ca\(^{2+}\), Ca\(^{2+}\) clearance mechanisms brought the Ca\(^{2+}\) concentration back down to the basal, resting level. Xia et al. (2007) hypothesized that the cAMP analog facilitates the opening of the CatSper channels, causing Ca\(^{2+}\) influx in the principal piece through the CatSper channels, initiating a tail to head propagation of Ca\(^{2+}\) (Xia et al., 2007). The main question they proposed is: can diffusion alone account for the sustained increase of Ca\(^{2+}\) in the head or is there some type of additional Ca\(^{2+}\) release that takes place due to the influx of Ca\(^{2+}\) from the CatSper channels?

We developed a 1-d reaction diffusion model to describe the CatSper mediated Ca\(^{2+}\) dynamics associated with hyperactivation (Olson et al., 2010), based on the experimental setup and results of Xia et al. (2007). The goal of this work was to introduce a mathematical model of Ca\(^{2+}\) dynamics in mammalian sperm relevant to hyperactivated
motility. With this model, we examined how the spatial and temporal evolution of Ca$^{2+}$ along the flagellar centerline depends upon diffusion, nonlinear fluxes, and the presence of a Ca$^{2+}$ store. The preliminary model assumed that the flagellar centerline was stationary and flat, and accounted for fluxes on the principal piece due to: (1) Ca$^{2+}$ influx through CatSper channels, (2) a constant leak flux, and (3) PMCA pumping Ca$^{2+}$ out of the cytosol. Following Wennemuth et al. (2003), the PMCA was assumed to be the primary mode of Ca$^{2+}$ clearance. Numerical simulations of this partial differential equation model demonstrated that diffusion alone could not account for a sustained increase in Ca$^{2+}$ concentration in the head, as observed in the experiments by Xia et al. (2007).

In order to achieve better agreement with these experimental results, the model was extended to account for additional flux terms at the site of the RNE, an internal Ca$^{2+}$ store in the neck of the sperm. The equations governing this system are as follows:

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial s^2} + J_{RNE, cyt} - J_{RNE, uptake} - J_{PMCA} + J_{leak} + J_{Cat} \quad (1)$$

$$\frac{\partial I}{\partial t} = D_I \frac{\partial^2 I}{\partial s^2} + IP_3^{\text{prod}} - IP_3^{\text{deg}} \quad (2)$$

Here, C is the Ca$^{2+}$ concentration C and I is the IP3 concentration I, s is the location along the flagellar centerline, and t is time. The ions Ca$^{2+}$ and IP3 have effective diffusion coefficients $D_C$ and $D_I$, respectively. In particular, the Ca$^{2+}$ fluxes $J_{PMCA}$, $J_{leak}$, and $J_{Cat}$ accounted for in this model (and also the initial starting model) are due to the PMCA, leak, and CatSper channels, respectively. Additional fluxes accounted for are due to a resequestration flux of Ca$^{2+}$ back into the RNE $J_{RNE, uptake}$, and a flux out of the RNE into the cytosol $J_{RNE, cyt}$, which is due to Ca$^{2+}$ release from the RNE, dependent on the local IP3 concentration, and a small leak flux. The extended model accounts for the local
concentration of IP₃, which changes due to production $IP₃_{prod}$ and degradation $IP₃_{deg}$. The Ca²⁺ release from the RNE was dependent on the local IP₃ and Ca²⁺ concentration. When IP₃-gated Ca²⁺ release from the RNE was accounted for, the results of the computational simulations agree with the qualitative trends of the experimental data. The model achieves a sustained increase of Ca²⁺ in the head, and exhibited a tail to head propagation of Ca²⁺, as shown in Figure 2 (Olson et al., 2010). Through the use of a simple mathematical model, we were able to demonstrate that diffusion alone cannot account for the sustained increase of Ca²⁺ in the head. We hope that this mathematical model motivates the performance of further experiments to understand the role of Ca²⁺ release from the RNE, the role of IP₃ and how its concentration evolves in time, as well as to determine kinetic rate parameters of channels and pumps relevant to Ca²⁺ dynamics.

**Role of mathematical models in the future**

In order for sperm to propel themselves forward, they use an undulatory flagellar motion, which is an emergent property of a complex system that couples the external fluid dynamics, passive elastic properties of the sperm structure, active force generation of the dynein arms, and chemical signaling. In order to fully understand how the sperm is able to reach the oocyte and swim efficiently through the reproductive tract by modifying its waveform, integrative models that account for each of these aspects are necessary (Fauci and Dillon, 2006). Models that account for the fluid-structure interaction assume the fluid flow is governed by the Stokes equations or the Navier Stokes equations. Using these equations, the flagellum exerts forces on the surrounding fluid, and the equations account for the fluid density and viscosity. Recent models have captured elements of the
full fluid-structure interaction (for example Gueron and Levit-Gurevich, 1998; Dillon et al., 2007; Fu et al., 2007).

Mathematical models that couple the relevant biochemistry with mechanics and hydrodynamics will be of great use in the future to understand the impact of Ca\(^{2+}\) dynamics on sperm motility. We have been working toward this end by exploring a model that couples Ca\(^{2+}\) dynamics along the moving flagellum, viscous fluid mechanics, and the elastic properties of the flagellum. We couple our previous calcium model for CatSper mediated Ca\(^{2+}\) dynamics (Olson et al., 2010) to a time-dependent, preferred curvature that drives flagellar bending. This target wave amplitude corresponding to the preferred curvature is chosen to depend on the local Ca\(^{2+}\) concentration. In order to reflect the dependence of dynein force generation on Ca\(^{2+}\), we used a modified Hill function to define the target amplitude \(b(s,t)\),

\[
b(s,t) = V_A \frac{Ca(s,t)}{Ca(s,t) + k_A}
\]

Here, \(k_A\) is a coefficient for receptor activation, \(V_A\) is the maximum amplitude, and \(Ca(s,t)\) is the Ca\(^{2+}\) concentration at time \(t\) and location along the flagellum \(s\). In order to account for an asymmetry in the model, we designate a principal and reverse bend direction by monitoring the curvature along the length of the flagellum, and we set \(k_A = k_A,1\) for positive curvature and \(k_A = k_A,2\). Choosing \(k_A,1 \neq k_A,2\) assumes that different sides of the flagellum have different binding affinities for Ca\(^{2+}\) or a different number of binding sites.

In this model, the waveform of the flagellum is not preset, but is an emergent property of the coupled system. By including a slight asymmetry in the curvature model that reflects a hypothesized asymmetry in the axoneme’s response to calcium, we observe
the transition from activated motility to hyperactivated motility (Olson et al., 2011).

Figure 3 shows some snapshots of a model flagellum as it achieves a swimming pattern characteristic of hyperactivation. We view this model as a starting point to investigate the biochemistry and mechanics of sperm motility. Future modeling efforts will involve accounting for discrete representations of the dynein arms (for example Dillon et al., 2007), whose activation kinetics will be governed by the evolving Ca\(^{2+}\) dynamics.

Authors’ Roles

S.D.O. wrote the initial draft of this invited review article; S.S.S. critically revised the biological portion of the draft; L.J.F. critically revised the mathematical portion. All three authors worked together on final revisions.

Funding: This work was supported by National Institutes of Health [1RO3HD062471-01] to SSS.
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Figure Legends

Figure 1: Ca\(^{2+}\) channels and pumps that have been immunolocalized in mammalian sperm. Arrows indicate the most common direction of Ca\(^{2+}\) movement caused by these entities in most cell types. The chief channels and pumps involved in hyperactivation are CatSper channels and plasma membrane Ca\(^{2+}\)-ATPase (PMCA), which are indicated by large arrows. Others that have been identified include: sarcoplasmic/endoplasmic reticular Ca\(^{2+}\)-ATPase (SERCA), IP\(_3\)-gated channels (IP\(_3\)); voltage-operated Ca\(^{2+}\) channels (VOCC), transient receptor potential Ca\(^{2+}\) channels (TRPC), secretory pathway Ca\(^{2+}\)-ATPase (SPCA), Na\(^{+}\)/Ca\(^{2+}\) exchanger (NCX), mitochondrial uniporter (MCU), and cyclic nucleotide-gated Ca\(^{2+}\) channels (CNG).

Figure 2: Simulations of the CatSper mediated Ca\(^{2+}\) dynamics model described in Olson et al. (2010) and based on the experimental results of Xia et al. (2007). At t = 0, a cell permeant cyclic nucleotide analog (8-Br-cAMP) was applied. The Ca\(^{2+}\) concentrations along the flagellum are shown, where x = 0 corresponds to the head and x = 100 µm corresponds to the endpiece of the flagellum. Panel (a) shows that Ca\(^{2+}\) first increases in the principal piece and then starts increasing in the neck, the location of the RNE. In this model, we account for IP\(_3\) gated Ca\(^{2+}\) release from the RNE. Concentrations at later times are shown in panel (b). When the CatSper channels close and Ca\(^{2+}\) clearance mechanisms bring the Ca\(^{2+}\) concentration back to the resting level.

Figure 3: Simulations of a swimming sperm in a 3-d viscous fluid where the CatSper mediated Ca\(^{2+}\) dynamics are accounted for and are coupled to the preferred curvature.
The solid black circle denotes the head side of the flagellum. Velocity vectors for the local fluid velocity are depicted in the plane of the beating flagellum and the calcium concentration along the length of the flagellum is shown. After application 8-Br-cAMP, the Ca\(^{2+}\) profiles (as in Figure 2) are determined by the partial differential equations accounting for release of Ca\(^{2+}\) from the RNE. These Ca\(^{2+}\) concentrations are then used to define a preferred curvature for the flagellar waveform using a modified Hill function for the amplitude. From left to right, top row: t=0, 1, 2 s and bottom row: t=4, 6, 8 s. Note how the waveform is modified as the Ca\(^{2+}\) concentration increases.
Figure 1
Figure 2

(a) 

(b)
Figure 3