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Ion Channels that Control Fertility in Mammalian Spermatozoa

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Abstract

Whole-cell voltage clamp of mammalian spermatozoa was first achieved in 2006. This technical advance, combined with genetic deletion strategies, makes unambiguous identification of sperm ion channel currents possible. This review summarizes the ion channel currents that have been directly measured in mammalian sperm, and their physiological roles in fertilization. The predominant currents are 1) a Ca^{2+}-selective current requiring expression of the 4 mCatSper genes, and 2) a delayed rectifier K^+ current with properties most similar to mSlo3. Intracellular alkalinization activates both channels and induces hyperactivated motility.

Keywords

CatSper; KSper; Ca^{2+}; hyperactivated sperm motility; patch clamp; sperm; Ca^{2+} channel

Introduction

Mature mammalian spermatozoa are quiescent in the male reproductive tract. Upon ejaculation, they become motile. As they move through the female reproductive tract, they become competent to fertilize the egg. During this period, called capacitation, spermatozoa begin to move progressively, develop hyperactive motility, and their acrosomes can react (Yanagimachi, 1994). Capacitation requires changes in intracellular pH ($[pH]_i$), and concentrations of Ca^{2+} ($[Ca^{2+}]_i$), and cAMP. The molecular mechanisms responsible for these changes are an active area of research.

$[Ca^{2+}]_i$ is increased either as Ca^{2+} enters the cytoplasm through plasma membrane ion channels, or as Ca^{2+} is released from intracellular stores. Many Ca^{2+}-permeable channels have been proposed to participate in sperm cell processes (Darszon *et al*., 2006), including classical voltage-gated Ca^{2+}- (Ca_V), transient receptor potential- (TRP), and cyclic nucleotide-gated- (CNG) channels. This review summarizes two particular ion currents in sperm cells, CatSper current ($I_{CatSper}$) and K^+ current in spermatozoa (I_{KSper}) that have been directly measured with the recently developed whole-sperm cell patch clamp (Kirichok *et al*., 2006).

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CatSpers1-4

The first member of the CatSper (Cation channel of sperm) family, CatSper1 was detected during searches for sequence homology to the voltage-gated Ca^{2+}-selective channels (Ca_V1-3) (Ren *et al*., 2001). The mouse *CatSper1* gene is located on mouse chromosome 19 and consists of 12 exons with an open reading frame (ORF) encoding a 686 amino acid protein. CatSper2 was discovered as a sperm cell-specific transcript using a signal peptide trapping method. Mouse chromosome 2 contains the *CatSper2* gene, encoding a protein of 588 amino acids in length (Quill *et al*., 2001). CatSper2 has at least 3 splice variants. The last two members of the family, CatSper3 and CatSper4, were found in database searches (Jin *et al*., 2005, Lobley *et al*., 2003, Qi *et al*., 2007). *CatSper3*, on mouse chromosomes 13, encodes a 395 amino acid polypeptide, while CatSper 4, on mouse chromosome 4, encodes a 442 amino acid protein. Both CatSper3 and CatSper4 have several potential splice variants. The four CatSper proteins have relatively low sequence identity in the transmembrane regions, ranging from 16 to 22%. Mouse CatSper orthologs have been found in all mammals examined (human (**Figure 1**), chimpanzee, dog, and rat), in sea squirt (*Ciona intestinalis*), and sea urchin (*Strongylocentrotus purpuratus*). CatSpers have not yet been identified in fish (*Fugu rubripes* and *Danio rerio*), flies (*Drosophila melanogaster*), worms (*Caenorhaditis elegans*), or plants (*Arabidopsis thaliana*).

Structural domains of CatSper proteins

The functional channel polypeptides with the closest similarity to CatSpers are the voltage-gated sodium channels (Na_VBP) in bacteria and the mammalian Ca_V family. The predicted topology of all 4 members of the CatSper family consists of cytoplasmic amino- and carboxyl-termini flanking 6 transmembrane (6TM)-spanning segments (S1-S6; **Figure 1**). Like other voltage-gated channels, these segments are arranged into 2 functionally distinct modules: the voltage sensor (S1-S4) and the pore-forming (S5-P loop-S6) domain. All known Ca_Vs have 4 repeats of the 6TM domain (arising from gene duplication of a single 6TM gene). Ca^{2+}-selectivity is suggested by negatively charged amino acids in the pore consensus sequence ([T/S]x[**D/E**]xW). CatSpers1-4 each contains a similar conserved motif, Tx**D**xW.

The S1-S4 segments of voltage-gated channels are called the voltage sensor domains (VSDs). The VSD contains 4 to 6 positively charged amino acids (arginine (R)/lysine (K)) spaced at helical turns (3 amino acid intervals) in the S4 segment. Voltage changes move the S4 segment, resulting in conformational changes that open and close (gate) the pore. CatSper1 and CatSper2 contain 4, while CatSper3 and CatSper4 have only 2, R/K residues in the S4 segment. This may be responsible for the reduced voltage dependence of the CatSper heterotetrameric channel.

The amino-termini of CatSper2, 3 and 4 are fairly well conserved, but CatSper1's amino terminus is remarkable for its abundance of histidine residues (49/250 amino acids) (Ren*et al*., 2001). The histidine rich amino terminus suggests that CatSper1's amino terminus might be involved in pH regulation of CatSper currents, although other functions are equally plausible. All four of the CatSper proteins contain a coiled-coil domain within their carboxyl-termini, suggesting that heteromultimerization involves these domains.

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Immunoprecipitation studies performed in *CatSper2-null* mice provide evidence that stable expression of CatSper1 protein in sperm requires CatSper2 (Carlson *et al*., 2005). Similarly, studies with CatSper1, 3, and 4 null mice demonstrated that stable CatSper3 and 4 required CatSper1 expression, supporting the heteromultimerization (**Figure 2**) of these subunits (Qi *et al*., 2007).

Tissue distribution and localization of CatSpers

Several laboratories have profiled gene expression of each member of the CatSper family by multi-tissue northern blot analysis. CatSper1, 2, 3 and 4 mRNA was detected exclusively in mouse and human testis (Jin *et al*., 2005, Qi *et al*., 2007, Quill *et al*., 2001, Ren *et al*., 2001). *In situ* hybridization studies of CatSper subtypes suggest that CatSpers are differentially transcribed during spermatogenesis. CatSper1 (Ren *et al*., 2001), CatSper3 and 4 (Jin *et al*., 2005, Qi *et al*., 2007, Schultz *et al*., 2003) transcripts are restricted to the late-stage germ-line cells (spermatids) in testes, while CatSper2 (Quill *et al*., 2001, Schultz *et al*., 2003) transcription begins in the early-stage of spermatogenesis (pachytene spermatocytes). Consistent with *in situ* hybridization studies, real time PCR demonstrated that the CatSper2 transcript was detected in the testis from P8 (postnatal day) mice, whereas transcription of CatSper1 started at P18. CatSper 3 and 4 mRNAs appeared in mouse testis at P15 (Li *et al*., 2007).

CatSpers1-4 protein localization by antibody staining has proven to be difficult due to lack of specific CatSpers2-4 antibodies. When CatSper subtype null mice are used as controls, CatSpers1-4 proteins are recognized only in testis. Within spermatozoa, CatSpers localize to the principal piece of the flagella (Jin *et al*., 2005, Qi *et al*., 2007, Quill *et al*., 2003, Ren *et al*., 2001). Genetically deleted mouse controls would be useful in testing the validity of the large number of other ion channel localization studies.

CatSperβ, an auxiliary subunit of the CatSper1 complex

Transgenic mice were made in which CatSper-HA-GFP was inserted on a *CatSper1-null* mice background (Li *et al*., 2007). This gene restored fertility in CatSper1 null mice, and the HA and GFP tags allowed for reliable protein purification. Using this strategy, two testis-specific proteins were identified: a heat-shock protein, HSP70-2, and a novel protein subsequently named CatSperβ. The mouse *CatSper* gene on chromosome 12, encodes an 1109 amino acid protein with its 2 transmembrane segments connected via a large extracellular (~1000 amino acid) domain. CatSperβ homologs are present in mammals and sea squirt (*C. intestinalis*), and like CatSper1, are absent in *C. elegans* and *D. melanogaster*. Immunoprecipitation studies with a native sample (or testicular membrane lysate) demonstrated that CatSper interacts with CatSper1, and like CatSper2-4, is missing in CatSper1-null spermatozoa (Liu *et al*., 2007). CatSperβ is localized to the principal piece of the sperm flagella. Taken together, these findings suggest that CatSperβ is an auxiliary subunit of the CatSper1 complex. Functional interactions and potential regulation of the CatSper complex by CatSper in spermatozoa are currently being studied.

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CatSpers form a Ca^{2+}-selective channel

To examine the basic functional properties of ion channels, they must be tested in voltage clamp under varying ionic conditions. Until recently, transmembrane voltage-clamp recordings in sperm were not possible. Thus, attempts were made to express CatSper proteins in heterologous systems. Despite much effort, heterologous expression of CatSper family members (in various mammalian cell lines and *Xenopus* oocytes), alone or in combination, failed to yield measurable current. However, initial measurements of Ca^{2+} influx in sperm cells were carried out using Ca^{2+}-sensitive fluorescent probes. cGMP (Ren *et al*., 2001) application or depolarization with alkaline K^+ solution increased $[Ca^{2+}]_i$, (Carlson *et al*., 2003, Xia *et al*., 2007). This inducible Ca^{2+} increase was abolished in sperm cells from *CatSper1*- and *CatSper2-null* mice, indicating that spermatozoa Ca^{2+} entry required CatSper proteins.

The recent development of patch clamp, by accessing the whole-spermatozoa via the cytoplasmic droplet, finally permitted characterization of CatSper currents. $I_{CatSper}$ is a weakly outwardly rectifying, pH-sensitive, Ca^{2+}-selective current (Kirichok *et al*., 2006). As for many Ca^{2+}-selective channels, barium (Ba^{2+}) was more permeant than Ca^{2+} while magnesium (Mg^{2+}) was impermeant. And, as for classical Ca_V channels, Ca^{2+} itself participates in Ca^{2+} selectivity by binding the pore: CatSper conductance was much larger in divalent free (monovalent) solutions.

$I_{CatSper}$ is weakly voltage dependent (Boltzmann slope ~4 compared to ~12 for highly voltage-dependent channels). $I_{CatSper}$ is, however, a very pH-sensitive current. CatSper's g-V curve is significantly shifted to more hyperpolarized potentials by alkaline pH, consistent with increased CatSper current after capacitating conditions that alkalinize the intracellular environment (pH 6.8 to the 7-8 range). An important advantage of employing spermatozoa over other cells is that, in addition to voltage clamp of intact sperm, sperm cell fragments (either the head and midpiece, or midpiece and principal piece), can be patch clamped separately (Kirichok *et al*., 2006, Qi *et al*., 2007). Using these methods, $I_{CatSper}$ was specifically localized to the sperm principal piece. $I_{CatSper}$ is absent in spermatozoa from *CatSper1-4-null* mice (Jin *et al*., 2007, Kirichok *et al*., 2006, Qi *et al*., 2007), while it remains unaltered in sperm cells from *wild-type* (*wt*) mice. In other experiments, CatSper current is unaltered in *Na^+/H^+ exchanger-null* mice (Qi *et al*., 2007) when intracellular pH is controlled.

Other Ca^{2+} permeant channels

A T type Ca_V current is small (<80 pA), but detectable in spermatocytes (Escoffier *et al*., 2007, Ren *et al*., 2001, Santi *et al*., 1996). The T-type Ca_V current is also present in *CatSper1-null* spermatocytes. This current is not detected in spermatocytes from *Ca_V3.2-null* mice, and had no effect on spermatogenesis or male fertility (Escoffier *et al*., 2007). Within the current resolution of patch clamp (~10 pA) and Ca^{2+} imaging (~100 nM), no purely voltage-gated Ca^{2+} influx typical of Ca_V channels was detected in spermatozoa from *CatSpers1-4*-null mice. Thus, it seems likely that classical Ca_V currents (CaV1-3, T, P/Q, N, L) are not significant in mature spermatozoa.

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Male mice lacking CatSpers1-4 are infertile

All four *CatSper* genes have now been deleted separately in mice. Homologous recombination was used to delete the first putative transmembrane segment (S1) of CatSper1 (Ren *et al*., 2001), S1-S3 of CatSper2 (Quill *et al*., 2003), and the putative pore region of CatSper3 and CatSper4 (Jin *et al*., 2007, Qi *et al*., 2007). Testicular histology, epididymal sperm count, and sperm morphology was unaffected in *CatSpers1-4-null* mice, indicating normal progression of spermatogenesis. Furthermore, there was no difference in the weight, life span, litter size, gross behavior, or mating behavior when *wt* and homozygous male mutant mice were compared. The striking phenotype of all *CatSpers1-4-null* mice is complete male infertility; female *CatSpers-null* mice have normal fertility. To date, some form of (nonsyndromic) male human infertility has been linked to a mutation in the *CatSper2* gene (Avidan *et al*., 2003), but other human CatSper mutations are likely to be found.

Several Ca_V proteins, as well as TRPC2 and CNGA3, have been detected by antibodies in mouse spermatozoa (Carlson *et al*., 2003, Darszon *et al*., 2006). Unfortunately, none of these currents were detected in whole-sperm voltage clamp of mouse epididymal sperm cells. In addition, mice carrying null mutations for Ca_V1.3 (Platzer *et al*., 2000), Ca_V2.2 (Ino *et al*., 2001), Ca_V2.3 (Saegusa *et al*., 2000), Ca_V3.1 (Kim *et al*., 2001) and Ca_V3.2 (Chen *et al*., 2003), TRPC2 (Stowers *et al*., 2002) and CNGA3 (Biel *et al*., 1999) have normal fertility. The *Ca_V1.2 null* mutant (Seisenberger *et al*., 2000) is embryonic lethal and *Ca_V2.1 null* pups die ~3-4 weeks after birth (Jun *et al*., 1999) before fertility can be examined. Thus, CatSper channels are the only known ion channels that specifically affect mammalian male fertility.

The acrosome reaction in *CatSper1-null* mice

Spermatozoa must release the acrosomal vesicle (remnant of the Golgi apparatus) contained in the head of the sperm upon contact with egg's *zona pellucida*. The acrosome reaction (AR) releases proteolytic enzymes from the head of the sperm cell that enable penetration of the oocyte extracellular matrix (*cumulus* cells and *zona pellucida*) (Yanagimachi, 1994). Although an increase in $[Ca^{2+}]_i$ is required for the AR, the source of this Ca^{2+} is not clear (Publicover *et al*., 2007). Interestingly, alkaline K^+ depolarization and cell-permeable cGMP application induced the AR in *CatSper1-null* spermatozoa. Alkaline K^+ depolarization did not measurably increase $[Ca^{2+}]_i$ in these *CatSper1-null* sperm cells (Xia *et al*., 2007). These data suggest that the AR does not require Ca^{2+} entry via plasma membrane ion channels.

Hyperactivated motility requires CatSpers1-4

Upon deposition in the vagina, immotile sperm acquire motility by contact with bicarbonate (HCO_3^-). Flagellar motion is symmetrical, low amplitude, and higher frequency when sperm cells are at the entrance of female reproductive tract. In the egg's vicinity, where pH is 7-8, sperm cells develop hyperactivated motility that is characterized by asymmetrical (whip-like), high amplitude, and lower frequency flagellar beating (Ho and Suarez, 2001). Hyperactivated motility, essential for penetration through egg's *zona pellucida*, requires elevation of Ca^{2+} in the sperm flagellum (Lindemann and Goltz, 1988).

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In vitro fertilization (IVF) studies showed that *CatSper1*- and *CatSper2-null* spermatozoa were incapable of fertilizing intact eggs (Quill *et al*., 2003, Ren *et al*., 2001). Mutant spermatozoa were able to initiate the fertilization process only after removal of the egg's *zona pellucida*. Since the acrosome reaction is normal in *CatSper1*- and *CatSper2-null* sperm cells, the most likely defect was in motility or hyperactivated motility. Ca^{2+}-independent motility was initially normal in *CatSpers1-4-null* spermatozoa, but mutant sperm cells failed to develop Ca^{2+}-dependent, hyperactivated motility (Carlson *et al*., 2005, Carlson *et al*., 2003, Jin *et al*., 2007, Qi *et al*., 2007). Ca^{2+} entry through CatSper channels is required for sperm hyperactivated motility and enhanced Ca^{2+} influx induced by alkalinization leads to increased flagellar bending. This result confirms the essential role of Ca^{2+} ions in sperm cell motility and fertility. Interestingly, CatSpers mutant spermatozoa display a gradual decrease in motility (Jin *et al*., 2007, Qi *et al*., 2007) and lower ATP levels compared to wt sperm cells (Xia *et al*., 2007), suggesting that Ca^{2+} influx through CatSper channels may regulate other signaling pathways such as Glyceraldehyde 3 phosphate dehydrogenase-S (GAPDS)-dependent glycolysis (Miki *et al*., 2004).

Thus far, we have discussed the major mammalian inward current in spermatozoa, $I_{CatSper}$. If $I_{CatSper}$ were the only active ion channel, the membrane potential would be the reversal potential for Ca^{2+} (E_{ca}; normally ~+150 mV). To balance $I_{CatSper}$, hyperpolarizing currents must also be present.

K^+ current in spermatozoa (I_{KSper})

Noncapacitated murine spermatozoon resting membrane potential is ~−30 mV. After capacitation, sperm cell hyperpolarize to ~−60 mV (Arnoult *et al*., 1999, Zeng *et al*., 1995). Using voltage-sensitive fluorophores, the capacitation-associated hyperpolarization was ascribed to an increase in K^+ permeability (Zeng *et al*., 1995) and block of epithelial sodium channel (Hernandez-Gonzalez *et al*., 2006).

In whole-sperm patch clamp, a constitutively active, weakly outwardly rectifying K^+ current was measured. This current (I_{KSper}) was specifically localized to the principal piece of the sperm flagellum (Navarro *et al*., 2007). I_{KSper} was reversibly blocked by quinine, EIPA, mibefradil, but clofilium block was irreversible in the time scale of the recordings. Surprisingly, 4-aminopyridine (4-AP), a nonselective K_V channel blocker, enhanced I_{KSper} and $I_{CatSper}$. This effect is a consequence of 4-AP's known alkalinization of the intracellular environment. Spermatozoon intracellular alkalinization, induced by NH_4Cl, strongly potentiated I_{KSper}. Average I_{KSper} increased ~8 fold when pH_i was elevated from 6 to 8. Thus, I_{KSper} is a pH-sensitive K^+ current activated *in vivo* by intracellular alkalinization.

Murine *Slo3* (Schreiber *et al*., 1998) gene is the most likely candidate responsible for I_{KSper}. mSlo3 is testis-specific and its ion channel properties resemble those of I_{Ksper}. mSlo3 is not functionally expressed in mammalian cell lines, but in *Xenopus* oocytes produces measurable currents potentiated by intracellular alkalinization. Currently, lack of specific antibodies available prevents immunocytochemical identification and localization of mSlo3. Deletion of the *Slo3* gene from the murine genome will be required to determine if I_{KSper} is encoded by *mSlo3*.

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I_{KSper} controls the sperm membrane potential

Current clamp experiments reveal the strong pH_i dependence of the sperm membrane potential. NH_4Cl-induced intracellular alkalinization (pH_i increased from 6 to ~8) hyperpolarized the resting membrane potential from ~0 mV to ~−54 mV (Navarro *et al*., 2007). This hyperpolarization was K^+ -dependent and blocked reversibly by quinine, EIPA and $BaCl_2$, and for prolonged durations by clofilium. Together with the lack of other observed currents, these data identify I_{KSper} as the dominant hyperpolarizing conductance within the physiological range. Thus I_{KSper} sets the spermatozoa resting membrane potential.

Integrated Model and Conclusion

The oviduct, where most capacitation occurs, is high in bicarbonate (~40 to 90 mM; (Maas *et al*., 1977, Tienthai *et al*., 2004)). Capacitation is defined phenomenologically as the acquisition of the sperm's ability to fertilize an egg, but includes changes in sperm membrane reorganization, an array of tyrosine phosporylation events, increases in $[Ca^{2+}]_i$ and membrane hyperpolarization (Yanagimachi, 1994). As a result, sperm cells change from progressive to hyperactivate motility and acquire the ability to acrosome react.

In vitro fertilization (IVF) procedures are possible in part because sperm capacitation can be reproduced *in vitro*. The essential condition for sperm cell *in vitro* capacitation is incubation at 37°C in a medium containing Ca^{2+}, 0.4% serum albumin and 20 mM HCO_3^- (Visconti *et al*., 1999b, Yanagimachi, 1994). Serum albumin allows cholesterol efflux from the sperm plasma membrane and perhaps induces sperm membrane reorganization (Visconti *et al*., 1999a). Bicarbonate (Gadella and Van Gestel, 2004) has a profound effect on mammalian sperm function, but the HCO_3^- entry pathway is not known. HCO_3^- directly activates the sperm-specific soluble adenylyl cyclase (sAC; **Figure 3**) (Chen *et al*., 2000). Activation of sAC increases cAMP levels, which in turn activates protein kinase A (PKA)-dependent phosphorylation cascades, and tyrosine phosphorylation (Visconti *et al*., 1995a, Visconti *et al*., 1995b). *sAC-null* mice are infertile, spermatozoa motility is severely impaired (Esposito *et al*., 2004), and they lack the pattern typical of tyrosine phosphorylation.

Activation of the sperm-specific Na^+/H^+ exchanger (sNHE), has been postulated to account for sperm intracellular alkalinization. Mice lacking this gene are sterile with severely decreased sperm motility (Wang *et al*., 2003). sNHE protein contains a voltage sensor domain and a cyclic-nucleotide binding site, suggesting that this protein may be regulated by cAMP and/ or membrane potential. Surprisingly, *sNHE-null* spermatozoa are deficient in sAC, which together with co-immunoprecipitation experiments indicates that sAC forms a complex with sNHE (Wang *et al*., 2007). It is still unknown whether cAMP directly or indirectly actives sNHE. It has not yet been technically possible to measure pH in the principal piece where sNHE is localized.

In summary, direct recordings of epididymal sperm cells under whole-cell voltage clamp reveals two primary currents, $I_{CatSper}$ and I_{KSper}. Intracellular alkalinization activates these two specific sperm-ion currents. $I_{CatSper}$ is responsible for the increase in $[Ca^{2+}]_i$ during capacitation that enables hyperactivated motility. *CatSpers1-4-null* mice are infertile and the

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spermatozoa fail to develop hyperactivated motility. Ca^{2+} influx through CatSper channels increases flagellar bending in a Ca^{2+}-dependent manner; perhaps (based on work in other cells, e.g. *Chlamydamonas*) by binding to calmodulin, activation of camodulin kinase, and phosphorylation of radial spoke protein(s) of the axoneme. Also, *CatSper1-null* spermatozoa have diminished intracellular ATP (Xia *et al*., 2007), suggesting that CatSper-specific Ca^{2+} entry may initiate flagellar glycolysis. However, Ca^{2+} also stimulates enzymes of the Kreb's cycle in mitochondria (see (Kirichok *et al*., 2004, Rizzuto *et al*., 2000)). Thus Ca^{2+} *via* $I_{CatSper}$, or combined with Ca^{2+} from intracellular stores, is likely to be required for the sustained ATP production needed for extended motility and hyperactivation. In contrast, I_{KSper} sets the sperm cell membrane potential and is responsible for the hyperpolarization associated with sperm capacitation. Currently there is no evidence for Ca_V, Cl^-, or cyclic nucleotide-gated currents from direct voltage clamp of mammalian epididymal sperm cells. To play a role in fertilization, these currents would have to be rapidly upregulated after exit from the epididymis. We have not extensively tested for transmitter-gated channels (known or unknown, e.g. chemoattractants), or receptor tyrosine kinase and G protein coupled receptor modulation of $I_{CatSper}$ or I_{KSper}. These studies will be interesting avenues for the future.

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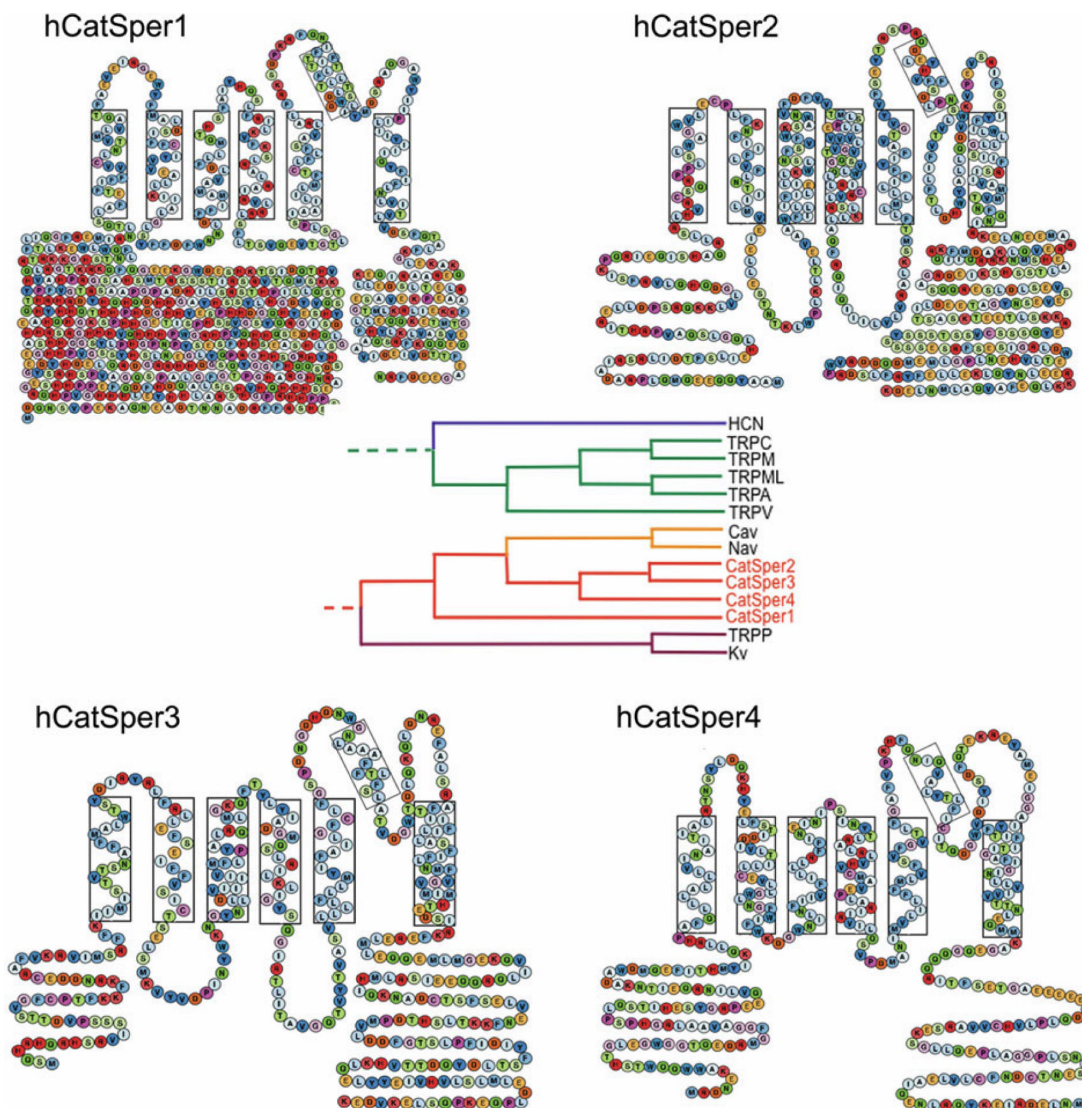

Figure 1. Human CatSper1-4 predicted secondary topology

CatSper1-4 have 6TM with a putative voltage sensor (S1-S4) and a pore (S5-S6) domain. Phylogenetic tree (anchored to NaChBac) is shown in the center. Boxes indicate putative transmembrane segments.

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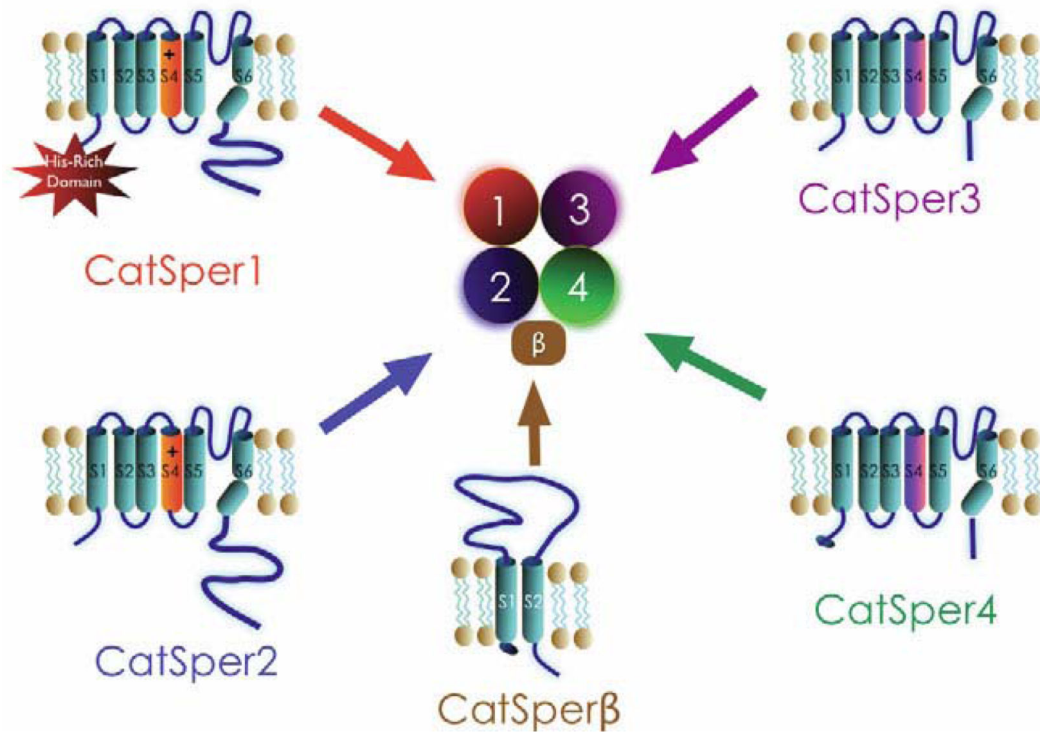

Figure 2. Heterotetramerization of the CatSper channel

The CatSper complex is formed from CatSpers1-4 protein subunits and an auxiliary subunit. The CatSper auxiliary subunit, CatSperβ, has 2 predicted transmembrane segments, separated by a large extracellular loop (~1000 amino acids).

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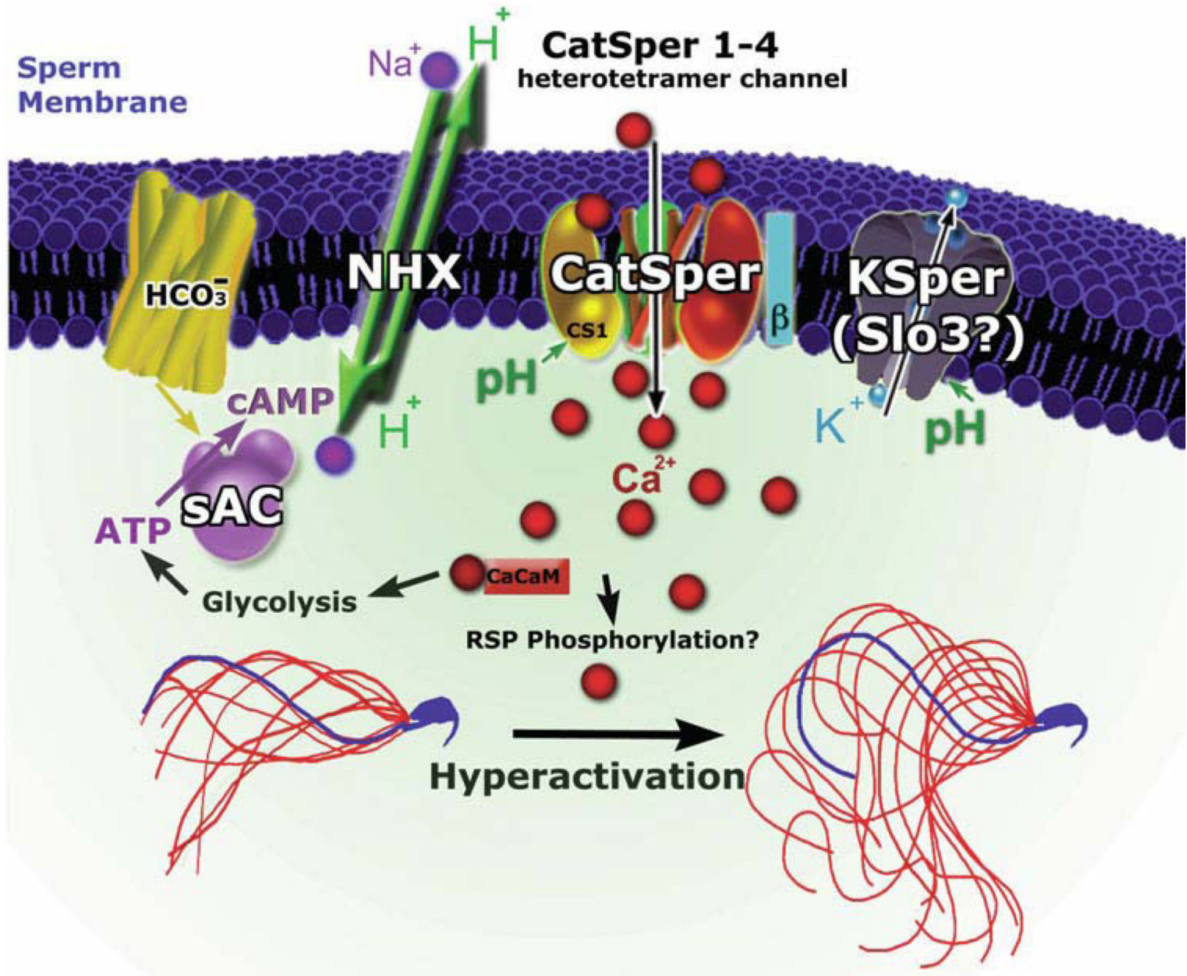

Figure 3. Spermatozoa ion channel activation and regulation integrated model

HCO_3^- ions bind and activate soluble adenylyl cyclase (sAC) that in turn increases cAMP synthesis. cAMP activates (direct or indirectly) the sperm specific Na^+/H^+ exchange (sNHE), thereby increasing intracellular pH. This intracellular alkalinization potently increases $I_{CatSper}$ and I_{KSper}. Alkalinization-mediated potentiation of I_{KSper} hyperpolarizes the membrane potential from ~0 to ~ −50 mV. Ca^{2+} entry through $I_{CatSper}$ induces a rise in $[Ca^{2+}]_I$, in turn activating Calmodulin and Calmodulin Kinase (CamK). These changes increase flagellar bending and enhance ATP production, thus increasing sperm endurance and hyperactivating sperm motility. Images of normal and hyperactivated motile sperm modified after Carlson, et al., 2005.