CatSperβ, a Novel Transmembrane Protein in the CatSper Channel Complex

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Four CatSper ion channel subunit genes (CatSpers 1–4) are required for sperm cell hyperactivation and male fertility. The four proteins assemble (presumably as a tetramer) to form a sperm-specific, alkalinization-activated Ca\(^{2+}\)-selective channel. We set out to identify proteins associating with CatSper that might help explain its unique role in spermatozoa. Using a transgenic approach, a CatSper1 complex was purified from mouse testis that contained heat shock protein 70-2, a testis-specific chaperone, and CatSperβ, a novel protein with two putative transmembrane-spanning domains. Like the CatSper ion channel subunits, CatSperβ was restricted to testis and localized to the principal piece of the sperm tail. CatSperβ protein is absent in CatSper1−/− sperm, suggesting that it is required for trafficking or formation of a stable channel complex. CatSperβ is the first identified auxiliary protein to the CatSper channel.

During mammalian fertilization, Ca\(^{2+}\) is crucial for sperm capacitation, motility, the acrosome reaction, sperm-egg fusion, and the activation of the fertilization wave in eggs (1). Depolarization, intracellular alkalinization, progesterone, cyclic nucleotides, and egg coat proteins trigger Ca\(^{2+}\) influx in mammalian sperm (1–4). The dominant Ca\(^{2+}\)-selective current in epididymal sperm is mediated via CatSper, although transient receptor potential (TRP), Ca\(_V\), and cyclic nucleotide-gated channel proteins have been detected in sperm cells and their precursors (see Refs. 5–7 for review). Of >20 ion channel genes disrupted in mice, only CatSper family mutations result in male infertility (6).

CatSpers1–4 are expressed in testis and localized primarily to the principal piece of sperm tail (8–12). CatSpers are most closely related to the six-transmembrane (TM)\(^2\) voltage-gated sodium channel (Na\(_V\)BP) in bacteria, with the next closest relatives being the large mammalian Ca\(_V\) and Na\(_V\) channels (13). CatSpers contain positively charged amino acids interspersed within their S4 transmembrane segments, suggesting they are voltage-sensitive channel subunits. CatSper1 is relatively unique and contains a remarkable abundance of histidine residues in its amino terminus, perhaps related to the known pH sensitivity of the CatSper Ca\(^{2+}\) current (14). CatSpers are also present in sea urchin and the ascidian Ciona intestinalis (15).

Targeted disruption of the mouse CatSper1 resulted in complete male infertility in an otherwise normal mouse (9). Although mutant mouse mating behavior, sperm count, and sperm cell morphology were indistinguishable from wild type (WT) mice, mutant sperm motility was abnormal. These sperm had reduced basal velocity and lacked vigorous beating and bending in the tail region. Mutant spermatozoa failed to fertilize intact eggs but could fertilize those in which their outer layers had been enzymatically removed (9). Further studies showed that CatSper null sperm cells could not be hyperactivated under physiological conditions (16, 17). Interestingly, depolarization evoked an increase in intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) in WT sperm cells, but not in CatSper1 null spermatozoa (16). The phenotype of CatSper2, 3, and 4−/− mice was indistinguishable from CatSper1−/− mice, and their sperm also lacked the hyperactivated motility needed for fertilization (12, 18). Whole sperm patch clamp of epididymal sperm showed that CatSper current is absent in CatSper1, 2, 3, and 4−/− mice (12, 14). Finally, CatSper genes appear to have similarly important roles in human fertility. Subfertile men with deficient sperm cell motility had significantly reduced expression of CatSper1 (19). CatSper2 has been implicated by linkage analysis in human asthenoteratozoospermia (20).

Despite the essential roles of CatSper proteins in sperm Ca\(^{2+}\) signaling and mammalian fertilization, detailed biophysical and structure-function studies have been hindered by the lack of function in heterologous expression systems essential for these studies. None of the four mammalian CatSper channel proteins expressed in heterologous expression systems, alone (HEK293 cells, Chinese hamster ovary-K1 cells) or in combination (Xenopus oocytes) (9), produced detectable i\(_{\text{CatSper}}\). Similarly, two CatSper homologs from sea urchin testis and two from ascidian C. intestinalis did not yield current when expressed in mammalian cells and Xenopus oocytes. In contrast, the functions of the majority of the several hundred channels (K\(^{+}\), Cl\(^{-}\), Ca\(^{2+}\), Na\(^{+}\), CNG (cyclic nucleotide-gated), and

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number(s) EF199807.

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2 The abbreviations used are: TM, transmembrane; Ca\(_V\), voltage-gated Ca\(^{2+}\) channel; eGFP, enhanced green fluorescent protein; HA, hemagglutinin; HEK, human embryonic kidney; HSP, heat shock protein; i\(_{\text{CatSper}}\), CatSper channel current; Na\(_V\), voltage-gated Na\(^{+}\) channel; ORF, open reading frame; WT, wild-type.

3 D. Ren and J. Xia, unpublished observations.
CatSper Channel β Subunit

transient receptor potential (TRP)) found in somatic cells have been faithfully reproduced in heterologous systems (21, 22).

One potential reason for the failure of functional heterologous expression is the lack of auxiliary subunits needed for proper channel trafficking, assembly, or gating. In this report, we describe a transgenic strategy to purify proteins associating with CatSper1. We found that a partial CatSper1 complex containing a genomic DNA fragment (genomic DNA library) was used to engineer a transgenic construct containing a genomic DNA fragment (genomic DNA library) was used to engineer a transgenic construct containing a genomic DNA fragment (~4 kb from an Nhel site) 5’ to the CatSper1 open reading frame (ORF), and a fragment of ~9 kb covering the whole ORF, and 0.5 kb 3’ to the ORF. A HA-eGFP fusion gene without a stop codon was synthesized by PCR using an eGFP vector (Clontech) as template. This mini-gene was inserted in-frame with the CatSper1 ORF (see Fig. 1 for detail). All fragments generated using PCR were sequenced to ensure that no unintended mutations were introduced. The insert, cloned in the pBluescript II SK vector (Stratagene), was excised, gel-purified, and used for pronuclear injection (Children’s Hospital, Boston, MA). Founders carrying the transgene, was excised, gel-purified, and used for pronuclear injection (Children’s Hospital, Boston, MA). Founders carrying the transgene but lacking WT CatSper1. Mice homozygous for the insertion were generated by crossing hemizygous mice and selected by semi-quantitative PCR and mating tests.

Ca²⁺ Imaging—Ca²⁺ imaging was as previously described (9). Briefly, caudal sperm were collected, loaded with Fura-2, and seeded onto coverslips coated with Cell-Tak (BD Biosciences). The ratiometric dye Fura-2 was used to minimize contributions from GFP in the transgenic sperm. Signals collected in the head region were analyzed. Imaging and analysis employed an inverted fluorescence microscope (IX-71; Olympus) with monochromator (DeltaRAM V; PTI), a cooled CCD camera (CoolSNAP HQ; Roper Scientific), data acquisition system, and control software (ImageMaster; PTI). Only cells that contributed from GFP in the transgenic sperm. Signals collected in the head region were analyzed. Imaging and analysis employed an inverted fluorescence microscope (IX-71; Olympus) with monochromator (DeltaRAM V; PTI), a cooled CCD camera (CoolSNAP HQ; Roper Scientific), data acquisition system, and control software (ImageMaster; PTI). Only cells that were evenly loaded with dye and motile were chosen for analysis (T = 20–25 °C).

Sperm Whole-cell Patch Clamp Recordings—Sperm whole-cell patch clamp recordings followed previously described methods (14). Corpus epididymal sperm were collected from 3–7-month-old mice. Giga seals were formed between glass pipettes (7–10 MΩ) and the cell membrane of sperm cytoplasmic droplets. Pipette solutions contained (in mM) 135 cesium-methanesulfonate, 5 CsCl, 5 HEPES, 10 EGTA, 5 Na₂ATP, and 0.5 Na₂GTP (pH 7.2 with CsOH). HS bath solution contained (in mM) 135 NaCl, 5 KCl, 2 CaCl₂, 1 MgSO₄, 20 HEPES, 5 glucose, 10 lactic acid, and 1 pyruvic acid (pH adjusted to 7.4 with NaOH). Divalent-free Na⁺ bath solution contained (in mM) 150 sodium gluconate, 20 HEPES, 5 HEDTA (pH 7.4 with NaOH).

In the absence of extracellular divalent ions, CatSper channels became permeable to monovalent cations (14), leading to an apparent reversal potential of ~0 mV in Fig. 2A. Recordings were made with an Axopatch 200B amplifier controlled by pCLAMP8.2 software through a Digidata 1322A interface (Axon). Signals were low pass-filtered at 1 kHz and sampled at 5 kHz.

Protein Purification—All purification steps were carried out at 4 °C unless otherwise stated. Frozen testes were homogenized in binding buffer (1.5 ml/testis) containing 50 mM NaH₂PO₄ and 300 mM NaCl, pH 8.0, supplemented with EDTA-free proteinase inhibitor mixture (protease inhibitor cocktail (PIC); Roche Applied Science). The homogenate was spun at 1,000 × g for 10 min at 4 °C. The supernatant was centrifuged at 100,000 × g for 50 min to obtain the microsomal fraction. ~100 mg of protein was solubilized for 1 h in 40 ml of buffer containing 50 mM NaH₂PO₄, 300 mM NaCl, 1× detergent and EDTA-free PIC, pH 8.0. Detergent contained 1% Nonidet P-40, 0.05% deoxycholate, and 0.1% SDS (radioimmune precipitation buffer) in one large scale purification, but only 1% Nonidet P-40 in several other such purifications. Following a 15-min spin, the supernatant was added to 1.5 ml of buffer-equilibrated cobalt resin and 1.6 ml of imidazole (1 M, pH 7.5), resulting in a final imidazole concentration of 37 mM. The large number of histidine residues in the amino terminus enabled CatSper1 to bind cobalt (9). After mixing 1 h, unbound protein was washed 2× using the same binding buffer supplemented with 40 mM imidazole, followed by wash in pH 6.5 buffer. Bound protein was eluted (22 °C) with 3 ml of elution buffer containing 50 mM NaH₂PO₄, 300 mM NaCl, 300 mM imidazole, 1× detergent, and EDTA-free PIC, pH 7.0. The eluate was concentrated to ~0.7 ml (YM-100; Centricron) and the 0.7 ml of concentrate added to 100 μl of buffer-equilibrated anti-HA affinity beads (Roche Applied Science) and 3.3 ml of IP buffer containing 150 mM NaCl, 2 mM EDTA, 1× EDTA-free PIC, 0.5× detergent, and 10 mM HEPES, pH 8.0, mixed 2 h, and washed 3× with IP buffer. Bound protein was eluted with 200 μl of HA peptide (5 mg/ml in IP buffer) for 15 min at 37 °C. The eluate was concentrated and resuspended in an lithium dodecyl sulfate (LDS) gel loading buffer (Invitrogen). Samples were heated for 15 min at 70 °C before being loaded onto a 4–12% Bis-Tris gradient gel (Invitrogen). After electrophoresis in SDS denaturing buffer, the gel was fixed and stained with Coomassie Blue (R-250) or silver stain for scaled down pilot purifications. Protein bands were excised and stored in 1% acetic acid before protein identification.

Protein Identification—Peptides from in-gel trypsin digestion were separated on a C18 column with a nano liquid chromatography system (Eksigent) and subsequently sequenced on-line using a nanospray/Qstar-XL mass spectrometer (ABI). Analyst QS software was used for data analysis and Mascot for data base searches (University of Pennsylvania Proteomics Facility).

Cloning of CatSperβ—The 12 peptide sequences (covering 109 amino acids) identified by mass spectrometry were used to search expressed sequence tag and genomic databases. Primers were designed according to the available sequences and used to amplify the whole ORF from mouse testis first
strand cDNA by PCR. PCR products were subcloned into a modified pTracer-CMV2 vector (Invitrogen) and multiple clones sequenced. Sequences were blasted against cDNA and genome databases to ensure that the clones selected for further analysis were free of mutations. The start of the ORF was unambiguously determined by the presence of an in-frame stop codon in the 5′-untranslated region. The predicted sequences of the human and C. intestinalis CatSperβ homologs were from NCBI data bases and the C. intestinalis cDNA data base (23).

**Multiple Tissue Reverse Transcription PCR**—PCR was performed according to standard protocols using commercial multiple panel cDNAs (Clontech) as templates. The sequences of the forward and reverse primers for CatSperβ amplification were (5′ to 3′) AGGGTACTGTTCGATAGGCTAC and ACAGTTGACTGGTAGGTGAGTCCAG, respectively. Samples were denatured for 2 min at 94 °C, followed by 35-cycle amplification, denature (20 s at 94 °C), annealing (20 s at 58 °C), and extension (30 s at 72 °C). Reactions were incubated at 72 °C for 10 min. Aliquots withdrawn from the reactions at 30 amplification cycles were also analyzed. Comparison between products after 30 and 35 cycles indicated no saturation. Mouse G3PDH gene was used as control for cDNA input.

**In Situ Hybridization**—Frozen mouse testis sections (~10-μm thick) were used for in situ staining. Single strand, digoxigenin-labeled RNA probes were synthesized from the double strand DNA templates (nucleotides 1940–3323) generated using PCR with primers attached to a T7 sequence. This fragment had no significant sequence similarity to other genes. A sense probe was used as a negative control. Hybridizations were washed, and signals were visualized using alkaline phosphate-conjugated anti-digoxigenin antibody and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate.

**Electrophoresis and Western Blotting**—Unless otherwise stated, pre-cast 4–12% Bis-Tris gradient gels without SDS (from Invitrogen) were used for electrophoresis. All the gels were run in electrophoresis buffer containing SDS. A fragment encoding amino acids 648–984 was cloned into the vector pET-32(a) and used for bacterial expression. The fusion protein was purified under denaturing condition and used for antibody production in rabbits. Because CatSper1 is only expressed in testis, anti-CatSperβ sera were preabsorbed against Sepharose beads conjugated with total protein from mouse brain and kidney to minimize non-specificity. Preabsorbed sera were used at 1:2000 for Western blots.

**Immunostaining**—Sperm were placed on coverslips and fixed with cold methanol at −20 °C for 10 min, followed by acetone for 1 min. Fixed sperm were blocked with 10% normal goat sera in phosphate-buffered saline for 30 min and incubated with anti-CatSperβ serum (1:50) at 4 °C overnight. After being washed three times with 1% Triton X-100 in phosphate-buffered saline, coverslips were incubated with Alex 594-conjugated goat anti-rabbit IgG secondary antibody (4 μg/ml) at 22 °C for 1 h and washed three times for 5 min each. Washed coverslips were mounted in 90% glycerol with anti-fade reagent and visualized under a Leica laser scanning confocal microscope. WT and CatSper1−/− sperm were processed identically.

**RESULTS**

**Transgenic Approach to CatSper1 Complex Purification**—Native ion channel plasma membrane proteins are of low abundance, requiring immunoprecipitation methods. Our anti-CatSper1 antibodies worked well for immunoblotting and immunostaining (9), but not for immunoprecipitation. To circumvent this problem we generated mice transgenic for CatSper1-HA-eGFP on the CatSper1−/− background. The mouse CatSper1 ORF covers 12 exons spanning ~9 kb; an HA epitope-tagged green fluorescence protein (HA-eGFP) encoding a mini ORF was inserted into the CatSper1 genomic DNA, immediately 5′ of the translational start site (Fig. 1, A and B). A genomic DNA fragment of ~4 kb 5′ of the first exon was used to drive the expression of the synthetic fusion gene. We established two independent lines with the transgene in the CatSper1−/− background (Fig. 1C). Both these lines were fertile, suggesting that the HA-eGFP-CatSper1 fusion protein fully rescued WT CatSper1 function. We did not observe any gross abnormality in the transgenic mice for a period of >4 years. This transgenic rescue experiment further confirmed that the male sterile phenotype in the CatSper1−/− mice was caused by the deficiency in the CatSper1 gene, but not by any potential spurious mutations introduced in the process of mutant generation.

Normal alkalinization-activated, voltage-dependent I_{CatSper} (Fig. 2) was recorded from HA-eGFP transgenic mouse epididymal sperm (n = 7), but was absent in all CatSper1−/− sperm (Ref. 14 and not shown). Fusion of the GFP protein to the amino terminus of CatSper1 did not abrogate the pH
suggest that the HA-eGFP-CatSper1 fusion gene functionally replaced the native CatSper1.

Commercial antibodies that efficiently precipitated the CatSper1 fusion protein from mouse testis were selected for use in fusion protein purification (Fig. 3A). In addition, the numerous amino-terminal histidine residues served as native metal binding sites. Indeed, CatSper1 bound well to a cobalt column under relatively stringent conditions (pH 6.5 wash with 1% Triton X-100, 40 mM imidazole). With these two “handles” on CatSper1, a simple strategy could be designed to purify the channel protein complex (Fig. 3B). The HA-eGFP-tagged CatSper1 protein solubilized from the transgenic testes was enriched on a cobalt column, followed by purification on an anti-HA antibody column. The protein eluted with the HA peptide was separated by SDS-PAGE. Proteins from CatSper1~−− (for small scale pilot purification) or WT (untagged CatSper1; for large scale purification) testes were used as negative controls. The purification procedure was highly efficient (silver stain detection with <5 mg of membrane protein starting material from 1–3 mice).

Identification of the CatSper1 Protein Complex—Three specific bands were identified (Fig. 3C). The bands from a purification using ~100 mg of protein (60 testes) were stained with Coomassie Blue, excised, trypsin-digested, and identified using mass spectrometry. Peptides from one band were identified as CatSper1 and GFP proteins. The second band was identified as testis-specific HSP70 (HSP70-2) (25). From the 12 peptides in the third band, a novel protein (CatSperβ) was identified. These three proteins (GFP-CatSper1, HSP70-2, and CatSperβ) were identified in two independent purifications. Using an HSP70-2-specific antibody for Western blot (25), we confirmed that HSP70-2 could be precipitated with anti-HA antibody from the transgenic mouse testis, presumably by the interaction between HSP70-2 and HA-eGFP-CatSper1, but not with anti-FLAG M2 antibody (negative control; not shown).

CatSperβ, a Novel Transmembrane Protein in the CatSper1 Complex—CatSperβ was identified from data base searches and reverse transcription PCR. The full-length mouse CatSperβ (GenBank™ access number EF199807) encodes an 1109-amino acid protein (126 kDa; calculated pl of 8.6; Fig. 4A). CatSperβ contains two clear transmembrane domains (6–22 of the NH2 terminus, and 1060–1076 at the carboxyl terminal domain; Fig. 4B). A large extracellular domain (~1000 amino acids) precedes the carboxyl terminal transmembrane segment. The predicted mouse intracellular carboxyl terminus is only 27 amino acids in length. Eight N-glycosylation sites are predicted in the polypeptide. The overall 2-TM topology is reminiscent of that of the large conductance K+ channel (BK) β subunits and the P2X receptor (ATP-gated channel) (26, 27), but CatSperβ (~126 kDa) is significantly larger (BK β subunits <30 kDa) (28). Data base searches indicate that CatSperβ is not predicted to be similar to any other protein of established function, although a putative extracellular fragment (Val-166-Gly-294) is weakly similar (42% similarity, 24% identity) to the extracellular loop of a P2X receptor (P2X3b, GenBank™ accession number NP_945337).

sensitivity of IcatSper because intracellular alkalization (induced by bath application of NH4Cl) readily potentiated IcatSper current (Fig. 2A). Membrane-permeant nucleotides increase [Ca2+], by an unknown process requiring CatSper1 expression (9, 24), although not via direct nucleotide activation of the CatSper channel (14). In HA-eGFP-CatSper1 transgenic sperm, application of cell-permeable cGMP (8-Br-cGMP, 2 mM) elicited an increase in [Ca2+], (Fig. 2B). Finally, based on observations from >100 litters over 4 years, the transgenic mice were fertile. Taken together, these data
Human CatSperβ (GenBank™ accession number AK126034) is located on chromosome 14. Mouse (C57BL/6) has two copies of CatSperβ, separated by ∼37 kb on chromosome 12. For reasons not fully understood, fertilization-specific proteins seem to be less conserved between species (29). Like CatSper1, CatSperβ is relatively poorly conserved between human and mouse (amino acid identity ∼56%; Fig. 4A). A CatSperβ homolog, CatSper Channel β Subunit

**FIGURE 3.** Purification of the CatSper1 protein complex. A, transgenic testis membrane proteins were solubilized and precipitated with anti-GFP or anti-HA antibodies. The eluate and flow-through were probed with anti-CatSper1 antibody. B, strategy used to purify the CatSper1-containing protein complex. C, the purified CatSper1 protein complex was separated by SDS-PAGE and stained with silver. Proteins from CatSper1 /- testes were used as controls. Three specific bands are indicated by arrows.

**FIGURE 4.** CatSperβ, a novel transmembrane protein isolated in the CatSper1 protein complex. A, alignment between the predicted mouse (upper) and human (lower) CatSperβ protein sequences. The eight putative glycosylation sites in the mouse sequence are indicated by *. The two predicted transmembrane domains (TM1, TM2) are boxed. The 12 peptide sequences identified by mass spectrophotometry are underlined. Amino acids 156–183 were identified as three peptides, 156–165, 166–173, and 174–183. B, hydrophilicity plot (window size = 11). Alternative TM prediction model suggests a third potential TM segment (indicated by arrow).
log in the marine chordate C. intestinalis is predicted to encode a 977-amino acid protein. The sequence identity between the mouse and C. intestinalis CatSperβ homologs is 21%, with highest homology in the region close to the carboxyl terminus.

Restricted Expression of CatSperβ mRNA in Testis—Among the eight adult tissues/organs and embryos of four developmental stages examined, only testis had detectable expression of CatSperβ mRNA (Fig. 5A). In situ mRNA antisense revealed robust, specific staining of CatSperβ in the seminiferous tubules; no significant signal was detected in the interstitial cells (Fig. 5B). Within the tubules, CatSperβ appears to be expressed in spermatocytes and spermatids, but not in spermatagonia (Fig. 5B), similar to CatSpers 1–4.

Disruption of CatSperβ Protein in CatSper1<sup>−/−</sup> Sperm—We developed a polyclonal antibody against amino acids 648–984 of CatSperβ protein. Taking advantage of the restricted expression of CatSperβ, nonspecific activity of the antibody was reduced by preabsorption of the antibody sera by total protein from mouse brain and kidney. The purified antibody specifically recognized a protein band from CatSperβ-transfected HEK293 cells, but not from mock-transfected cells. B, interaction between CatSperβ and HA-eGFP-CatSper1 transgenic mice were immunoprecipitated with anti-GFP or anti-FLAG M2 antibody (negative control) and blotted with anti-CatSperβ antibody. The lower bands are presumed immunoglobulins (lg). C, disruption of CatSperβ protein in sperm, but not in testis, in the CatSper1 mutant mice. Total sperm (7.5 × 10<sup>7</sup> cells) (upper) or testis (50 μg) (lower) proteins prepared from WT (+/+), CatSper1<sup>−/−</sup>, and the HA-eGFP-CatSper1 transgenic (Tg; in CatSper1<sup>−/−</sup> background) mice were blotted with anti-CatSperβ antibody. Protein expressed from CatSperβ-transfected HEK293 cells was loaded on the same gel as the molecular weight reference (lane ctrl). Prolonged exposure did not reveal a CatSperβ band in the CatSper1<sup>−/−</sup> lane (not shown). The migrations of CatSperβ in panels B and C were aberrant due to lower SDS content of the commercial gel compared with our own in panel A. D, immunofluorescence detection with anti-CatSperβ antibody in WT (+/+) and CatSper1<sup>−/−</sup> sperm. Immunofluorescence (in green) localizes specifically to the principal piece. Scale bar, 10 μm.

In mouse sperm, the CatSperβ antibody recognized a single major band migrating at the same position as recombinant CatSperβ protein from HEK293 cells (Fig. 6C). Interestingly, CatSperβ was undetectable in the CatSper1 mutant sperm, but was present in CatSper1 mutant testes. In HA-eGFP-CatSper1 transgenic mice, CatSperβ protein was again detected in the mutant sperm (Fig. 6C). Like CatSper1, CatSperβ protein was localized to the sperm principal piece and was largely absent in the CatSper1<sup>−/−</sup> sperm (Fig. 6D). These data suggest that CatSperβ protein is colocalized with, and dependent upon,
CatSper1 protein. Because CatSper2, 3, and 4 antibodies are not as specific for their targets by immunohistochemistry, CatSperβ dependence on these proteins will be examined in more detail using CatSper 2, 3, and 4−/− mice.

DISCUSSION

We used a tagged CatSper1 transgenic mouse to enable unambiguous purification of CatSper1-associating proteins from testes. Previous work has suggested that CatSper1−4 form a heterotetrameric complex surrounding the Ca2+ selective pore (12). In this report, we have shown that a novel auxiliary subunit, CatSperβ, accompanies CatSper proteins. We suggest that CatSperβ is a subunit of the CatSper complex based on its in vitro co-purification, in vivo colocalization, disappearance in CatSper1−/− sperm, and reappearance in sperm from mice rescued with tagged CatSper1. Interestingly both CatSper and CatSperβ are lacking in Caenorhabditis elegans and Drosophila but both appear in the ascidian C. intestinalis, suggesting that the origin of this molecular complex structure can be traced back to before the divergence among deuterostomes.

The CatSper channel complex is reminiscent of the maxi-K Ca2+ activated K+ channel, which consists of a pore-forming α subunit (7-TM) and a 2-TM β subunit. Many other channel complexes have transmembrane auxiliary subunits. In CaV channels, the single TM α2/β subunit not only increases channel protein surface expression but also affects kinetics (30). The 1-TM β subunit of NaV channels has a short intracellular tail but large extracellular domains that also mediate cell-cell interaction (31, 32). The CatSperβ protein is predicted to have a large extracellular fragment (~1000 amino acids) with several putative N-glycosylation sites, and we speculate that these extracellular domains could be “sensors” for sperm interactions with other cell types or surfaces.

Not surprisingly, heat shock proteins have been implicated in channel assembly, including the HERG K+ channel (33), the CIC-2 Cl− channel (34), and the CFTR Cl− channel (35). Here, we find that the testis-specific HSP70-2 purifies with CatSper1, suggesting that sperm-specific membrane proteins such as channels require unique chaperones for their correct folding/assembly or trafficking. Unfortunately, preliminary experiments in which HSP70-2, CatSperβ, and all four CatSper subunits were expressed in Xenopus oocytes did not yield functional currents. Similarly, we did not observe changes in the subcellular localization of CatSper1 protein when co-expressed with CatSperβ in cultured HEK293 cells. Functional expression of sperm-specific proteins commonly fails in heterologous systems, as it does in proteins specific to other ciliated structures (e.g. olfactory receptors) (36). We can only speculate as to why this might be so; functional heterologous CatSper channel formation may require 1) additional unknown protein subunits, 2) an intact intraflagellar transport system (37), 3) the unique lipid composition of sperm plasma membrane (38), 4) sperm-specific cytosolic composition (39), or 5) all the above. Whatever the reasons may be for this complexity, the CatSper protein complex appears to consist of some of the most unique and diverse elements in the field of ion channels.

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