Core course 2017

Research in my lab
- Contraceptive vaccine Nov 1
- Biosensors Nov 3

Practical bioinformatics
- Using NCBI Nov 6
- Molecular modeling Nov 8
Global population is growing exponentially!
The overpopulation problem, up close.
CatSper

- 4 subunits + 5 associated proteins
- All 9 required for male fertility
- Responsible for sperm hyperactive motility
- Expressed exclusively in sperm
- 6-transmembrane Voltage-gated calcium channel (Ca\textsubscript{\text{v}})
- Heterotetramer
- Not glycosylated
--- > candidate for contraceptive vaccine
- Structure not known.
Homolog sequences for each CatSper subunit were identified by searching the RefSeq database using BLASTp and restricting the search to mammals, using the Entrez search string "cation channel sperm-associated protein [n]" where n was 1, 2, 3, 4 respectively in four BLASTp searches. Sequences with the annotation "LOW QUALITY SEQUENCE" were removed.

Sequences were aligned in UGENE using MUSCLE in the default mode. Any sequences lacking any transmembrane helix domain were removed. Sequences were pruned to a maximum 90% identity.

Transmembrane regions are roughly aligned between CatSPers with the VS domain (s4) aligned.
Conserved cysteines in mammalian CatSper

Voltage Sensitive domain ——— Pore domain

1 2 3 4

S1 s1s2 S2 S3 s3+s4 S4 linker helix S5 s5p P-helix ps6 S6

1 2 3 4

C362 S393 C210 C499

C96 C210 C197

C114 C170 C233
6 TM helices

>gi|21314844|ref|NP_647462.1| cation channel sperm-associated protein 1 [Mus musculus]

MDQSSRDEFYHETHPGSQPSPHQPHPHPTLHRPNGVYDSDPQHGMFPQPYQOHGFHNQELQHLEFSDHSAHHYQQDRAGVSTLPNSHAYGSHEPQHEPSGPRIDPNNPHQDDPHEPSELPSSTTSQHCTHQQYHERSHHLNPQNRDHDATISYRSSTFRYSIAPFSSQERPHLHALDHHH
EGHHAHSHGEHHEHKEQHRYHDHMHMMHIIHRSPASAQLSHKSHTLATSFSHVGSKSTASGARYTFGARSQIFQRAGSRESLRESLQSEGEDDHQKRCRQRAKHAHTGNIQLWKEKISHLQLGLQQMLSTQS
LGQETTIFIVCLNTVILVAQTFTELEIRGEWYMVLDIIFLSILYVEAVLKLIALGLEYFYDPWNNLDFIMMVADLDFVLQINSLSSYNHSLFRLKFKSMRLRAIRVRRRLSSTLHENVATLGSGLPSITAITLTMTCCLFLSVRLAFFSDPKFQDNFITLTLFTMRTLDDWSLLYIDRNAQGAWYIIPIILMICIVIQYFILNLVIAVLVDNFMQALLKGLKKEVLEQARVHEKLDDSLTDLKNADANQMTEEAHKMQLI
EGMFGMTKQRVLHFQFLQLVAAVEQHQKFRSQAYVIDLVDMAFEAGDDYKG

Results from TMHMM server:
Homologs of CatSper with known structures

Arcobacter butzleri Cav
Vertebrate TRPV (capsaicin, cold)

heterotetramer has 6 "arrangomers"

4-fold tandem fusion
Cys in all 4 mouse CatSper mapped onto acrobacter monomer --> homotetramer

Which conserved Cys could be interacting?
Disulfides 1 and 2.

Can't cross-pair because of different depths.
hanging conserved Cys?

What could they be doing?
Conserved cysteines in mammalian CatSper
Transmembrane Cys can still form self-reacting SS when it mutates to a new position. Therefore, variable position conserved Cys are self-reacting. Single position conserved cys are cross-reacting.
**fixed evolutionary position** is consistent with **unlikely** mutational pathway

**variable evolutionary position** is consistent with **easy** mutational pathway

Variable position means self-reacting.
Conserved cysteines in mammalian CatSper

Ten representative sequences from mammals for each of the four CatSper α subunits were aligned (UGENE, MUSCLE) to the structurally-aligned homology model (MOE) of the mouse CatSper sequences.
Two-row model for super-quaternary structure

C4 N-term domain inserts between C1 and C2 N-term domains, creating a linear chain of tetramers, leaving the one remaining SH (C3 N-term domain) exposed. Single rows pair in anti-parallel fashion to give two-row model.
Two-row Catsper displays C4 N-term domain. If it self-associates, then antiparallel rows can stack,

19.4nm measured 90° from row direction, between a3a4 loops.
WT mouse cauda epididymal sperm cell #1, zoom-in

annulus

20nm thick

100nm separation

Acc.V  Spot  Magn  Det  WD  Exp
10.0 kV  3.0  54532x  SE  10.6  1
CatSper1-KO cauda epididymal sperm #2, zoom-in
3D STORM images of CatSper I in WT sperm

CatSper is quadrilaterally arranged in rows of width <100nm along the flagella.
Sperm competition


Chicago


Transmembrane Cys can still form self-reacting SS when it mutates to a new position. Therefore, variable position conserved Cys are self-reacting. Single position conserved cys are cross-reacting.
Aligned blocks from two regions of a multiple sequence alignment of 27 mammalian CatSper β’s. Mouse numbering is given above. The CXC motif is 80% conserved in the first site, 100% conserved in the second.
C-terminal sequences from 32 mammalian CatSper δ’s. Arrows mark where CXXC and CXC motifs are seen. Most species have the C-terminal extension. Those that have it conserve the motif.
Figure 4. In-solution immunostaining of live spermatozoa by CatSper antibodies. (A) Transmembrane topology of CatSper proteins showing extracellular epitopes (colored). Confocal images of (B) mouse sperm by anti-CatSperε, (C) human sperm by anti-CatSper1, and (D,D') human sperm by anti-CatSper4 recognizing two different regions.
fixed mouse sperm + anti-CatSper 4 (green), DAPI nuclear stain (blue)

Jean-Ju Chung, Yale Med. March 2016
Human Papilloma virus

- Antigenic carrier
- FDA-approved (Gardasil, Cerverix)
- Virus-like particles contain no DNA
- Folds and assembles with inserts.
- Chimeric proteins raise antibodies against inserts.
sperm

(a)

CatSper

(b)

epitope

(c)

immunity

(f)

female

(e)

VLP

(d)
Pregnancies by Intention Status

Nearly half of U.S. pregnancies are unintended.

- 55% Intended
- 27% Mistimed
- 18% Unwanted

www.guttmacher.org
Two-row Catsper displays C4 N-term domain. If it self-associates, then antiparallel rows can stack,

19.4nm measured 90° from row direction, between a3a4 loops.
Figure 6. Inhibition of motility and fertility by CatSper antibodies against extracellular epitopes of CatSper 1(a3a4) and epsilon (CSε). (A) Motility analysis of capacitated mouse sperm in the Presence of 20 ug/ml antibodies by CASA. (B) In vitro fertilization performed with COC eggs. Antibodies are treated either during sperm capacitation or fertilization at 20 ug/ml (unpublished).
HPV surface
Figure 2. Stereo image of three capsomeres (pentamers) of HPV based on the cryo-EM structure (3J6R, left). Brightly colored loops indicate the three insertion points, PNNNK (pink), SETTY(yellow), and SAYAAN (cyan). Sites are accessible to antibodies, widely separated from each other, and are known to fold correctly with insertions.
Figure 3. Transmission electron micrograph of the clarified lysate of wt L1 VLPs from human cell culture (HEK-293TT), uranyl acetate was used as a contrast agent. Scale bar is 60nm. Image by Haixin Sui, Wadsworth labs.