HEP (2007) 179:53–75
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TRPC2: Molecular Biology and Functional Importance

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Abstract TRPC (canonical transient receptor potential) channels are the closest mammalian homologs of *Drosophila* TRP and TRP-like channels. TRPCs are rather nonselective Ca^{2+} permeable cation channels and affect cell functions through their ability to mediate Ca^{2+} entry into cells and their action to collapse the plasma membrane potentials. In neurons the latter function leads to action potentials. The mammalian genome codes for seven TRPCs of which TRPC2 is the largest with the most restricted pattern of expression and has several alternatively spliced variants. Expressed in model cells, TRPC2 mediates both receptor- and store depletion-triggered Ca^{2+} entry. TRPC2 is unique among TRPCs in that its complete gene has been lost from the Old World monkey and human genomes, in which its remnants constitute a pseudogene. Physiological roles for TRPC2 have been studied in mature sperm and the vomeronasal sensory system. In sperm, TRPC2 is activated by the sperm's interaction with the oocyte's zona pellucida, leading to entry of Ca^{2+} and activation of the acrosome reaction. In the vomeronasal sensory organ (VNO), TRPC2 was found to constitute the transduction channel activated through signaling cascade initiated by the interaction of pheromones with V1R and V2R G protein-coupled receptors on the dendrites of the sensory neurons. V1Rs and V2Rs, the latter working in conjunction with class I MHC molecules, activate G_i- and G_o-type G proteins which in turn trigger activation of TRPC2, initiating an axon potential that travels to the axonal terminals. The signal is then projected to the glomeruli of the auxiliary olfactory bulb from where it is carried first to the amygdala and then to higher cortical cognition centers. Immunocytochemistry and gene deletion studies have shown that (1) the V2R-G_o-MHCIb-β2m pathway mediates male aggressive

behavior in response to pheromones; (2) the V1R-G_{i2} pathway mediates mating partner recognition, and (3) these differences have an anatomical correlate in that these functional components are located in anatomically distinct compartments of the VNO. Interestingly, these anatomically segregated signaling pathways use a common transduction channel, TRPC2.

Keywords TRP · TRPC2 · G protein · Acrosome reaction · Vomeronasal organ

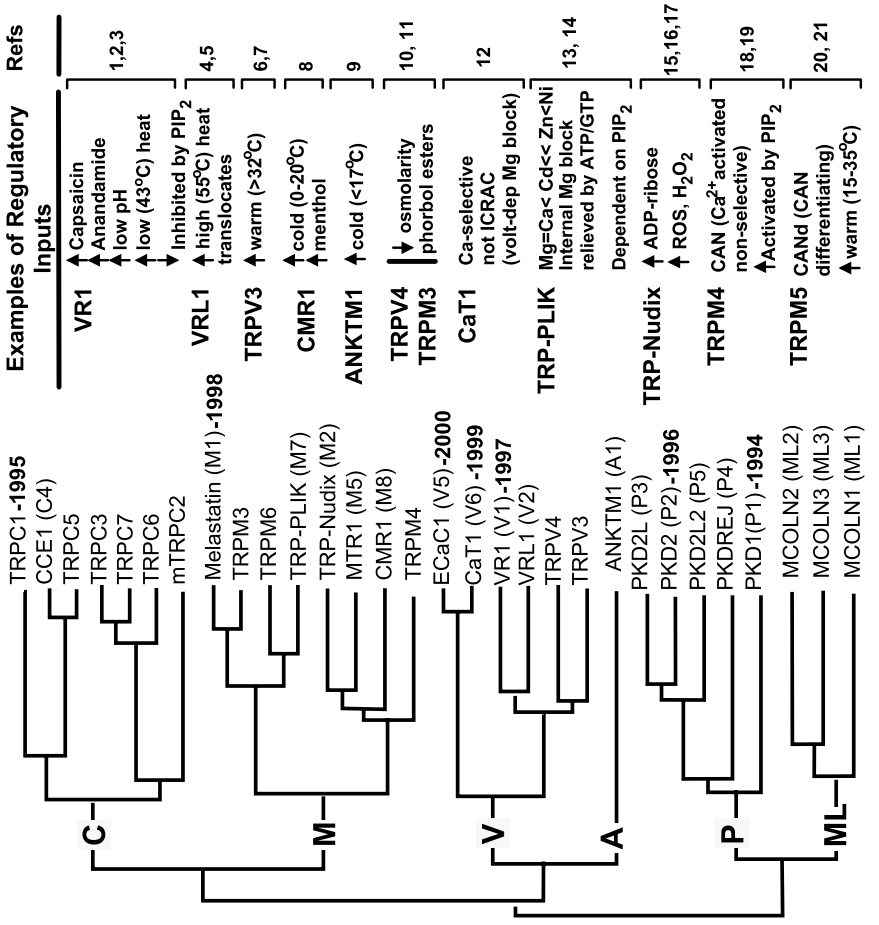
1 The TRP Family of Cation Channels

Activation of the Gq-phospholipase C (PLC) signaling results in hydrolysis of phosphoinositol bisphosphate (PIP_2) into two second messengers—diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3)—followed by IP_3-induced Ca^{2+} release from intracellular stores. Further, the depletion of intracellular Ca^{2+} stores activates Ca^{2+} permeable cation channels in the plasma membrane.

Mammalian homologs of the fly transient receptor potential (*trp*) channel, canonical TRPs (TRPCs), have been postulated as the pore-forming molecules through which store depletion-activated Ca^{2+} entry takes place (Birnbaumer et al. 1996). To date, seven subfamilies of TRP and TRP-like channels have been identified, and are found to be activated by an astounding set of diverse mechanisms and ligands. Figure 1 illustrates the phylogenetic relations among the TRP family of ion channels and lists some of signals and factors that

Fig. 1 (*Left*) Phylogenetic tree of TRP channels. The ion channel domains of TRP channels (reviewed in Birnbaumer et al. 2003) were analyzed by the GrowTree routine of the Wisconsin GCG Molecular Biology Package. Individual trees were constructed for the TRP C, M, V, P, ML and A subfamilies. Separately, a tree was constructed with the ion channel domains of one member of each of the subfamilies [TRPC3, TRPM2, TRPV1, PKD2 (TRPP2), TRPML1, TRPA1 (ANKTM1)], and used as anchoring branches for the families. Human sequences were used for all except the TRPC2, for which the mouse TRP channel domain sequence was used. The years that members of TRP subfamily were discovered are indicated in *bold* next to the channel names. (*Right*) The diverse regulatory inputs for different TRP channels. References (Ref): 1, Caterina et al. 1997; 2, Zygmunt et al. 1993; 3, Prescott and Julius 2003; 4, Caterina et al. 1999; 5, Kanzaki et al. 1999; 6, Peier et al. 2002; 7, Smith et al. 2002; 8, McKemy et al. 2003; 9, Story et al. 2003; 10, Guler et al. 2002; 11, Grimm et al. 2003; 12, Hoenderop et al. 1999; 13, Montilh-Zoller et al. 2003; 14, Runnels et al. 2002; 15, Perraud et al. 2001; 16, Wehage et al. 2002; 17, Hara et al. 2002; 18, Launay et al. 2002; 19, Nilius et al. 2006; 20, Hofmann et al. 2003; 21, Talavera et al. 2005

regulate their activity. While we have a somewhat better understanding of the physiological relevance of TRP-like channels (V, M, P, ML and A subfamilies), the role and the mechanism of activation of the TRPC class of channels is, for the most part, still incompletely understood. One exception is TRPC2, which was proposed to be the candidate cation entry channel that plays major roles in two distinct pathways: store-operated Ca^{2+} entry during acrosome reaction and pheromone-induced vomeronasal signaling. In this chapter, we review the properties of the TRPC2 channel and the current understanding of its physiological significance.



2
The TRPC2 Channel

2.1
Expression, Structure, and Regulation

The existence of the mouse TRPC2 gene was first reported by Zhu et al. (1996). This was followed by the cloning of bovine (Wissenbach et al. 1998), mouse (Vannier et al. 1999), and rat (Liman et al. 1999) complementary DNAs (cDNAs). Phylogenetic analysis of mammalian TRPC2s showed that the genes encode uninterrupted open reading frames (ORFs) with the potential of forming active channels in all vertebrates up to New World monkeys but no longer encode functional ORFs in Old World monkeys, apes and humans, in which the TRPC2 is a pseudogene (Liman and Innan 2003; Yildirim et al. 2003).

The mouse TRPC2 gene is found on chromosome 7 in a region that is syntenic to human chromosome 11p15.3–15.4. Rather than finding a full TRPC2 gene in this location of the human chromosome 11, we found the genomic region to encode a TRPC2-like transcript. Mapping the human cDNA to the human chromosome 11 sequence revealed an intron–exon structure similar to that of the mouse gene spanning from exons 14 to 21, with the exception that the sequence corresponding to exon 16, coding for the fifth transmembrane segment and half of the pore region, is absent. A search for TRPC2 sequences elsewhere in the human genome was negative, except for locating sequences similar to those comprising a fusion of mouse exons 2 and 3 approximately 70 Mb upstream of the sequences coding for the TRPC2-like transcript (Yildirim et al. 2003; Fig. 2).

Four variants, apparently having their origin in alternative splicing of the primary transcript, have been described for mouse TRPC2. They have been named TRPC2a, TRPC2b (Vannier et al. 1999), TRPC2α, and TRPC2β (Hofmann et al. 2000). The a and b forms correspond to the cDNAs originally reported as clones 14 and 17, respectively. They code for proteins of 1,172 and 1,072 aa, respectively, differing in their N termini. The shorter, TRPC2b (clone 17), differs from TRPC2a in its first 11 aa, with amino acids 12–1,072 being identical to amino acids 112–1,172 of TRPC2a. The α and β forms of TRPC2, reported by Hofmann et al. (2000), are identical to amino acids 287–1,172 of the TRPC2a. Codon 283 codes for the first P of the *P*QP motif located at the 5′-end of exon 10 (Fig. 3). Thus, the α, β, and a forms share sequences encoded in exons 10–21, differing upstream of exon 10. Amino acids upstream of PQP in TRPC2α and β are Met and Met-Asp-Pro-Leu-Ser (MDPLS), respectively. The TRPC2α cDNA was reported with 1,004 nt of 5′-untranslated sequence. The sequence alignment of the TRPC2a and TRPC2α cDNAs revealed that the first 713 nt of the 5′-untranslated sequence of TRPC2α are identical to those sections of the TRPC2a cDNA that are derived from exons 3–8, the remaining 291 nt just before the initiator ATG of TRPC2α are identical to the 5′-end of intron H

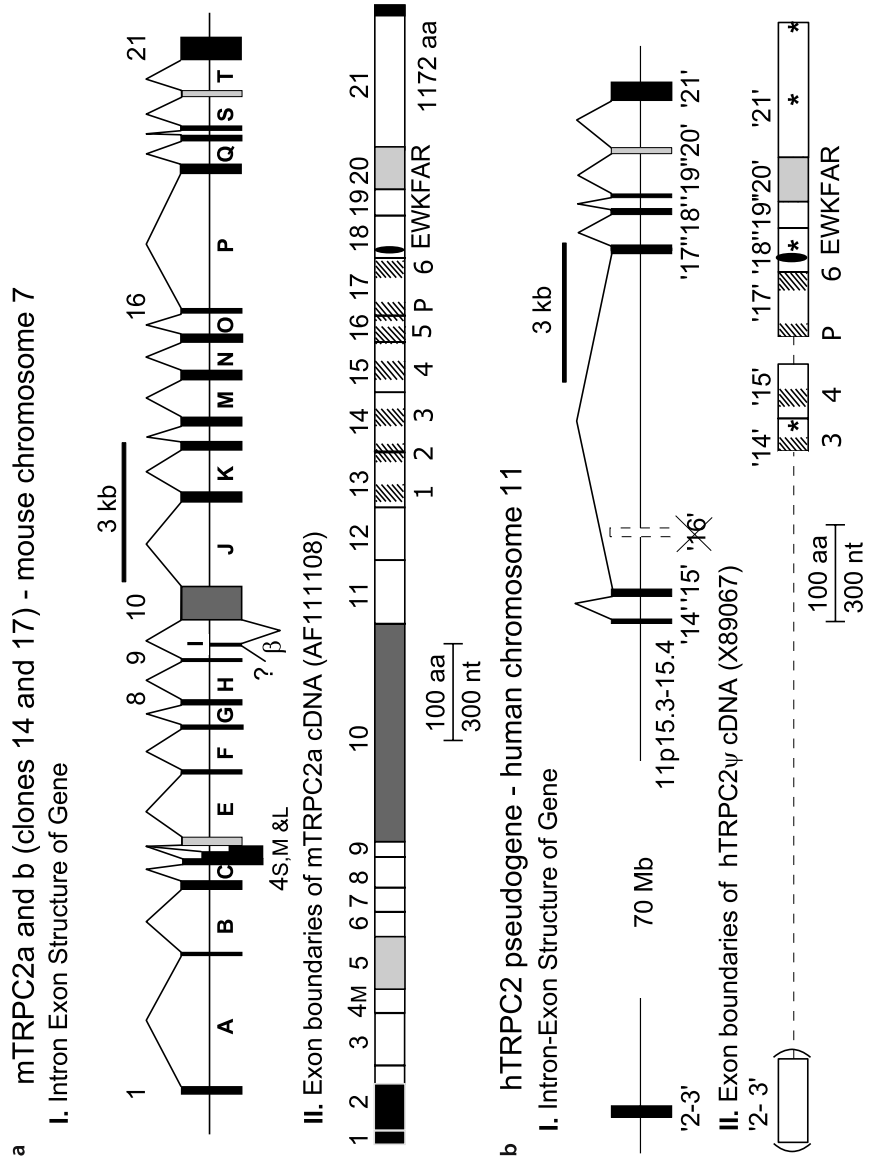


Fig. 2 a,b Comparison of the mouse chromosome 7 region harboring the TRPC2 gene to the syntenic region of human chromosome 11 harboring the TRPC2 pseudogene. **a** Intron–exon distribution along the chromosome is shown *above* the diagram of the cDNA with its exon boundaries. **b** Intron–exon distribution along the human chromosome is shown *above* the diagram of the human cDNA. Note that it leaves as undecided (*?*) the existence of an additional exon with a separate promoter located 5′ to exon β (between exons 9 and 10). The human Trpc2 sequences can be found in the complements to regions 63,880 to 74,537 of genomic contig NT_035090 and 2,272,990 to 2,336,104 of contig NT_033927 (reprinted from Yildirim et al. 2003 with permission)

between exons 8 and 9 (Fig. 3aII). It is noteworthy that the mouse TRPC2β is nearly identical to the rat TRPC2 cDNA, expressed in the vomeronasal sensory organ (VNO) (Liman et al. 1999). The ORF of the rat TRPC2β cDNA codes for a protein that is almost identical to TRPC2a from amino acids 289–1,172, beginning with MDPLSP, where the P corresponds to the second P of the PQP motif of mouse exon 10. As is the case for mouse TRPC2β, the upstream nucleotides reported for rat TRPC2 are wholly represented within the genomic sequence upstream of exon 10, giving no further information as to whether the cDNA is derived from a bona fide mRNA or not. Further tests will be required to settle this issue.

Kyte–Doolittle analysis of the amino acid sequence of TRPC2 protein identifies seven hydrophobic regions with the potential to form six transmembrane segments. The long N-terminus of TRPC2 was shown to contain ankyrin (reviewed in Birnbaumer et al. 2003), calmodulin (CaM) (Yildirim et al. 2003), and enkurin (Sutton et al. 2004) binding domains. In analogy to all other TRPCs, TRPC2 also has a CaM binding site on its C-terminus (Tang et al. 2001; Yildirim et al. 2003). In addition, the mouse TRPC2 C-terminus has binding site for junctate, an IP_3R-associated protein (Treves et al. 2004; Stamboulian et al. 2005). The role of these domains in channel function is not clear at this time.

Upon expression in model cells, the mouse TRPC2 variants a and b are activated by both mere store depletion and activation of a G_q-coupled receptor (Vannier et al. 1999; Fig. 4). It is also noteworthy that neither the murine TRPC2α nor the murine TRPC2β cDNAs encode a protein capable of forming active channels. Instead, when observed by confocal microscopy, TRPC2α and TRPC2β fail to reach the plasma membrane (Hofmann et al. 2000).

2.2 Functional Roles

2.2.1 The Acrosome Reaction

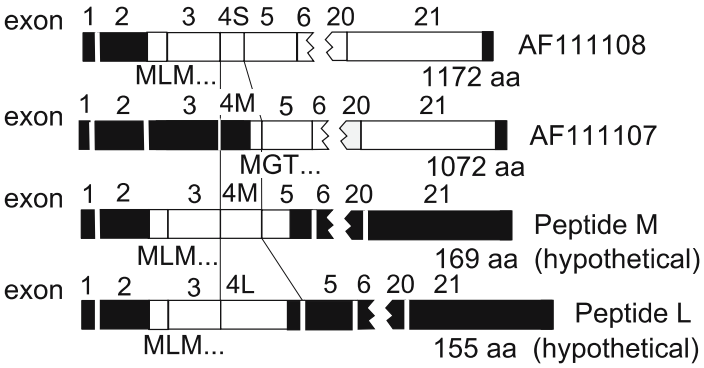
In mammals, oocyte fertilization by a sperm cell involves a preparatory acrosome reaction triggered by the interaction of the sperm head with zona pellucida proteins surrounding the oocyte (Fig. 5a). The acrosome reaction

Fig. 3 a Diagrams of ORF in mature TRPC2 transcripts based on RT-PCR results reported by Yildirim et al. (2003) and on α and β cDNAs reported by Hofmann et al. (2000). **b** Alignment of deduced amino acid sequences of ORFs encoded in the TRPC2 transcripts with exons 4S, 4M, and 4L. *Top line* of alignment is master sequence; -, amino acids identical to TRPC2a in TRPC2b, peptide L and peptide M, gap; *, stop; |, exon boundaries (reprinted from Yildirim et al. 2003 with permission)

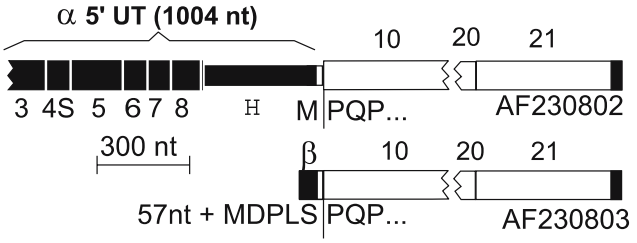
a

Predicted mTRPC2 ORFs based on cDNA and RT-PCR Analysis

I. formed by exons 1 through 21
using either exon 4S (86 nt), 4M (165 nt) or 4L (273 nt)



II. formed by exons α (1007 nt) plus 10 through 21,
or exons β (72 nt) plus 10 through 21



b

Deduced amino acid sequence of putative peptides M (exon 4M) and L(exon 4L)

```
        exon 2                    |exon 3
TRPC2a MLMSRTDSKS GKNRSGVRMF KD|GDFLTPAS GESWDRLRLT CSQPFTRHQS  50
Pept.M ---------- ---------- --|-------- ---------- ----------  50
Pept.L ---------- ---------- --|-------- ---------- ----------  50
                                        |exon 4
TRPC2a FGLAFLRVRS SLGSLADPVV DPSAPGSSGL NQ|NSTDVLES DPRPWLTNPS 100
Pept.M ---------- ---------- ---------- --|-------- ---------- 100
Pept.L ---------- ---------- ---------- --|-------- ---------- 100

TRPC2a IRRTFFPDPQ T......... ..........  .......... ..........  111
Pept.M ---------- -YVPAVDISQ GQGHIAHGHK NPSRGPL... ..........  137
Pept.L ---------- -YVPAVDISQ GQGHIAHGHK NPSRGPLVSH LRSKHVRMAN  150
TRPC2b                                                 MGTK    4
            |exon 5
TRPC2a .......|SKT EISELKGMLK QLQPGPLGRA ARMVLSAARK (+1028aa) 1172
Pept.M .......|EHQ GNFRAQGYVE AVAARASGAG SPHGAFCCP*             169
Pept.L YVSFK*                                                  155
TRPC2b THPVVPW|--- ---------- ---------- ---------  (+1028aa) 1072
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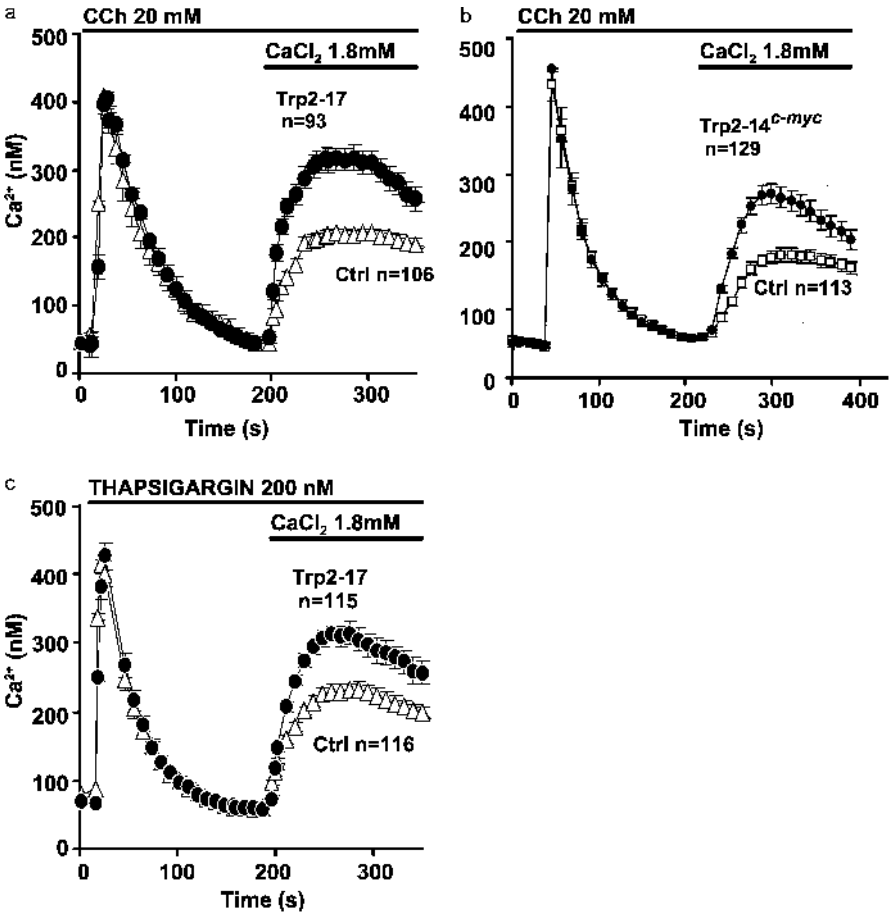


Fig. 4 a–c Expression of TRPC2 in COS-M6 cells enhances receptor-operated and store-operated Ca^{2+} entry. Ca^{2+} transients induced by carbachol (*CCh*) in the presence of extracellular Ca^{2+} induces capacitative calcium entry (CCE) that is significantly enhanced in COS-M6 cells expressing either **a** TRP2-17 or **b** TRP2-14 tagged at its C terminus with the epitope EQKLISEEDL that is recognized by the 9E10 anti-*myc* monoclonal antibody. **c** Thapsigargin-induced store depletion stimulates CCE in *TRP2-17* expressing COS-M6 cells. For details see Vannier et al. 1999 (adapted from Vannier et al. 1999 with permission)

is an exocytotic reaction that depends on Ca^{2+} influx from the extracellular milieu culminating with the release the acrosome's content, i.e., of enzymes that facilitate the penetration of the sperm head through the zona pellucida and injection of the male genome into the oocyte's cytoplasm. The zona pellucida is a glycoprotein envelope composed of three main glycoproteins termed ZP1, ZP2, and ZP3 (reviewed in Primakoff and Myles 2002; Jovine et al. 2005). Of these, ZP3 is the main activator of the acro-

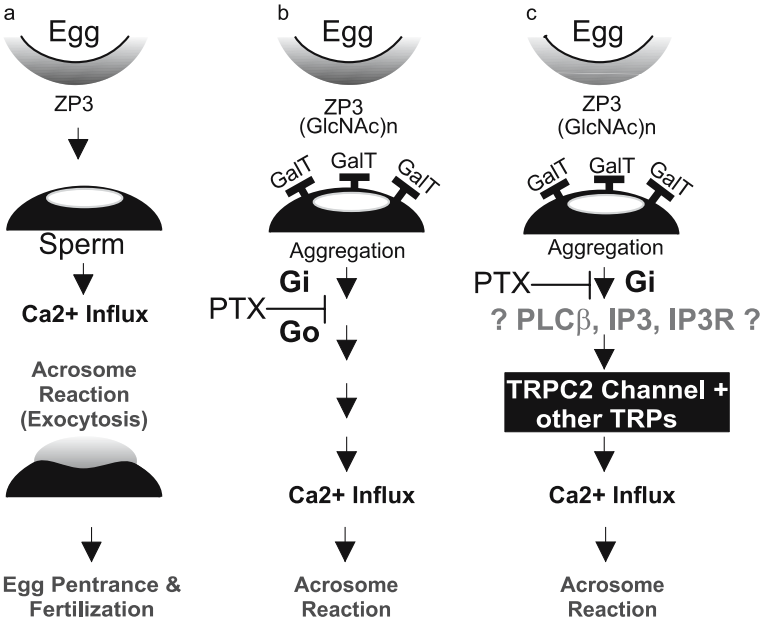


Fig. 5 a Interaction of sperm head with ZP3 protein of zona pellucida of the egg triggers Ca^{2+} influx and an exocytotic, acrosome reaction. **b** ZP3 of zona pellucida interacts with galactosyltransferase (GalT) promoting its clustering and aggregation and resulting in a pertussis toxin (PTX)-sensitive Ca^{2+} influx, which implicates involvement of G_i/G_o proteins in acrosome reaction (Endo et al. 1987; Miller et al. 1992). **c** The PTX-sensitive step is mediated by G_i (no G_o was detected in sperm heads), which activates a TRPC2 channel presumably through involvement of PLCβ, DAG, and IP_3–IP_3R (Jungnickel et al. 2001)

some reaction (Bleil and Wassarman 1983; Fig. 5b). A range of intracellular signaling cascades is triggered during the acrosome reaction. These include aggregation of sperm surface galactosyltransferase (GalT), activation of pertussis toxin-sensitive G proteins (Wilde et al. 1992; Ward et al. 1992; Ward and Kopf 1993), and activation of a voltage-independent cation channel that induces membrane depolarization leading to activation of a voltage-dependent T-type calcium channel that causes a transient increase in intracellular Ca^{2+} levels (Florman et al. 1992; Arnoult et al. 1996, 1999; Wassarman 1999). The transient phase is followed by a sustained phase of elevated intracellular Ca^{2+} (Florman et al. 1989; Lee and Storey 1989; Shirakawa and Miyazaki 1999). The mechanism leading to the sustained Ca^{2+} phase during the acrosome reaction was analyzed in capacitated sperm by Fura-2 Ca^{2+} imaging experiments in which application of thapsigargin caused store-operated Ca^{2+} entry into sperm. This suggested a role for store-operated Ca^{2+} entry channels in ZP3-induced acrosome reaction (O'Toole et al. 2000; Fig. 5b).

Among the members of TRPC channels, TRPC2 transcripts were detected in mouse testis by Northern blot analysis (Vannier et al. 1999), which made this channel a potential candidate for regulation of store-operated Ca^{2+} entry during the acrosome reaction (Fig. 5c). In 2001, using an antibody raised against the second extracellular loop between transmembrane domains 4 and 5 of TRPC2 (anti-RDAS antibody), Jungnickel et al. (2001) located TRPC2 by immunofluorescence on the anterior part of sperm heads. They also provided evidence that the anti-RDAS antibody inhibited Ca^{2+} entry into fluo3-loaded sperm cells induced either by thapsigargin or ZP3. The active participation of TRPC2 in the physiologic activation of the acrosome reaction was further supported by showing that the anti-RDAS antibody not only blocked Ca^{2+} entry but also the acrosome reaction itself (Jungnickel et al. 2001). Walensky and Snyder (1995) had shown earlier the presence of IP_3Rs in the acrosome cap. The presence of IP_3R and TRPC2 in close proximity is consistent with the possibility that TRPC2 may be activated according to the protein–protein interaction model of TRPC channel activation (Kiselyov et al. 1998; Birnbaumer et al. 2000). However, when the role of intracellular Ca^{2+} pools was analyzed by applying Ca^{2+} ionophores, ionomycin, and A23187, the Ca^{2+} released from intracellular stores was not sufficient to drive the acrosome reaction and required extracellular Ca^{2+} (O'Toole et al. 2000). While no doubt important, TRPC2 is not essential under in vivo conditions, as TRPC2-deficient mice are fertile (Stowers et al. 2002; Leypold et al. 2002). This last observation suggests that other TRPCs or TRP-related channels are involved in modulating Ca^{2+} entry during the acrosome reaction (Wissenbach et al. 1998; Trevino et al. 2001; Castellano et al. 2003). Thus, the mechanism of how ZP3 signaling induces store depletion still needs to be elucidated.

2.2.2 Gender-Specific Sexual Behavior

2.2.2.1 Transduction of Pheromone Signals

The mammalian olfactory system contains sensory neurons that are found in two different locations; the main olfactory epithelium of the nose (MOE) and the VNO. These two types of sensory neurons belong to two neuronal networks that differ in their function and their molecular makeup. From the functional viewpoint, MOE neurons respond to volatile odors, whereas VNO neurons respond to nonvolatile "odors" including pheromones, and are important for sexual behavior (reviewed in Buck 2000). The olfactory signals perceived by the MOE are sent to the main olfactory bulb (MOB) where axons of cells expressing a given olfactory receptor converge in glomeruli (Ressler et al. 1994; Vassar et al. 1994). From there, mitral cells project to the olfactory cortex leading to various cognitive and emotional responses as well as measured thoughts and behaviors.

In contrast, the axons from neurons of the VNO are projected to the glomeruli of the accessory olfactory bulb (AOB), from where projections extend to the amygdala and the hypothalamus in which behaviors such as social recognition, neuroendocrine function, and reproductive behavior are conceived (Keverne 1999; Dulac 2000). The detection of pheromones by the VNO neurons occurs via two classes of G protein-coupled receptors (GPCRs), V1Rs, that are restricted to the apical zone of the VNO and are co-expressed with $G_{i2}\alpha$, and V2Rs that are expressed in the basal zone together with $G_o\alpha$, class Ib major histocompatibility complex (MHC1b) proteins and β2 microglobulin (β2m) (Dulac and Axel 1995; Herrada and Dulac 1997; Berghard and Buck 1996; Jia and Halpern 1996; Saito et al. 1998). Immunohistochemical analysis of VNO sections and immunoaffinity chromatography analysis of VNO extracts showed that V2Rs and MHC1b molecules interact (Ishii et al. 2003; Loconto et al. 2003), leading to the proposal that these two types of molecules might function together during chemosensory perception (Loconto et al. 2003). In support, Leinders-Zufall and colleagues (2004) showed that peptides that bind to MHC molecules function as signals of genetic individuality that are required for mate recognition and thus influence social behavior.

Studies on the olfactory transduction cascade in the MOE neurons provided evidence that odors induce an inward cationic current that causes membrane depolarization and generate an action potential (Trotier and MacLeod 1983; Firestein and Werblin 1989; Firestein et al. 1990). The components of this cascade include a family of 500–1,000 odorant receptors that activate the heterotrimeric G protein G_{olf} (Buck and Axel 1991; Raming et al. 1993), G_{olf} (Pace and Lancet 1986; Jones and Reed 1989), the G_{olf} G protein, adenylyl cyclase III [activated by $G_{olf}\alpha$ (Pace et al. 1985; Sklar et al. 1986; Bakalyar and Reed 1990)], and the olfactory nucleotide (cAMP)-gated channel (oCNG) (Nakamura and Gold 1987; Firestein et al. 1991; Liman and Buck 1994; Fig. 6). In contrast, VNO neurons use a different set of signaling molecules for the transduction of chemical stimuli (Berghard et al. 1996; Liman and Corey 1996), in which activation of the V1R or V2R GPCRs activate $G_{i2}\alpha$ or $G_o\alpha$, respectively (Dulac and Axel 1995; Herrada and Dulac 1997; Berghard and Buck 1996; Jia and Halpern 1996; Saito et al. 1998; Fig. 7a). V1Rs belong to the rhodopsin or class I type of GPCRs, while V2Rs belong to the class 3 family of GPCRs, to which metabotropic glutamate receptors (mGluRs), GABA-B receptors and Ca^{2+} sensing GPCRs belong. Activated G_i or G_o, either through GTP–Gα or Gβγ, in turn stimulate the PLCβ pathway causing synthesis of the DAG and IP_3 second messengers.

2.2.2.2 TRPC2: The Candidate Transduction Channel Responsible for Transducing Vomeronasal Signaling

The evidence for the identity of the ion channels that mediate this signaling pathway with involvement of PLC in VNO neurons came from Dulac and

colleagues in 1999. These authors cloned the rat TRPC2 (rTRPC2) cDNA from rat VNO and characterized its spatial expression pattern both at the mRNA and protein levels (Liman et al. 1999; Fig. 7b, 3–5). These analyses showed that rTRPC2 was exclusively expressed in both apical and basal zones of VNO, particularly at the dendritic tips of the vomeronasal sensory neurons (VSNs) suggesting a role for rTRPC2 in sensory signal transduction. A follow-up study by Menco et al. (2001) supported these findings and showed by light and electron microscopy that the rTRPC2 gene product localizes to the microvilli of rat vomeronasal receptor cells. These are the sites of VNO cells that interact with odorants and pheromones. These authors also showed that the sites of TRPC2 expression are enriched in $G_{i2}\alpha$ and $G_o\alpha$ (Menco et al. 2001), with $G_{i2}\alpha$ being found in the apical zone where V1R receptors are found, and $G_o\alpha$ being found in the basal zone where V2R receptors and the MHC1b molecules had been found (Fig. 7b, 6–9).

The TRPC2 gene was inactivated by homologous recombination independently by two groups in order to investigate the significance of the channel in VNO-mediated sensory responses and attendant behaviors (Leypold et al. 2002; Stowers et al. 2002). As listed in Table 1, analyses of the phenotype of $TRPC2^{-/-}$ mice provided evidence that TRPC2 is a component of the VNO signaling pathway(s) that detects male-specific pheromones, mediates gender-specific behavior, and has a role in male–male aggression (Leypold et al. 2002; Stowers et al. 2002). These data and the analysis of $\beta 2m^{-/-}$ mice has shed light on the structural components that make up the functional units in VNO (Loconto et al. 2003; Fig. 8).

The findings from the phenotypic analysis of $TRPC2^{-/-}$ mice made TRPC2 a candidate ion channel that contributes to the VSN signaling. To elucidate this concept at the molecular level, Zufall and colleagues investigated the existence and characteristics of PLC-activated cation channels by performing inside-out recordings from plasma membranes of the dendritic tips of VSNs in the presence of a plasma membrane permeable DAG analog 1-stearoyl-2-arachidonoyl-*sn*-glycerol (SAG) in the bath solution (Lucas et al. 2003). They

Fig. 6 a,b Signal transduction in the olfactory epithelium. **a** Odor (*circle*) stimulates olfactory receptor (*OR*), which activates olfactory G protein (G_{olf}) triggering the adenylyl cyclase (*AC*)-cAMP *ON* signal. cAMP activates the olfactory cyclic nucleotide gated channels (*cNGC*) which results in two responses: an action potential and entry of Ca^{2+} which via Ca^{2+}/CaM and CaMKII inhibits the adenylyl cyclase, thereby terminating the signaling process. **b** Localization of the messenger RNA (mRNA) of signal transduction components of the olfactory epithelium; odorant receptors (*OR*) (adapted from Ressler et al. 1993 with permission), G_{olf}, oCNC1, oCNC2, ACIII (adapted from Berghard et al. 1996 with permission). *OE*, olfactory epithelium; *OB*, olfactory bulb; *VNO*, vomeronasal organ; *AOB*, accessory olfactory bulb

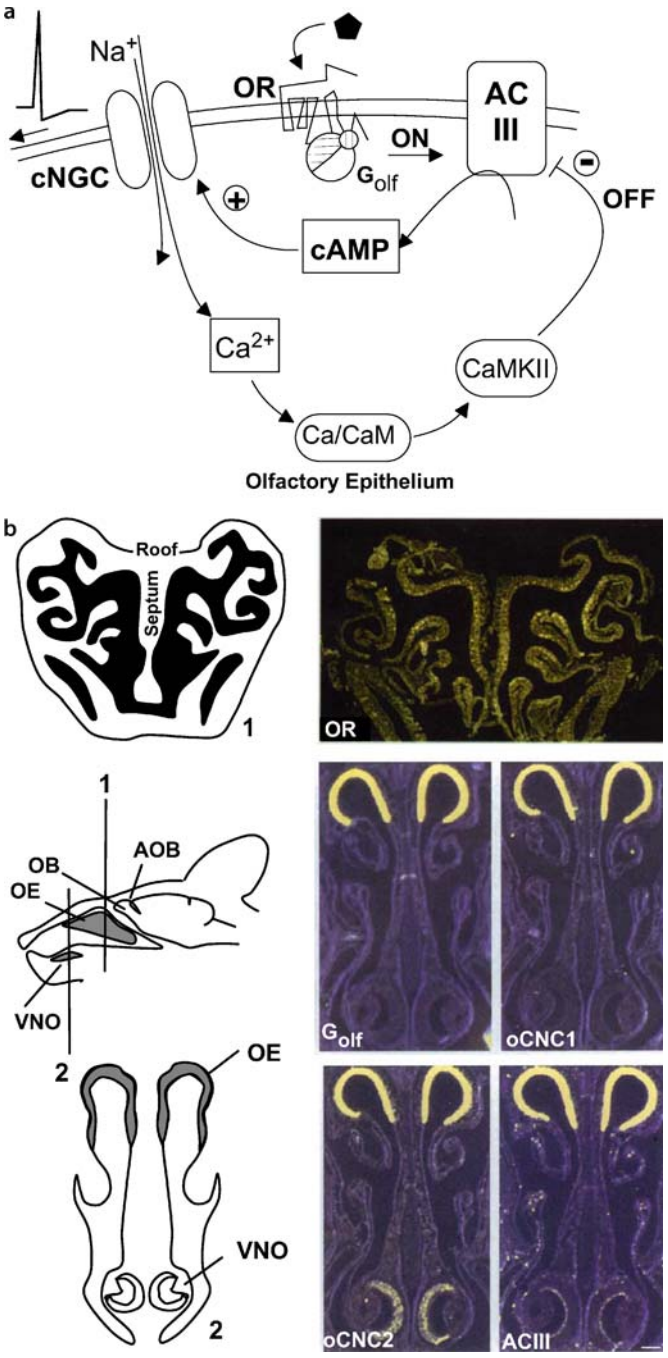

a
Na+
OR
AC
III
ON
cNGC
Golf
OFF
cAMP
Ca2+
CaMKII
Ca/CaM
Olfactory Epithelium
b
Roof
Septum
1
OR
1
AOB
OB
OE
VNO
Golf
oCNC1
2
OE
VNO
2
oCNC2
ACIII

Table 1 Phenotype of the TRPC2$^{-/-}$ mice. (From studies of Stowers et al. 2002; Leypold et al. 2002)

Abolished sensory response to pheromone cues in urine
Abolished pheromone-evoked male–male aggression
Abolished maternal aggression against intruders
Males display loss of gender discrimination for mating
Males display sexual behavior toward male intruders
Males display defects in territorial markings
Normal testosterone production by males
Normal male–female mating behavior

found a sustained inward current, which could not be activated by Ca^{2+} alone or by application of 50–100 μM IP_3 to the bath, suggesting that the activation pathway is not through IP_3R signaling or store-depletion. The SAG-induced currents had a conductance of 42 pS, and extracellular Ca^{2+}, Mg^{2+}, ATP, or GTP was not necessary for the development of this current. The characteristics of this cation channel are listed in Table 2.

In order to test whether the TRPC2 channel is involved in the generation of this DAG-induced current, plasma membranes from the dendritic tips of VSNs of TRPC2$^{-/-}$ mice were used to record SAG-induced currents (Lucas et al. 2003). These recordings showed that ablation of the TRPC2 gene reduced the SAG-induced current, indicating that TRPC2 is a component of the signaling

Fig. 7 a,b Signal transduction in the VNO. **a** In the apical zone (*left*), the TRPC2 transduction channel is activated by the V1R-$G_{i2}\alpha$-triggered stimulation of PLCβ. In the basal zone (*right*), the V2R.MHCIb (M10).β2m complex stimulates PLC via activation of $G_o\alpha$. This results in the activation of the TRPC2 vomeronasal transduction channel. **b** Localization of the signal transduction components of the vomeronasal olfactory system. *B1–2*, V1R (adapted from Dulac and Axel 1995) and $G_{i2}\alpha$ (adapted from Matsunami and Buck 1997) colocalize in the VNO apical zone. *B3* Rat TRPC2 mRNA is found in both apical and basal zones of the VNO (adapted from Liman et al. 1999 with permission). *B4–5*, Rat TRPC2 and $G_{i2}\alpha$ protein localizes to the dendritic tips of the VNO neurons (adapted from Liman et al. 1999 with permission). *B6–9*, V2R, $G_o\alpha$ (adapted from Matsunami and Buck 1997 with permission), M10, and β2m (adapted from Ishii et al. 2003 with permission) mRNAs are found in basal zone of the VNO. *VNO*, vomeronasal organ; *AOB*, accessory olfactory bulb; *D*, dorsal, *P*, posterior, *V*, ventral, *A*, anterior

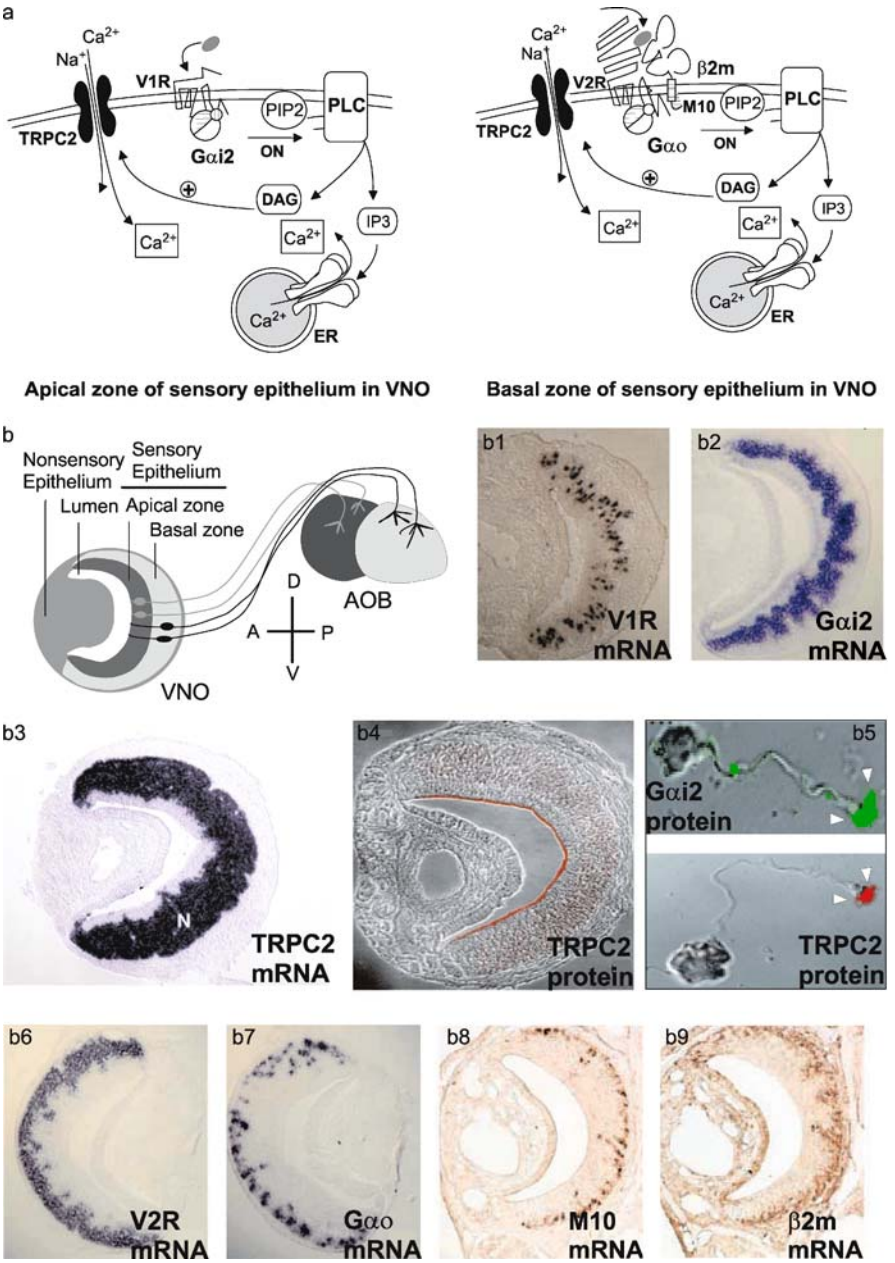

a
Ca2+
Na+
V1R
TRPC2
PIP2
PLC
Gαi2
ON
DAG
IP3
Ca2+
ER
Apical zone of sensory epithelium in VNO
V2R
β2m
M10
Gαo
Basal zone of sensory epithelium in VNO
b
Nonsensory Epithelium
Sensory Epithelium
Lumen
Apical zone
Basal zone
AOB
VNO
D
A
P
V
b1
V1R mRNA
b2
Gαi2 mRNA
b3
N
TRPC2 mRNA
b4
TRPC2 protein
b5
Gαi2 protein
TRPC2 protein
b6
V2R mRNA
b7
Gαo mRNA
b8
M10 mRNA
b9
β2m mRNA

Table 2 Properties of the PLC-activated cation channel at the dendritic tips of VSNs. (These observations were reported by Lucas et al. 2003)

Conductance	Ion permeability	Cation current can be induced by	Pheromone-induced cation current is insensitive to	Pheromone/SAG-induced cation current is sensitive to
42 pS	Na^+, Ca^{2+}, Cs^+ But not NMDG	Pheromone cues in urine DAG analogs SAG, OAG, DOG But not by OG, IP_3, store depletion Cyclooxygenase inhibitor, indomethacin (50 μM) Lipoxygenase inhibitor, nordihydroguaiaretic acid (100 or 500 μM)	PLC antagonist, U-73122 2-ABP	DAG lipase inhibitor, RHC-80267 PLA_2 inhibitor, isotetrandrine PI3 kinase inhibitor, LY-29002 PKC inhibitors, staurosporine or calphostin C

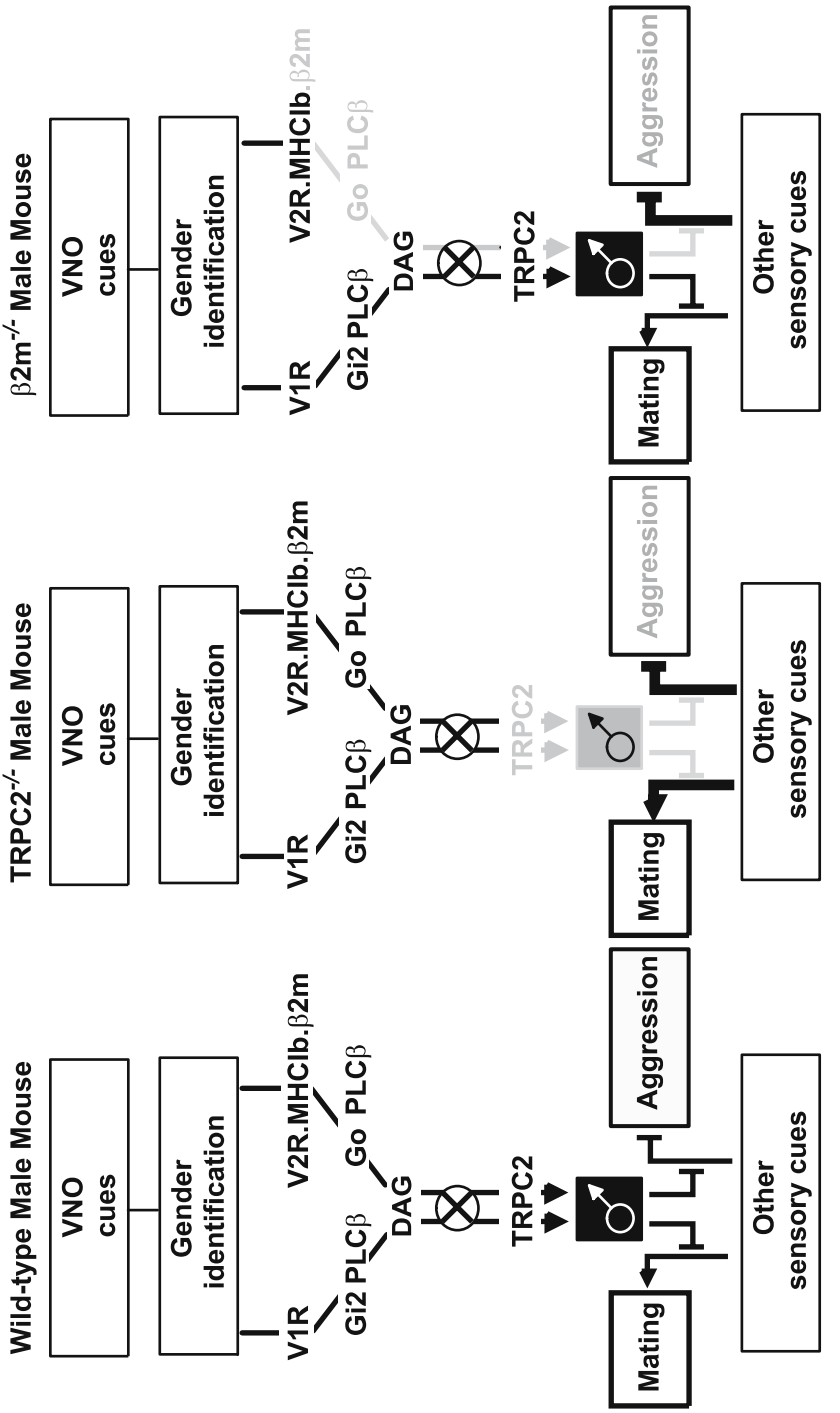
2-ABP, 2-Aminoethoxydiphenyl borate; DOG, 1,2-dioctanoyl-*sn*-glycerol; NMDG, *N*-methyl-D-glucamine; OAG, 1-oleoyl-2-acetyl-*sn*-glycerol; OG, 1-oleoyl-glycerol; $PLA_{2,}$ phospholipase A_2; SAG, 1-stearoyl-2-arachidonoyl-*sn*-glycerol; VSN, vomeronasal sensory neurons

cascade in sensory transduction. A reduction, but not a full loss, of the SAG- or urine-induced current is indicative that one or more cation channels are involved in this process, presumably other members of the TRPC family, which might interact with TRPC2 to regulate Ca^{2+} entry. A candidate partner for TRPC2 is the TRPC6 channel, which has been shown to be regulated by DAG (Hofmann et al. 1999; Chu et al. 2004).

3 Concluding Remarks: One Channel with two Distinct Roles?

The findings from the analysis of the sperm acrosome reaction, the sensory transduction in the VNO neurons and the characterization of $TRPC2^{-/-}$ mice

Fig. 8 Basal and apical vomeronasal zones mediate distinct behavioral responses using separate receptor/G protein systems but a common TRPC2 transduction channel (adapted from Dulac and Torello 2003 with permission)


Wild-type Male Mouse
VNO cues
Gender identification
V1R
Gi2
PLCβ
V2R.MHCIb.β2m
Go
PLCβ
DAG
TRPC2
Mating
Aggression
Other sensory cues
TRPC2−/− Male Mouse
VNO cues
Gender identification
V1R
Gi2
PLCβ
V2R.MHCIb.β2m
Go
PLCβ
DAG
TRPC2
Mating
Aggression
Other sensory cues
β2m−/− Male Mouse
VNO cues
Gender identification
V1R
Gi2
PLCβ
V2R.MHCIb.β2m
Go
PLCβ
DAG
TRPC2
Mating
Aggression
Other sensory cues

provided evidence that the TRPC2 channel is important for both sperm function and vomeronasal pheromone sensing. Yet the main role in these two important functions appears to differ. In the acrosome reaction the channel shows characteristics of a store-operated Ca^{2+} entry channel supplying the Ca^{2+} required to trigger exocytosis. In sensory neurons, on the other hand, the channel shows characteristics of a depolarizing cation channel that triggers an action potential. Whether Ca^{2+} entering into the neurons during depolarization also plays an additional role is not known. In neurons the TRPC2 channel appears to be activated by DAG in response to stimulation of PLC activity (Jungnickel et al. 2001; Lucas et al. 2003). In sperm, DAG did not induce an acrosome reaction (Stamboulian et al. 2005), suggesting that the mode of activation of the TRPC2 channel might be cell-specific and might require different partners for signaling to proceed. Thus, while advances are being made in identifying the components that make up different signaling pathways in different cells, the molecular details of the process by which TRPC2 is being activated remain in need of further clarification.

Acknowledgements Supported by the Intramural Research Program of the NIH, and NIEHS.

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