# $Ca^{2+}$-activated $Cl^-$ channels at a glance

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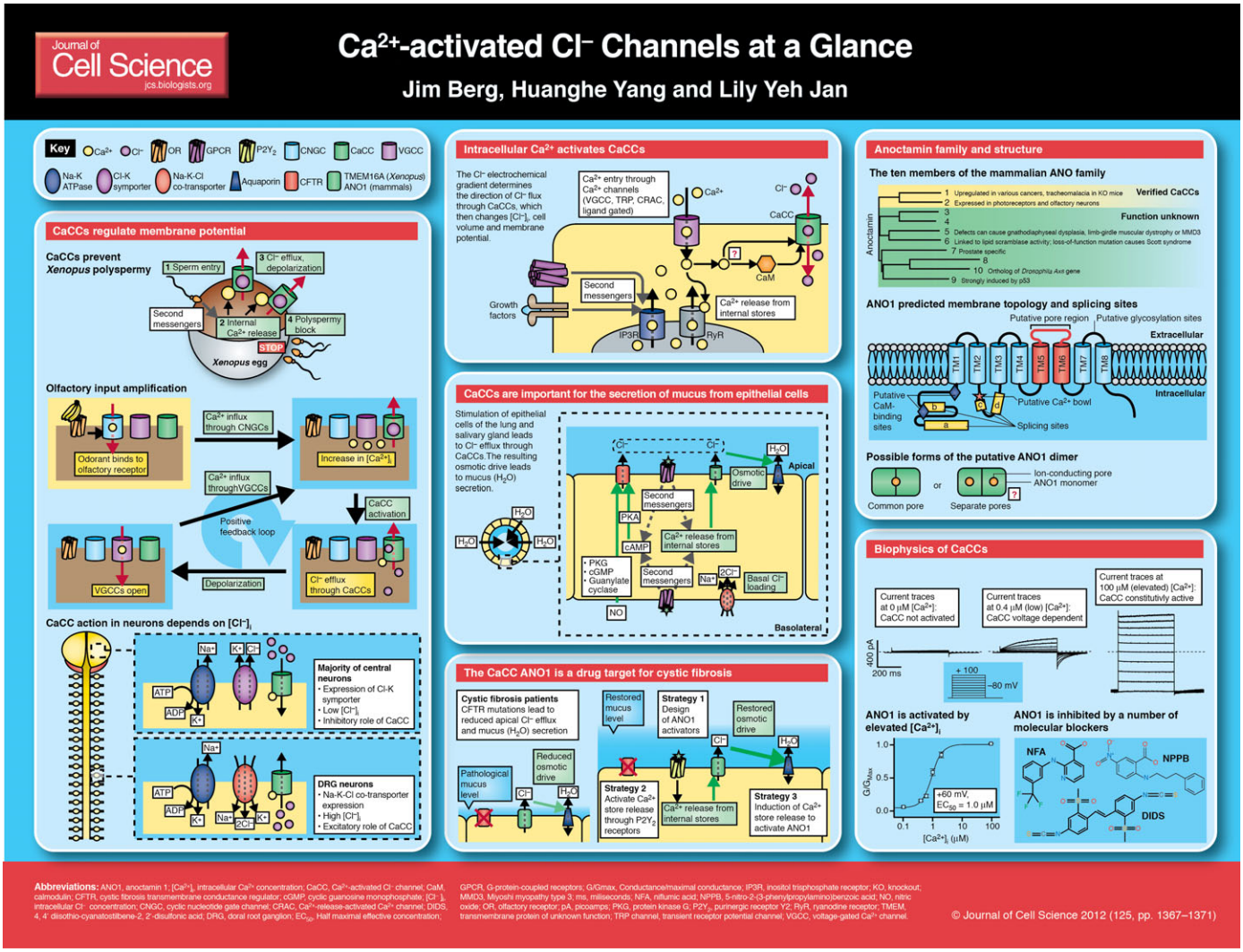
$Cl^-$ channels are membrane proteins that are responsible for the passive flow of $Cl^-$ into and out of the cell. The $Ca^{2+}$-activated $Cl^-$ channels (CaCCs) are attracting a lot of attention lately. The molecular identity of these channels remained elusive (for a review, see Hartzell et al., 2005) until anoctamin 1 and anoctamin 2 (in mammals ANO1 and ANO2, respectively; in *Xenopus* TMEM16A and TMEM16B, respectively) were identified as CaCCs in 2008 (Yang et al., 2008; Schroeder et al., 2008; Caputo et al., 2008). ANO1 and ANO2 possess the same properties as endogenous CaCCs, which have been observed in cells of various organisms over the past three decades; these properties include anion selectivity, submicromolar sensitivity to intracellular $Ca^{2+}$, voltage activation at low $Ca^{2+}$ concentrations and inhibition by the pharmacological agents niflumic acid (NFA) and 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB). In this Cell Science at a Glance article, we first outline the cell physiology and biophysical characteristics of CaCC, with a focus on the anoctamin family of transmembrane proteins in different species; then we discuss the implications of ANO1 identification on studying diseases that arise from CaCC defects, as well as the possibility of targeting CaCC channels as a therapeutic agent for other diseases.

## CaCCs influence cellular electrical activity from algae to neurons

CaCCs have a long evolutionary history – in algae, they are responsible for propagation of the action potential that, following an external stimulus, signals for the cell to terminate its usual protoplasmic streaming (Fromm and Lautner, 2007; Shiina and Tazawa, 1987). In *Xenopus* oocytes, rapid $Cl^-$ efflux through CaCCs that are activated following an encounter with a single sperm depolarizes the membrane, thereby preventing the entry of additional sperm (see poster 'CaCCs regulate membrane potential') (Cross,




*(See poster insert)*

1981). *Xenopus* CaCC has been well-characterized electrophysiologically and was the foundation for the expression cloning of TMEM16A (Schroeder et al., 2008).

CaCCs have also been described in many mammalian excitable cells – one example is the interstitial cells of Cajal (ICC) of the intestinal tract. These cells have an electrical pace-making activity that leads to the generation of a slow wave, which is propagated to the smooth muscle of the intestine to cause gastrointestinal tract peristalsis (Sanders et al., 2006). Because CaCC blockers inhibit the activity of this slow wave (Hirst et al., 2002), it is hypothesized that CaCCs are involved in its generation. This hypothesis was recently supported by reports of high levels of ANO1 expression in the ICC (Zhu et al., 2009; Huang et al., 2009), and the lack of any slow-wave activity or rhythmic contraction of smooth muscles in the digestive tract of *Ano1* knockout mice (Huang et al., 2009; Hwang et al., 2009).

CaCCs are also found on the membrane of numerous different types of smooth muscle cells (Large and Wang, 1996), where the opening of CaCCs leads to depolarization of the membrane, followed by opening of voltage-gated $Ca^{2+}$ channels (VGCCs), and subsequent contraction. The initial trigger for CaCC opening is unknown, but the channels might be activated by an agonist-induced increase in the intracellular $Ca^{2+}$ concentration $[Ca^{2+}]_i$ (Large and Wang, 1996). In smooth muscle cells, CaCCs also open following the release of intracellular $Ca^{2+}$ from internal stores (a $Ca^{2+}$ spark). The CaCC opening creates a spontaneous transient inward current (STIC), which can lead to increased contractility of rhythmically active smooth muscle cells (Bao et al., 2008). ANO1 is likely to be the CaCC responsible for this current, as it is expressed at a high level in both arterial and tracheal smooth muscle (Davis et al., 2010; Manoury et al., 2010; Huang et al., 2009). In addition, siRNA knockdown of *Ano1* results in a lack of arterial smooth muscle CaCCs in rats (Manoury et al., 2010), and *Ano1*-knockout mice show abnormal trachealis musculature (Rock et al., 2008).

Shortly after the discovery of CaCCs in *Xenopus* oocytes, a similar current was found in salamander retinal rod cells (Bader and Bertrand, 1982). This was the first of a number of studies to show that the CaCC current is a component of sensory processing. In retinal sensory cells, the activation of CaCCs is likely to stabilize the membrane during synaptic activity (Lalonde et al., 2008). However, the molecular identity of the retinal CaCC remains unresolved, because both TMEM16A (Mercer et al., 2011) and ANO2 (Stöhr et al., 2009) have been found at the photoreceptor synapses of salamander and mice, respectively.

In contrast to the stabilizing effect of CaCC activation in the retina, CaCC activity in olfactory, mechanically activated and temperature-sensitive neurons is predicted to be excitatory so as to amplify sensory transduction (Kleene, 2008; Mayer, 1985). In olfactory sensory neurons, the binding of an odorant to a receptor leads to the intracellular production of cyclic nucleotides that activate a cyclic nucleotide gated (CNG) cation channel. The subsequent $Ca^{2+}$ influx both depolarizes the cell and activates CaCCs, leading to $Cl^-$ efflux and an amplification of the membrane response (see poster 'CaCCs regulate membrane potential') (Kleene, 2008). The molecular identity of the olfactory CaCC has been clearly demonstrated to be ANO2, because the olfactory CaCC current is completely abolished in the *Ano2*-knockout mouse (Billig et al., 2011). The same study also casts doubt on the requirement of CaCC activity for olfaction, because electrical responses to odorants are only reduced by ~40% in *Ano2*-knockout mice, which appear to exhibit normal olfactory behavior (Billig et al., 2011).

In neurons, the role of CaCC depends on the intracellular $Cl^-$ concentration $[Cl^-]_i$, which is determined by the expression of $Cl^-$ transporters on the cell membrane (see poster 'CaCCs regulate membrane potential'). The action of CaCCs expressed in adult mouse central neurons (Llano et al., 1991) is predicted to be inhibitory, because – owing to the expression of a Cl-K symporter – these neurons have a very low $[Cl^-]_i$, (Ben-Ari et al., 2007). However, immature central neurons and adult peripheral neurons express a Na-K-Cl co-transporter (Ben-Ari et al., 2007; Sung et al., 2000), leading to elevated $[Ca^{2+}]_i$ and an excitatory role for $Ca^{2+}$. In 1985, Mayer described a neuronal CaCC current in a subset of dorsal root ganglion (DRG) sensory neurons, in which $Ca^{2+}$ influx due to stimulation caused CaCC activation, leading to prolonged depolarization and occasionally to burst firing (Mayer, 1985). The CaCC current is upregulated in DRG neurons following axotomy (Sánchez-Vives and Gallego, 1994; André et al., 2003), suggesting that CaCCs have a role in neuropathic pain or nerve regeneration. In addition, in injured DRG neurons, the activity of $Cl^-$ channels generally results in further excitation (Sung et al., 2000), which might enhance CaCC effects, leading to increased neuropathic pain. The molecular identity of the CaCC in DRG neurons has yet to be determined, because Boudes and colleagues observed the expression of both ANO1 and ANO2 in the DRG of the mouse (Boudes et al., 2009). However, in cultured small DRG neurons, it has also been reported that siRNA-mediated knockdown of *Ano1* substantially reduces the induction of the CaCC current in response to the inflammatory agent bradykinin (Liu et al., 2010). Additional studies are required to fully understand the role of the CaCC current in sensory transduction of DRG neurons.

## CaCCs control fluid secretion

In addition to their influence on the electrical properties of excitable cells, CaCCs also have a substantial impact on an entirely different cellular process in many different tissues – the flow of $H_2O$ out of secretory cells.

Acinar cells line the ducts of the salivary and lachrymal glands as well as the pancreas (Melvin et al., 2005; Hartzell et al., 2005). Acinar cells are highly polarized and secrete fluid from their apical side in a CaCC-dependent manner. $Cl^-$ is pumped into the basal end of the cells through a cation-$Cl^-$ co-transporter that is coupled to a $Cl^-/HCO_3$ exchanger. Upon stimulation and increase of internal $[Ca^{2+}]$, $Cl^-$ exits the apical end of the cell through CaCCs, creating an osmotic drive for $H_2O$ molecules to follow (Melvin et al., 2005). Data collected from salivary acinar cells reveal the presence of a CaCC with properties very similar to those of ANO1 and, furthermore, ANO1 is expressed at a high level in these cells (Romanenko et al., 2010). Additional support for a role of ANO1 in salivary function comes from *Ano1*-knockout mice – which show impaired fluid secretion (Ousingsawat et al., 2009), and siRNA-mediated *Ano1* knockdown in salivary glands – which reduces the production of saliva (Yang et al., 2008).

Epithelial cells of the lung and gastrointestinal tract are organized in a

manner similar to acinar cells, but the role of the CaCC current in these cells is less clear. Their apical surface expresses cAMP-dependent cystic fibrosis transmembrane conductance regulator (CFTR), a $Cl^-$ channel that is a key determinant in fluid secretion, as shown by the severe defects observed in patients with cystic fibrosis (see poster 'Biophysics of CaCCs') (Anderson et al., 1991). It seems that ANO1 is also involved in this process, because functional studies in *Ano1*-knockout mice have shown a reduction of stimulation-induced $Cl^-$ secretion in colon and trachea epithelia (Ousingsawat et al., 2009; Rock et al., 2009). The interplay between CaCC and CFTR is intriguing, because both channels induce stimulation-dependent fluid secretion, albeit by different intracellular pathways. Indeed, as discussed below, the CaCC is a putative drug target in patients suffering from cystic fibrosis patients with impaired CFTR function.

## Structure–function analysis of ANO1 and ANO2

A number of recent structure–function studies have focused on the ANO1 CaCC. Mutations of the predicted pore region – a putative re-entrant loop between transmembrane domain 5 and 6 – substantially affect its ion selectivity (Yang et al., 2008), and biochemical studies have indicated that the channel probably exists as a homodimer (see poster 'Anoctamin family and structure') (Fallah et al., 2011; Sheridan et al., 2011). The human *ANO1* gene contains four alternatively spliced segments (designated a, b, c and d), which are not predicted to alter the overall membrane topology but change the biophysical properties of the resulting channels (Caputo et al., 2008; Ferrera et al., 2009).

CaCCs are highly sensitive to changes in $[Ca^{2+}]_i$ (see poster 'Biophysics of CaCCs'). $Ca^{2+}$ binding appears to be cooperative (Kuruma, 1999; Yang et al., 2008), suggesting that multiple $Ca^{2+}$-binding sites exist; however, the ANO1 sequence does not reveal any canonical $Ca^{2+}$-binding motifs. A sequence in the first intracellular loop that resembles the '$Ca^{2+}$ bowl' observed in large conductance $Ca^{2+}$-activated $K^+$ (BK) channels (Schreiber and Salkoff, 1997) was proposed to be a $Ca^{2+}$-binding site (Duran et al., 2010). However, ablating this sequence does not dramatically alter the $Ca^{2+}$ sensitivity of the channel, making this sequence less likely to comprise the $Ca^{2+}$ sensor (Xiao et al., 2011).

Alternatively, the $Ca^{2+}$ sensor of CaCCs might be an accessory protein, such as calmodulin (see poster 'Intracellular $Ca^{2+}$ activates CaCCs'). Calmodulin is involved in a number of cellular processes and has specifically been demonstrated to determine the $Ca^{2+}$ sensitivity of the small conductance $Ca^{2+}$-activated $K^+$ (SK) channel (Xia et al., 1998). Dominant-negative forms of calmodulin were found to reduce the native CaCC activity in olfactory sensory neurons (Kaneko et al., 2006). However, a recent biophysical study of ANO2, identified as the olfactory sensory CaCC (Hengl et al., 2010), failed to show that calmodulin has any effect on ANO2 activity (Pifferi et al., 2009). This debate is far from over, as a more recent study found an association between ANO2 and calmodulin – both biochemically and functionally (Tian et al., 2011).

One of the hallmarks of the endogenous CaCC current is that it is voltage gated at low (submicromolar) $[Ca^{2+}]$ (see poster 'Biophysics of CaCCs'). Using HEK-293 cells expressing mouse ANO1, Xiao et al. found that the channel is gated synergistically by $Ca^{2+}$ and voltage: When $[Ca^{2+}]_i$ is raised from 1 μM to 2 μM, the voltage that is required to activate ~50% of the channels shifts from +64mV to –81 mV. At the same time, the cooperativity of $Ca^{2+}$ binding leading to channel activation is enhanced twofold at +100 mV compared with that at –100 mV (Hill coefficient of 2.0 compared with 1.0, respectively). (Xiao et al., 2011). The precise element within the channel that senses voltage has not yet been determined, although it has been proposed that a string of four glutamate residues on the first intracellular loop can account for some of the sensitivity (Xiao et al., 2011).

## Function of other anoctamin family members

ANO1 and ANO2 belong to a ten-member family of proteins that are predicted to have eight transmembrane domains, with both their N- and C- termini located on the intracellular face of the membrane. In heterologous expression systems, most of the remaining anoctamin family members seem to be expressed intracellularly (Schreiber et al., 2010), hampering direct electrophysiological characterization to test whether they also function as ion channels. One exception is ANO6, which is robustly expressed on the surface of many different cell types (Schreiber et al., 2010). However, the electrophysiological characterization of ANO6, on the basis of siRNA assays carried out in the same laboratory, offered three different conclusions: (1) ANO6 is a volume-regulated $Cl^-$ channel involved in regulatory volume decrease (RVD) (Almaca et al., 2009), (2) it is a CaCC (Schreiber et al., 2010) and, (3) ANO6 is an outward rectifying $Cl^-$ channel (ORCC) without $Ca^{2+}$ sensitivity (Martins et al., 2011). Besides these contradictory findings, the recent observations that link ANO6 with phospholipid scramblase activity in blood cells and the subsequent hemostasis disorder known as Scott syndrome (Suzuki et al., 2010; Castoldi et al., 2011) make this protein even more intriguing. Notwithstanding the importance of further studies to clarify the properties and function of ANO6, one thing is clear: ANO6 must differ from the ANO1 CaCC, because the heterologous expression of ANO6 enhances phospholipid scramblase activity to expose phosphatidylserine that usually resides in the inner leaflet to the outer leaflet of the plasma membrane of B-cells – however, this does not occur when ANO1 is heterologously expressed (Suzuki et al., 2010).

## CaCCs and human disease

Since the discovery of ANO1, there has been much excitement with regard to possible therapeutic applications, and some of the approaches have been straightforward. For instance, owing to its high level of expression in the ICC, staining of ANO1 can be used to detect ICC depletion – which is observed in many gastrointestinal disorders – thereby replacing staining of other, less specific, ICC markers (Kashyap et al., 2011). Some approaches have been more complex. Because both ANO1 and CFTR are expressed on the apical surface of lung epithelial cells, ANO1 activation is being examined as a possible surrogate for CFTR. In cystic fibrosis patients, mutations of CFTR lead to decreased apical $Cl^-$ flow and, thus, a reduced fluid secretion. If ANO1 activity can be enhanced in these cells, fluid secretion might return to normal levels (see poster 'The CaCC ANO1 is a drug target for cystic fibrosis'). In terms of drug targets, ANO1 can either be targeted directly by activators, or the $[Ca^{2+}]_i$ can be increased to indirectly activate ANO1 channels. In

fact, two drugs that use the latter strategy are now undergoing clinical trials in cystic fibrosis patients (Cuthbert, 2011). One compound, INS37217, activates apical purinergic receptor $Y_2$ ($P2Y_2$), which leads to internal $Ca^{2+}$ release and subsequent activation of ANO1. In the other approach, the antibiotic duramycin creates $Ca^{2+}$-permeable pores that let $Ca^{2+}$ into the cell and release $Ca^{2+}$ from intracellular stores to activate ANO1. When ANO1 is studied further, more avenues of its activation might be revealed.

Because ANO1 had shown to be highly expressed in a number of different cancer tissues (West et al., 2004), it was of interest even before it was found to be a CaCC. ANO1 expression correlates with the development of metastasis, and reducing ANO1 levels by RNAi or inhibiting its functionality with channel blockers reduces the migration of cultured cancer cells (Ayoub et al., 2010). Although this observation is promising for cancer research, it also highlights potential problems if used for cystic fibrosis therapies that involve ANO1 activation, as discussed above.

Further members of the anoctamin family – other than ANO1 and ANO2 – have been implicated in a number of diseases, although none of them has so far been identified as being a CaCC. Similar to ANO1, ANO7 has a role in cancer, specifically prostate cancer (Bera et al., 2004). A set of dominant mutations in ANO5 was linked to the rare skeletal condition gnathodiaphyseal dysplasia (Tsutsumi et al., 2004), whereas recessive mutations were found to lead to limb–girdle muscular dystrophy or Miyoshi myopathy (Bolduc et al., 2010). ANO10 is mutated in patients with cerebellar ataxia (Vermeer et al., 2010), which suggests a role for ANO10 as an ion channel in the nervous system; and ANO6 mutations were found in patients with the rare blood disorder Scott syndrome, which is caused by a defect in phospholipid scrambling activity (Suzuki et al., 2010; Castoldi et al., 2011). It is unlikely that all of these effects are the result of defects in $Cl^-$ channel activity, but more studies are required to fully understand the molecular and physiological functions of the anoctamin family of proteins.

## Perspectives

Research into CaCCs has, for a long time, relied heavily on carefully controlled electrophysiology experiments. However, this changed recently, following the identification of ANO1 and ANO2 as CaCCs with characteristics that match those of the endogenous current – at present, the CaCC field is in an exciting state of discovery. As our knowledge of ANO1 and ANO2, and their expression profiles becomes more complete, we will be able to identify further potential therapeutic targets and employ the high-throughput screening of small molecules that modulate the activity of these proteins (Namkung et al., 2011a; Namkung et al., 2011b). These screens might result in the identification of specific inhibitors and activators to be used in the study of CaCCs in native tissues – something the field has been lacking thus far – and, importantly, might uncover therapeutic CaCC targets for the treatment of cystic fibrosis and other diseases.

Although the identification of ANO1 and ANO2 as CaCCs has provided a leap in our understanding of the physiology of $Cl^-$ channels, a number of questions still remain. We must determine whether there are other CaCC candidates that have not yet been identified. In addition to the characterization of the rest of the anoctamin family, other transmembrane proteins of unknown function should be examined to determine whether they also have CaCC characteristics, or characteristics of other unidentified $Cl^-$ channels. However, identification of appropriate cellular cofactors might be necessary to study these channels in cultured cell expression systems. Finally, the crystal structure of the ANO1 CaCC might answer questions on transmembrane helical packing and the molecular mechanism of ion permeability – which, at present, can only be speculated upon. This structural information, together with any physiology learned in the meantime, will help in the development of ANO1 as a therapeutic target for disease.

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## References

**Almaça, J., Tian, Y., Aldehni, F., Ousingsawat, J., Kongsuphol, P., Rock, J. R., Harfe, B. D., Schreiber, R. and Kunzelmann, K.** (2009). TMEM16 proteins produce volume-regulated chloride currents that are reduced in mice lacking TMEM16A. *J. Biol. Chem.* **284**, 28571-28578.

**Anderson, M. P., Gregory, R. J., Thompson, S., Souza, D. W., Paul, S., Mulligan, R. C., Smith, A. E. and Welsh, M. J.** (1991). Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science* **253**, 202-205.

**André, S., Boukhaddaoui, H., Campo, B., Al-Jumaily, M., Mayeux, V., Greuet, D., Valmier, J. and Scamps, F.** (2003). Axotomy-induced expression of calcium-activated chloride current in subpopulations of mouse dorsal root ganglion neurons. *J. Neurophysiol.* **90**, 3764-3773.

**Ayoub, C., Wasylyk, C., Li, Y., Thomas, E., Marisa, L., Robé, A., Roux, M., Abecassis, J., de Reyniès, A. and Wasylyk, B.** (2010). ANO1 amplification and expression in HNSCC with a high propensity for future distant metastasis and its functions in HNSCC cell lines. *Br. J. Cancer* **103**, 715-726.

**Bader, C. and Bertrand, D.** (1982). Voltage-activated and calcium-activated currents studied in solitary rod inner segments from the salamander retina. *J. Physiol.* **331**, 253-284.

**Bao, R., Lifshitz, L. M., Tuft, R. A., Bellvé, K., Fogarty, K. E. and ZhuGe, R.** (2008). A close association of RyRs with highly dense clusters of Ca2+-activated Cl- channels underlies the activation of STICs by Ca2+ sparks in mouse airway smooth muscle. *J. Gen. Physiol.* **132**, 145-160.

**Ben-Ari, Y., Gaiarsa, J. L., Tyzio, R. and Khazipov, R.** (2007). GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol. Rev.* **87**, 1215-1284.

**Bera, T. K., Das, S., Maeda, H., Beers, R., Wolfgang, C. D., Kumar, V., Hahn, Y., Lee, B. and Pastan, I.** (2004). NGEP, a gene encoding a membrane protein detected only in prostate cancer and normal prostate. *Proc. Natl. Acad. Sci. USA.* **101**, 3059-3064.

**Billig, G. M., Pál, B., Fidzinski, P. and Jentsch, T. J.** (2011). Ca2+-activated Cl− currents are dispensable for olfaction. *Nat. Neurosci.* **14**, 763-769.

**Bolduc, V., Marlow, G., Boycott, K. M., Saleki, K., Inoue, H., Kroon, J., Itakura, M., Robitaille, Y., Parent, L., Baas, F. et al.** (2010). Recessive mutations in the putative calcium-activated chloride channel Anoctamin 5 cause proximal LGMD2L and distal MMD3 muscular dystrophies. *Am. J. Hum. Genet.* **86**, 213-221.

**Boudes, M., Sar, C., Menigoz, A., Hilaire, C., Péquignot, M. O., Kozlenkov, A., Marmorstein, A., Carroll, P., Valmier, J. and Scamps, F.** (2009). Best1 is a gene regulated by nerve injury and required for Ca2+-activated Cl- current expression in axotomized sensory neurons. *J. Neurosci.* **29**, 10063-10071.

**Caputo, A., Caci, E., Ferrera, L. and Pedemonte, N.** (2008). TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. *Science* **322**, 590-594.

**Castoldi, E., Collins, P. W., Williamson, P. L. and Bevers, E. M.** (2011). Compound heterozygosity for 2 novel TMEM16F mutations in a patient with Scott syndrome. *Blood* **117**, 4399-4400.

**Cross, N. L.** (1981). Initiation of the activation potential by an increase in intracellular calcium in eggs of the frog, *Rana pipiens*. *Dev. Biol.* **85**, 380-384.

**Cuthbert, A. W.** (2011). New horizons in the treatment of cystic fibrosis. *Br. J. Pharmacol.* **163**, 173-183.

**Davis, A. J., Forrest, A. S., Jepps, T. A., Valencik, M. L., Wiwchar, M., Singer, C. A., Sones, W. R., Greenwood, I. A. and Leblanc, N.** (2010). Expression profile and protein translation of TMEM16A in murine smooth muscle. *Am. J. Physiol. Cell Physiol.* **299**, C948-C959.

**Duran, C., Thompson, C. H., Xiao, Q. and Hartzell, H. C.** (2010). Chloride channels: often enigmatic, rarely predictable. *Annu. Rev. Physiol.* **72**, 95-121.

**Fallah, G., Romer, T., Detro-Dassen, S., Braam, U., Markwardt, F. and Schmalzing, G.** (2011). TMEM16A(a)/anoctamin-1 shares a homodimeric architecture with CLC chloride channels. *Mol. Cell. Proteomics* **10**, M110.004697.

**Ferrera, L., Caputo, A., Ubby, I., Bussani, E., Zegarra-Moran, O., Ravazzolo, R., Pagani, F. and Galietta, L. J. V.** (2009). Regulation of TMEM16A chloride channel properties by alternative splicing. *J. Biol. Chem.* **284**, 33360-33368.

**Fromm, J. and Lautner, S.** (2007). Electrical signals and their physiological significance in plants. *Plant Cell Environ.* **30**, 249-257.
**Hartzell, C., Putzier, I. and Arreola, J.** (2005). Calcium-activated chloride channels. *Annu. Rev. Physiol.* **67**, 719-758.
**Hengl, T., Kaneko, H., Dauner, K., Vocke, K., Frings, S. and Möhrlen, F.** (2010). Molecular components of signal amplification in olfactory sensory cilia. *Proc. Natl. Acad. Sci. USA* **107**, 6052-6057.
**Hirst, G. D. S., Bramich, N. J., Teramoto, N., Suzuki, H. and Edwards, F. R.** (2002). Regenerative component of slow waves in the guinea-pig gastric antrum involves a delayed increase in [Ca2+]i and Cl- channels. *J. Physiol. Lond.* **540**, 907-919.
**Huang, F., Rock, J. R., Harfe, B. D., Cheng, T., Huang, X., Jan, Y. N. and Jan, L. Y.** (2009). Studies on expression and function of the TMEM16A calcium-activated chloride channel. *Proc. Natl. Acad. Sci. USA* **106**, 21413-21418.
**Hwang, S. J., Blair, P. J. A., Britton, F. C., O'Driscoll, K. E., Hennig, G., Bayguinov, Y. R., Rock, J. R., Harfe, B. D., Sanders, K. M. and Ward, S. M.** (2009). Expression of anoctamin 1/TMEM16A by interstitial cells of Cajal is fundamental for slow wave activity in gastrointestinal muscles. *J. Physiol. Lond.* **587**, 4887-4904.
**Kaneko, H., Möhrlen, F. and Frings, S.** (2006). Calmodulin contributes to gating control in olfactory calcium-activated chloride channels. *J. Gen. Physiol.* **127**, 737-748.
**Kashyap, P., Gomez-Pinilla, P. J., Pozo, M. J., Cima, R. R., Dozois, E. J., Larson, D. W., Ordog, T., Gibbons, S. J. and Farrugia, G.** (2011). Immunoreactivity for Ano1 detects depletion of Kit-positive interstitial cells of Cajal in patients with slow transit constipation. *Neurogastroenterol. Motil.* **23**, 760-765.
**Kleene, S.** (2008). The electrochemical basis of odor transduction in vertebrate olfactory cilia. *Chem. Senses* **33**, 839-859.
**Kuruma, A.** (1999). Bimodal Control of a Ca2+-activated Cl- Channel by Different Ca2+ Signals. *J. Gen. Physiol.* **115**, 59-80.
**Lalonde, M. R., Kelly, M. E. and Barnes, S.** (2008). Calcium-activated chloride channels in the retina. *Channels (Austin)* **2**, 252-260.
**Large, W. A. and Wang, Q.** (1996). Characteristics and physiological role of the Ca(2+)-activated Cl-conductance in smooth muscle. *Am. J. Physiol.* **271**, C435-C454.
**Liu, B., Linley, J. E., Du, X., Zhang, X., Ooi, L., Zhang, H. and Gamper, N.** (2010). The acute nociceptive signals induced by bradykinin in rat sensory neurons are mediated by inhibition of M-type K+ channels and activation of Ca2+-activated Cl- channels. *J. Clin. Invest.* **120**, 1240-1252.
**Llano, I., Leresche, N. and Marty, A.** (1991). Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. *Neuron* **6**, 565-574.
**Manoury, B., Tamuleviciute, A. and Tammaro, P.** (2010). TMEM16A/anoctamin 1 protein mediates calcium-activated chloride currents in pulmonary arterial smooth muscle cells. *J. Physiol. Lond.* **588**, 2305-2314.
**Martins, J. R., Faria, D., Kongsuphol, P., Reisch, B., Schreiber, R. and Kunzelmann, K.** (2011). Anoctamin 6 is an essential component of the outwardly rectifying chloride channel. *Proc. Natl. Acad. Sci. USA* **108**, 18168-18172.
**Mayer, M.** (1985). A calcium-activated chloride current generates the after-depolarization of rat sensory neurones in culture. *J. Physiol. Lond.* **364**, 217-239.
**Melvin, J., Yule, D., Shuttleworth, T. and Begenisich, T.** (2005). Regulation of fluid and electrolyte secretion in salivary gland acinar cells. *Annu. Rev. Physiol.* **67**, 445-469.
**Mercer, A. J., Rabl, K., Riccardi, G. E., Brecha, N. C., Stella, S. L. and Thoreson, W. B.** (2011). Location of release sites and calcium-activated chloride channels relative to calcium channels at the photoreceptor ribbon synapse. *J. Neurophysiol.* **105**, 321-335.
**Namkung, W., Phuan, P. W. and Verkman, A. S.** (2011a). TMEM16A inhibitors reveal TMEM16A as a minor component of calcium-activated chloride channel conductance in airway and intestinal epithelial cells. *J. Biol. Chem.* **286**, 2365-2374.
**Namkung, W., Yao, Z., Finkbeiner, W. E. and Verkman, A. S.** (2011b). Small-molecule activators of TMEM16A, a calcium-activated chloride channel, stimulate epithelial chloride secretion and intestinal contraction. *FASEB J.* **25**, 4048-4062.
**Ousingsawat, J., Martins, J. R., Schreiber, R., Rock, J. R., Harfe, B. D. and Kunzelmann, K.** (2009). Loss of TMEM16A causes a defect in epithelial Ca2+-dependent chloride transport. *J. Biol. Chem.* **284**, 28698-28703.
**Pifferi, S., Dibattista, M. and Menini, A.** (2009). TMEM16B induces chloride currents activated by calcium in mammalian cells. *Pflugers Arch.* **458**, 1023-1038.
**Rock, J. R., Futtner, C. R. and Harfe, B. D.** (2008). The transmembrane protein TMEM16A is required for normal development of the murine trachea. *Dev. Biol.* **321**, 141-149.
**Rock, J. R., O'Neal, W. K., Gabriel, S. E., Randell, S. H., Harfe, B. D., Boucher, R. C. and Grubb, B. R.** (2009). Transmembrane protein 16A (TMEM16A) is a Ca2+-regulated Cl- secretory channel in mouse airways. *J. Biol. Chem.* **284**, 14875-14880.
**Romanenko, V. G., Catalán, M. A., Brown, D. A., Putzier, I., Hartzell, H. C., Marmorstein, A. D., Gonzalez-Begne, M., Rock, J. R., Harfe, B. D. and Melvin, J. E.** (2010). Tmem16A encodes the Ca2+-activated Cl- channel in mouse submandibular salivary gland acinar cells. *J. Biol. Chem.* **285**, 12990-13001.
**Sánchez-Vives, M. V. and Gallego, R.** (1994). Calcium-dependent chloride current induced by axotomy in rat sympathetic neurons. *J. Physiol. Lond.* **475**, 391-400.
**Sanders, K. M., Koh, S. D. and Ward, S. M.** (2006). Interstitial cells of cajal as pacemakers in the gastrointestinal tract. *Annu. Rev. Physiol.* **68**, 307-343.
**Schreiber, M. and Salkoff, L.** (1997). A novel calcium-sensing domain in the BK channel. *Biophys. J.* **73**, 1355-1363.
**Schreiber, R., Uliyakina, I., Kongsuphol, P., Warth, R., Mirza, M., Martins, J. R. and Kunzelmann, K.** (2010). Expression and function of epithelial anoctamins. *J. Biol. Chem.* **285**, 7838-7845.
**Schroeder, B., Cheng, T., Jan, Y. N. and Jan, L. Y.** (2008). Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. *Cell* **134**, 1019-1029.
**Sheridan, J. T., Worthington, E. N., Yu, K., Gabriel, S. E., Hartzell, H. C. and Tarran, R.** (2011). Characterization of the oligomeric structure of the Ca(2+)-activated Cl- channel Ano1/TMEM16A. *J. Biol. Chem.* **286**, 1381-1388.
**Shiina, T. and Tazawa, M.** (1987). Ca2+-activated Cl− channel in plasmalemma of Nitellopsis obtusa. *J. Membr. Biol.* **99**, 137-146.
**Stöhr, H., Heisig, J. B., Benz, P. M., Schöberl, S., Milenkovic, V. M., Strauss, O., Aartsen, W. M., Wijnholds, J., Weber, B. H. F. and Schulz, H. L.** (2009). TMEM16B, a novel protein with calcium-dependent chloride channel activity, associates with a presynaptic protein complex in photoreceptor terminals. *J. Neurosci.* **29**, 6809-6818.
**Sung, K., Kirby, M. and McDonald, M.** (2000). Abnormal GABAA receptor-mediated currents in dorsal root ganglion neurons isolated from Na-K-2Cl cotransporter null mice. *J. Neurosci.* **20**, 7531-7538.
**Suzuki, J., Umeda, M., Sims, P. J. and Nagata, S.** (2010). Calcium-dependent phospholipid scrambling by TMEM16F. *Nature* **468**, 834-838.
**Tian, Y., Kongsuphol, P., Hug, M., Ousingsawat, J., Witzgall, R., Schreiber, R. and Kunzelmann, K.** (2011). Calmodulin-dependent activation of the epithelial calcium-dependent chloride channel TMEM16A. *FASEB J.* **25**, 1058-1068.
**Tsutsumi, S., Kamata, N., Vokes, T. J., Maruoka, Y., Nakakuki, K., Enomoto, S., Omura, K., Amagasa, T., Nagayama, M., Saito-Ohara, F. et al.** (2004). The novel gene encoding a putative transmembrane protein is mutated in gnathodiaphyseal dysplasia (GDD). *Am. J. Hum. Genet.* **74**, 1255-1261.
**Vermeer, S., Hoischen, A., Meijer, R. P. P., Gilissen, C., Neveling, K., Wieskamp, N., de Brouwer, A., Koenig, M., Anheim, M., Assoum, M. et al.** (2010). Targeted next-generation sequencing of a 12.5 Mb homozygous region reveals ANO10 mutations in patients with autosomal-recessive cerebellar ataxia. *Am. J. Hum. Genet.* **87**, 813-819.
**West, R. B., Corless, C. L., Chen, X., Rubin, B. P., Subramanian, S., Montgomery, K., Zhu, S., Ball, C. A., Nielsen, T. O., Patel, R. et al.** (2004). The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. *Am. J. Pathol.* **165**, 107-113.
**Xia, X. M., Fakler, B., Rivard, A., Wayman, G., Johnson-Pais, T., Keen, J. E., Ishii, T., Hirschberg, B., Bond, C. T., Lutsenko, S. et al.** (1998). Mechanism of calcium gating in small-conductance calcium-activated potassium channels. *Nature* **395**, 503-507.
**Xiao, Q., Yu, K., Perez-Cornejo, P., Cui, Y., Arreola, J. and Hartzell, H. C.** (2011). Voltage- and calcium-dependent gating of TMEM16A/Ano1 chloride channels are physically coupled by the first intracellular loop. *Proc. Natl. Acad. Sci. USA* **108**, 8891-8896.
**Yang, Y. D., Cho, H., Koo, J. Y., Tak, M. H., Cho, Y., Shim, W.-S., Park, S. P., Lee, J., Lee, B., Kim, B.-M. et al.** (2008). TMEM16A confers receptor-activated calcium-dependent chloride conductance. *Nature* **455**, 1210-1215.
**Zhu, M., Kim, T., Ro, S. and Yan, W.** (2009). A Ca2+-activated Cl− conductance in interstitial cells of Cajal linked to slow wave currents and pacemaker activity. *J. Physiol.* **587**, 4905-4918.