The TMEM16 Protein Family: A New Class of Chloride Channels?

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ABSTRACT Cl^- channels play important roles in many physiological processes, including transepithelial ion absorption and secretion, smooth and skeletal muscle contraction, neuronal excitability, sensory perception, and cell volume regulation. The molecular identity of many types of Cl^- channels is still unknown. Recently, three research groups have arrived independently at the identification of TMEM16A (also known as anoctamin-1) as a membrane protein strongly related to the activity of Ca^{2+}-activated Cl^- channels (CaCCs). Site-specific mutagenesis of TMEM16A alters the properties of the channels, thus suggesting that TMEM16A forms, at least in part, the CaCC. TMEM16A is a member of a family that includes nine other membrane proteins. All TMEM16 proteins have a similar structure, with eight putative transmembrane domains and cytosolic amino- and carboxy-termini. TMEM16B expression also evokes the appearance of CaCCs, but with biophysical characteristics (voltage dependence, unitary conductance) different from those associated to TMEM16A. The roles of the other TMEM16 proteins are still unknown. The study of TMEM16 proteins may lead to identification of novel molecular mechanisms underlying ion transport and channel gating by voltage and Ca^{2+}.

INTRODUCTION

Ion channels permeable to Cl^- and other anions are key proteins of all cells, involved in housekeeping roles such as cell volume regulation or in more specialized physiological functions such as excitability, contraction, and transepithelial electrolyte/fluid transport (1). All these functions are carried out by a plethora of different Cl^- channels whose molecular identity is only partially known. For many years, studies based mostly on patch-clamp technique have reported the existence of Cl^-/anion channels characterized by different biophysical properties, mechanism of regulation, and pharmacological sensitivity (1,2). Mechanisms of activation include direct binding by extracellular ligands, elevation of intracellular Ca^{2+}, cAMP-dependent phosphorylation, membrane potential changes (hyperpolarization or depolarization), and cell swelling. A fraction of these channels have been identified at the molecular level: cystic fibrosis transmembrane conductance regulator (CFTR) as the cAMP-activated channel of epithelial cells, gamma aminobutyric acid (GABA)- and glycine-activated ionotropic receptors of inhibitory synapses, ClC-1 in skeletal muscle, ClC-Ka and ClC-Kb of kidney and inner ear, and the ubiquitous ClC-2 (1,2). The identity of other Cl^- channel types has remained elusive and quite controversial.

Among these more obscure membrane proteins, Ca^{2+}-activated Cl^- channels (CaCCs) have attracted the attention of many investigators who have tried to give them a molecular identity (3,4). CaCCs participate in many important physiological processes, including epithelial fluid secretion, smooth muscle contraction, and olfactory transduction (Fig. 1). Previous attempts to identify the CaCC indicated that CLCA (5), ClC-3 (6), bestrophins (7,8), and Tweety (9) are possible candidates. However, expression of such proteins generates Cl^- currents that are actually different from those of classical CaCCs (3,10). Indeed, one of the most common characteristics of native CaCCs in many tissues and cell types is a characteristic voltage dependence that is modulated by intracellular Ca^{2+} (3,4,11,12,13). At nonmaximal Ca^{2+} concentrations, such CaCC channels are activated and deactivated by positive and negative membrane potentials, respectively. Consequently, the current-voltage relationship is outwardly rectifying. With maximal Ca^{2+}, on the other hand, the channels are fully activated at all membrane potentials and the current-voltage relationship becomes linear. Such properties were not recapitulated by any of the postulated CaCC candidates. The simplest explanation for this discrepancy is that these candidate proteins represent different types of Cl^- channels (e.g., bestrophins) or no channels at all. For example, it is now believed that CLCA proteins are actually cell adhesion molecules whose relationship with Cl^- channels is indirect (14).

Recently, three research groups have independently identified TMEM16A as a strong candidate for the CaCC (15–17).

TMEM16A ENTERS THE SCENE

Yang and colleagues (15) identified TMEM16A by performing a bioinformatic analysis looking for novel membrane proteins with more than two transmembrane domains. Heterologous expression of TMEM16A, which was initially cloned from mouse retina, resulted in the appearance of Ca^{2+}-activated Cl^- channels. Furthermore, silencing of TMEM16A in vivo caused inhibition of salivary gland secretion, a classical example of a function involving CaCCs (15).

TMEM16A was also found by expression cloning (16). In fact, expression cloning in *Xenopus laevis* oocytes is

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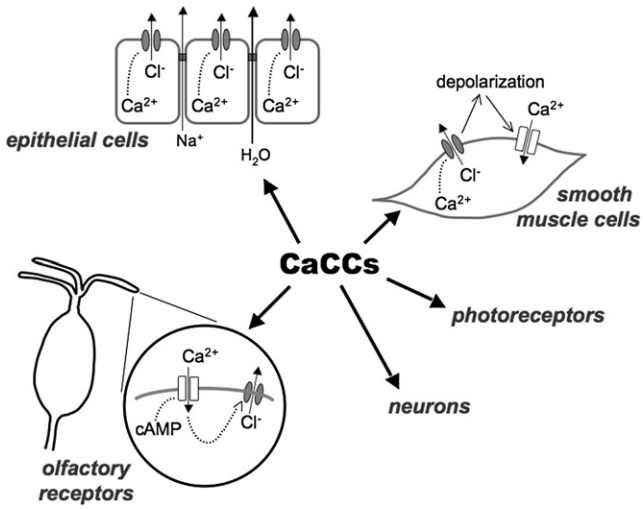

FIGURE 1 Physiological roles of CaCCs. In epithelial cells, activation of CaCCs by intracellular Ca^{2+} elevation leads to Cl^- secretion followed by transepithelial transport of Na^+ and water. In smooth muscle cells, activation of CaCCs is part of an amplification mechanism. Intracellular Ca^{2+} increase by extracellular stimuli activates CaCCs and Cl^- efflux. The resulting membrane depolarization opens voltage-dependent Ca^{2+} channels that cause a further intracellular Ca^{2+} increase, thus potentiating contraction. Another amplification mechanism occurs in olfactory receptors, where the initial Ca^{2+} increase is triggered by cAMP-gated channels. CaCC is also involved in phototransduction and regulation of neuronal excitability.

a classical strategy for identifying many types of ion channels, such as ClC-0 (18) and ENaC (19). Typically, this approach involves the expression in oocytes of large collections (libraries) of coding sequences derived from a tissue with high expression of a specific ion channel. The process is repeated on progressively smaller pools of sequences until a single clone coding for the searched channel is identified. This strategy was not possible for CaCCs because *Xenopus* oocytes already have a high endogenous expression of these channels. However, Schroeder and colleagues (16) realized that oocytes from a different amphibian, the Axolotl salamander (*Ambystoma mexicanum*), are devoid of CaCC activity. This finding paved the way for a classical expression cloning approach that resulted in the identification of a novel protein, TMEM16A, as the CaCC channel or a subunit thereof (16).

In our laboratory, we arrived at the same conclusion regarding TMEM16A using a functional genomics approach (17) (Fig. 2). We started from the observation that interleukin-4 (IL-4) upregulates Ca^{2+}-dependent Cl^- secretion in human bronchial epithelial cells (20). These data suggested a mechanism of action based on the upregulation by IL-4 of the gene coding for CaCCs. Therefore, we performed a global gene expression analysis by microarrays to identify genes upregulated by IL-4 and coding for membrane proteins with unknown function. The microarray analysis identified TMEM16A as a candidate. TMEM16A was then confirmed by a combination of experiments involving gene silencing with siRNA and overexpression by transfection (17).

Interestingly, all three studies reported that heterologous expression of TMEM16A in different cell types always generated Cl^- currents with the classical properties of CaCCs, e.g., slow activation at positive membrane potentials and inhibition by niflumic acid, 5-nitro-2-(phenylpropylamino)-benzoate (NPPB), and other CaCC blockers. The fact that three research teams arrived at the same conclusion using different strategies seems good evidence in favor of TMEM16A as a protein strongly related to CaCC activity.

PROPERTIES OF TMEM16A-ASSOCIATED Cl^- CURRENTS

The most striking feature of the TMEM16A protein is the consistent finding that its expression in different cell systems

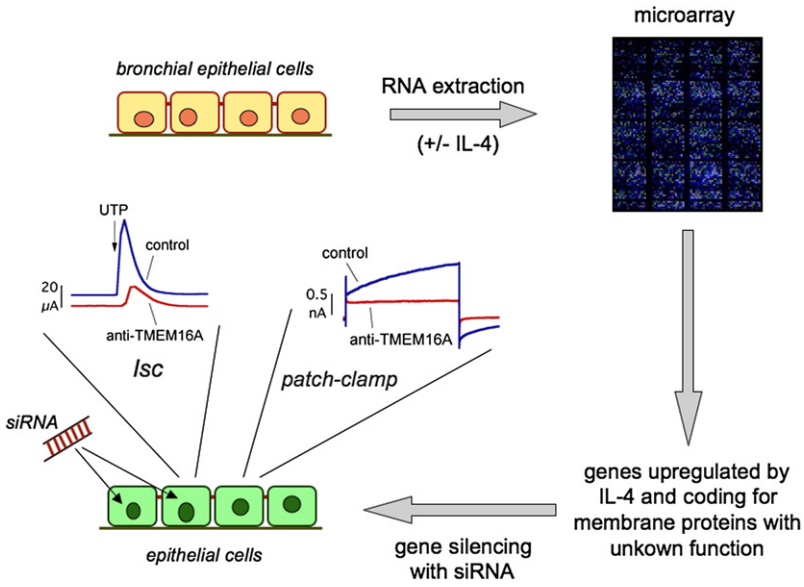

FIGURE 2 Identification of TMEM16A by functional genomics. Differentiated human bronchial epithelial cells respond to a 24-h IL-4 treatment with an upregulation of Ca^{2+}-depedent Cl^- secretion, presumably by hyperexpression of the gene coding for CaCC. Global gene expression analysis was carried out by microarrays to identify CaCC candidates, i.e., genes upregulated by IL-4 and coding for putative membrane proteins with unknown function. Candidate genes were silenced with siRNA in epithelial cells with endogenous CaCC activity. Functional analysis by short-circuit current (Isc) recording across whole epithelia or by the whole-cell patch-clamp technique showed that silencing of TMEM16A caused reduction of CaCC activity.

(HEK-293 and FRT cells and Axolotl oocytes) always leads to the appearance of voltage-dependent Ca^{2+}-activated Cl^- channels very similar to classical CaCCs (15–17,21). At non-maximal Ca^{2+} concentrations, voltage pulses to positive membrane potentials elicit slow-activating currents. This effect is reversible, as the return to negative membrane potentials causes a deactivation of the current. Accordingly, the steady-state current-voltage relationship under this condition is outwardly rectifying. However, elevation of intracellular Ca^{2+} to micromolar concentrations progressively shifts the voltage-dependent activation of the channel to negative values. At maximal concentration of Ca^{2+}, the channels become fully active at all membrane potentials, and consequently, the relaxations after voltage steps disappear. Yang et al. found that, similar to the case for native CaCC channels, the apparent affinity of TMEM16A-associated Cl^- channels for Ca^{2+} is affected by membrane potential (15). Indeed, the half-effective Ca^{2+} concentration is 2.6 μM at −60 mV and 0.4 μM at +60 mV. This property may be attributable to a Ca^{2+}-binding site lying within a region of the protein that senses the transmembrane electric field. Another possibility is that the affinity of the binding site for Ca^{2+} is modified by voltage using an allosteric mechanism.

Also, the ion selectivity of TMEM16A-associated channels reported by Yang et al. (15) resembles, at least in part, that of CaCC channels. In particular, cells expressing TMEM16A show channels with the following permeability sequence to anions: $NO_3^- > I^- > Br^- > Cl^- > F^-$ (15). This sequence is similar, although not identical, to that reported for native CaCCs (3,22,23). Interestingly, Schroeder et al. report that the channels induced by TMEM16A expression do not have a fixed ion selectivity (16). Rather, the ion permeability changes within the same cell depending on the state of channel activity. This finding suggests the existence of multiple open states of the channel, each characterized by a different degree of ion selectivity.

A third parameter to describe Cl^- channels is their sensitivity to pharmacological modulators. Compared to cation channels, most Cl^- channels suffer from a relative lack of specific inhibitors or activators (1,2). However, there is a panel of inhibitors that may be used to generate a fingerprint of pharmacological sensitivity for each Cl^- channel. For example, CaCCs are typically blocked by niflumic acid and NPPB at low micromolar concentrations (3). NPPB and niflumic acid, but not the selective CFTR inhibitor-172, strongly inhibit TMEM16A-dependent Cl^- channels (17). The only discrepancy is in the case of tamoxifen, a poorly specific channel inhibitor. This compound was reported to be active by Yang and colleagues (15), but Schroeder and co-workers (16) found it to be inactive.

Regarding the mechanism of regulation, TMEM16A-dependent Cl^- channels appear to be activated by all experimental maneuvers that result in an intracellular Ca^{2+} increase (15–17). Such maneuvers include intracellular dialysis with a high-Ca^{2+} solution utilizing a patch-clamp pipette, stimulation with a Ca^{2+} ionophore, receptor-mediated Ca^{2+} elevation, or perfusion of the cytosolic side of membrane patches during inside-out patch-clamp recordings. These findings clearly indicate that TMEM16A-dependent channels are activated by Ca^{2+}, but they do not clarify the mechanism through which Ca^{2+} activates the channels. Actually, this is an unresolved issue also for native CaCCs (3). Indeed, various studies have arrived at discrepant results. It has been reported that CaCCs are activated through calmodulin (24), or by means of Ca^{2+}-dependent phosphorylation (25,26). In smooth muscle cells, phosphorylation has even an inhibitory mechanism (27,28).

STRUCTURE-FUNCTION OF TMEM16A (OR, IS TMEM16A A CHANNEL?)

TMEM16A is part of a family of proteins that includes nine other members named as TMEM16B-K (29). The primary sequence identity is relatively high for TMEM16B (~60%) but decreases progressively with the other TMEM16 proteins, so that TMEM16F, G, H, J, and K are only 20–30% identical. All TMEM16 proteins have a similar putative topology, consisting of eight transmembrane segments and cytosolic N- and C-termini (Fig. 3). Because of this topology and their role in anion transport, TMEM16 proteins were recently named anoctamins. Interestingly, the transmembrane segments are the regions of TMEM16 proteins showing maximal conservation. For example, the sequence of the putative sixth transmembrane domain of human

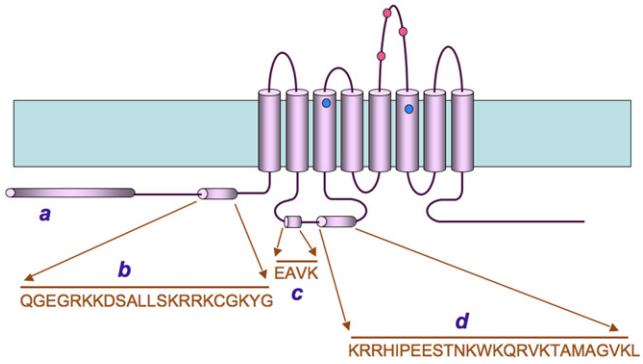

FIGURE 3 Structure of TMEM16A protein. The protein has eight putative transmembrane domains and intracellular N- and C-termini. Various isoforms are generated by inclusion or skipping of four alternative segments labeled *a* (116 residues), *b* (22 residues), *c* (4 residues), and *d* (26 residues). The full protein, TMEM16A(*abcd*), containing all four segments, is 1008 amino acids long. The sequence of segments *b*, *c*, and *d* is shown. Colored circles show amino acids whose mutagenesis leads to alteration of ion channel properties (voltage dependence and/or ion selectivity). Blue circles, R563 and Q757 in the third and sixth transmembrane domains (17); red circles, R669, K693, and K716 (R621, K645, and K668 in Yang et al. (15)). According to results obtained for these three amino acids (15), the region between the fifth and sixth transmembrane domains may not be entirely extracellular, as shown in the figure, but may form a reentrant loop that constitutes part of the channel pore.

TMEM16A (anoctamin-1 or ANO1) is 50% identical to that of TMEM16K (anoctamin-10, ANO10). However, the sequence identity decreases to ~20% when the protein is considered as a whole. The region between the fifth and sixth transmembrane segments, instead of being fully extracellular, as shown in Fig. 3, may form a reentrant loop important for the formation of the channel pore. Indeed, mutagenesis of positively charged amino acids localized in this region altered ion selectivity of the channels, thus enhancing their permeability to cations (15). Altered ion selectivity and voltage dependence were also observed by mutagenesis of an arginine and a glutamine in the third and sixth transmembrane domains, respectively (17) (Fig. 3). These results suggest that various regions of the TMEM16A protein are directly or indirectly involved in determining the properties of Ca^{2+}-activated Cl^- channels.

Another interesting characteristic of the TMEM16A protein is the presence of multiple isoforms that originate by alternative splicing (17). We have described the existence of a basic minimal isoform, termed TMEM16A(0), formed by 840 residues and containing all putative transmembrane segments. This structure can be expanded by addition/inclusion of four alternative regions called segments *a* (116 residues), *b* (22 residues), *c* (4 residues), and *d* (26 residues) (Fig. 3). Segment *a* is added at the N-terminus, thus extending the cytosolic portion of the protein. Segment *b* is included before the first transmembrane domain. Segments *c* and *d* are instead localized in the first intracellular loop connecting the second and third transmembrane domains. It is worth noting that TMEM16A(0) is functional and able to transport anions (17). However, in contrast to other isoforms, such as TMEM16A(*abc*) and TMEM16A(*abcd*), it is devoid of voltage-dependent activation. This finding suggests that alternative splicing is a mechanism to regulate channel properties and may be the basis for generation of different CaCC types with different voltage dependence and Ca^{2+} sensitivity.

Interestingly the studies carried out so far on TMEM16A have not led to the clarification of the mechanism of activation by Ca^{2+}. The TMEM16A sequence does not clearly show similarity to known domains involved in direct Ca^{2+} binding (e.g., EF-hands) or motifs suggesting binding to calmodulin. It is possible that either Ca^{2+} dependence is mediated by another protein or TMEM16A has noncanonical domains involved in regulation by Ca^{2+}. In this respect, we can hypothesize that regions of TMEM16A protein rich in negatively charged residues are involved in Ca^{2+} binding. For example, there is a cluster of four glutamic acid residues in the first intracellular loop connecting the second and third transmembrane domains, right before the site of insertion of segment *c*. Actually, inclusion of the microexon corresponding to segment *c* adds a fifth glutamic acid residue. This concentration of negative charges may resemble the Ca^{2+} bowl of large-conductance Ca^{2+}-activated K^+ channels (30). However, we have to consider that coordination of Ca^{2+} ions may also involve the carbonyls of the peptidic backbone. Therefore, the site for Ca^{2+} binding may not be easily predicted. Demonstration that TMEM16A is a Ca^{2+}-regulated molecule may require extensive mutagenesis studies spanning large sections of the whole protein.

In summary, the high similarity between TMEM16A-dependent currents and CaCCs suggests that the TMEM16A protein is a channel. However, although the data obtained so far are exciting and encouraging, it is important to be cautious, particularly considering previous claims about other membrane proteins as Cl^- channels. In fact, various scenarios are still possible. TMEM16A may be the only protein needed to form the CaCC channel, or it may be just part of the channel, thus requiring assembly with other proteins yet to be identified. Finally, although this is less likely, we cannot exclude entirely the possibility that TMEM16A is not actually part of the channel but is instead required for activating or trafficking of the real CaCC. As an example, we cite the STIM-1 protein (31), which is important for the function of the Ca^{2+} channel activated by store depletion. By sensing the endoplasmic reticulum Ca^{2+} content, STIM-1 works as a regulator of the real channel represented by the Orai-1 protein (32).

It is not simple to define absolutely the criteria needed to conclude that a given membrane protein is an ion channel. Alteration of intrinsic ion channel properties by site-specific mutagenesis is considered a good proof that a given protein is an ion channel. Actually, as stated above, amino acid changes in the sequence of TMEM16A have resulted in the modification of voltage dependence and ion selectivity of the associated Cl^- channels (15,17). These findings suggest that mutations directly alter the structure and function of the pore and of the voltage sensor of the CaCC channel. However, considering the small number of sites that have been mutated so far, it is still possible that the observed effects are caused by intramolecular or intermolecular allosteric mechanisms. Actually, Schroeder et al. have suggested the existence of multiple open-channel states, each characterized by a different ion selectivity (16). If this is the case, mutations of sites involved in channel gating may lead to an alteration of the apparent ion selectivity of macroscopic currents, thus leading to the erroneous conclusion that those sites form the channel pore.

It is probable that progressive accumulation of mutagenesis studies from various laboratories will provide further evidence for TMEM16A as a CaCC channel. Also, reconstitution of TMEM16A protein in cell-free artificial membranes may be a way to demonstrate the ion channel function. These and other types of studies will need also to clarify whether the CaCC is a TMEM16A monomer, a TMEM16A homo-oligomer, or a heteromultimeric complex formed by TMEM16A in combination with other proteins (possibly of the TMEM16 family). In the latter case, tissue-specific differences in the assembly of CaCCs could account for the wide variety of CaCC properties reported in many studies.

THE OTHER TMEM16 PROTEINS

The probable role of TMEM16A as an ion channel suggests that the other TMEM16 proteins are also involved in ion transport. Indeed, Schroeder et al. showed that heterologous expression of TMEM16B elicits the appearance of Ca^{2+}-activated Cl^- currents (16). This finding was later confirmed by three other groups (33–35). In particular, TMEM16B (anoctamin-2) is expressed in photoreceptors (33) and in the cilium of olfactory cells (35), where it appears to play a special role in sensory transduction.

Interestingly, TMEM16B shows some significant functional differences compared to TMEM16A. The channels associated with TMEM16B have a unitary conductance ~10-fold reduced relative to TMEM16A: 0.8 pS vs 8.3 pS (15,35). Also, TMEM16B expression evokes Cl^- currents that have kinetics faster than those of TMEM16A. Under conditions of nonmaximal activation by Ca^{2+}, membrane depolarization elicits TMEM16B-dependent currents that rapidly increase, reaching a new steady state in <50 ms. The time constant for current activation at $+100$ mV is only 4 ms (34). In contrast, TMEM16A-dependent currents show, upon depolarization, a slow activation that takes hundreds of milliseconds to stabilize (16,17). Finally, TMEM16B is less Ca^{2+}-sensitive. The half-effective Ca^{2+} concentrations for TMEM16B and TMEM16A at positive membrane potentials are in the micromolar and the nanomolar ranges, respectively (15,34,35). It appears also that the apparent Ca^{2+} affinity for TMEM16B is less affected by membrane potential than that of TMEM16A (34,35). For example, the half-effective Ca^{2+} concentration for TMEM16B is 4.9 μM at -50 mV and 3.3 μM at $+50$ mV (34). In contrast, TMEM16A shows a near-sixfold difference in apparent Ca^{2+} affinity when changing the membrane potential to a similar extent (15). The permeability sequence for anions measured for TMEM16B-dependent currents is $SCN^- > I^- > NO_3^- > Br^- > Cl^-$ (34).

Differences in the properties of the currents associated with TMEM16A and TMEM16B may be very useful in understanding the structure-function relationship. Comparison of the primary structure of the two proteins, by demonstrating similarity or divergence, may lead to identification of the domains involved in Ca^{2+} dependence, voltage sensitivity, and anion transport.

So far, much less is known for the other TMEM16 proteins. According to databases, TMEM16C is particularly expressed in the nervous system but there are no indications about its physiological role. TMEM16E (anoctamin-5), also known as GDD1, is mutated in a human genetic disease called gnathodiaphyseal dysplasia, a possible calcification disorder of the bone (36). TMEM16F and TMEM16K have ubiquitous expression, whereas TMEM16G seems specifically expressed in prostate. Among all TMEM16 proteins, TMEM16H is the one with the most intriguing structural characteristics. The region between the fifth and sixth transmembrane domains is rich in negative charges. In particular, it contains a stretch of 20 contiguous residues of glutamic and aspartic acid. The cytosolic carboxy-terminus is instead extremely abundant in prolines (56 out of 282 total residues).

We do not know at the moment whether the TMEM16 proteins, other than TMEM16A and TMEM16B, are also associated with Cl^- channels. It is possible that they represent different types of ion channels activated by other types of physiological stimuli. There is still a plethora of anion channels whose molecular identity remains to be clarified. Probably the most wanted protein in this field now is the volume-regulated anion channel (VRAC, also known as VSOAC). This channel was previously associated with other proteins such as the P-glycoprotein, ClC-3, and pICln, but its molecular identity has actually remained unresolved (1). It is tempting to postulate that VRAC is related to one of the proteins of the TMEM16 family, an hypothesis that is worth being tested in future studies.

PERSPECTIVES

The clarification of the physiological role of TMEM16 proteins will probably benefit from the development and study of animal models. A knockout mouse for TMEM16A is already available (37). Actually, it was generated even before knowing the involvement of TMEM16A in ion transport. Interestingly, this animal shows a severe phenotype characterized by altered formation of tracheal cartilage rings (37). This alteration probably causes a tracheal collapse and suffocation of the animal at early stages after birth. However, it is probable that careful examination of these animals will reveal further alterations in other organs. At the moment, the reason for tracheal cartilage abnormalities is unknown. The TMEM16A knockout mouse shows also a strongly reduced Ca^{2+}-dependent Cl^- secretion and accumulation of mucus in the airways (38,39). The former defect supports the role of TMEM16A as a Ca^{2+}-activated Cl^- channel in epithelial cells. The latter defect is instead a tantalizing finding suggesting that the phenotype of TMEM16A knockout mice mimics, at least in part, the lung disease of cystic fibrosis (CF) patients. CF is a severe genetic disease caused by mutations that impair the function of the CFTR epithelial Cl^- channel (40). CFTR loss of function causes an imbalance in ion/fluid transport, resulting in severe dehydration of the airway surface, accumulation of mucus, and colonization of the airways by antibiotic-resistant bacteria (40). It is well known in the CF field that CFTR knockout mice have no CF-like disease in their lungs. This characteristic has been explained by postulating that CFTR knockout mice have a Cl^- channel whose activity may compensate for the lack of CFTR. It is possible that TMEM16A is that channel. Indeed, the absence of TMEM16A function in knockout mice significantly impairs mucociliary transport (39). Therefore, TMEM16A has an important role in the

physiology of airway epithelium and is a possible pharmacological target to circumvent the Cl^- transport defect in CF patients.

TMEM16A is also a potential drug target for other human diseases. The role of CaCCs in smooth muscle contraction is the rationale to look for TMEM16A modulators as possible drugs for the treatment of asthma, hypertension, and gastrointestinal motility disorders. However, to avoid undesired side effects, developers of new drugs targeting TMEM16A have to take into account its expression and role in many tissues and organs. The profiling of alternative splicing in different tissues and the characterization of pharmacological sensitivity of the various TMEM16A isoforms may help to design tissue-specific drugs.

Another intriguing characteristic of TMEM16 proteins, and of TMEM16A in particular, is their overexpression in human cancers. In fact, TMEM16A was already known to oncologists by various names—DOG-1, ORAOV2, and TAOS-2—because of its high expression levels in various tumors, particularly of the gastrointestinal tract (41–43). The relationship between cancer and a protein with a role in Cl^- transport is not clear. CaCCs may be important in proliferation, migration, and resistance of cancer cells to apoptotic stimuli. In such a case, TMEM16A may become an important target for antitumor therapies. However, we cannot exclude the possibility that overexpression of TMEM16A derives from amplification of the chromosomal region containing the gene (11q13). This event may be favored because the same region contains other genes involved in cancer cell survival, proliferation, and invasiveness.

In conclusion, the identification of TMEM16A and TMEM16B as possible Ca^{2+}-dependent Cl^- channels is an exciting novelty in the field of ion transport physiology and membrane protein biophysics. Investigation of the structure-function relationship may reveal novel molecular mechanisms and domains developed by nature to transport ions across the plasma membrane and to sense changes in membrane potential and cytosolic Ca^{2+} concentration. The prospect of defining the role of the other TMEM16 proteins is similarly interesting. It is possible that these proteins represent other types of ion channels. Alternatively, as found for ClC proteins, it is possible that the TMEM16 family includes members with different functions (44). Some may be channels, whereas others may be transporters or have unexpected properties.

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