Oluwatobiloba Oguntoyinbo
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Structure and Function of TMEM 16A (Anoctamin)

INTRODUCTION

The first calcium-activated chloride channels (CaCCs) were discovered in Xenopus oocytes[1]. he protein is widely expressed and its primary physiological role is to mediate and regulate anion penetration of the membrane in response to an increase in intracellular Ca_2^{+2}[2]. For many years, research in the field was slowed by the significant ambiguity and debate surrounding the molecular identification of CaCCs. In 2008, three distinct research teams discovered that TMEM16A, one of these orphan proteins, is a part of the Ca^{2+}-activated Cl channel, CaCC[1]. Ten members of the TMEM16 family which all have a high degree of sequence conservation, are found in vertebrates. The majority of these are thought to behave as Ca^{2+}-activated lipid scramblases with non-selective ion channel activity, which catalyze lipid shuffling between leaflets inside the bilayer in an ATP-independent manner. Of these, TMEM16A and TMEM16B are CaCCs. The following are typical attributes of the channel: 1) Outward rectification of the steady-state current-voltage relationship caused by activation and deactivation at positive and negative membrane potentials, respectively; and 2) cytosolic Ca^{2+} activation with an apparent affinity that is boosted by a change in the membrane potential to positive values[3].

As the most traditional CaCCs, TMEM16A exhibits conventional CaCC traits such voltage-dependent activation and Ca^{2+}[4]. In addition to calcium ion, voltage, and anion control, recent research have discovered that phosphatidylinositol 4, 5-bisphosphate [PI(4,5)P2] regulates TMEM16A[5]. Currently, epithelial, neuronal, smooth muscle, vascular endothelial, and cardiac cells have been found to express TMEM16A[1]. TMEM16A's widespread distribution across various tissues suggests that it has diverse physiological functions. Research has demonstrated that TMEM16A plays a role in regulating neuronal signaling, as well as airway and exocrine gland

secretion, smooth muscle contraction, and rhythmic movements of the gastrointestinal system. The diversity of TMEM16A's physiological roles is indicated by the protein's widespread distribution in many tissues. According to studies, TMEM16A regulates smooth muscle contraction, rhythmic gastrointestinal motions, exocrine gland secretion, neuronal signaling, and airway secretion[6]. Additionally, TMEM16A is linked to cancer. According to research, some cancer cells, such as head and neck squamous cell carcinoma, gastric cancer, gastrointestinal stromal tumors, and colon cancer, have abnormally elevated levels of the gene TMEM16A. Inhibiting TMEM16A can prevent the growth, migration, and invasion of cancer cells. It is crucial to comprehend the structural and functional relationships of TMEM16A in order to use it as a target in the treatment of diseases, even if multiple TMEM16A regulators have been identified through high-throughput screening[7]. Here, I review existing literature in an effort to describe the most recent studies on the structure of TMEM16A and how it connects to its functions.

The Overall Structure of The TMEM16A Channel

Chemical cross-linking, nondenaturing polyacrylamide gel electrophoresis (PAGE), and electromobility shift experiments carried out by Sheridan et al. demonstrated that TMEM16A occurs as a dimer[8]. Additionally, Fallah et al. (2011) evaluated the homodimeric structure of TMEM16A using blue native PAGE and chemical cross-linking. Hence, biochemical tests were used to identify the quaternary structure of TMEM16A[9]. The first high-resolution structure of the TMEM16 family was revealed by Brunner et al. in 2014 for a TMEM16 homologue from Nectria haematococca (nhTMEM16)[1].

Before the TMEM16A structure was discovered, the structure of nhTMEM16 served as a crucial point of comparison for us to comprehend the TMEM16A structure and offered the opportunity for homology modeling of TMEM16A. The TMEM16A sequence has segments a, b, c, and d that

can be translated into proteins with anywhere between 712 and 1006 amino acids. The first 116 amino acids of alternatively spliced segment A are crucial for TMEM16A activity. Progressively reduction in TMEM16A expression and function was accompanied by stepwise shortening of the N-terminus. Segments b (located at the N-terminus and containing 22 amino acids) and c (located in the first intracellular loop and including EAVK) may be components of protein domains involved in voltage and Ca^{2+} sensing. Segment b's absence has a significant impact on how sensitive TMEM16A is to Ca^{2+}. The apparent Ca^{2+} sensitivity is reduced by almost 50 times when segment C is deleted, and the voltage-dependent activation of the channel is improved. The 26 amino acid segment d doesn't seem to change the activity of the TMEM16A channel. However, segment b and segment d's alternate splicing might work closely together. On the one hand, the d segment is typically left out of mature transcripts in the five tissues—liver, placenta, prostate, thyroid, and trachea—that include the b segment. On the other hand, skipping of segment b is connected to inclusion of segment d in some tissues but not all of them[1].

Recently, single particle cryoelectron microscopy has revealed the high-resolution protein structure of TMEM16A. First disclosed10 was the Mus musculus TMEM16A (PDB ID: 5NL2) with a resolution of 6.6Å[10]. The subunit cavity site, which is sealed from the membrane to prevent lipid ingress and only permits ion permeation, is where nhTMEM16 differs most from other TMEMs. The structural distinctions between lipid scramblase and ion channels in the TMEM16 family are made known for the first time by this structure. Two study publications were independently published in Nature in December 2017 by two different groups which reported the double Ca^{2+} binding state (5OYB, 6BGI), the single Ca^{2+} binding state (6BGJ), and the Ca^{2+} free state (5OYG) were among the four high-resolution TMEM16A structures that they discovered[1]. Among these, 5OYB has the greatest resolution and most comprehensive crystal structure of

TMEM16A (Fig. 1a and b). The nhTMEM16 and the mTMEM16A have a similar general design. Long stretches of amino acids connecting the a-helices a1-a2, a5-a6, and a9-a10 engage with one another on the extracellular side to generate a folded domain that is made up of coil regions with secondary structure elements interleaved and is supported by four disulfide bridges. The cell membrane is perforated by ten transmembrane helices, two of which are conserved Ca^{2+} binding sites and a closed ion channel. The N-terminal domain on the intracellular side has a ferredoxin-like fold that is similar to the corresponding region in the nhTMEM16 scramblase (Fig. 1a and b)[1].

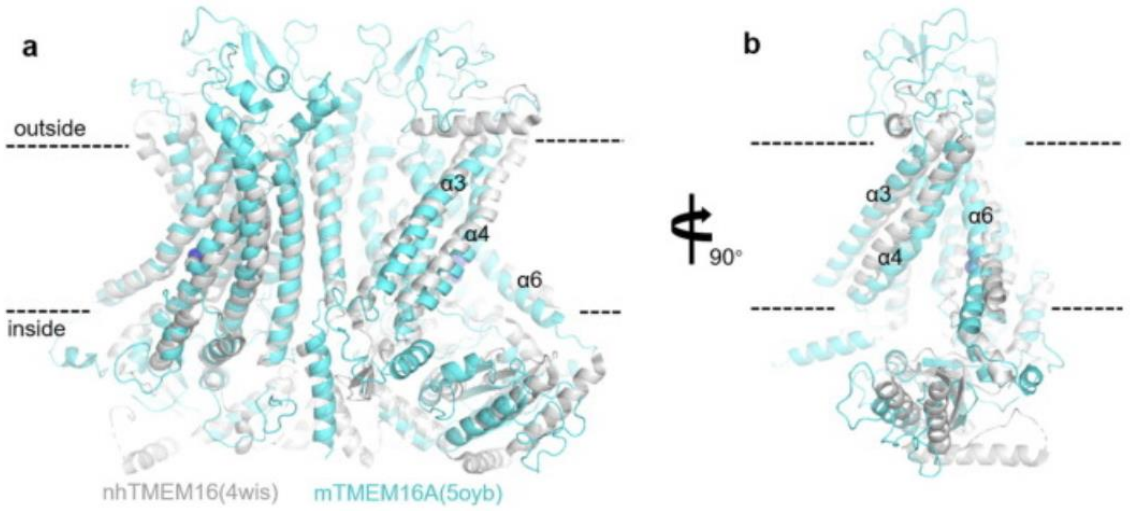


Fig. 1 Illustration of the TMEM16A channel's Ca2+-bound structure (cyan) with a lipid scrambler (nhTMEM16; gray).

Ca^{2+} binding sites

In TMEM16A, Ca2+-dependent gating is mostly regulated by Ca2+ binding. The first intracellular 23 loop structure (EEEEEAVK), which was discovered by Xiao and Cui (2014) via site-directed mutagenesis, is crucial for both Ca2+ and voltage sensitivity. 444EEEE/AAAA447 mutant altered voltage dependency, whereas 448EAVK deletion dramatically decreased Ca2+ affinity[11]. Due to slower channel activation, TMEM16A lacking EAVK exhibits poorer steady-state calcium

sensitivity. This suggests that the calcium sensitivity of TMEM16A current activation and deactivation depends on EAVK. Additionally, glutamine replacement of E702 and E705 of 7 in TMEM16A shown a considerable reduction in calcium sensitivity[12]. Also, Paulino et al. (2017) discovered that altering seven amino acids in the 68 (N650, N651, E654, E702, E705, E734, D738) decreased the efficacy of Ca2+, indicating that these acidic residues can coordinate with Ca2+[10].

Pore domain

The position of the TMEM16A pore domain is crucial to comprehending how TMEM16A mediates and regulates anion permeation. The scramblase structures of the TMEM16 family, which includes TMEM16K, TM16F, afTMEM16, and nhTMEM16, demonstrate that each subunit's "subunit cavity" offers a sufficient route for polar lipid headgroups to travel through the bilayer's hydrophobic core13. The pore domain in the mTMEM16A that facilitates ion permeation has the form of a "subunit cavity." But in relation to the TMEM16 lipid scramblase, this area, particularly TM 3, 4, and 6, experiences a conformational change (Fig. 1b)[1].

PI(4,5)P2 binding sites

The major signaling lipid phosphatidylinositol (4,5)-bisphosphates [PI(4,5)P2] is a crucial regulator of numerous cation channels and a number of signaling lipids. Through experimental mutagenesis and molecular modeling, Yu et al. discovered three PI(4,5)P2binding sites in TMEM16A, revealing the mechanism by which PI(4,5)P2 regulates TMEM16A[5]. These three sites are made up of five basic residues each (R433, K430, R429, R437, K313), seven (K659, R662, R665, R668, R682, R683, K684), and three (R461, K480, R484). Experimental evidence shows that these residues have varying degrees of influence on how TMEM16A reacts to PI (4,5)P2. (Fig.

2a). Near the membrane-cytoplasmic interface, where there is a high electrostatic potential, these sites are evenly distributed (Fig. 2b). Due to the negative charge of PI(4,5)P2, its binding site typically has two or more positively charged residues.[1].

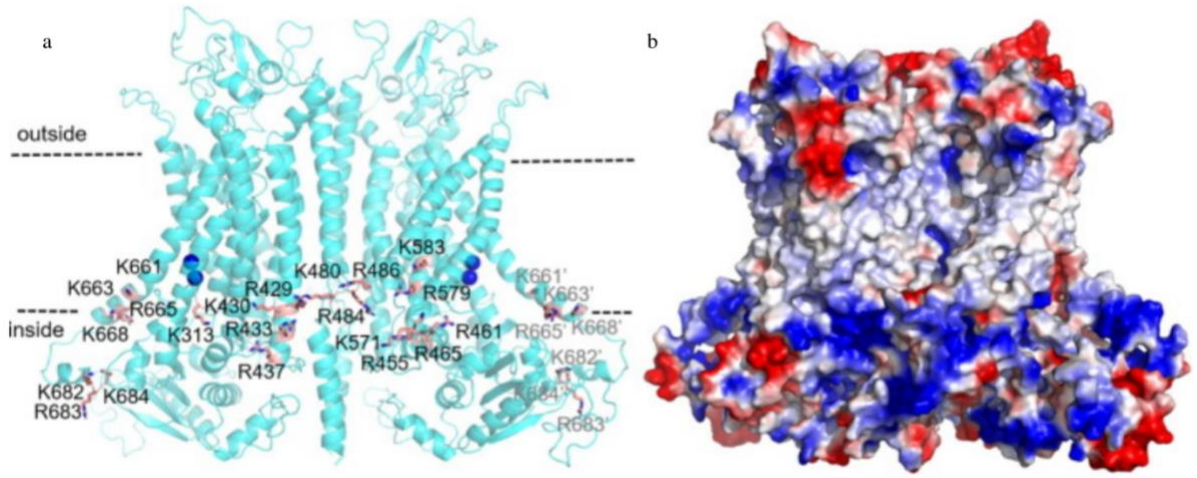


Fig. 2 (a) PI(4,5)P2 binding sites - Only the amino acids that can be presented on this side are shown, and important amino acids are denoted by sticks. (b) The TMEM16A electrostatic surface. Blue (+50 kT/e), and red (50 kT/e).

Calcium-dependent conformational transition

The structure of TMEM16A in various states may hold clues to the allosteric mechanism behind Ca2+ activation. The revelation of several homologous structure details offers a significant and comprehensive reference for the study of the TMEM16A channel. Paulino et al. explained the activation mechanism of mTMEM16A based on the TMEM16A structure in the Ca2+ free (5OYG) and binding (5OYB) states (Fig. 3)[10]. The inner half of TM6, which serves as a gating factor throughout the activation process, is where there is the most visible variation between the two structures. The Ca2+ ions at the binding site attract E654, which causes the lower half of TM6 to reorient and results in the transition of this fragment from a-helix to p-helix. In summary, TM6 is a key component of TMEM16A channel gating, especially E654 and K645 on TM6 play a crucial role in Ca2+ binding and ion permeation[1].

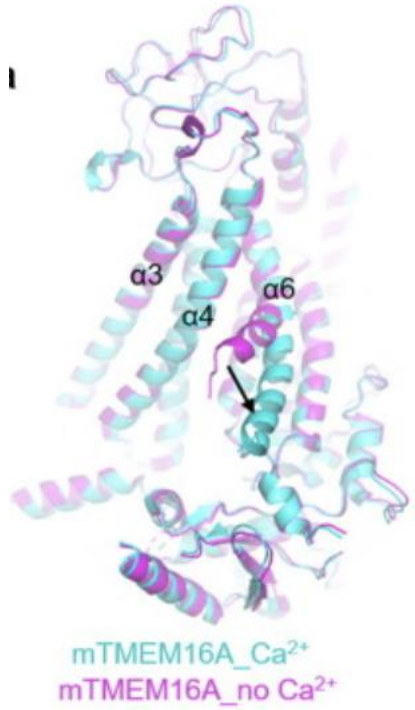


Fig. 3. TMEM16A's Ca2+-bound structure is cyan, while its Ca2+-free structure is shown in magenta.

Electrostatic properties of TMEM16A

The APBS tool's calculation of the protein's electric field and electrostatic surface potential reveals that the Holo state's positive electric field is stronger than the Apo state's in the neck and intracellular vestibule regions. The enrichment of chloride ions and the promotion of chloride ion penetration are made possible by a strong positive electric field. The TMEM16A protein's electrostatic surface potential also confirms a crucial point. The environment is exposed to the Apo state's Ca^{2+}-binding site, which has an electrostatic surface potential that is electronegative and electrostatically complementary to Ca2+. The Holo state structure's Ca^{2+}-binding site is enclosed between TMs 6 and 8, which is primarily due to the bottom portion of TM6 moving toward TM7 and TM8. These findings show the electrostatic characteristics of the various TMEM16A channel states and point to a possible physical relationship between the channel structure and Ca^{2+} binding and chloride ion penetration[12].

Conclusion

The TMEM16A calcium-activated chloride channel exhibits calcium- and membrane potential-dependent activation. The channel is considered to be a target for the therapy of diseases like hypertension, cystic fibrosis, and lung cancer because of its widespread expression and contribution to a number of physiological processes. It is crucial to fully comprehend TMEM16A's structural features, as was done above, in order to determine its physiological and pathological functions. As a result, this will serve as a crucial foundation for the development of medications that target TMEM16A.

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