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PERSPECTIVES

Time for TMEM?

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Plasma membrane anion channels have been among the most difficult of proteins to identify at the molecular level. Unlike their positive cousins the Na^+, K^+ and Ca^{2+} channels, a lack of high affinity ligands has hampered both their purification and molecular characterization. The task has been further complicated by the finding that some proteins thought to be anion channels turn out not to be channels at all, instead functioning as transporters, proteases or signalling molecules. Despite these hurdles, several families of both verifiable and putative anion channels have been identified. Of these rare membrane proteins, perhaps those most refractory to formal identification have been the calcium-activated chloride channels or CaCCs. Calcium-activated chloride currents are widely expressed and easy to record. Importantly, robust CaCC currents can be recorded from airway cells making them of significant clinical interest as an alternative pathway for airway chloride secretion in cystic fibrosis (CF) patients. Understanding and manipulating these channels would provide a mechanism to increase the depth of airway surface fluid, thus potentially correcting the airway dehydration which is the consequence of a dysfunctional CFTR, and which is the underlying cause for the decreased lung function and persistent infections associated with CF. However, these currents and channels have proved notoriously difficult to tie down to any particular protein. Several different genes have been previously proposed to encode putative CaCCs, including the CLCA family, members of which seem to have protease and cell adhesive properties (Bothe *et al.* 2011), and the bestrophins, proteins previously thought to be very good candidates but which may in fact regulate CaCCs by affecting intracellular calcium handling (Kunzelmann *et al.* 2011).

The most recent pretenders to the title of true CaCC are the anoctamins or TMEM family. These proteins, originally identified in bronchial epithelial cells, *Xenopus* oocytes and murine eye, have so far passed most of the challenges with which they have been presented. The anoctamin/TMEM family currently consists of 10 members, although only the first two are associated with significant calcium-activated chloride channel activity (two other members seem to have some ability to act as CaCCs), with the majority having as yet no known function (Ferrara *et al.* 2010). When heterologously expressed, TMEM16A is associated with expression of a robust outwardly rectifying calcium and time-dependent anion current; siRNA silencing abolishes this current while a TMEM16A knockout mouse exhibits significantly reduced airway CaCC activity (Rock *et al.* 2009). However, a recent report that a blocker of TMEM16A ($T16_{inh}$-A01) was only partially effective against human airway CaCC, while nearly abolishing TMEM16A activity in a heterologous expression system (Namkung *et al.* 2011) has cast doubt on the role of this protein in the human airway.

The function of TMEM16A in human airway has now been re-visited in a study by Scudieri *et al.* (2012) in the current issue of *The Journal of Physiology*. These authors report that while expression of TMEM16A in normal and CF primary airway epithelial cells and a CF bronchial epithelial cell line is relatively poor under control conditions, the small calcium-activated chloride current is abolished by siRNAs directed against human TMEM16A. These data suggest that TMEM16A is responsible for basal CaCC activity. However, the critical data were obtained in cells exposed to IL4, a key cytokine involved in the development of Th2 cells and the generation of IgE, which is central to the allergic response and the pathogenesis of asthma. In cells pretreated for 24 h with IL4, calcium-sensitive chloride currents were increased by 8- to 10-fold. This increase in current was matched by an increase in TMEM16A expression. However, the effects of IL4 on current were only partially inhibited by $T16_{inh}$-A01. In addition, some residual current remained following siRNA silencing, consistent with the expression of a non-TMEM16A current in the airway cells, the molecular identity of which is currently unknown.

Exposure to IL4 was associated with an increased amount of TMEM16A expression at the apical membrane, although in the airway this seemed to be restricted to a subset of epithelial cells. It turned out that the cells showing the highest expression of TMEM16A were goblet cells, primarily responsible for the secretion of mucus. IL4 is known to cause mucus cell metaplasia, and this is characterized by increased expression of MUC5AC. Scudieri *et al.* report that following a prolonged (72 h) incubation with IL4, 60% of TMEM16A positive cells also expressed MUC5AC, although with shorter incubation times far fewer cells showed this association, and untreated cells exhibited nearly no co-expression. In contrast the ciliated airway cells expressed very little TMEM16A. Because MUC5AC expression seems to lag behind the maximal increases in CaCC activity the authors propose that IL4 causes increased expression of TMEM16A in a subset of cells (potentially Clara cells), which then acquire the goblet cell phenotype. Expression of an apical chloride channel in these cells or in the mucus granule membrane would facilitate wash-out of mucus into the pericilliary fluid layer. Interestingly, mucus granules contain significant amounts of ATP which could increase TMEM16A activity by binding to cell surface purinergic receptors activating the calcium signalling cascade. Consistent with this possibility, niflumic acid, which also inhibits CaCCs, prevents degranulation of airway mucus granules (Kondo *et al.* 2012). A second recent study has similarly reported that TMEM16A is involved in airway mucus secretion and is up-regulated in a murine model of asthma. Furthermore this study demonstrated that TMEM16A may also be involved in the increased contractility (hyperesponsiveness) of airway smooth muscle, an additional characteristic of asthma (Huang *et al.* 2012). Manipulation of this channel would therefore seem to be an attractive therapeutic strategy in airway diseases such as asthma and COPD in which inflammation and increased mucus production are prevalent. Whether or not this channel will also prove to be a useful target to increase airway fluid secretion in CF remains to be determined.

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