Chloride secretion, anoctamin 1 and Ca$^{2+}$ signaling

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Ten paralogues proteins form the anoctamin family (Ano1-10, TMEM16A-K). It was shown for Ano1, 2, and 6 that they can produce Ca$^{2+}$ activated Cl$^-$ channels, 1,2 while Ano6 and other anoctamins have additional functions such as scrambling of membrane phospholipids. 3 Ano1 is now regarded as the important apical Ca$^{2+}$ activated Cl$^-$ channel that is in charge of Ca$^{2+}$ dependent Cl$^-$ secretion; although it’s pronounced outward rectification would prefer Cl$^-$ movement into the cell, rather than out of the cell. Whether there is substantial contribution of other anoctamin paralogues to Ca$^{2+}$ dependent Cl$^-$ secretion is currently not known. It is well established that epithelial Cl$^-$ secretion is mainly due to an increase in intracellular cAMP and activation of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl$^-$ channel. It makes entirely sense that cAMP is the big player, since it is well known that cAMP does not only activate luminal CFTR channels through protein kinase A dependent phosphorylation, but in parallel also activates basolateral Cl$^-$ uptake through Na$^+$/2Cl$^-$/K$^+$ cotransporters (NKCC1), and recycling of K$^+$ by basolateral K$^+$ channels (KCNQ1) (Fig. 1). Only concerted activation of channels and transporters in luminal and basolateral membrane allow for sustained electrolyte secretion.

Compared to cAMP-controlled secretion, Cl$^-$ transport activated by increase in intracellular Ca$^{2+}$ has been shown to be much more transient, probably because of only transient activation of luminal Ano1 Cl$^-$ channels, but also because basolateral uptake by NKCC1 is not activated, at least not to the same level as for cAMP-stimulation. This asks for the true contribution of Ca$^{2+}$ mediated secretion in airways and gut. Interestingly, we know for quite some time that CFTR and Ca$^{2+}$ dependent Cl$^-$ currents somehow interfere. This has been confirmed recently by showing that CFTR and Ano1 functionally and molecularly interact. 4 In these experiments it was never possible to add both seemingly independent Cl$^-$ conductances on top of each other. Maybe this is due to the fact that CFTR is not only controlled by cAMP, but also by intracellular Ca$^{2+}$ levels. Is the effect of Ca$^{2+}$ on CFTR even more important for secretion than Ca$^{2+}$ activation of Ano1? Therefore CFTR was suspected to live a secret life as Ca$^{2+}$ activated Cl$^-$ channel. 5

Our recent work on the naive intestinal epithelium from wildtype and intestinal Ano1 null mice suggests that Ano1 is localized in or close to the basolateral rather than in the apical membrane. Ano1 supports Cl$^-$ secretion by enhancing Ca$^{2+}$ signaling though G protein coupled receptor (GPCR), which may improve activation of basolateral K$^+$ channels (Fig. 1). This will increase the driving force for Cl$^-$ exit through luminal CFTR. 6 Thus the situation in mouse intestinal epithelial cells would be comparable to that found in human cells. Here we know already for some time that CFTR is the only relevant luminal Cl$^-$ channel. 7 How does Ano1 control intracellular Ca$^{2+}$ signals? As hypothesized before, 6 i) Ano1 may control Ca$^{2+}$ release from the endoplasmic reticulum (ER), or ii) may glue Ca$^{2+}$ stores to the basolateral membrane (similar to the yeast protein Isp2, which shares considerable homology with Ano1), or iii) may somehow control Ca$^{2+}$ influx pathways. It has been shown earlier that TRP channels (such as TRPV4 and TRPC2) and Ano1 interact, and we and others even reported cation permeability for anoctamins. 2,8 We found that in the presence of cations during patch clamp experiments, the reversal potential
for overexpressed anoctamin 1 currents was never identical with a pure Cl\textsuperscript{−} equilibrium potential, but suggested some “contaminating” cation currents. The possibility that Ano1 also conducts Ca\textsuperscript{2+} or translocates Ca\textsuperscript{2+} influx channels to the plasma membrane, cannot be ruled out. We proposed earlier that Cl\textsuperscript{−} channels are rather passive transport elements, inasmuch as Cl\textsuperscript{−} ions simply escort “leading” cations, such as Na\textsuperscript{+} (transported by the epithelial Na\textsuperscript{+} channel ENaC), K\textsuperscript{+} (conducted by K\textsuperscript{+} channels), and Ca\textsuperscript{2+} (IP3-receptor, store operated Ca\textsuperscript{2+} influx channels), thereby compensating charge movement and facilitating transport. Anoctamins could serve as those counter ion channels.

For Cl\textsuperscript{−} secretion by airway epithelial cells, the contribution of Ano1, and its true cellular function still remains to be determined. It has been reported earlier that in airway epithelial cells individual apical and basolateral Ca\textsuperscript{2+} signaling exists, with separate apical and basolateral ER Ca\textsuperscript{2+} stores that independently activate channels in the apical and basolateral membrane. Although evidence has been provided for luminal expression of Ano1 in airway epithelial cells, it is still necessary to determine whether Ano1 is actually located in the luminal bilayer, or in an ER-compartment in close proximity to the apical membrane. Although functional reconstitution of overexpressed Ano1 has been reported, it is essential to examine whether Ano1 operates as a secretory Ca\textsuperscript{2+} activated Cl\textsuperscript{−} channel, and without the help of additional proteins. This is particularly important, since Ano1 may be targeted pharmacologically, as an alternative secretory Cl\textsuperscript{−} channel and as a replacement to CFTR, which is not functional in cystic fibrosis. With the help of potent small molecule activators for Ano1, it may be possible to get sufficient Cl\textsuperscript{−} and HCO\textsubscript{3}\textsuperscript{−} secretion going in CF airways, and to overcome this devastating lung disease.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 1. Hypothetical models for the roles of Ano1 in intestinal (left) and airway (right) epithelium. CFTR is activated through intracellular cAMP and Ca\textsuperscript{2+} dependent second messenger pathways. In intestinal epithelial cells, Ano1 may support Ca\textsuperscript{2+} dependent Cl\textsuperscript{−} secretion by facilitating basolateral Ca\textsuperscript{2+} signaling (left). In airway epithelial cells, Ano1 is located in, or close to the apical membrane. There it may operate as a Cl\textsuperscript{−} exit channel independent of CFTR, or may control apical Ca\textsuperscript{2+} signaling.