7 Summary

Proteinbiochemical Structural Analyses of the Orthologous CLCA-proteins equine eCLCA1 and murine mCLCA3: A Contribution to the Understanding of their Operation

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Members of the CLCA-family represent an emerging protein family and are among the most promising molecular candidates for the calcium-activated chloride conductivity observed in several tissues. In particular, the murine mCLCA3 and the equine eCLCA1 have been identified as clinically relevant molecules in diseases with secretory dysfunctions. Their induction of goblet cell metaplasia in diseases like asthma and recurrent airway obstruction is of particular biomedical relevance. Functional analyses have indicated that these CLCA-proteins evoke a calcium-activated chloride conductance when heterologously expressed. However, it is not yet clear whether the CLCA proteins form chloride channels *per se* or function as mediators of other, yet unkown chloride channels. A four or five integral transmembrane model has initially been proposed for CLCA proteins. Such a protein structure could potentially form a transmembrane conductive pathway for anions. However, recent immunohistochemical and immune electron-microscopical analyses detected the mCLCA3-protein in the extracellular mucous layer, consistent with a secreted protein.

This thesis was designed to address the issue of whether mCLCA3 und eCLCA1 are integral transmembrane proteins and act as real anion channels or wether they are secretory proteins. In this study, systematic computer-based und biochemical analyses were conducted. First, the amino acid sequences of the two proteins were screened for potential transmembrane domains using several computer algorithms. Subsequently, confocal microscopy on mCLCA3-transfected cells revealed that the mCLCA3-protein was intracellularly localized in secretory vesicles without any association with the plasma membrane. In addition, the intracellular trafficking was investigated by pulse chase experiments in transfected and metabolically labeled mammalian cells. The locations of the proteins were then characterized by westernblot analyses and the glycosylation patterns of the proteins were determined. The results show that the characteristic cleavage of the eCLCA1 und mCLCA3 precursor proteins takes place in the endoplasmic reticulum. Both cleavage products of the mCLCA3-protein are released as

glycosylated, mature proteins into the extracellular space. This was similarly confirmed for the eCLCA1-protein. Thus, due to the lack of any transmembrane domains, these proteins are unable to form anion channels on their own. These results strongly suggest that eCLCA1 und mCLCA3 are secretory glycoproteins rather than transmembrane molecules. As such they could interact with other chloride channel proteins to induce the chloride conductivity observed in several previous experiments. Moreover, they could act as signalling molecules to evoke goblet cell metaplasia characteristic for complex airway diseases including asthma and recurrent airway obstructions. Future studies will have to address the interaction partners and the mechanisms responsible for their indirect induction of chloride secretion, goblet cell metaplasia and mucin secretion.