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Cloning of Slc26a6 from murine small intestine for cystic fibrosis studies

Bicarbonate secretion in the small intestine is important for protecting the intestinal epithelium against stomach acid and providing the proper pH for digestive enzymes and mucus. In the disease cystic fibrosis, bicarbonate secretion is deficient and contributes to the pathogenesis of intestinal disease. Bicarbonate transport is mediated by the cystic fibrosis transmembrane regulator (CFTR), the Cl channel that is defective in cystic fibrosis and that works in concert with a Cl/HCO<&sub3> exchanger. However, the specific identity of the Cl/HCO<&sub3> exchanger working with CFTR is unknown. Recently, studies have localized 2 members of the sulfate permease family (Slc26a3, Slc26a6) to the apical membrane and these proteins have Cl/HCO<&sub3> activity. Because of the difficulties in studying intestinal transport in humans, a CF mouse model was developed that has intestinal disease similar to CF patients. However less is known about the murine homologues of CFTR and these Cl/HCO<&sub3> exchanger proteins. Slc26a3 has been successfully cloned, expressed, and examined in isolation for interactions with mCFTR. Murine Slc26a6 has also been cloned from the mouse kidney, but different splice variants are expressed in the intestine. Therefore, to allow the study of the properties of Slc26a6 in isolation, primers modified from published sequences and Accuprime Pfx DNA Polymerase were used to amplify a single band approximately 2700 base pairs in length from murine small intestinal cDNA. Sequencing primers were designed every 500 bp based on the published sequence of Slc26a6 and the overlapping sequences combined to generate the entire sequence. This sequence was blasted to the published sequence of Slc26a6 to confirm the identity of the clone. The sequence will be subcloned into a bicistronic plasmid expressing DsRed2 and transiently expressed in CHO cells. The ion transport substrates, sensitivity to known inhibitors of Cl/HCO<&sub3> exchangers, and electrogenicity of the transporter will be tested by measuring changes in intracellular pH.