NHE3 and its signaling complexes: role in acute regulation, cytoskeletal association and protein-protein interactions

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The small intestinal and renal proximal tubule brush boder (BB) Na/H exchanger NHE3 is presented to illustrate how a transport protein is highly regulated. NHE3 is both stimulated and inhibited from basal as part of digestion and is inhibited in diarrheal diseases. It exists in large, multiprotein complexes that change dynamically as part of digestion. These complexes are formed *via* protein-protein interactions in the C-terminal 377 aa of NHE3 with at least 9 binding partners identified. As with many proteins, NHE3 is attached to the cytoskeleton; what is unusual is that it attaches at two sites in the C-terminus. NHE3 binds ezrin directly and also indirectly *via* binding to the NHERF family (Na/H exchanger Regulatory Factor) of 4 related, multi-PDZ domain apical domain proteins (NHERF1-4). NHERF binding is to several aa in the middle of the NHE3 C-terminus and is involved in NHE3 complex formation, acute NHE3 regulation (both stimulation and inhibition) and limiting mobility in the BB, but does not affect basal trafficking or percent in the plasma membrane. The 4 NHERF family proteins contribute to different regulatory processes. This appears to be due to their differential localization and the different binding partners they bring into NHE3 complexes. NHERF proteins are required for cAMP, cGMP and Ca^{2+} inhibition of NHE3 and LPA stimulation. In these effects, they help form different NHE3 complexes which change as part of acute regulation. Direct ezrin binding to NHE3 also affects NHE3 function, but acts in a complimentary fashion to NHERF binding. Direct ezrin binding is to a C-terminal domain that is α-helical and occurs at 3 positively charged aa. Direct ezrin binding is necessary for basal and acute NHE3 regulation and acts by affecting the percent of NHE3 on the plasma membrane under basal conditions as well as the acute changes in rates of endocytic recycling and delivery to the plasma membrane of newly synthesized NHE3 while having less effects on initial rates of endocytosis. However, it does allow more prolonged endocytosis, probably by allowing delivery to the intervillus clefts of microvillar NHE3. Direct ezrin binding is not involved in NHE3 complex formation. Direct ezrin binding is necessary for mobility of NHE3 in the BB, perhaps *via* attachment to a motor protein. Limited BB mobility under basal conditions is markedly increased as part of acute NHE3 regulation. This dynamic aspect of BB NHE3 function is dependent on NHERF association and may be due to transient disassociation of NHE3 from the cytoskeleton during both addition of NHE3 to the BB (stimulation) and removal (inhibition). NHE3 is an example of an acutely regulated transporter that exists in large complexes, which dynamically change as part of signaling, and associates with cytoskeleton in a dynamic manner. The cytoskeletal association has at least two components, which contribute differently but in a complimentary manner to NHE3 regulation.