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NHERF- 1 Modulates Intestinal NaPi transporter NaPi-2b expression in Apical Microvilli.

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The regulation of phosphate (Pi) homeostasis is maintained by the coordinated function of the renal and intestinal phosphate transporters. Several PDZ (PSD-95/discs large/ZO-1 homologous) domain proteins, including NHERF-1, PDZK1, ShanK2, and PIST play an important role in the regulation of the renal sodium-phosphate (NaPi) co-transporters (NaPi-2a and NaPi-2c). The main mediator of intestinal sodium dependent transcellular Pi transport, NaPi-2b, also contains a PDZ-binding motif consensus in the C-terminal region. However, interactions of the transporter NaPi-2b with PDZ proteins have been not described and their potential role in regulation of the intestinal transporter is not known. For this purpose we performed studies with knock-out (KO) mice models and cell culture to determine a potential role for NHERF-1 and PDZK1 in the regulation of NaPi-2b.

To study the putative interaction between NaPi-2b and PDZ proteins we determined the Forster Resonance Energy Transference (FRET) by using Fluorescence Lifetime Imaging Microscopy (FLIM). OK cells, an extensively used proximal tubule model, and CaCo-2$_{BBE}$ cells, an enterocyte cell model, were used to perform this technique. First, expression of EGFP-NaPi-2b was confirmed in the microvilli of both cell types, and images along a single microvillus were obtained with the novel Modulation Tracking (MT) method. Cells co-expressing EGFP-NaPi-2b and mCherry-NHERF-1 were analyzed by FLIM-FRET technique revealing significant FRET between NaPi-2b and NHERF-1. Parallel studies between the pair NaPi-2b and PDZK1 proteins resulted in non occurrence of FRET. To evaluate the functional significance of these results we study NaPi-2b expression and activity in NHERF1 KO and PDZK1 KO mice models, where we found that adaptation to a low Pi diet of NaPi2b was markedly impaired in the NHERF-1 KO mice but not in the PDZK1 KO.

Our results therefore suggest an important role of NHERF1 in modulation of NaPi-2b expression or stability in the microvilli of the mouse intestine.

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