Reassessing the roles of the house-keeping sodium-dependent inorganic phosphate transporters PiT1 and PiT2

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ABSTRACT

The present PhD dissertation is based on experimental work carried out at The Institute of Clinical Medicine and The Department of Molecular Biology, University of Aarhus. The work was conducted to gain further insight into structural and functional aspects of the type III NaP_i transporters and gammaretroviral receptors, human PiT1 and PiT2.

Combined studies of the dual functions allowed for identification of several negatively charged amino acids putatively located in transmembrane domains and in extracellular loops of the PiT proteins as being critical for P_i transport function. Analyses of the identified transmembranic positioned amino acids in a PiT2 background suggest that they are responsible for the coupling of Na^+ import to P_i transport. Moreover, the identified extracellularly positioned amino acids are located in two homologous sequences defining the PiT family (the PiT family signature sequence), but their functions are yet to be determined.

Analyses of the basal transport characteristics of the PiT proteins revealed that they were both high-affinity P_i transporters, albeit showing different transport kinetics, and that they both sustained NaP_i uptake at a wide pH range. Moreover, Ca^{2+} or Ca^{2+} and Mg^{2+} were found to increase PiT2- and PiT1-mediated NaP_i uptake, respectively, but neither caution was critical for NaP_i transport functions.

Acute high P_i levels induce down-regulation of PiT2 and we found PiT2 to be down-regulated via clathrin-coated pits; an event which relies on PiT2 specific sequences that are intracellularly positioned. PiT1 was also shown to be down-regulated by acute high P_i levels, but the pathway exploited by PiT1 remains to be identified. Interestingly, PiT2 NaP_i transport knock-out mutants were down-regulated as wild-type PiT2 at acute high P_i levels implying that the PiT2 protein can function as a P_i sensor besides being a NaP_i transporter.

In summary, the obtained results provide new insight into PiT1 and PiT2 transport mechanisms and regulation of the PiT proteins.

The PiT proteins are ubiquitously expressed and have therefore been assigned housekeeping P_i transport functions. Recently, they have, however, been implicated in specialized functions as, e.g., the complex formation of bone. In addition, they have been implicated in development of pathologic conditions, e.g., calcification of blood vessels as observed in diabetics and patients suffering from chronic kidney disease. In these patients, the ectopic calcification correlates with elevated P_i and calcium levels in the blood. Thus, our results directly show a role of these risk factors of ectopic calcification in the regulation of PiT1 and PiT2 and their P_i transport function and they can hopefully contribute to shed further light upon the mechanisms behind ectopic calcification.