

Mechanism of regulation of blood phosphate level: regulation of surface expression the renal Na/Pi-cotransporter

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Summary

Phosphate (Pi) reabsorption at the renal proximal tubules play an important role in the homeostasis of Pi concentration in the body. Pi reabsorption is performed by the type-2 Na/Pi-cotransporter expressed on the apical surface of the epithelial cells of the proximal tubule. The Pi uptake is regulated by changing the expression level of Na/Pi-cotransporter, rather than by changing the activity of the transporter. However, little is known about how the cells increase or decrease the level of the transporter. For the first step toward the complete understanding of the mechanism of regulation of Na/Pi-cotransporter, we studied whether endocytosis signals exist in the mouse type-2 Na/Pi-cotransporter, Na/Pi-7.

Na/Pi-7 is thought to have 8 transmembrane domains, with both N- and C-termini exposed to the cytoplasm. Therefore, Na/Pi-7 has 5 cytoplasmic regions including N- and C-termini; C1, C2, C3, C4 and C5, from N- to C-terminus. We made chimeric proteins which have each of Na/Pi cytoplasmic regions to study which of them to have endocytosis signals. Only the chimera with C4 was endocytosed when expressed in HeLa cells, suggesting the existence of an endocytosis signal in C4. When the amino acid sequence of C4 was examined, we found YWRF sequence, a possible tyrosine-based sorting signal, at the C-terminus of C4.

Tyrosine-based sorting signals act as the sorting signal through the interaction with  $\mu$  subunits of AP complexes. This interaction concentrates the molecules with tyrosine-based sorting signals in their cytoplasmic region in clathrin coated vesicles. Our results suggest that Na/Pi-7 has a tyrosine-based sorting signal in the C4 region, and that this signal act as the endocytosis signal for Na/Pi-7. The present study was done by using chimeric molecules having excised cytoplasmic regions of Na/Pi-7, and further studies have to be done to see whether YWRF signal act as the endocytosis signal in the context of the whole Na/Pi molecule.