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REVIEW ARTICLE

The Renal Type IIa Na/P_i Cotransporter

Structure–Function Relationships

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Abstract

The type IIa Na/P_i cotransporter mediates proximal tubular brush-border membrane secondary active phosphate (P_i) flux. It is rate limiting in tubular P_i reabsorption and, thus, a final target in many physiological and pathophysiological situations of altered renal P_i handling *(1–4).* In the present short review, we will briefly summarize our current knowledge about the transport mechanism (cycle) as well as particular regions of the transporter protein ("molecular domains") that potentially determine transport characteristics.

Index Entries: Phosphate; brush border; kidney; proximal tubule; cotransport.

TRANSPORT CYCLE

The transport cycle (*see* Fig. 1) has been characterized by either tracer uptake or electrophysiological measurements performed after expression of the cloned type IIa Na/P_i transporter in *Xenopus laevis* oocytes *(1,5–8).* A comparison of P_i uptake, Na^+ uptake, and charge movements indicate an overall three Na^+ to one P_i stoichiometry *(7).* Although monovalent P_i can be transported, divalent P_i is the preferred substrate *(7,9–11).* This explains part of the observed pH dependence of proximal tubular P_i reabsorption *(1).* However, changing pH has additional effects (e.g., on the reorientation of the empty carrier and on the interaction with Na^+ ions) *(1,4,9).* The reorientation of the empty carrier involves the apparent transfer of one negative charge *(5).* Under normal physiological conditions, the first step involves an interaction of the carrier with one Na^+ ion on the external surface *(5,9).* In the absence of the transport substrate (P_i), a Na^+-dependent leak or slippage (approx 10% of fully loaded carrier) can be observed *(1,5).* In the presence of P_i the transporter interacts with the transport substrate (P_i) followed by the loading of two additional Na^+ ions *(1,5).* Thus, the translocation of the fully loaded carrier is, *per se,* an electroneutral process; that is, the observed charge transfer within the

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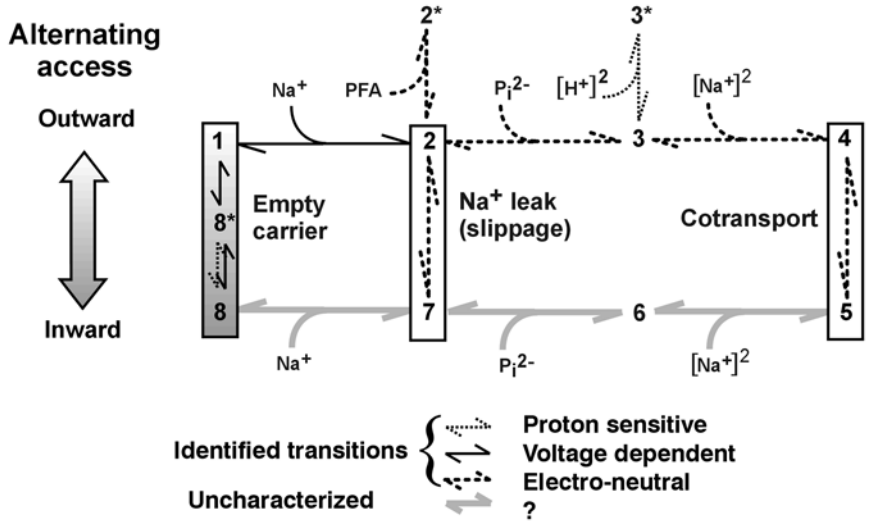

Fig. 1. Transport cycle of type IIa Na/P_i-cotransporter activity. The transporter interacts first with one Na^+, followed by interaction with P_i and two additional Na^+ ions. The translocation of the fully loaded carrier is an electroneutral process, whereas reorientation of the empty transporter is associated with a net-charge translocation. Phosphonoformic acid (PFA) blocks the carrier by interacting with the P_i-binding step. Protons reduce transport activity by interfering with the translocation of the unloaded carrier, with the Na^+ interactions as well as with the availability of divalent P_i. For further discussion, *see* the text. (Based on data presented in refs. *5* and *9*.)

transport cycle is the result of the reorientation of the unloaded carrier *(1,5).* Phosphonoformic acid (PFA), a well-known inhibitor of this transport activity, interacts with the P_i-binding site and is apparently not (or only at an extremely slow rate) transported *(1,5,12).*

FUNCTIONAL REGIONS

The type IIa Na/P_i cotransporter has at least eight transmembrane domains (TMDs) with cytoplasmic amino and carboxy-termini (Fig. 2). This prediction is based on epitope insertion/antibody accessibility *(13)* and cysteine insertion/accessibility studies *(14–17).* Functional regions (i.e., affecting transport activity) were identified by two separate approaches: cysteine insertion/modification [cysteine scanning *(14–17)*] and chimera construction *(18,19).* The latter is based on the different transport properties of type IIa (renal) and type IIb (intestinal) Na/P_i cotransporters *(1,5,20,21).*

Three amino acids in the predicted third extracellular loop are responsible for the increased transport rates at alkaline pH values of type IIa as compared to type IIb-mediated transport activity *(19).* This conclusion is based on experiments involving chimera constructions, sequence comparisons, and site-directed mutagenesis *(19).* A similar strategy suggested that amino acid residues in the predicted fifth transmembrane domain codetermine the lower apparent affinity for Na^+ interaction in type IIa-mediated, as compared to type IIb-mediated, transport activity *(18).*

Cysteine modification experiments provided information on structure–function relationships in the type IIa cotransporter *(15–17).* In addition to two used glycosylation sites *(22),* the predicted second extracellular loop contains a functionally important disulfide bridge *(14,23)* (Fig. 2). The transporter might be cleaved between the two glycosylation sites and is (with an intact disulfide bridge) still functional *(24–26);* Ehnes et al., in preparation). The predicted third

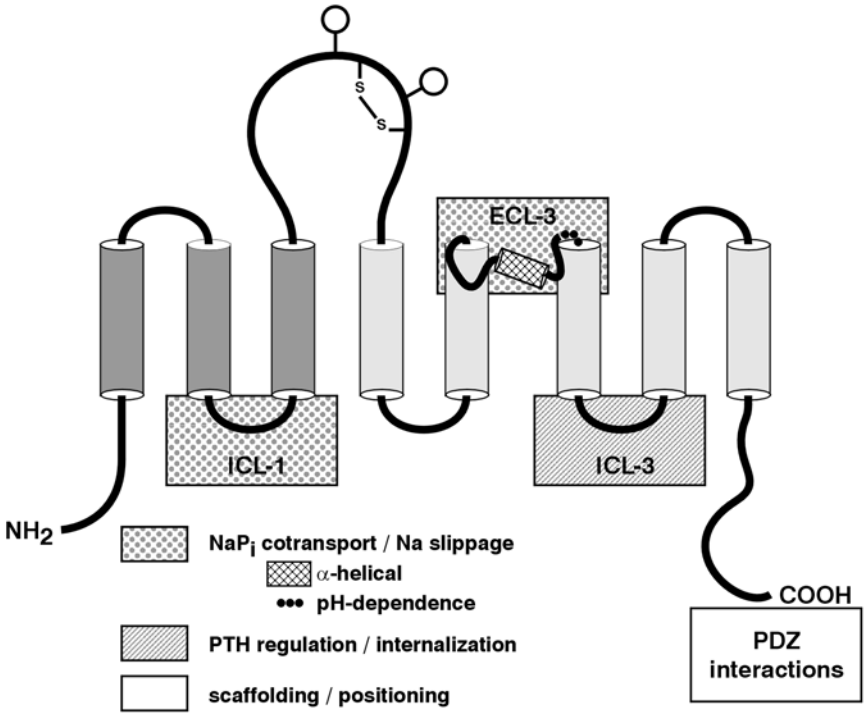

Fig. 2. Membrane topology and functional domains of the type IIa Na/P_i cotransporter. The transporter contains at least eight TMDs with cytoplasmic amino- and carboxy-termini. The predicted first intracellular and third extracellular loops contain functionally important domains and might be "re-entrant" to form a "permeation pore." In addition, the predicted third intracellular loop contains sequences for regulatory internalization, the carboxy-terminus sequences required for apical expression, in part involving an interaction with PDZ-binding proteins. For further discussion, *see* the text.

extracellular loop is thus far, functionally the most important: Cysteine insertion/modification (scanning) leads to transport inhibition depending on the amino acid position and conditions of application (time, concentration, side of membrane) of the cysteine-modifying reagent *(15,16).* We concluded that this region is in part membrane associated ("re-entrant") and α-helical and undergoes transport-dependent conformational changes *(15,16)* (Fig. 2). Intramolecular sequence comparisons indicated that the predicted first intracellular loop shows sequence homology to the predicted third extracellular loop *(14,17).* Cysteine insertion/modification in this region suggested a functional importance of these amino acid residues, also which were shown to be preferentially accessible from the cell interior *(17).* Thus, we propose that the first intracellular together with the third extracellular loop forms a putative "permeation pore" *(17)* (Fig. 2).

MONOMERIC VS MULTIMERIC FUNCTION

Radiation/inactivation studies suggested that Na/P_i-cotransport function may require a homotetrameric organization *(27).* The cysteine insertion/modification resulted in fully inhibitable transporter constructs *(15)* (Fig. 3, S460C). This provided an opportunity to re-evaluate the question of multimeric requirements. We have used two different protocols *(28):* First, mixing wild-type and mutant transporters resulted in inhibitable transport activity directly related to the mixing ratio. Second, fusion proteins (concatamers) comprising two

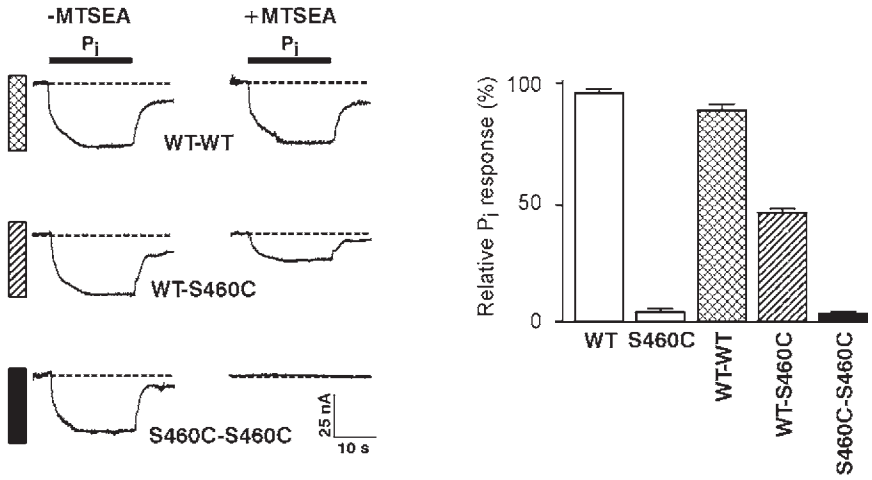

Fig. 3. "Monomeric" function of the type IIa Na/P_i cotransporter. Cysteine scanning studies resulted in a mutant cotransporter (S460C) fully inhibited by a cysteine modification reagent (MTSEA). Linking two transporter units (concatamers/tandem) resulted in a noninhibitable construct if two wild-type transporters are linked (WT–WT), 50% inhibition if wild type and mutant (WT–S460C), and full inhibition if two mutant transporters (S460C–S460C) are fused. For further discussion, *see* the text. Left: typical tracings obtained by electrophysiology after addition of 1 m*M* P_i before and after incubation in the cysteine modifying reagent MTSEA; right: summary of several independent experiments. (Based on data presented in ref. *28*.)

identical mutants (S460C–S460C), mixed wild type (WT) and mutant (WT–S460C), and two WT components (WT–WT), gave respectively 50%, 100%, and 0% loss of transport activity after Cys modification *(28)* (Fig. 3). These findings are consistent with each monomer-mediating Na/P_i cotransport, but does not exclude that the transporter can be clustered in "multimeric" complexes. Thus, the larger functional molecular weight observed in the radiation/inactivation studies could suggest that the transporter is part of a larger complex also containing "specific" interacting proteins *(29).*

CONCLUSIONS

The type IIa Na/P_i cotransporter mediates a $3Na^+ : 1HPO_4^{2-}$ cotransport and is a functional monomer. The transporter is separated by a large glycosylated extracellular loop into two functional domains containing "reentrant" loops (Fig. 2). The transporter can be cleaved at the extracellular loop without functional influence if stabilized by a disulfide bridge. In addition, sites important for regulatory internalization (predicted third intracellular loop) and for apical expression including scaffolding mechanisms (COOH-terminal sequences) have been identified (*29–32;* Gisler et al., in preparation) (Fig. 2).

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