# A single residue controls the substrate-induced conductance in the glutamine transporter SNAT3 (slc38a3)

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The glutamine transporter SNAT3 plays a pivotal role in several organs (Mackenzie & Erickson, 2004). In the liver it is involved in the uptake of glutamine, which serves as a central donor for ammonia dispatched in the urea cycle. In the brain SNAT3 releases glutamine from astrocytes and thereby forms part of the glutamate-glutamine cycle. In the kidney SNAT3 takes up glutamine into epithelial cells of the proximal tubule, where it is the principle donor of ammonia released in the urine to control plasma pH. The Mechanism of SNAT3 involves the cotransport of $1Na^+$ together with the substrate and the antiport of $1H^+$ (Broer *et al.*, 2002). As a result the SNAT3 transporter could be regarded as a glutamine-dependent $Na^+/H^+$ antiporter. Although the overall mechanism of the transporter is electroneutral, substrate-induced currents are observed when the transporter is expressed in *Xenopus laevis* oocytes.* These 'uncoupled' currents are carried by cations or protons, depending on the extracellular pH (Schneider *et al.*, 2007). To understand coupled and uncoupled movements of ions in the glutamine transporter SNAT3 we characterised site-directed mutants of the transporter. To identify residues that could be involved in $Na^+$-binding and translocation we compared the sequences of proton-coupled transporters (solute carrier family [slc] 36) with those of $Na^+$-coupled transporters (slc38). Residue Asn76 was selected for further analysis and changed into aspartate, glutamate, glutamine, histidine or serine. All of the mutants showed residual uptake of glutamine ranging from 10% to 40% of that of the wildtype. Subsequently, mutants were analysed in more detail by recording substrate-dependent currents, substrate-independent currents and by recording the intracellular pH using pH-sensitive microelectrodes. Mutation of Asn76 into any other residue completely abolished substrate-induced currents at pH 7.4, where they are largely carried by cations and at pH 8.4 where they are largely carried by protons. The glutamine-dependent $Na^+/H^+$ exchange activity by contrast was still readily detectable confirming the residual uptake of radioactive glutamine. The Asn76Gln replacement in particular showed significant coupled transport but no substrate-induced currents. Replacement of Asn76 by aspartate not only abolished substrate-induced currents but caused partial suppression of a substrate-independent conductance observed at pH 8.4. Removal of $Na^+$ renders wildtype and mutant transporters non-functional, due to the $Na^+$ cotransport mechanism. However, addition of glutamine in the absence of $Na^+$ caused a huge increase of the membrane conductance in case of the Asn76Asp mutation but not in the wildtype. A model explaining these observations in terms of an alternate access mechanism of the SNAT3 protein has been developed.

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*Procedures to remove oocytes were approved by the animal ethics committee of the ANU and the state of Rheinland-Pfalz.