Regulation of the glutamine transporter SN1 (SNAT3)

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The glutamine transporter SN1 is mainly expressed in hepatocytes and in brain astrocytes. It is involved in both uptake and efflux of glutamine and its activity is tightly regulated. Transport of glutamine via SN1 is coupled to the cotransport of 1 Na^+ and the antiport $1H^+$ (Chaudhry *et al.*, 1999). As a result glutamine transport is electroneutral and its preferred direction is governed by extracellular pH and intracellular Na^+ concentration. In addition, SN1 is allosterically regulated, becoming inactive at acidic pH (Broer *et al.*, 2002).

In the brain SN1 mediates the release of glutamine from astrocytes, which is used as a precursor for neurotransmitter glutamate biosynthesis in neurons. Increased extracellular glutamate concentrations induced a rapid increase of SN1 activity in astrocytes. The upregulation was not caused by activation of ionotropic or metabotropic glutamate receptors but required uptake of glutamate into astrocytes. Experiments in *Xenopus* oocytes* suggest that glutamate may act as a direct regulator of SN1 activity.

Severalfold evidence suggests that protein trafficking is a major mechanism by which SN1 activity is regulated. In *Xenopus* oocytes SN1 activity rapidly decreased after treatment of oocytes with phorbolester. Confocal microscopy of oocytes expressing a GFP-SN1 construct revealed that loss of activity was accompanied by a retrieval of the transporter from the plasma membrane. Retrieval of SN1 was specific but did not involve phosphorylation of the transporter. A similar downregulation by incubation with phorbolester was observed in cultured HepG2 cells but not in primary cultures of brain astrocytes.

A possible mechanism for the retrieval of transporter may involve ubiquitination followed by degradation of the transport protein. Coexpression of SN1 with the ubiquitin ligase Nedd4-2 reduced the transport activity of SN1, a downregulation that was abrogated by coexpression of protein kinase sgk1 or protein kinase B (PKB) (Boehmer *et al.*, 2003). Coexpression of sgk1 or PKB together with SN1 resulted in an increase of the transport activity.

Taken together these data provide evidence for a regulation of SN1 by plasma membrane trafficking. The actual components of the signal transduction pathways, however, are likely to differ between cell types.

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*Animal experimentation protocols were approved by the Australian National University.