

Sensing and signalling pathways involved in the adaptive regulation of the SNAT2 amino acid transporter

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Abstract. Nutrient sensing and signalling mechanisms controlling the System A transporter SNAT2 have been assessed. SNAT2 appears to be an amino acid (AA) sensor which exerts autoregulatory control of transcription and, potentially, transporter turnover. An additional sensor for large neutral AA may also control SNAT2 through the JNK pathway. (www.actabiomedica.it)

Key words: Amino acid transport, Nutrient sensing; MAPK

Introduction

The dynamic control of nutrient transport is of fundamental physiological importance. Robust, multi-tiered regulatory networks exist to co-ordinate nutrient exchange in response to tissue-specific and systemic signals. The expression and activity of the System A transporter SNAT2 is stimulated during AA deprivation through a process known as adaptive regulation (1). We have dissected the pathways that control SNAT2 expression and find evidence for the parallel action of two AA sensors and several signalling intermediates. SNAT2 expression is regulated at the level of transcription and protein turnover. Furthermore, data is provided supporting a bifunctional role for SNAT2 as both an AA transporter and an AA receptor.

Materials and methods

System A activity was measured using 10 μM ^{14}C -MeAIB. L6 cells were grown as described (2) and HeLa cells grown in DMEM / 10%FBS / 1% antibiotic. The SNAT2 promoter was cloned from rat DNA and placed into pC3luc. Rat SNAT2 and

SNAT5 were tagged by PCR and placed in pCDNA6. Chicken anti-SNAT2 antibodies were raised to an N-terminal epitope (2).

Outcomes

AA deprivation of L6 myotubes led to a 4-fold stimulation of the transport of the System A substrate Me-AIB (10 μM ; 4 hour stimulation) and was accompanied by an equivalent increase in SNAT2 expression (2). L6 cells were stably transfected with a luciferase reporter construct containing domains of the SNAT2 promoter (-1kb to +1kb) known to respond to AA limitation (3). AA deprivation for 7h led to a modest (35%) increase in SNAT2-luciferase activity in these cells.

To test whether AA exerted post-translational control over SNAT2, HeLa cells were transfected with V5-tagged SNAT constructs. AA deprivation for 8h led to a marked increase in SNAT2-V5 immunoreactivity, without affecting the expression of a SNAT5-V5 construct from an identical expression vector. A chimera between the cytoplasmic N-terminus of SNAT2 and the C-terminus of SNAT5 rendered the expression of the latter sensitive to AA availability.

AA sensing events relevant to System A regulation were addressed by incubating L6 cells with individual AA (2 mM) for 4h. Me-AIB transport was measured subsequent to these treatments and compared with AA-starved control cells. AA that are known to interact with SNAT2 prevented adaptive regulation and the expression of SNAT2 (eg. Ala, Gln, Me-AIB). Additionally, aromatic AA prevented adaptive regulation at concentrations where they do not significantly bind to SNAT2.

A variety of kinase inhibitors were provided to L6 cells during the adaptation protocol. SP600125, an inhibitor of JNK, prevented adaptive regulation and JNK was stimulated in AA-deprived cells (see also (4)). JNK activation was inhibited by aromatic AA and by a subset of the System A substrates. Conversely, System A substrates were identified which did not affect JNK phosphorylation, but which did repress the System A transporter (eg. sarcosine, Me-AIB). The latter effect occurred at AA concentrations reflecting the extracellular binding affinity of SNAT2. Consistent with a role for SNAT2 in AA sensing, shRNA directed against SNAT2 led to the derepression of the SNAT2-luciferase reporter in HeLa cells.

Discussion/Conclusions

By characterising adaptive regulation, we have isolated two sensor/effector pathways that control SNAT2 expression in mammalian cells and shown that SNAT2 is controlled at the level of transcription and protein turnover.

References

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