Functional characterisation of the novel human equilibrative nucleoside transporter (hENT3) located in intracellular membranes

Kay Barnes, Ralph Hyde, Sophie Foppolo and Stephen A. Baldwin

Introduction
Both hENT3 and mENT3 are members of the equilibrative nucleoside transporter family. These transporters play key roles in the uptake of precursors for nucleotide synthesis by salvage pathways in a number of cell types, for example, bone marrow and brain. They also regulate the concentration of adenosine available to cell surface purinoreceptors, thus influencing coronary blood flow, inflammation and neurotransmission. Family members are predicted to share a common topology of 11 transmembrane (TM) α-helices, with a cytoplasmic N-terminus and a large cytoplasmic loop linking TM6 and TM7. Unlike other family members, hENT3 has a long (51 residue) hydrophilic N-terminal region preceding TM1 that possesses a putative dileucine-based endosomal/lysosomal targeting motif (DE)XXXL(LI). We propose that hENT3, unlike other family members located at the cell surface, functions intracellularly.

Subcellular distribution of hENT3
To examine the role of the dileucine motif in the subcellular distribution of the transporter, a comparison was made of green fluorescent protein (GFP) fusion proteins bearing wild type hENT3 (GFP-hENT3), hENT3 lacking the first 36 residues of the N-terminal region (GFP-hENT3ΔN), or hENT3 in which the dileucine motif at positions 31 and 32 had been replaced by alanine residues (GFP-hENT3AA)(Fig.1).

Fig. 1 Role of the N-terminal dileucine motif in determining the subcellular distribution of hENT3 A-C, distribution of fluorescence in HeLa cells expressing GFP fusion proteins bearing the wild-type hENT3 (GFP-hENT3) (A), hENT3 lacking the first 36 residues of the N-terminal region (GFP-hENT3ΔN) (B), or hENT3 in which the dileucine motif at positions 31 and 32 had been replaced by alanine residues (GFP-hENT3AA) (C). Each image corresponds to one representative deconvolved optical section. Scale bars 10 µm. D-F, distribution of fluorescence in cryosections of oocytes injected with RNA transcri pts encoding GFP-hENT3 (D) or GFP-hENT3AA (E) or injected with water alone (F). Scale bars, 20 µm.

The GFP protein-tagged forms of the full-length hENT3 protein were found to be predominantly intracellular proteins that co-localised, in part, with lysosomal markers in cultured human HeLa cells. Truncation of the hydrophilic N-terminal region or mutation of the dileucine motif to alanine caused the protein to be relocated to the cell surface in human cells and in Xenopus oocytes.
**Characterisation of transport activity**

By using mRNA transcripts of the dileucine mutant hENT3AA injected into *Xenopus* oocytes, the protein was shown to be a low affinity sodium-ion independent nucleoside transporter that could also transport adenine. Its activity was strongly dependent upon pH, and the optimum pH value of 5.5 probably reflected the location of the transporter in acidic, intracellular compartments (Fig. 2).

![Graph](image)

**Fig. 2 pH dependence of hENT3-mediated adenosine transport.** Uptake of $^{14}$C-labelled adenosine (20 µM, 20 °C, 5 min) in oocytes injected with the hENT3AA RNA transcripts or water alone was measured in transport medium containing 100 mM sodium chloride and buffered at pH values ranging from 5.0 to 8.5.

**Collaborators**

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**Publications**


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