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INTRINSIC ACTIVITY

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MEETING ABSTRACT

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A new role of the N-terminus in folding and intracellular trafficking of the human creatine transporter 1

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Background: The human creatine transporter 1 (CRT1, SLC6A8) is a member of the sodium-dependent neurotransmitter transporter (NTT) protein family. Creatine transporter deficiency (CTD) has been associated with a number of disorders, ranging from epilepsy to mental retardation, autism, development delay, behavior problems, motor dysfunction or gastrointestinal symptoms. Folding and trafficking defects in SLC6 proteins frequently lead to pathological conditions; *e.g.* mutations in the human creatine, dopamine and GABA transporters trigger CTD, parkinsonism and epilepsy, respectively. Treatment with the chemical chaperone 4-phenylbutyrate (4-PBA) rescues the surface expression and uptake activity of several folding-deficient CTD variants. Hence, it is vital to decipher the arcane molecular machinery behind the protein folding and intracellular trafficking of hCRT1.

Methods: The following methods were used: (i) mutagenesis, to create serial truncation mutants along the N-tail, as well as single/double point mutations in other regions of hCRT1 (QuikChange kit); (ii) biochemical (western blotting and immunoprecipitation) and pharmacological characterization (specific [^{3}H]creatine uptake assays) of wildtype and mutant transporters in transiently transfected HEK 293 cells; (iii) immunocytochemistry analysis of C- and N-tail yellow fluorescent protein (YFP)-tagged hCRT1 and several serial truncation mutants' localization at the cell surface and ER, respectively.

Results: Using biochemical and pharmacological approaches, we observed that CRT1 is the only NTT intolerant to introducing a YFP tag at its N-terminus, *i.e.* resulting in ER retention. We generated serial truncations along the N-tail of CRT1 and found that the truncated mutant of ΔN51-CRT1 abolished creatine uptake. In addition, N-tail YFP-tagged hCRT1 and ΔN60-CRT1 mutants are located in ER in transiently transfected HEK 293 cells.

Discussion: Our findings break a hallmark rule, previously established for SLC6 transporter relatives of hCRT1, that their amino tails are virtually dispensable to their folding and trafficking (cell surface expression) or even their substrate uptake activity. Our data provide novel insights into the molecular and physiological features underlying the non-conforming folding and trafficking routes of CRT1. These ought to impart crucial details relevant to the role and regulation of CRT1 in disease (*e.g.* CTD or cancer).

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