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Imaging studies on transmembrane topology of CLN3

Batten disease belongs to the family of neuronal ceroid lipofuscinoses (NCLs), a group of neurodegenerative storage disorders, characterized by pathological accumulation of autofluorescent material in the lysosomes. The juvenile form of neuronal ceroid lipofuscinosis (JNCL) is caused by mutations in CLN3, an approximately 55 kDa multispan transmembrane protein of unknown function. Several topological models of CLN3 have been proposed, basing on various experimental methods and in silico predictions. However, no conclusive structure exists up to date. In this work, the suggested models were tested using the FRET-FLIM technique. A library of tagged CLN3 constructs was created by random insertion of a GFP using a transposomic approach. The inserts were replaced with a myc-epitope. Chimeric constructs were created by introducing GFP as a second tag positioned close to the C-terminus of CLN3-myc clones. FRET-FLIM was measured between the GFP-tag (donor) and myc-epitope labelled with antibodies conjugated to a red dye (acceptor). Additionally, information about battenin topology was obtained from experiments on the CLN3-myc constructs. Selective permeabilisation of the cellular membranes with differential concentrations of digitonin was performed to control the cytoplasmic vs. luminal position on the clones. Co-localisation study of the CLN3-myc constructs with LAMP1 lysosomal marker revealed possible vulnerable regions of CLN3, which react to tagging with mislocalisation. In silico predictions were correlated with the experimental findings. Based on the results of this work, a new model of battenin structure was proposed, suggesting six transmembrane domains, both termini in the cytosol and two hydrophobic helices parallel to the cytosolic face of the lipid bilayer.