THE ROLE OF THE APP COPPER BINDING DOMAIN IN REGULATING APP METABOLISM AND STRUCTURE

L. Spoerri\textsuperscript{1,2}, L.J. Vella\textsuperscript{1,2}, C.L. Pham\textsuperscript{1,2}, K.J. Barnham\textsuperscript{1,2,3}, R. Cappai\textsuperscript{1,2}

\textsuperscript{1} The University of Melbourne, \textsuperscript{2} Bio21 Molecular Science & Biotechnology Institute, \textsuperscript{3} The Mental Health Research Institute of Victoria, Parkville, VIC, Australia

Two major pathological characteristics of Alzheimer's disease (AD) include accumulation of β-amyloid (Aβ), a toxic peptide derived from the Amyloid Precursor Protein (APP), and abnormal copper distribution in the brain. A relationship exists between copper and APP, whereby copper can modulate APP processing and APP can regulate copper homeostasis. A better understanding of the molecular mechanisms involved in these events could lead to novel therapeutics to treat the disease. The aim of this work was to investigate the sequence activity relationship of the putative copper binding residues, previously identified by NMR and crystallography, on APP metabolism by performing mutagenesis studies in HEK293 cell lines overexpressing APP WT and APP CuBD mutations. This investigation was complemented by structural studies employing yeast expressed recombinant APP CuBD. Using a combination of single, double and triple mutations of the three APP CuBD histidines we demonstrate that each one of these residues is important for APP metabolism and structural stability. In particular His149 and His151 play a crucial and mutually redundant role in these processes. Mutating these three histidines to asparagines results in APP retention in the ER and impaired APP maturation and processing in the HEK293 cell line, presumably due to the formation of APP aggregates/oligomers. Mutation of the histidines in recombinant protein results in protein aggregation/oligomerization and secondary structural changes that correlate with the observations in the cell line. This data provide novel information on how the CuBD affects APP structural stability and processing.