Calcyon interacts with clathrin adaptor proteins AP-1, AP-2, and AP-3 and accelerates post-Golgi trafficking of VSVG

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Calcyon is a single transmembrane protein that interacts with clathrin light chain and stimulates clathrin mediated endocytosis [1]. The clathrin adaptor protein (AP) complexes AP-1, AP-2, AP-3, and AP-4 are hetero-tetramers. Their ‘mu’ subunits interact with YXXØ type motifs. As the calcyon cytoplasmic domain contains two such tyrosine motifs we tested whether AP mu subunits bind to calcyon. GST pull down assays suggests that calcyon can directly interact with mu1, mu2 as well as the ubiquitous and neuronal isoforms of mu3, 3A and 3B, respectively. Yeast 2 hybrid assays confirmed the direct interaction between calcyon and mu1, mu2, mu3A and 3B subunits of AP. Further GST-calcyon effectively pulled down AP-1, AP-2, and AP-3 from wild type brain lysates, and FLAG-calcyon immunoprecipitated the γ subunit of AP-1, α subunit of AP-2, and δ subunit of AP-3 from Cal⁴Œ [2] brain lysate. Pull down experiments with tyrosine motif point mutations or C terminal truncations suggest that the second tyrosine motif of calcyon is essential for interacting with AP. AP’s regulate internalization of cargo as well as the trafficking of cargo from the trans-Golgi network to vesicles. Neuronal endocytic protein of 21kD (NEEP21), a member of NEEP21/calcyon/P19 gene family [3], regulates transcytosis of cargos from the dendritic to axonal compartment of neurons. Given the evidence that NEEP21 is involved in neuronal transcytosis and that calcyon interacts with clathrin as well as AP-1, AP-2, and AP-3, I tested whether calcyon plays a role in transcytosis. Chasing the trafficking of the G protein of vesicular stomatotitis virus (VSVG) by ‘temperature block and release assay’ suggests that calcyon accelerates post-Golgi trafficking of VSVG. Taken together my data suggests that calcyon directly interacts with AP via a YXXØ type motif and accelerates post-Golgi trafficking.