

## The Role of Ubiquitin in M2 Muscarinic Acetylcholine Receptor Endocytic Pathways

Muscarinic acetylcholine receptors (mAChRs) are well known as key regulators of many physiological processes in mammalian systems. A lot of attention has recently been given to cholinergic signalling implications in higher brain functions such as memory formation, whereas impaired muscarinic neurotransmission may contribute to cognitive defects and devastating memory loss. Furthermore, loss of cell surface M2 AchR subtype from the cholinergic neurones of acetylcholinesterase knockout mice contributes to memory defects. These changes in receptor density in part reflect receptor downregulation via the endocytic pathway. Since acetylcholinesterase inhibitors are the only FDA approved treatment for Alzheimer's disease the regulation of M2-AchR remain a promising target for CNS disorders like Alzheimer's disease and Schizophrenia (1). However, despite much research into GPCR trafficking, very little is known about the processes and implications of mAChRs degradation.

We hypothesize that manipulating trafficking may enhance existing acetylcholine based therapies for cognitive disorders. Better understanding of mAChRs trafficking and whether these can be regulated both in vitro and in vivo may provide a new spectrum of therapeutic targets for the treatment of cognitive disorders. The objectives of this project are to study different aspects endocytic trafficking of mAChR and molecular mechanisms that control the post-endocytic fate of these receptors. In the proposed study we use the epitope FLAG-M2-ACh receptor to study its compartmentalization and association with adaptor proteins that have previously implicated in the trafficking of other GPCRs (2).

Characterization of M2-AchR endocytosis, recycling and downregulation was performed on HEK293 cell line using a combination of confocal microscopy, western blotting and FACS fluorescent flow cytometry. We show that M2-AChR undergoes agonist-induced internalization in a dynamin-dependent and clathrin-dependent manner, before rapidly being targeted to acidic lysosomal compartments where receptor proteolysis takes place. As downregulation of many receptors is mediated by posttranslational modifications with addition of ubiquitin, we wanted to test if M2 requires ubiquitination in order to transit the endolysosome pathway. We used a traditional strategy of replacing all 29 intracellular lysine residues with arginine. This change in primary sequence prevents receptors trafficking to the late endosome/MVB without affecting the rate of endocytosis implying that the M2 subtype requires ubiquitin modification and functional ESCRT complexes for efficient degradation. Modulating the specific proteins involved in the M2-AchR ubiquitination could provide new therapies designed to regulate receptor signalling by controlling surface expression and endocytic pathways in specific brain areas.

1. Volpicelli-Daley LA *et al.* (2003). *Ann Neurol.* **53** (6): 788–96.
2. J.N. Hislop *et al.* (2009). *J. Biol. Chem.* **284**: 19361–70.