

Cutting edge novel technologies to investigate lysosomal channels and transporters important in recovery post-autophagy

Supervisory team:

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Project description:

Autophagy is a fundamental function of cells that governs the response to starvation, infection and protein mis-processing. It is central to cellular and organism health, directly impacts upon healthy ageing and is demonstrably impaired in cancers, metabolic conditions and neurodegenerative diseases. The process of clearing damaged mitochondria or toxic protein aggregates within the cell is called macroautophagy. Here the cell engulfs the target within a double membrane that originates from the endoplasmic reticulum and transports it to the lysosome where it fuses in a calcium (Ca^{2+}) dependent manner; the cargo is destroyed by proteases contained within the acidic lysosomal lumen. This is where our current knowledge of autophagy ends. However, it is clear that the lysosome must somehow recover from this process, as the macroautophagy delivers a cargo of cytoplasm that would de-acidify and depolarise the lysosome. This would shut down the degradative and recycling capacity of the lysosome and impair one of the fundamental roles of autophagy, to allow the cell to survive during periods of starvation. Clearly, in a healthy cell, the lysosome does recover, the question we are asking with this project is how does it do this?

It is remarkable that this process has never been studied, in part, we believe, owing to the difficulty in isolating and studying lysosomal ion channel and transporter function. The process of maintaining membrane potential depends upon ion transport, as occurs during neuronal action potentials. The lysosomal membrane functions in the same way, but is under-studied and has never been followed during an event such as autophagy and post-autophagy recovery. We have developed cutting edge techniques to magnetically purify lysosomes and utilise them across a host of automated electrophysiology/patch clamping instruments that, in the UK, exists only in Cardiff. With this project we will measure how lysosomal ion fluxes are altered during and post-autophagy in magnetically purified lysosomes from cells where chemical treatments are used to induce or arrest autophagy. We will determine how these ion fluxes alter lysosomal membrane potential, and how this impacts on lysosomal solute transporter function. Together, these data will provide the first evidence not only of how the lysosome recovers post-autophagy, but how this process governs the cellular response to starvation by restoring lysosomal transporter function to enable macromolecular recycling and protein synthesis. We will use our data to generate models to fill this gap in our understanding of autophagy, a truly critical cellular process.