Early uptake of EGF and transferrin visualised using Correlative Light Electron Microscopy (CLEM)

Supported by an RMS Summer Studentship award, I spent time in the lab of Prof. Paul Verkade at the University of Bristol to study the early uptake mechanisms of Transferrin (Tf) and Epidermal Growth Factor (EGF) using a combination of microscopy techniques. These early steps of internalisation are still not completely understood. We applied Correlative Light Electron Microscopy (CLEM) to combine fluorescence light microscopy with the high resolution and structural background of electron microscopy.

Internalisation experiments

A549 cells were grown in cell imaging dishes where the glass coverslip has a finder pattern embossed on it to allow retraction at the light and electron microscopy level. Cells were serum-starved to express more receptors on the surface and subsequently incubated at 37°C with EGF (1:10) and Tf (1:20) tagged probes that are visible in both the light and electron microscope. EGF-biotin was bound to streptavidin-labelled Quantum dots 655 (6x12 nm rods) and Tf was bound to Alexa488-5nm gold. After incubation, samples were fixed in 4% PFA. Confocal Laser Scanning Microscopy was used to obtain light microscopy images. Cells were then processed for EM by crosslinking lipids with 1% osmium tetroxide, crosslinking nucleic acids with uranyl acetate and dehydrating with 80, 90, 96 and 100% ethanol. Samples were then embedded in Epon resin. Sections were cut 1mm x 1mm x 300nm with an ultramicrotome.

Imaging

All the microscopy experiments were performed in the Wolfson Bioimaging Facility. Electron micrographs were obtained with 120kV and 200kV TEMs. Fluorescence images were obtained using a confocal microscope and analysed using FIJI. Tomograms were created using eTom for IMOD (University of Colorado, Boulder).

Results and discussion

Light microscopy images of cells incubated with probes for 0 and 10 minutes were acquired, and a suitable cell was chosen for CLEM. The chosen cell exhibited punctate fluorescence around its peripheral and juxtamembrane regions, indicative of some degree of receptor internalisation as would be expected 10 minutes after probe application (Figure 1). Low levels of colocalisation suggest receptors have already begun segregating into distinct endosomal vesicles, either through recycling or degradative pathways.