Summer Studentship Report

Benjamin Hicks

Benjamin, who studies at the University of Bristol, was one of six undergraduates who carried out summer projects in 2019 with the help of a £2,000 RMS Summer Studentship award. Here is his report for in**focus**

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 retracing 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

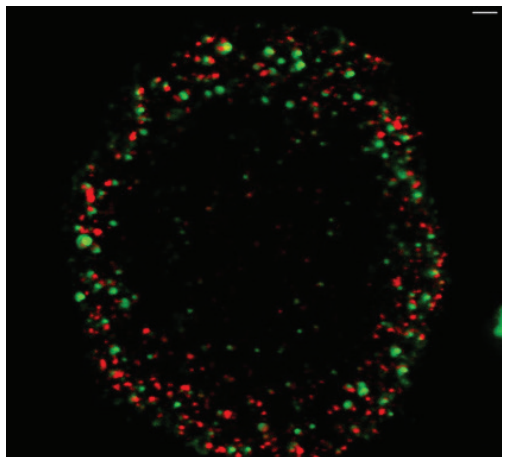


Figure 1. Light microscopy image of cell after 10 min probe internalization. Green puncta are Tf-Alexa488 probes and red foci are EGF-QDs.

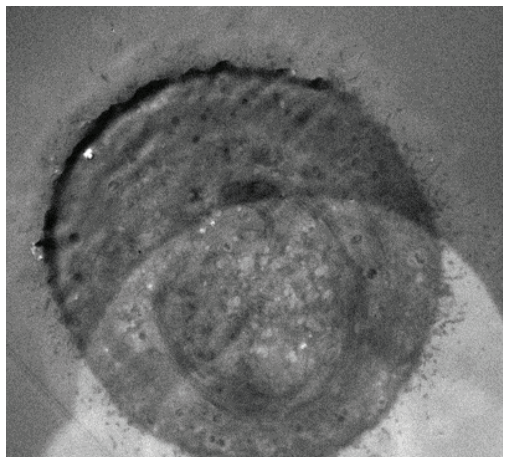


Figure 2: Low magnification EM image of chosen cell.

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

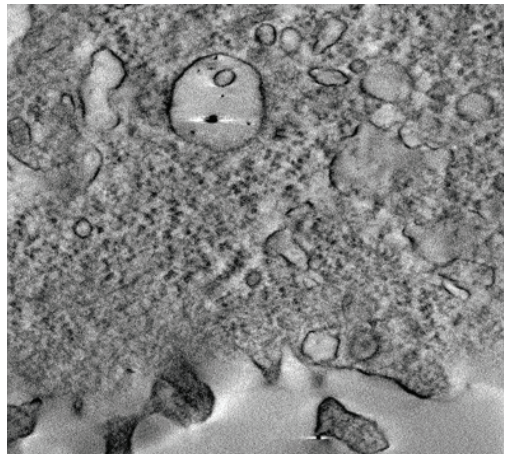


Figure 3. EM image of region of interest with early endosome (top) and plasma membrane (bottom).

TEMs. Fluorescence images were obtained using a confocal microscope and analysed using FIJI. Tomograms were created using eTomo for IMOD (University of Colorado, Boulder)

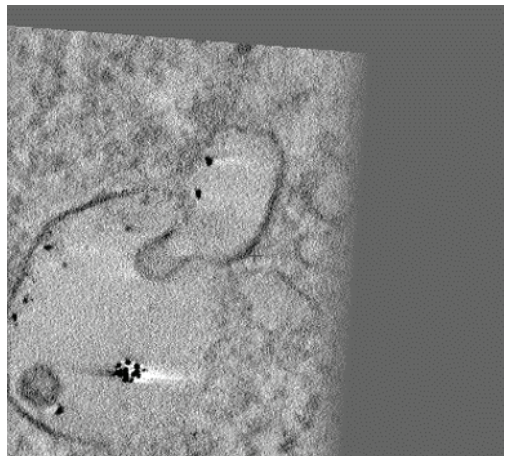


Figure 4. Tomography slice showing a large early-endosome and smaller compartment fusing to it.

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.

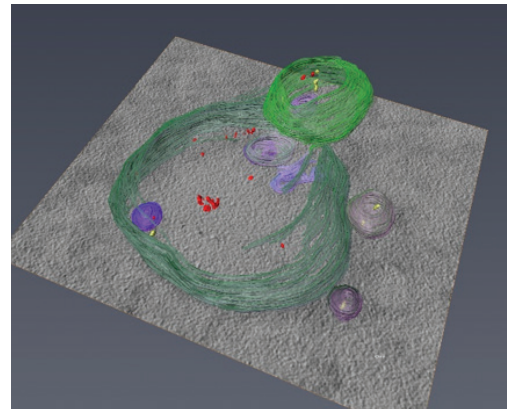
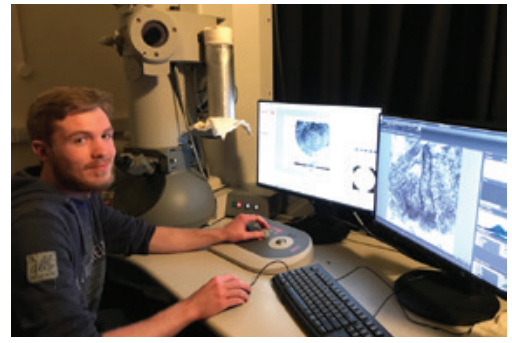


Figure 5. Tomography model of two fusing early-endosomal compartments containing ILVs and probes.

After processing for EM, we localised the chosen cell in the section via the finder pattern (Figure 2) and found a region of interest near the plasma membrane which seemingly consisted of a single early-endosome containing one or both types of probes (Figure 3). We then performed tomography to visualise the region across a range of tilt angles along one axis. This revealed this existence of a second, smaller endosomal compartment potentially fusing with the larger one, as well as smaller structures presumed to be intra-luminal vesicles (Figure 4).

Finally, I created a model from the tomography data (Figure 5). EGF probes are visibly larger than Tf probes, being 6-12nm rods and therefore this allowed me to label them accordingly. A striking feature of the structure is a large cluster of Tf probes in the largest endosomal compartment. Whether these are membrane-bound via their cognate receptors is unclear as the membrane was not well defined in that region. An interesting observation is that probes are predominantly segregated into separate compartments.



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