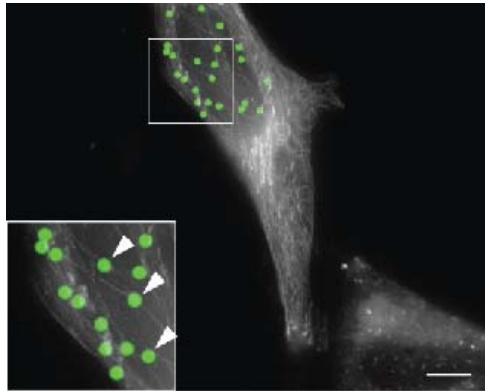
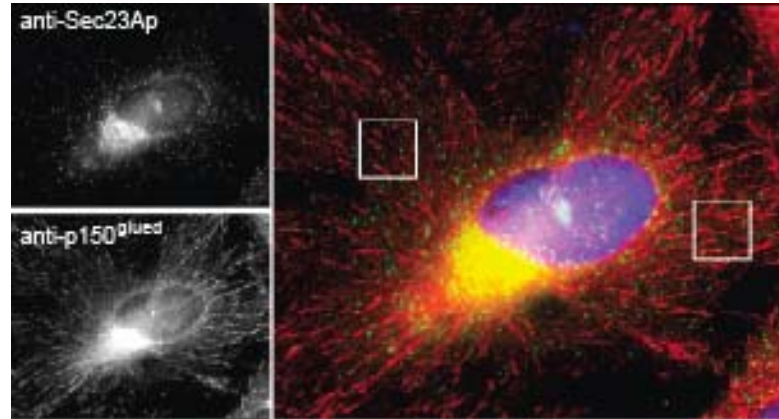


ER-to-Golgi membrane traffic



1/ HeLa cells with pseudo-coloured ERES in green circles; continuous tracks of GFP-p150^{Glued} reveal the paths taken by polymerizing microtubules, which can be seen to grow directly through ERES (arrowheads). Scale bar: 10µm



2/ Colocalization of COPII and p150^{Glued} in HeLa cells.
 Green: COPII label (Sec23Ap)
 Red: dynactin label (p150^{Glued})
 Blue: DNA label
 Scale bar: 5µm

Dr David Stephens and colleagues from the [Department of Biochemistry](#) at the University of Bristol, are interested in protein secretion mechanisms in mammalian cells. Investigating the way in which proteins exit the ER and are transported to the Golgi apparatus is essential as disruption of this pathway is involved in many human diseases. Cargo export from the ER is mediated by the COPII coat complex. The Stephens lab are studying the mechanisms and regulation of COPII and VTC formation, as well as VTC movement along the microtubule cytoskeleton.

Cargo concentrates into COPII-coated ER export sites (ERES); COPII vesicles then bud and uncoat before forming vesicular-tubular transport carriers (VTC) which then move in a dynein and dynactin dependent manner along microtubules towards the Golgi. To investigate how COPII localizes and maintains in ERES, researchers used live cell imaging techniques with YFP-Sec23p to label COPII and Rhodamine-Tubulin to label microtubules. Images, acquired using an Olympus IX-70 and TILL Photonics widefield imaging system and imported into **Volocity** and analyzed with **Volocity Visualization**, showed that ERES localize in vicinity to microtubules.

The following step was to understand how VTCs link to microtubules and how their motility is regulated. Time-lapse imaging analysis allowed Dr Stephens and colleagues to prove that microtubules continue to polymerize after passing one or more ERES and that the association of ERES with microtubules lasted for several minutes at least. This indicates that growing microtubules are targeted towards ERES consistent with a "search-and-capture" mechanism. Image 1 shows a maximum intensity (extended focus) projection from a time-lapse sequence of HeLa cells expressing GFP-labelled microtubule plus ends and reveals direct evidence of microtubule migration towards and through GFP-labelled ERES.

In addition, this work identified one of the COPII-dependent mediators involved in ERES/microtubule association as p150^{Glued}, a component of the dynactin complex that interacts with the dynein motor and also binds to microtubules. Image 2 is an immunofluorescence analysis of fixed cells that reveals colocalization of COPII with p150^{Glued}. This suggests the presence of a protein interaction network that functions to coordinate COPII vesicle formation with VTC generation and transport. Measurement of the velocity of individual VTCs using particle tracking in **Volocity Classification** and time lapse imaging, indicated that although inhibition of the COPII-dynactin interaction caused changes in COPII dynamics, the mobility and speed of VTC's was not affected. This confirms a role of dynactin at early stage of COPII function.

Using **Volocity** has allowed the Stephens lab to suggest a mechanism by which membranes of the early secretory pathway can be linked to motors and microtubules for subsequent organization and movement to the Golgi apparatus.