

## 4D-IMAGING OF TUBULO-VESICULAR CARRIER FORMATION IN THE ENDOCYTIC PATHWAY

Jez G. Carlton, Colin G. Blackmore\* Peter J. Cullen

Department of Biochemistry,

University of Bristol, BS8 1TD, UK

\*PerkinElmer Life & Analytical Sciences,

Seer Green, HP9 2FX, UK

E-mail : j.carlton@bristol.ac.uk

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The sorting endosome exists in cells as an intracellular sorting network. This organelle receives cargo input from both the TGN and the plasma membrane, and dispatches these cargo to a variety of subcellular destinations, including the lysosome, the recycling endosome and the TGN. Cargo removal from the sorting endosome is thought to proceed through the generation of tubular and vesicular transport carriers from the sorting endosome [1]. Selective incorporation of cargo into these carriers ensures that cargo is delivered to, and remains at, its correct subcellular destination. Sorting nexin-1 (SNX1) is the prototypical member of a family of sorting nexins, a group of peripheral membrane proteins defined by the presence of a phosphoinositide binding PX domain [2]. In the case of SNX1, it has been shown that the endogenous protein functions in the return of mannose six-phosphate receptors from endosomes to the TGN [3]. Studies of the SNX1-decorated compartment presents particular difficulties for study as the labelled structures are small (~50-500nm), move rapidly in 3-dimensions, and overexpression of this protein is known to cause endosomal disruption and mis-sorting of cargo.

Dual spinning disk confocal microscopy was used to study formation and trafficking of tubulovesicular structures labelled with GFP-SNX1 expressed in HeLa cells. Rapid 4-D imaging (of 3 volumes per second) over short periods of time revealed stochastic movement of most SNX1-labelled vesicles. Extended timelapse imaging demonstrated net movement of vesicles towards the perinuclear region of cells, which is consistent with their role in retrieval of cargo from endosomes to the TGN [3].

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