doi:10.1017/S1431927611001073
Microsc. Microanal. 17 (Suppl 2), 2011

A high precision survey of the molecular dynamics of mammalian clathrin mediated endocytosis.

Marcus J. Taylor[1], David Perrais[2], Christien J. Merrifield[1,*]

[1]MRC Laboratory of Molecular Biology, Hills Road Cambridge, CB2 2QH

[2]Centre National de la Recherche Scientifique, Université de Bordeaux, UMR 5091, Bordeaux, France.

*Correspondence : cjm@mrc-lmb.cam.ac.uk

Abstract.

Dual color total internal reflection fluorescence microscopy (TIR-FM) is a powerful tool for decoding the molecular dynamics of clathrin mediated endocytosis (CME). Typically, the recruitment of a fluorescent protein (FP) tagged endocytic protein was referenced to the disappearance of spot-like clathrin coated structure (CCS), but the precision of spot-like CCS disappearance as a marker for canonical CME remained unknown. Here we have used a TIR-FM based imaging assay to detect scission events with a resolution of ~2s. We found scission events engulfed comparable amounts of transferrin receptor cargo at CCS of different sizes and CCS did not always disappear following scission. We measured the recruitment dynamics of 34 types of endocytic protein to scission events: Abp1, ACK1, amphiphysin1, APPL1, Arp3, BIN1, CALM, Cip4, clathrin light chain (Clc), cofilin, coronin1B, cortactin, dynamin1&2, endophilin2, Eps15, Eps8, Epsin2, Fbp17, FCHo1&2, GAK, Hip1R, lifeAct, Mu2 subunit of the AP2 complex, myosin1E, myosinVI, NECAP, NWASP, OCRL1, Rab5, SNX9, synaptojanin2β1 and syndapin2. For each protein we aligned ~1000 recruitment profiles to their respective scission events and constructed characteristic 'recruitment signatures' which were grouped, as for yeast, to reveal the modular organization of mammalian CME. A detailed analysis revealed the unanticipated recruitment dynamics of SNX9, Fbp17 and Cip4 and showed the same set of proteins was recruited, in the same order, to scission events at CCS of different sizes and lifetimes. Collectively these data reveal the fine grained temporal structure of CME and suggest a simplified canonical model of mammalian CME in which the same

core mechanism of CME, involving actin, operates at CCS of diverse size and lifetimes.

https://doi.org/10.1017/S1431927611001073 Published online by Cambridge University Press