Remodeling of endosomal membranes by branched actin networks

Emmanuèle Helfer[1], **Alexis Gautreau**[2]

[1] *Centre Interdisciplinaire de Nanoscience de Marseille, Aix-Marseille Université – CNRS UMR7325, Campus de Luminy, Case 913, 13288 Marseille cedex 9, France*
[2] *Ecole Polytechnique, Route de Saclay, 91128 Palaiseau cedex, France*
helfer@cinam.univ-mrs.fr

Sorting of cargoes in endosomes occurs through their selective enrichment into sorting platforms, where transport intermediates are generated. The **WASH complex**, which was recently discovered as an actin nucleator (NPF), directly binds to lipids, activates the Arp2/3 complex and hence polymerization of branched actin onto such sorting platforms, and contributes to endosomal fission [1].

We previously showed in living cells that **branched actin polymerization plays a role into the organization of the WASH complex into microdomains at the surface of endosomes** [2]. Preventing the generation of branched actin networks induced endosomal accumulation of the WASH complex, and inhibited the dynamic cytosol/endosome exchange of the WASH complex. We also observed that discrete WASH domains coalesce upon actin depolymerization. Our results suggest a feedback loop process, in which the branched actin network, i.e. the product of the WASH complex, regulates dynamically and spatially WASH organization at the endosomal membrane (Figure). Moreover, the coalescence behavior of WASH domains raises the hypothesis of the potential role of the WASH complex in lipid repartitioning, as it was shown to bind specific lipids [3]. Such membrane reorganization may induce line tension at the proteo-lipidic domain boundary and thus provide a dynamin-independent contribution to membrane scission [4].

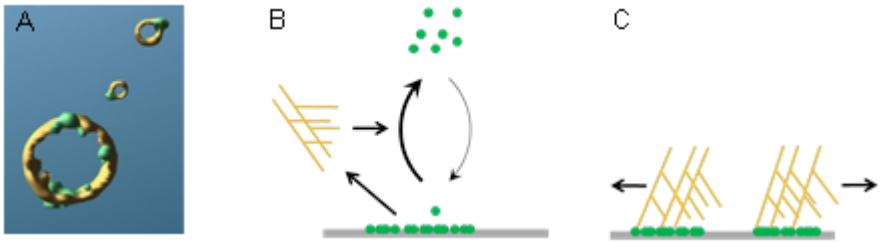

Figure: A) 3D-reconstruction of WASH domains (in green) on endosomes (in yellow) in cells [image from E. Derivery]. B) The branched actin network (in yellow) limits the recruitment of WASH (in green) to the endosomal membrane. C) The branched actin network associated to a WASH domain prevents its coalescence with a neighbor domain.

We are developing **a bottom-up approach to reconstitute the WASH/actin machinery on synthetic membranes**. Such *in vitro* biomimetic approaches allow controlling biochemical and physical parameters (like protein surface density or membrane composition), and have proven to enable understanding molecular mechanisms occurring *in vivo* [5,6]. We and others have confirmed that the retromer complex (CSC) is the endosomal receptor of the WASH complex and characterized their interaction [7,8]. We now anchor artificially CSC to either supported bilayers or giant unilamellar vesicles (GUVs) in order to recruit physiologically the WASH complex to the membranes. We are studying the distribution of

both protein complexes at the membrane surfaces, using fluorescence microscopy techniques, as function of biochemical and biophysical parameters. The next step consists in adding proteins, like actin and the Arp2/3 complex, to generate a branched actin structure from the WASH domains. From the changes due to the presence of actin we aim to decipher how the actin network regulates proteo-lipidic domains formed by the WASH complex, the CSC and the associated lipids. On the long term, we intend to reconstitute the WASH-induced fission of membrane tubules pulled from GUVs.

Keywords: WASH complex, actin, membrane, proteo-lipidic domain, biomimetism

[1] T. S. Gomez and D. D. Billadeau, *A FAM21-containing WASH complex regulates retromer-dependent sorting*, Dev Cell **17**, 699 (2009); E. Derivery, C. Sousa, J. J. Gautier, B. Lombard, D. Loew, A. Gautreau, *The Arp2/3 activator WASH controls the fission of endosomes through a large multiprotein complex*, Dev Cell **17**, 712 (2009)

[2] E. Derivery, E. Helfer, V. Henriot, A. Gautreau, *Actin polymerization controls the organization of WASH domains at the surface of endosomes*, PLoS One **7**, e39774 (2012)

[3] D. Jia, T. S. Gomez, Z. Metlagel, J. Umetani, Z. Otwinowski, M. K. Rosen, D. D. Billadeau, *WASH and WAVE actin regulators of the Wiskott-Aldrich syndrome protein (WASP) family are controlled by analogous structurally related complexes*, Proc Natl Acad Sci U S A **107**, 10442 (2010)

[4] W. Römer, L.-L. Pontani, B. Sorre, C. Rentero, L. Berland, V. Chambon, C. Lamaze, P. Bassereau, C. Sykes, K. Gaus, L. Johannes, *Actin dynamics drive membrane reorganization and scission in clathrin-independent endocytosis*, Cell **140**, 540 (2010)

[5] T. P. Loisel, R. Boujemaa, D. Pantaloni, M.-F. Carlier, Reconstitution of actin-based motility of Listeria and Shigella using pure proteins, Nature **401**, 613 (1999); S. Wiesner, E. Helfer, D. Didry, G. Ducouret, F. Lafuma, M.-F. Carlier, D. Pantaloni, A biomimetic motility assay provides insight into the mechanism of actin-based motility, J Cell Biol **160**, 387 (2003)

[6] L. Trichet, O. Campas, C. Sykes, J. Plastino, *VASP governs actin dynamics by modulating filament anchoring*, Biophys J **92**, 1081(2007); V. Delatour, E. Helfer, D. Didry, K. H. D. Lê, J.-F. Gaucher, M.-F. Carlier, G. Romet-Lemonne, *Arp2/3 controls the motile behavior of N-WASP-functionalized GUVs and modulates N-WASP surface distribution by mediating transient links with actin filaments*, Biophys J **94**, 4890 (2008)

[7] E. Helfer, M. E. Harbour, V. Henriot, G. Lakisic, C. Sousa-Blin, L. Volceanov, M. N.J. Seaman, A. Gautreau, *Endosomal recruitment of the WASH complex: active sequences and mutations impairing interaction with the retromer*, Biol. Cell **105**, 1 (2013)

[8] D. Jia, T. S. Gomez, D. D. Billadeau, M. K. Rosen, *Multiple repeat elements within the FAM21 tail link the WASH actin regulatory complex to the retromer*, Mol Biol Cell **23**, 2352 (2012)