Harnessing actin assembly for endocytosis

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Abstract

The role of actin in clathrin-mediated endocytosis is being studied in yeast and mammalian cells. Live cell fluorescence imaging of tagged endocytic proteins reveals the spatio-dynamics of the endocytic pathway. Genome editing in mammalian cells is used to create gene fusions of endocytic proteins to fluorescent proteins to preserve natural expression levels and minimize pathway perturbation. Actin is recruited late in the pathway, typically after dynamin, to drive membrane invagination and scission. Because proteins are not being overexpressed, it is possible to use the fluorescence from tagged proteins to count the number of specific proteins present at endocytic sites. Enough dynamins are recruited to endocytic sites to encircle the vesicle neck approximately once. The yeast endocytic pathway consists of over 60 proteins. A molecular-genetic gene fusion strategy is being used to identify the essential functions in this pathway. A module consisting of WASP, type 1 myosin and WIP/Vrp1, among other proteins, can be replaced by a single WASP-Myosin fusion protein, identifying the minimal functional unit. A current focus in the yeast studies is on the roles for post-translational modifications of endocytic proteins in regulation of the pathway.