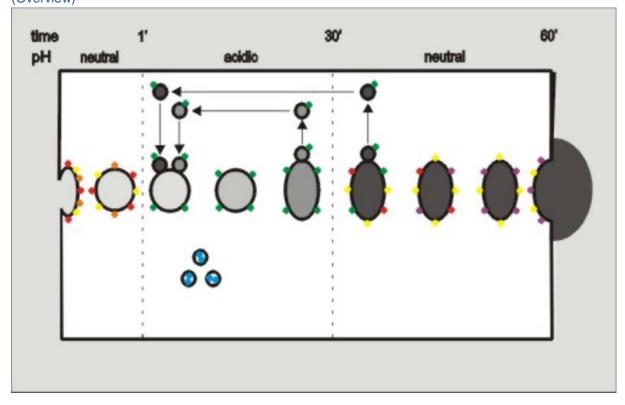
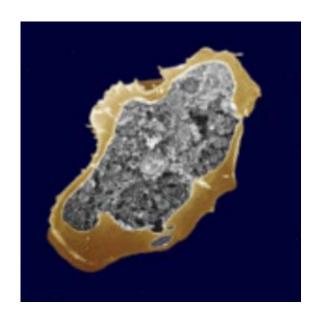
## Proteins involved in endocytic transit (Overview)



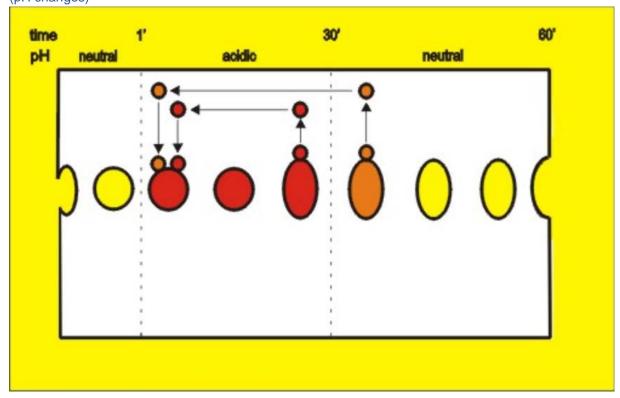
**Top:** Representation of a Dictyostelium cell (white box) in liquid medium (gray). Between endocytosis (left) and exocytosis (right), the medium is concentrated (light to dark gray). Proteins that contribute to endocytic transit are represented by colored dots.

**Right:** Scanning electron micrograph of a cell where the plasma-membrane (gold) has been removed to reveal the internal organisation.

**Reference:** Maniak, M.: Endocytic transit in Dictyostelium. Protoplasma. 210, 25-30, 1999



# Proteins involved in endocytic transit (pH changes)

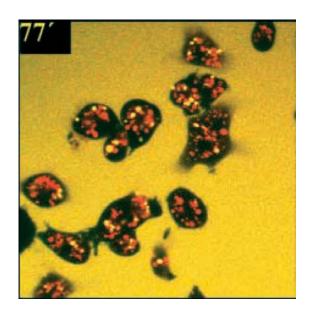


**Top:** Representation of pH-changes during endocytic progression.

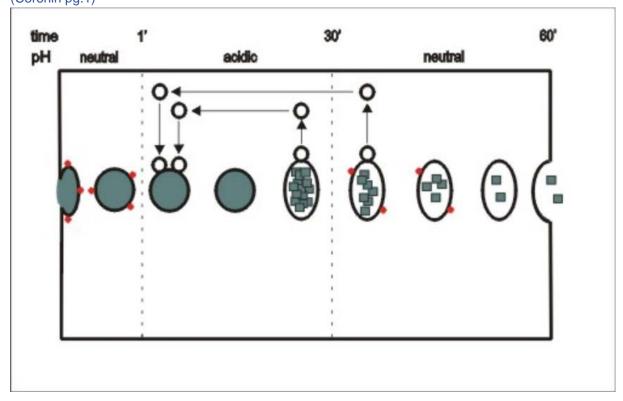
**Right:** Cells in pH-indicator medium. Red vesicles are acidic; yellow vacuoles are neutral.

**Function:** The proton-transporting ATPase associates with the endosomal membrane during the acidic phase.

**Reference:** Maniak, M.: Fluid-phase uptake and transit in axenic Dictyostelium cells. Biochem. Biophys. Acta, 1525, 197 - 204, 2001



# Proteins involved in endocytic transit (Coronin pg.1)

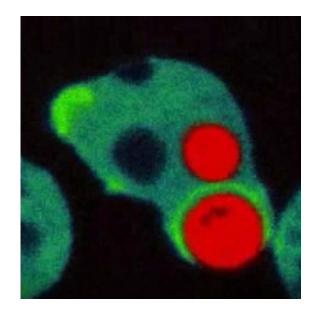


**Top:** Coronin (red dots) in particle uptake, degradation, and exocytosis phases.

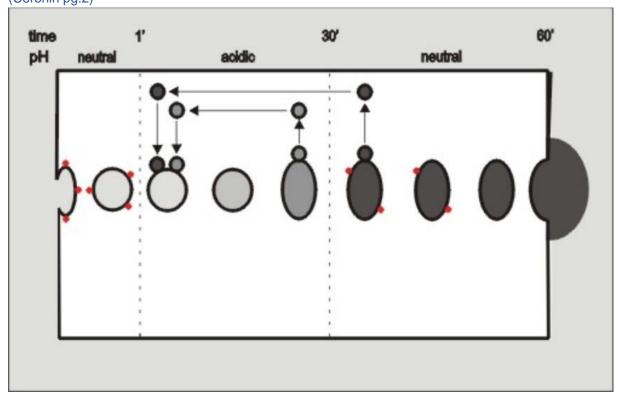
**Right:** Coronin-GFP (green) accumulates at the site of phagocytic uptake (red particle).

**Function:** A mutant lacking coronin has a reduced efficiency of phagocytosis.

**Reference:** Maniak, M., Rauchenberger, R., Albrecht, R., Murphy, J. & Gerisch, G.: Coronin involved in phagocytosis: dynamics of particle-induced relocalization visualized by a green fluorescent protein tag. Cell 83, 915-924, 1995



# Proteins involved in endocytic transit (Coronin pg.2)

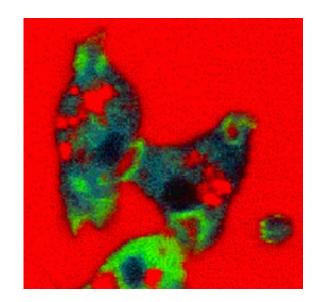


**Top:** Coronin (red dots) during uptake and transit of fluid-phase marker.

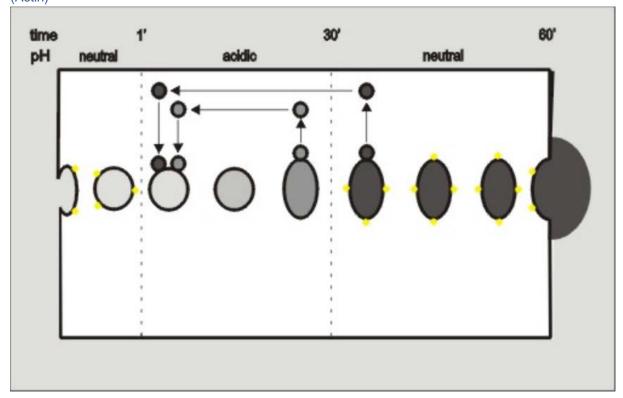
**Right:** Accumulation of coronin-GFP (green) at the site of macropinocytosis.

**Function:** A coronin null-mutant is reduced in fluid-phase uptake.

**Reference:** Hacker, U., Albrecht, R. & Maniak, M.: Fluid-phase uptake by macropinocytosis in Dictyostelium. J. Cell Sci. 110, 105-112, 1997



### Proteins involved in endocytic transit (Actin)



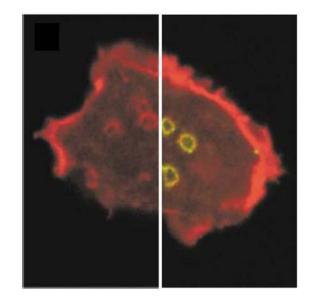
**Top:** Actin (yellow dots) on nascent (light-gray) and mature (dark-gray) endosomes.

**Right:** Coincidence of actin (red) with an endosomal marker protein (green).

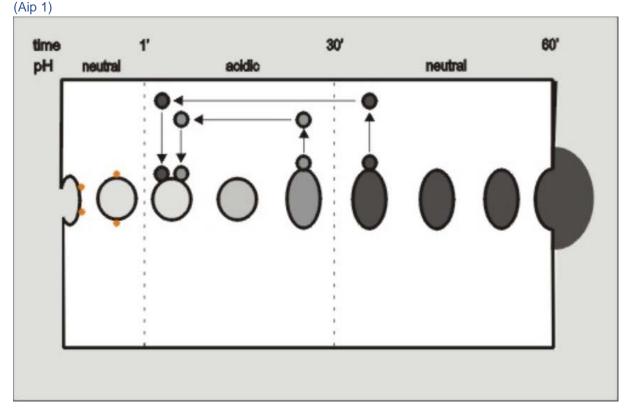
**Function:** When actin is removed from mature endosomes by the means of site-directed depolymerisation, brought about by expressing a vacuolin-myc-cofilin fusion, endosomes aggregate into large clusters.

**Reference:** Drengk, A., et al.: A coat of filamentous actin prevents clustering of late endosomal vacuoles in vivo. Curr. Biol. 13, 1814 - 1819, 2003

**Reference:** Rauchenberger, et al.: Coronin and vacuolin identify consecutive stages of a late, actin-coated endocytic compartment in Dictyostelium. Current Biology 7, 215-218, 1997



#### Proteins involved in endocytic transit

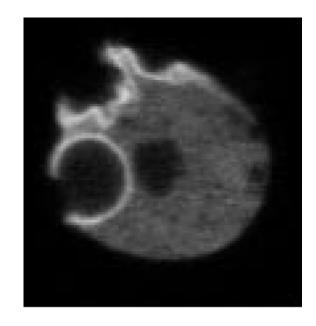


**Top:** Actin interacting protein 1 (Aip1, orange dots) on nascent endosomes.

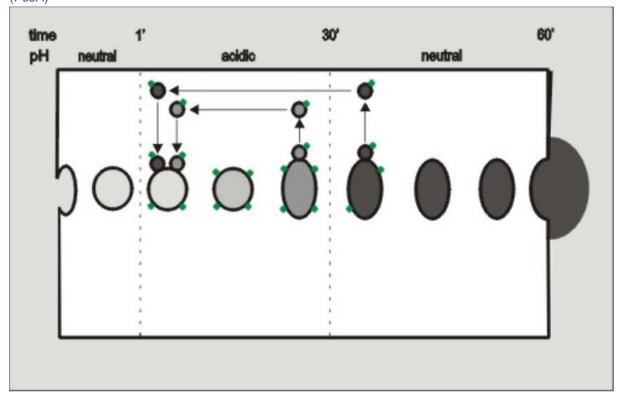
**Right:** Aip 1-GFP expressing cell during phagocytosis of a yeast particle.

**Function:** Cells bearing a targeted disruption of Aip 1 are reduced in phagocytosis and macropinocytosis.

Reference: Konzok, A., Weber, I., Simmeth, E., Hacker, U., Maniak, M. & Müller-Taubenberger, A.: DAip1, a Dicytostelium homologue of the yeast actin-interacting protein Aip1, is involved in endocytosis, cytokinesis and motility. J. Cell Biol. 146, 453-464, 1999.



### Proteins involved in endocytic transit (FcsA)

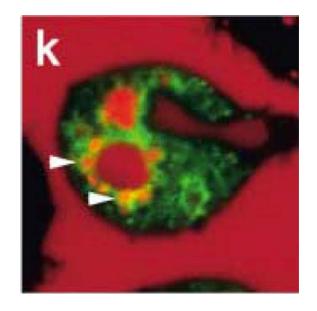


**Top:** Long chain fatty acyl CoA synthetase 1 (FcsA, green dots) on acidic endosomes and recycling vesicles.

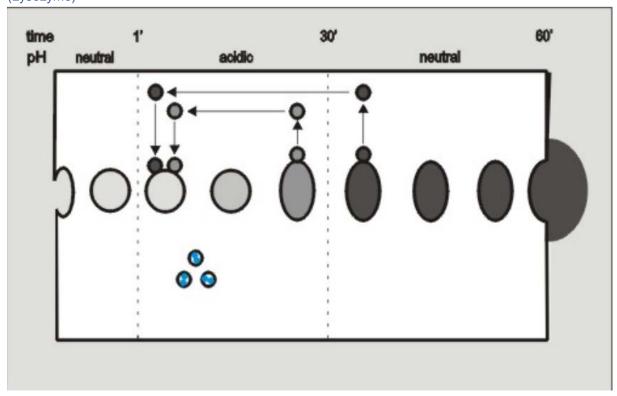
**Right:** FcsA-GFP (green) on (large) endosomes and (small) recycling vesicles (both red).

**Function:** The uptake of fatty acids from food particles across the membrane of the endosome is inefficient in a fcsA mutant.

**Reference:** von Löhneysen, K., Pawolleck, N., Rühling, H., and Maniak, M.: A Dictyostelium Long Chain Fatty Acyl Coenzyme A Synthetase mediates fatty acid retrieval from endosomes. Eur.J. Cell Biol., 82, 505 - 514, 2003



### Proteins involved in endocytic transit (Lysozyme)

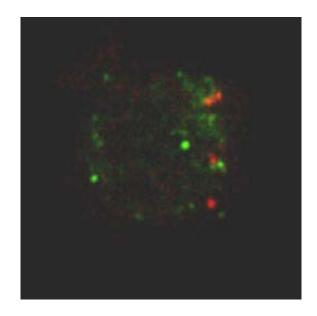


**Top:** Vesicles containing lysozyme (blue dots).

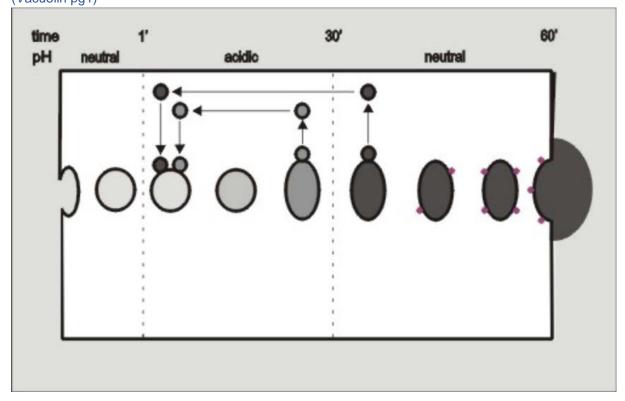
**Right:** Immunofluorescence image of a cell expressing lysozyme-GFP (green) stained for lysosomal esterase (red).

**Function:** Lysozyme degrades bacteria in vitro. A mutant lacking the major lysozyme isoform compensates by increased phagocytosis.

Reference: Müller, I. Subert, N., Otto, H. Herbst, R., Rühling, H., Maniak, M. & Leippe, M.: A Dictyostelium mutant with reduced lysozyme levels compensates by increased phagocytic activity. J. Biol. Chem. 280, 10435-10443, 2005



### Proteins involved in endocytic transit (Vacuolin pg1)

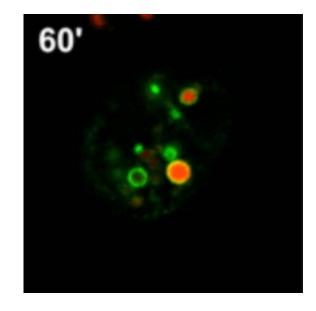


**Top:** Vacuolin (purple dots), a protein related to flotillin/reggie from vertebrates is a marker for late, neutral endosomes

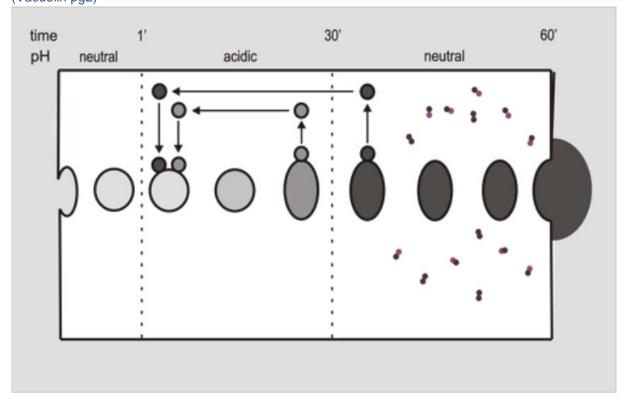
**Right:** Fluid-phase-marker (red) reaches the GFP-vacuolin decorated endosome after one hour.

**Function:** Cells lacking the gene encoding vacuolin B have a single giant neutral endosome and a prolonged transit of endocytic cargo. Vacuolin A mutants are phenotypically normal.

**Reference:** Rauchenberger, et al.: Coronin and vacuolin identify consecutive stages of a late, actin-coated endocytic compartment in Dictyostelium. Current Biology 7, 215-218, 1997 **Reference:** Jenne, N., et al.: Targeted gene disruption reveals a role for vacuolin B in the late endocytic pathway and exocytosis. J. Cell Sci. 111, 61-70, 1998.



### Proteins involved in endocytic transit (Vacuolin pg2)

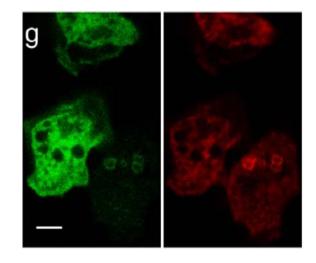


**Top:** Overexpression of the vacuolin oligomerisation domain (brown dots) removes endogenous vacuolin (purple dots) from the endosomal membrane

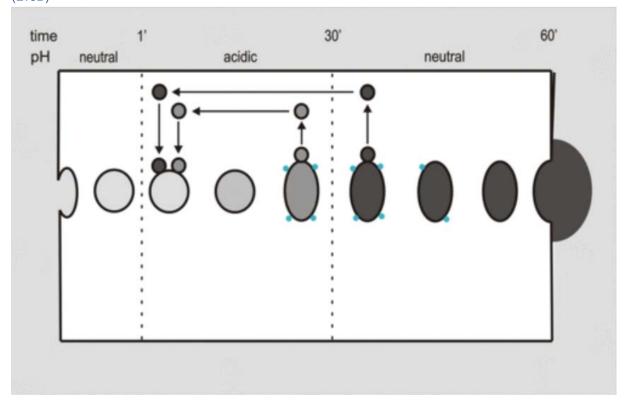
**Right:** Three cells expressing the oligmerization domain (green) at different levels. Only the weakest cell (lower center) shows typical rings of endogenous vacuolin (red). In the other cells endogenous vacuolin becomes dispersed in the cytoplasm.

**Function:** Oligomerisation provides stronger target affinity to otherwise weakly binding PHB domains such as in vacuolin or in mammalian flotillin.

**Reference:** Wienke, D., Drengk, A., Schmauch, C., Jenne, N. & Maniak, M.: Vacuolin, a flotillin/reggie-related protein from Dictyostelium oligomerizes for endosome association. Eur. J. Cell Biol., 85, 991-1000, 2006



## Proteins involved in endocytic transit (LvsB)



**Top:** The LvsB-protein (light blue dots) decorates endosomes at the border between acidic and neutral stage.

**Right:** Red acidic vesicles and green vacuoles are small in wild-type cells (left) and greatly enlarged in the LvsB-mutant (right).

**Function:** The LvsB-protein prevents inappropriate fusion events between acidic and neutral endosomes.

**Reference:** Kypri, E., Schmauch, C., Maniak, M. & De Lozanne, A.: The BEACH protein LvsB is localized on lysosomes and postlysosomes and limits their fusion with early endosomes. Traffic 8, 774 - 783, 2007

