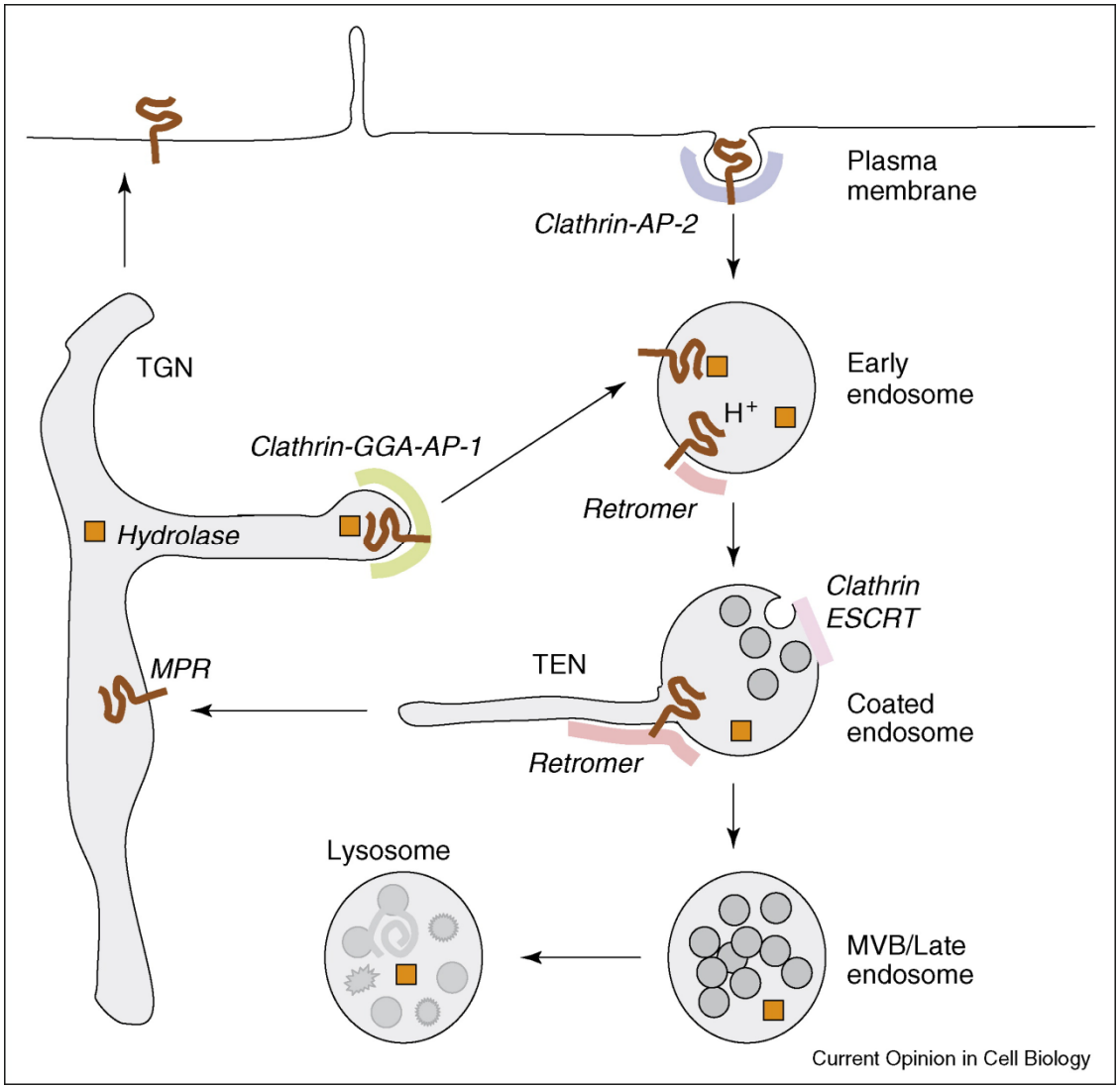
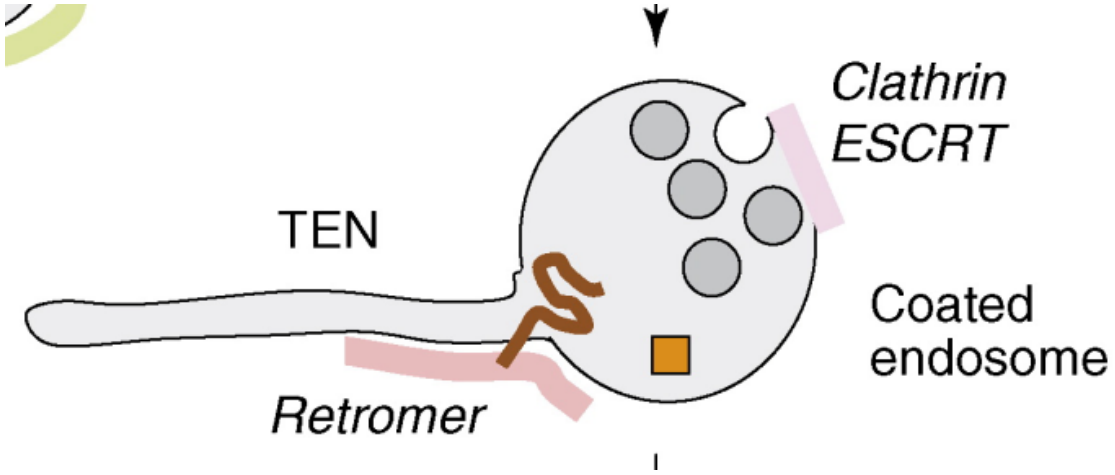
Retromer and ESCRTs

Coats of the late endosomal system: Retromer and ESCRT



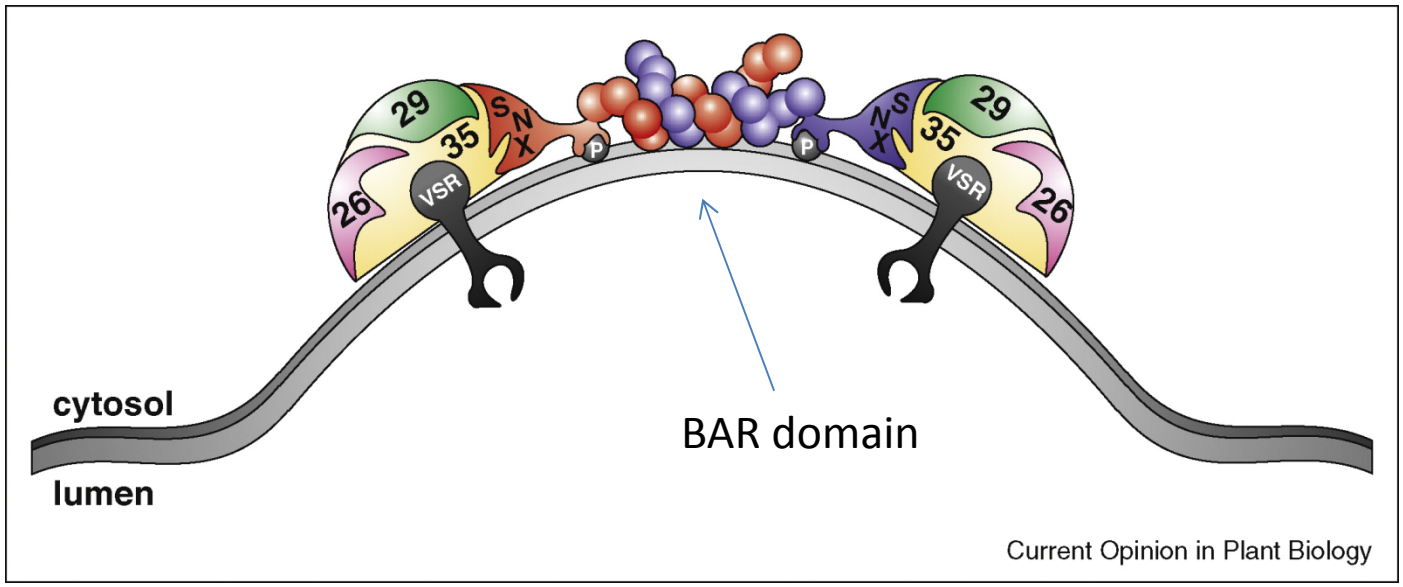
Pause to consider



How do these two traffic pathways differ from ones we've seen before?

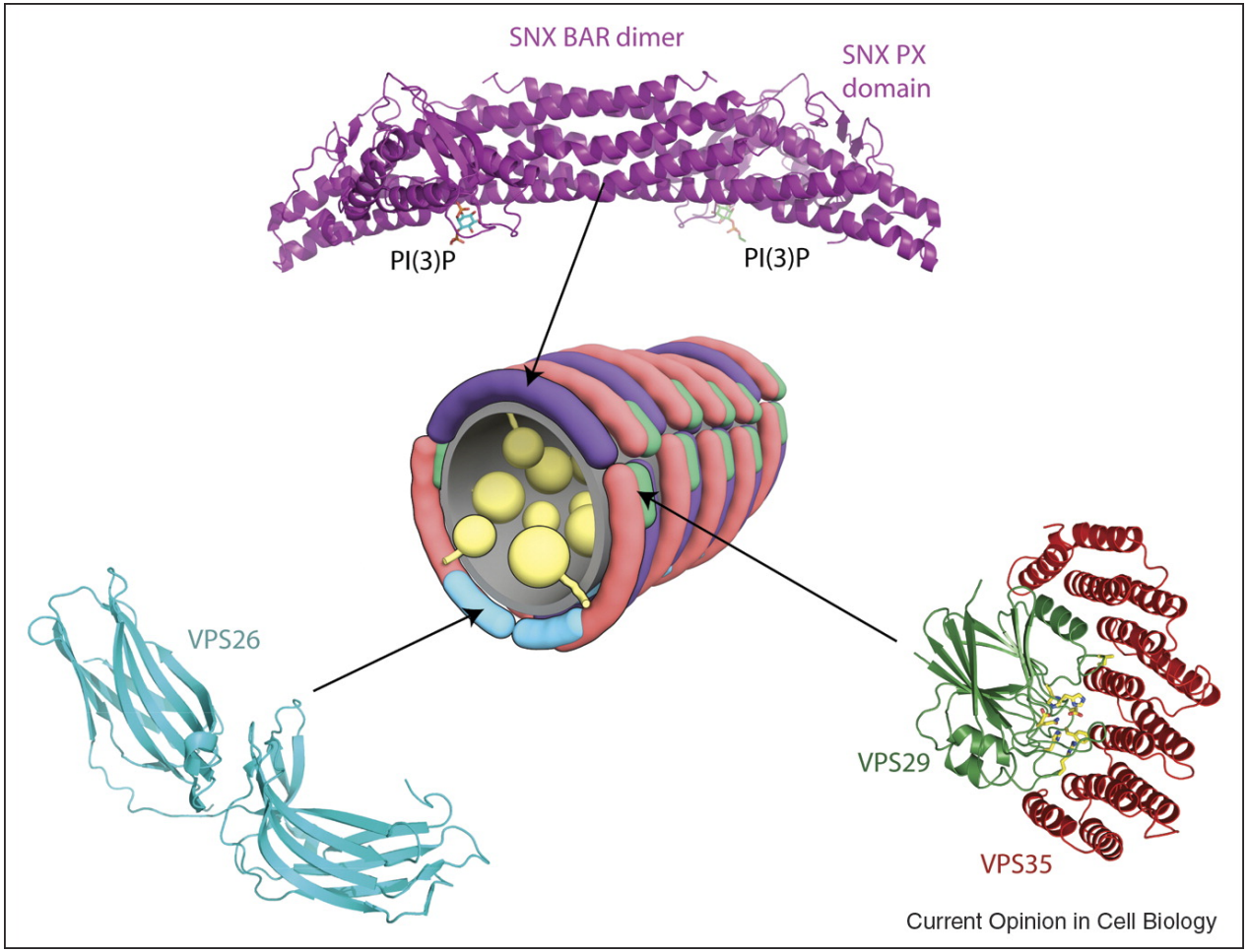
What are the architectural challenges for MVB formation? for retromer tubules?

Retromer schematic



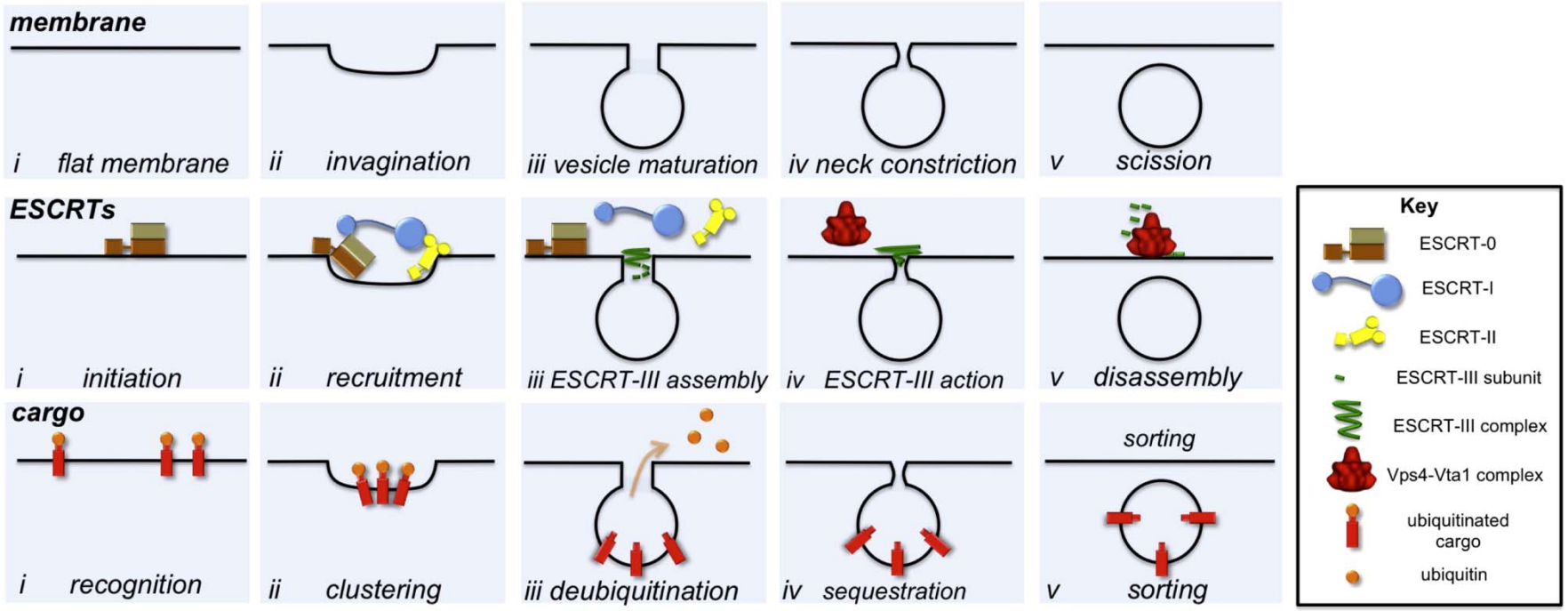
Schellmann & Pimpl 2009

Retromer Schematic



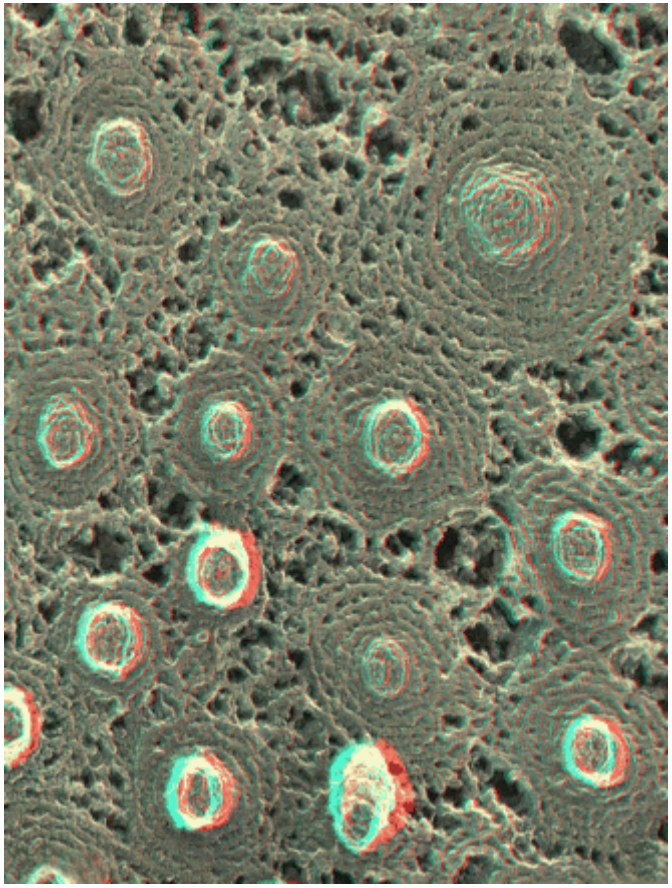
Bonifacino & Hurley, 2008

ESCRT

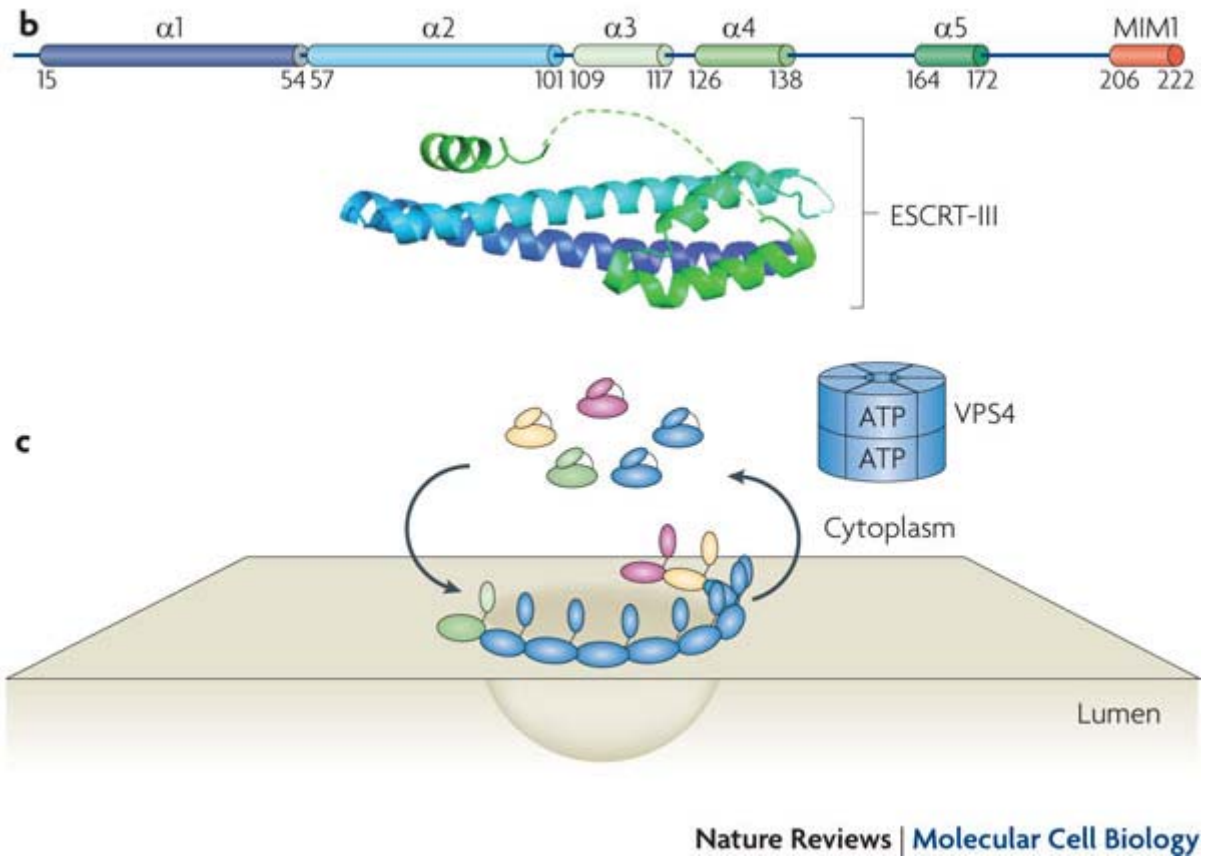


Henne et al 2011

Top view of assembled ESCRTIII



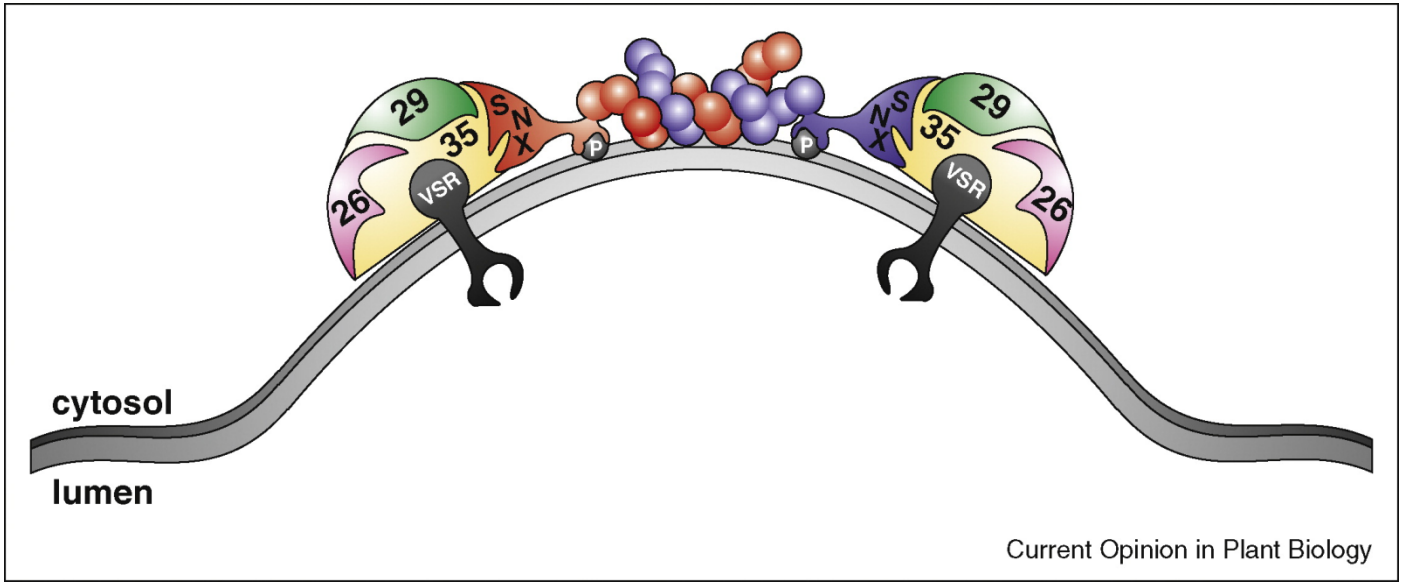
ESCRT schematic



Hurley and Hanson 2010

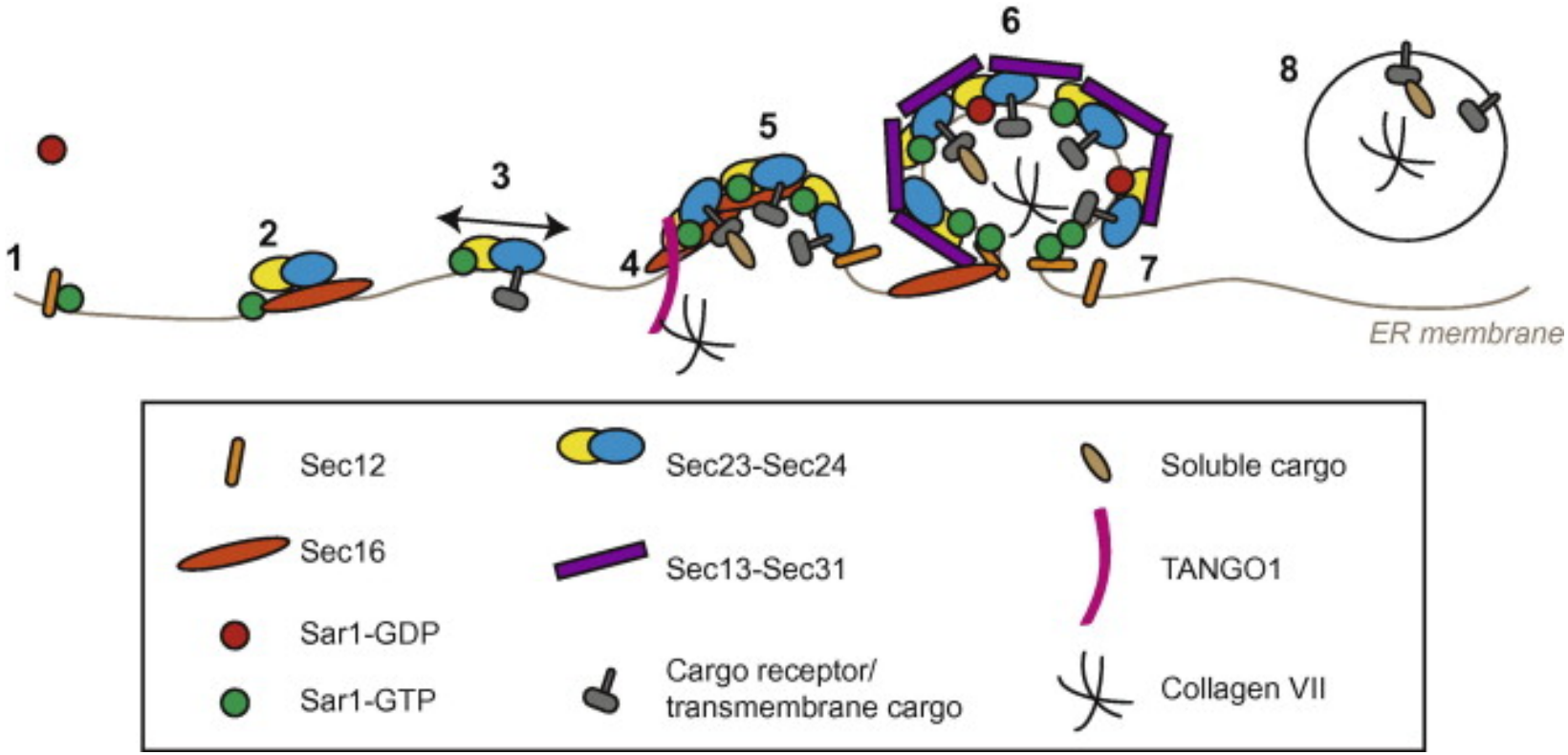
Coat recruitment

How are coats recruited?



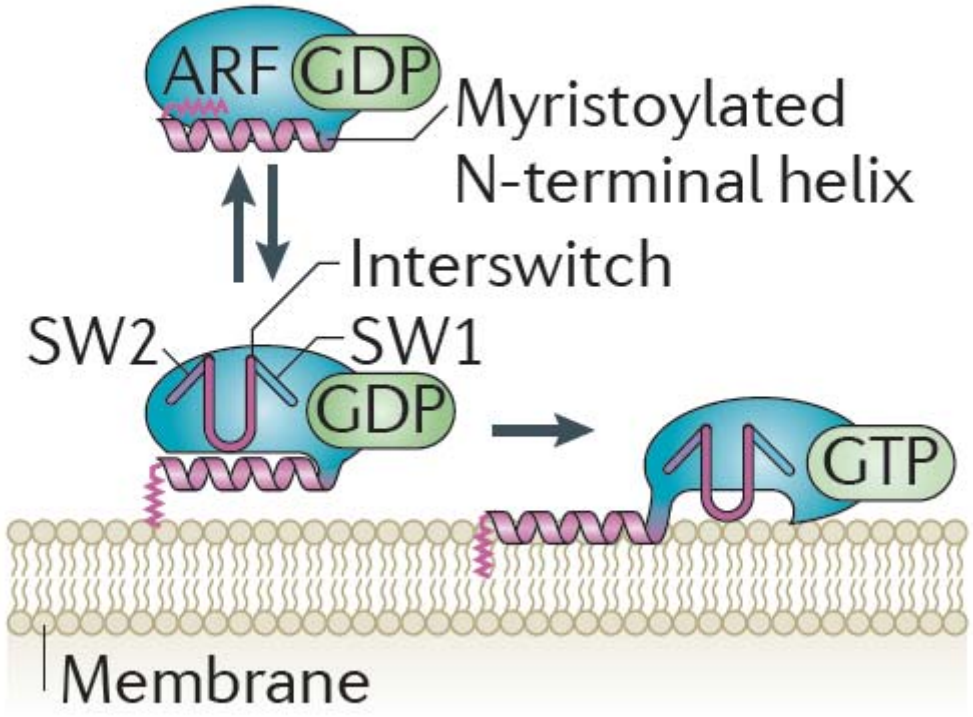
Schellmann & Pimpl 2009

Recruitment of COPII by Sar1



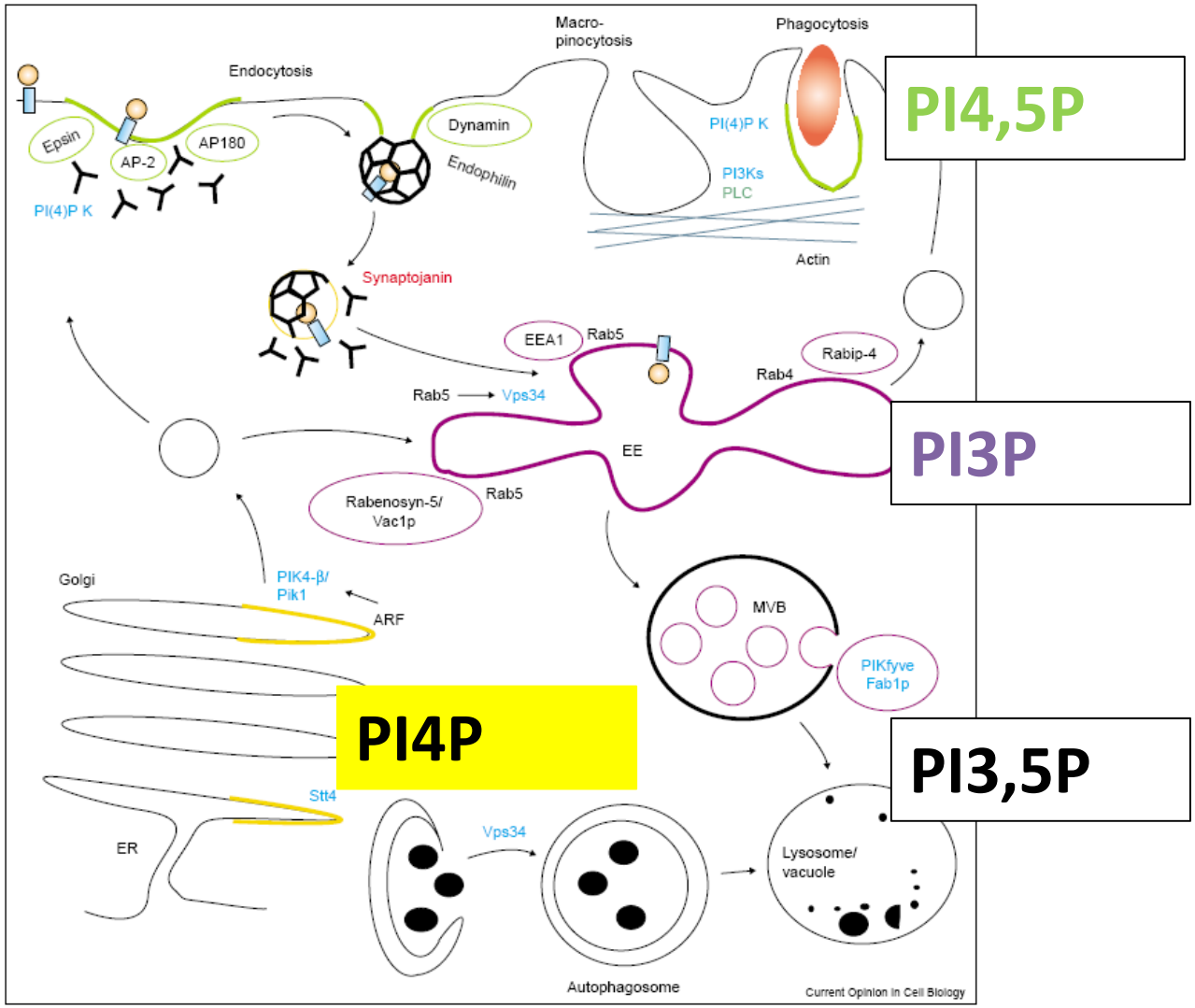
Budnik & Stevens 2009

GTP-exchange factors allow membrane association of ARF family GTPases



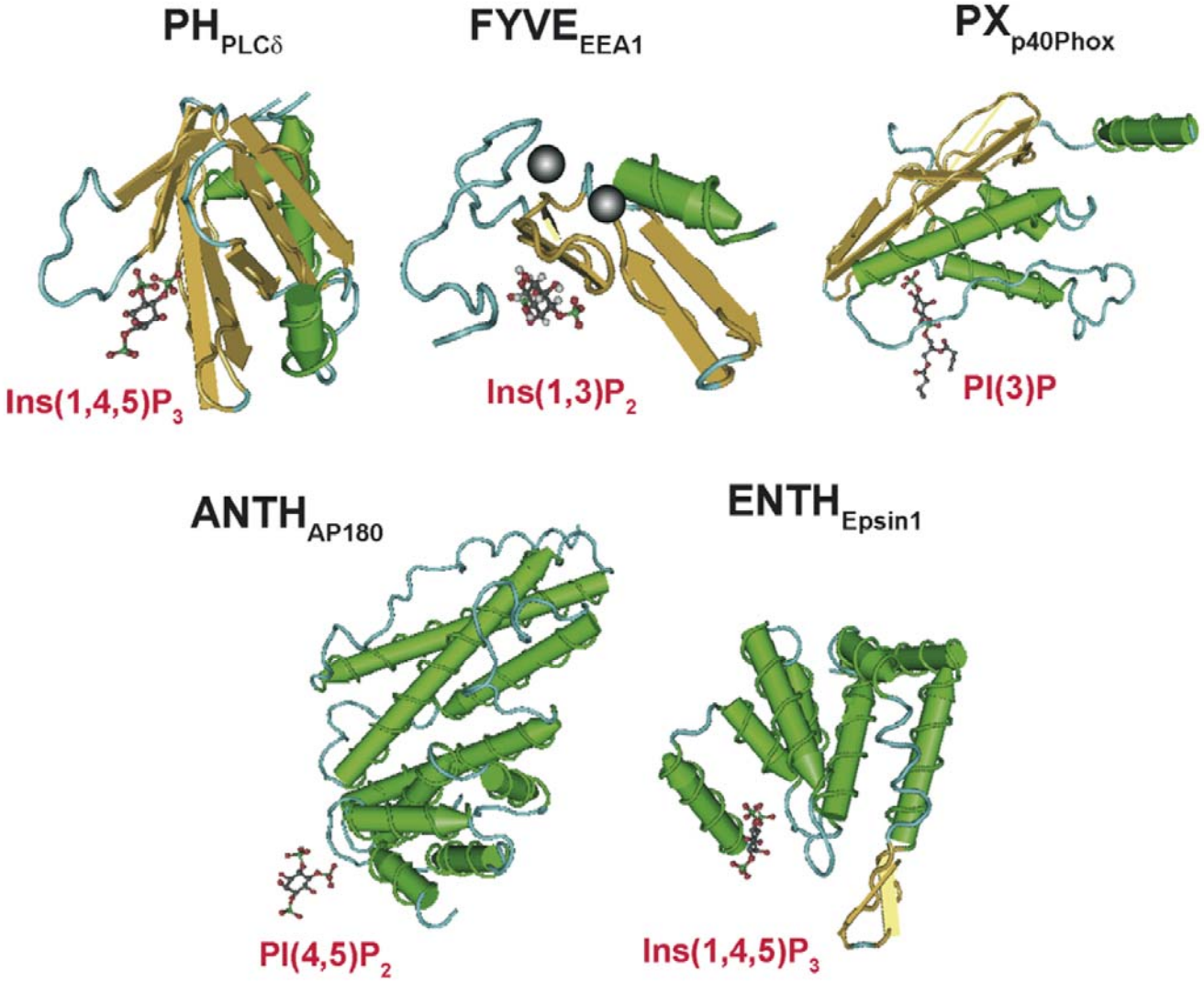
Donaldson & Jackson 2011

Recruitment of coats by lipids



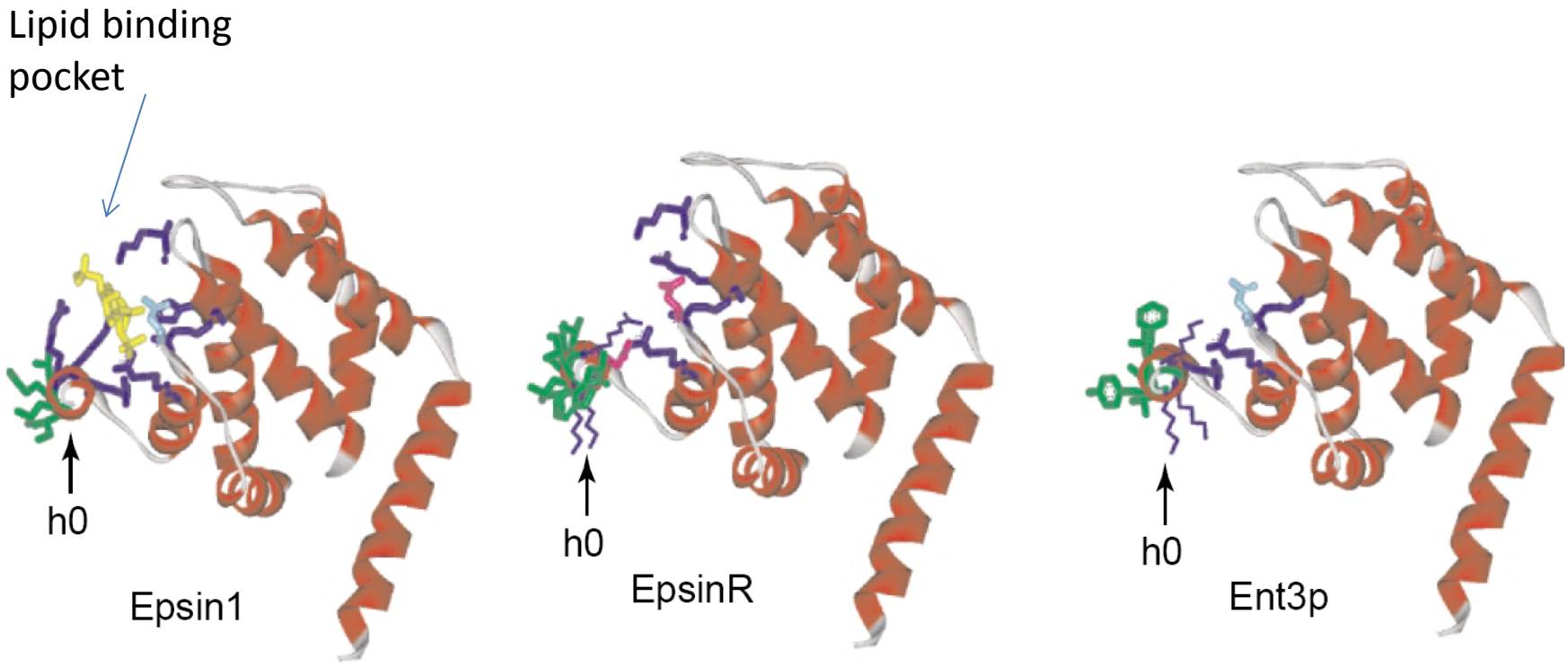
Simonsen et al.

Common PIP binding domains



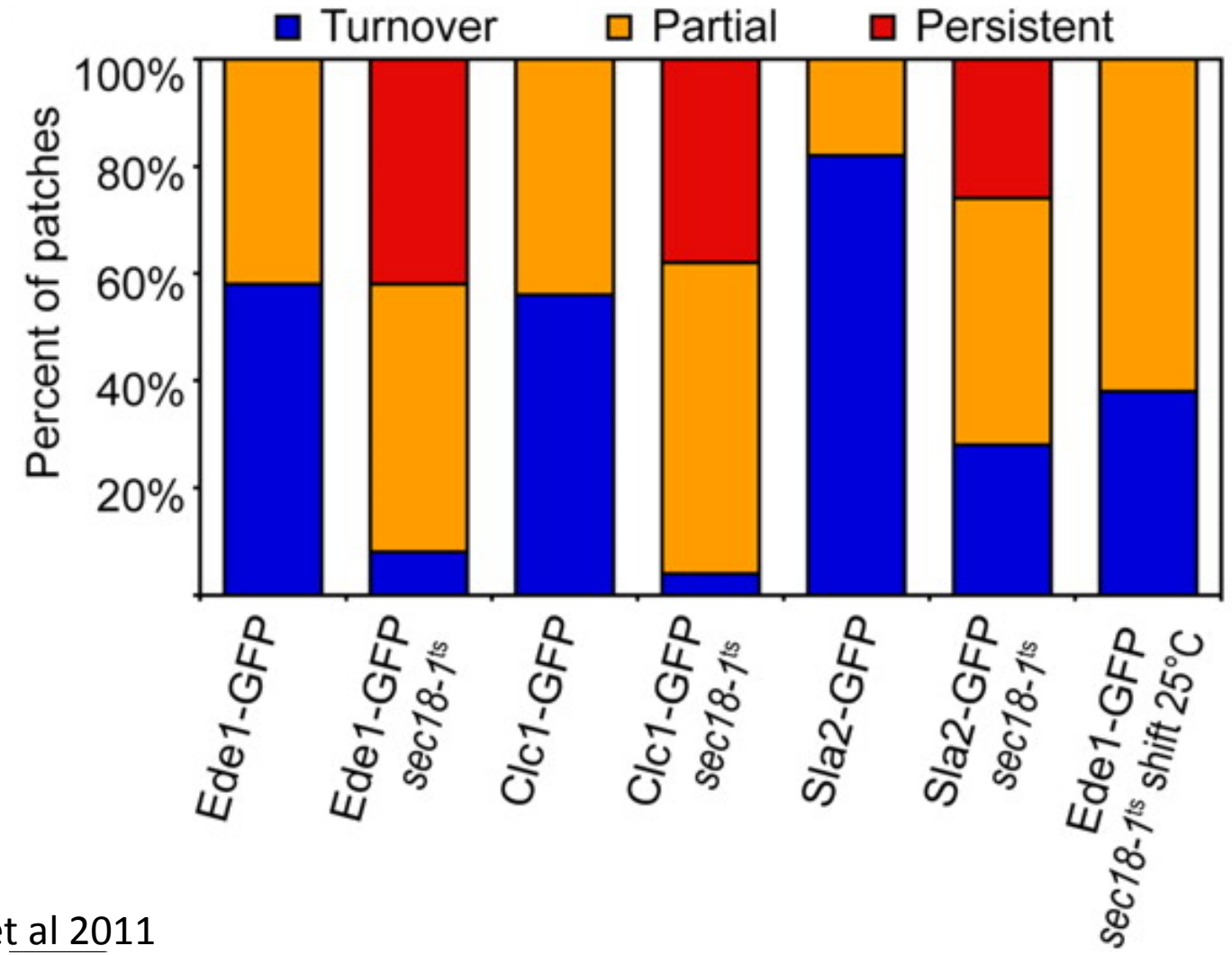
Kraub & Hauck 2008

Individual domain families have diversified



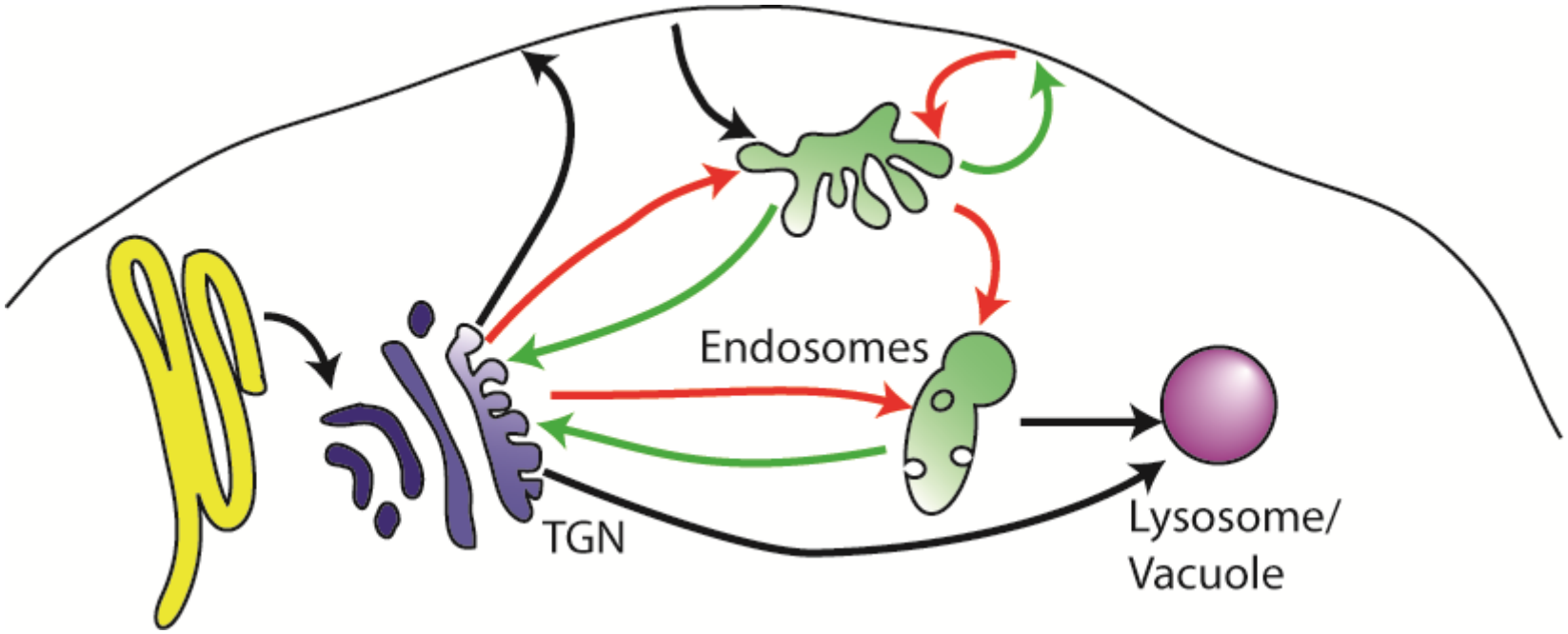
Duncan & Payne 2003

Recruitment of coats by cargo

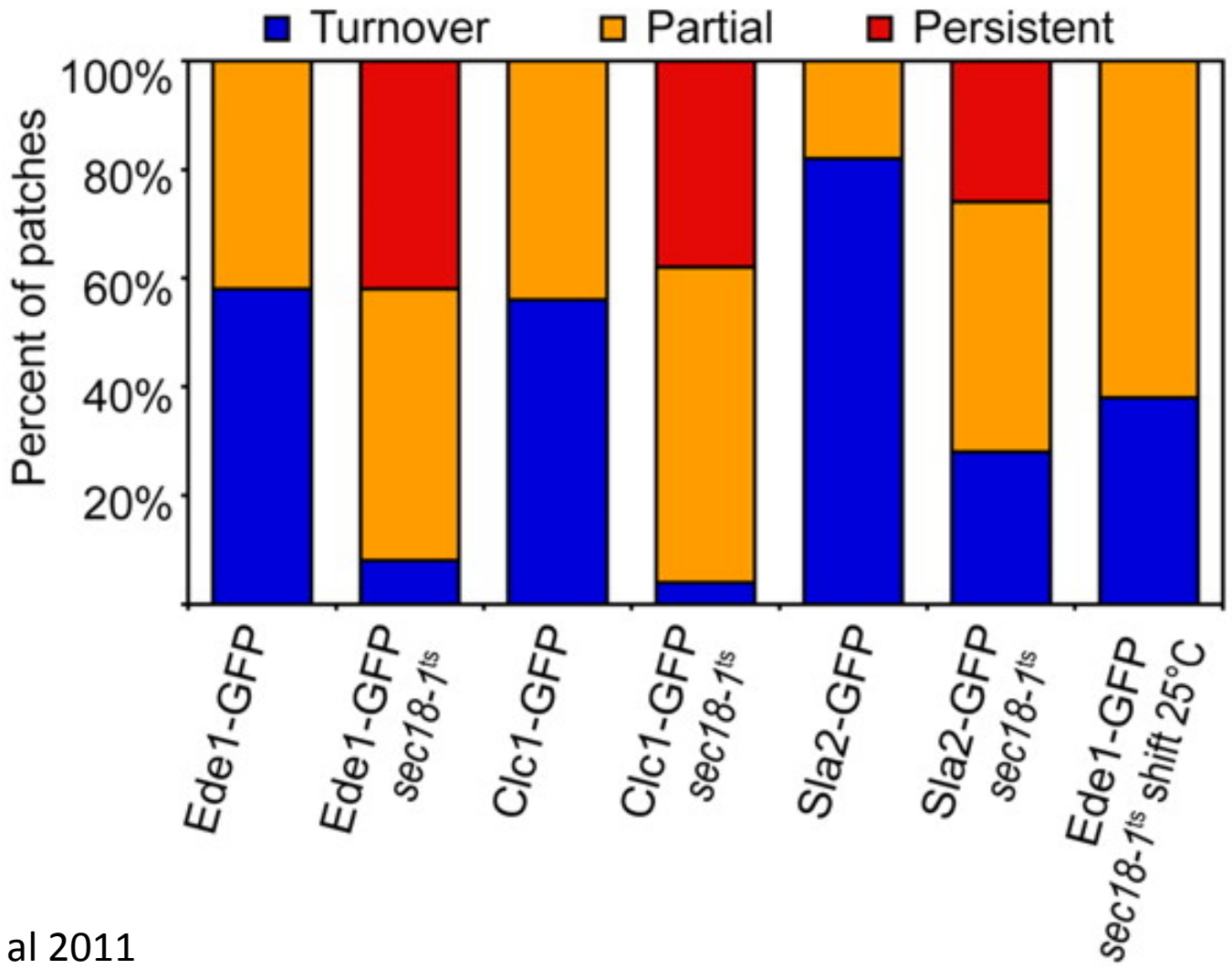


Carroll et al 2011

Sec18-ts depletes cargo

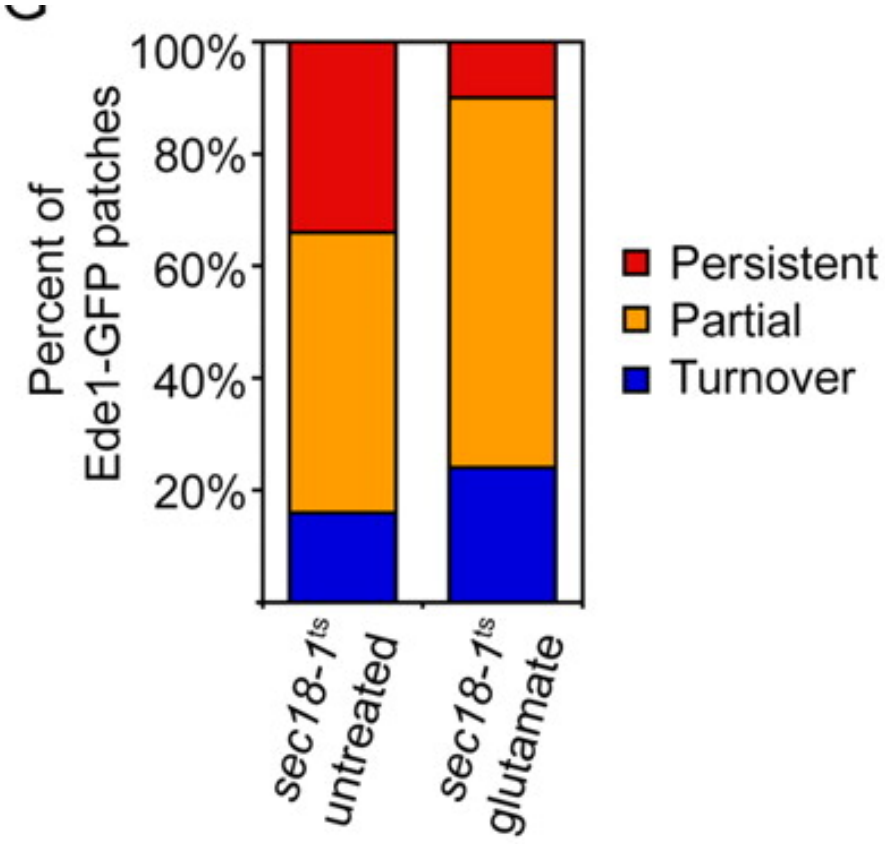


Cargo depletion reduces endocytic structures



Carroll et al 2011

Induction of endocytosis in depleted cells stimulates coat assembly



Carroll et al 2011

Common cargo motifs

YXXϕ–clathrin adaptors

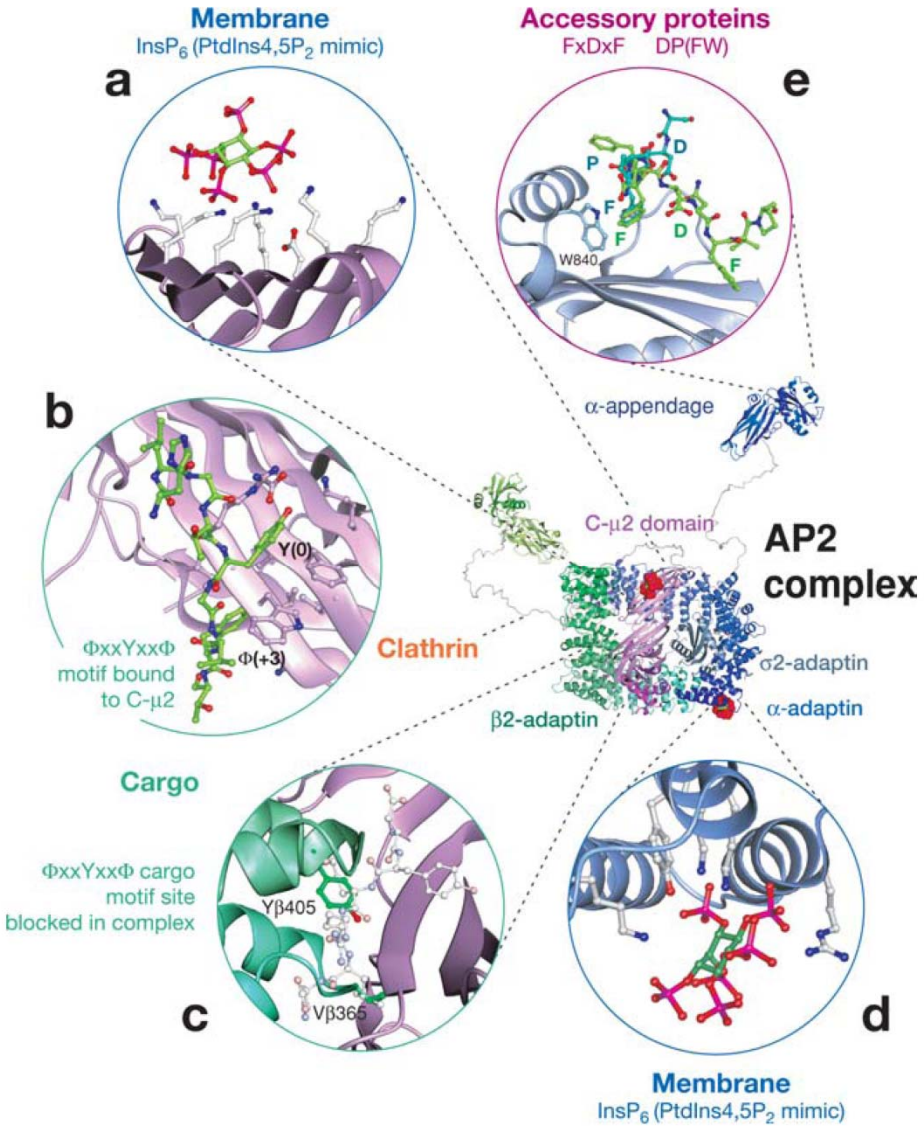
Acidic dileucine [DE]xxxLL-clathrin adaptors

NPXY-endocytic clathrin adaptors

Ubiquitin-clathrin adaptors, ESCRTs

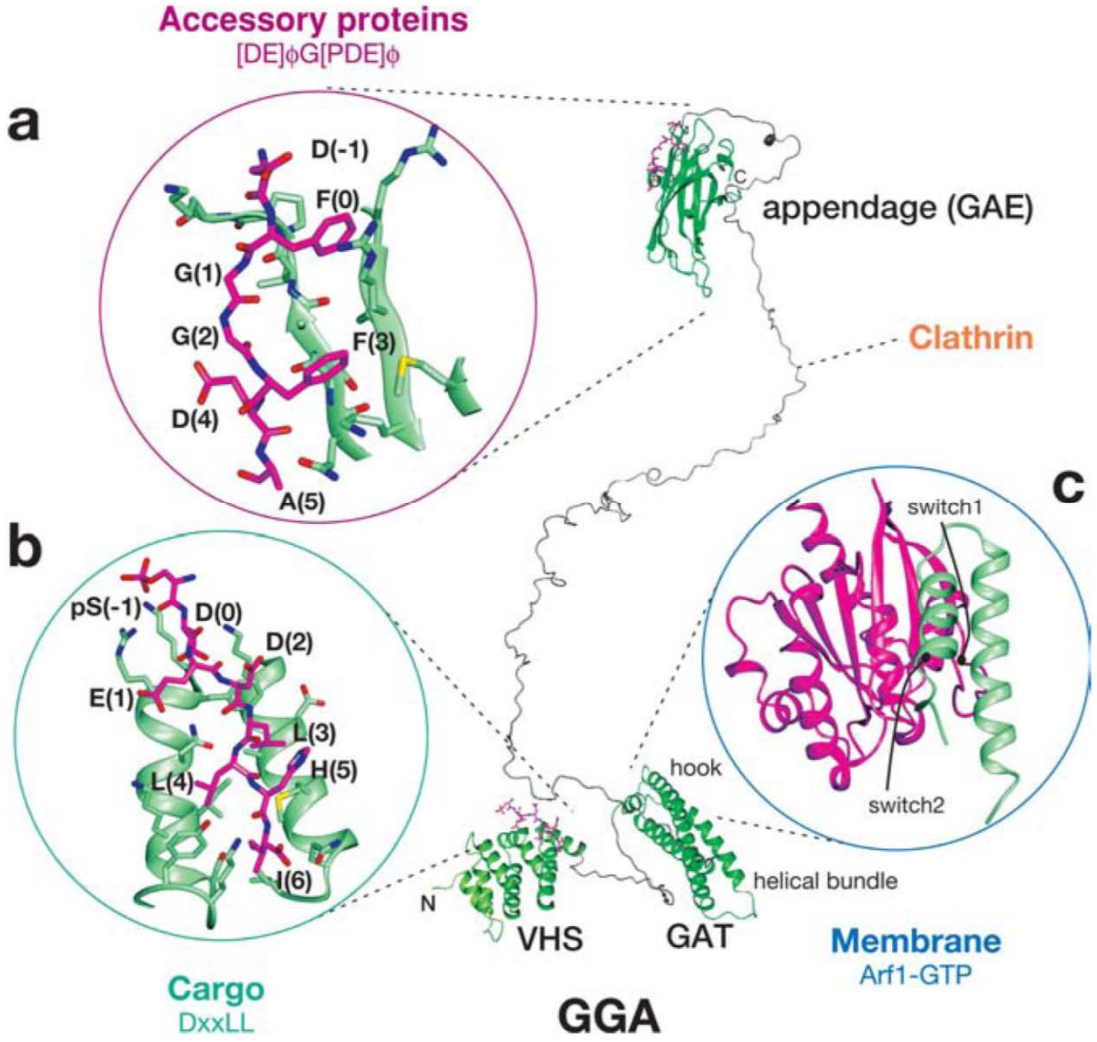
See Pandey 2010 for a more comprehensive list

Cargo binding sites



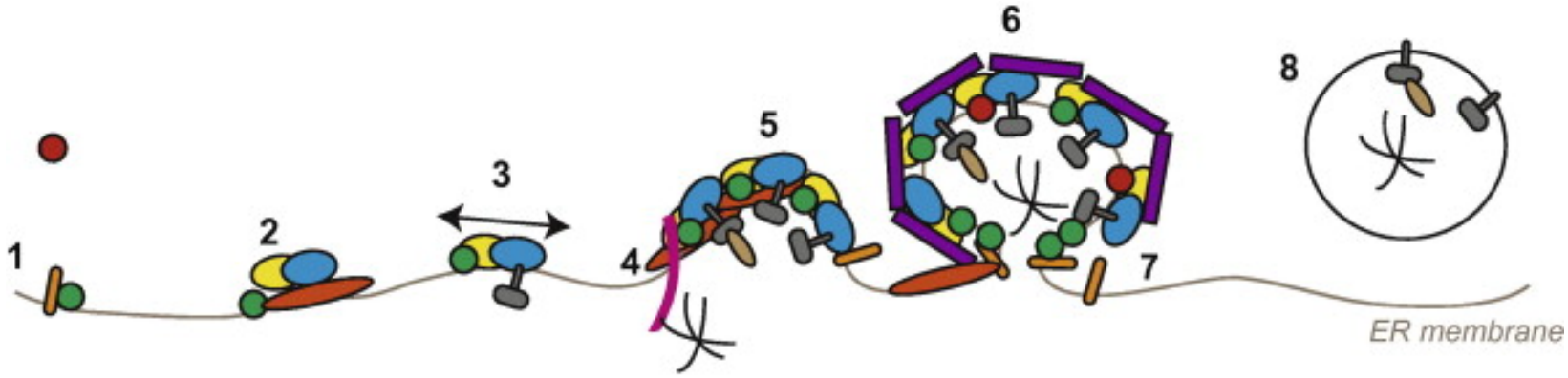
Owen et al 2004

Cargo binding sites



Owen et al 2004

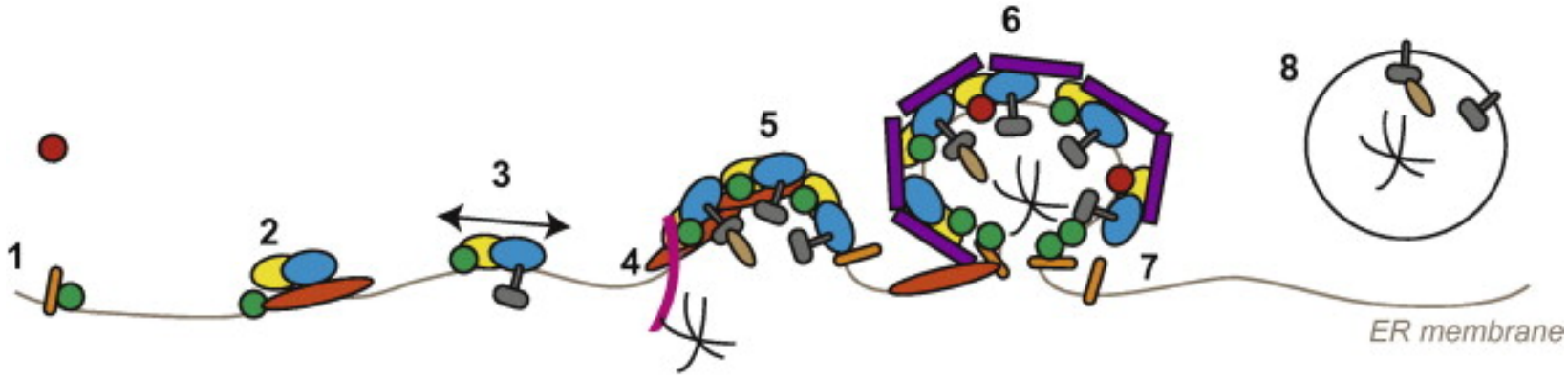
Pause to consider



How can we experimentally determine recruiting factors?

Budnik & Stevens 2009

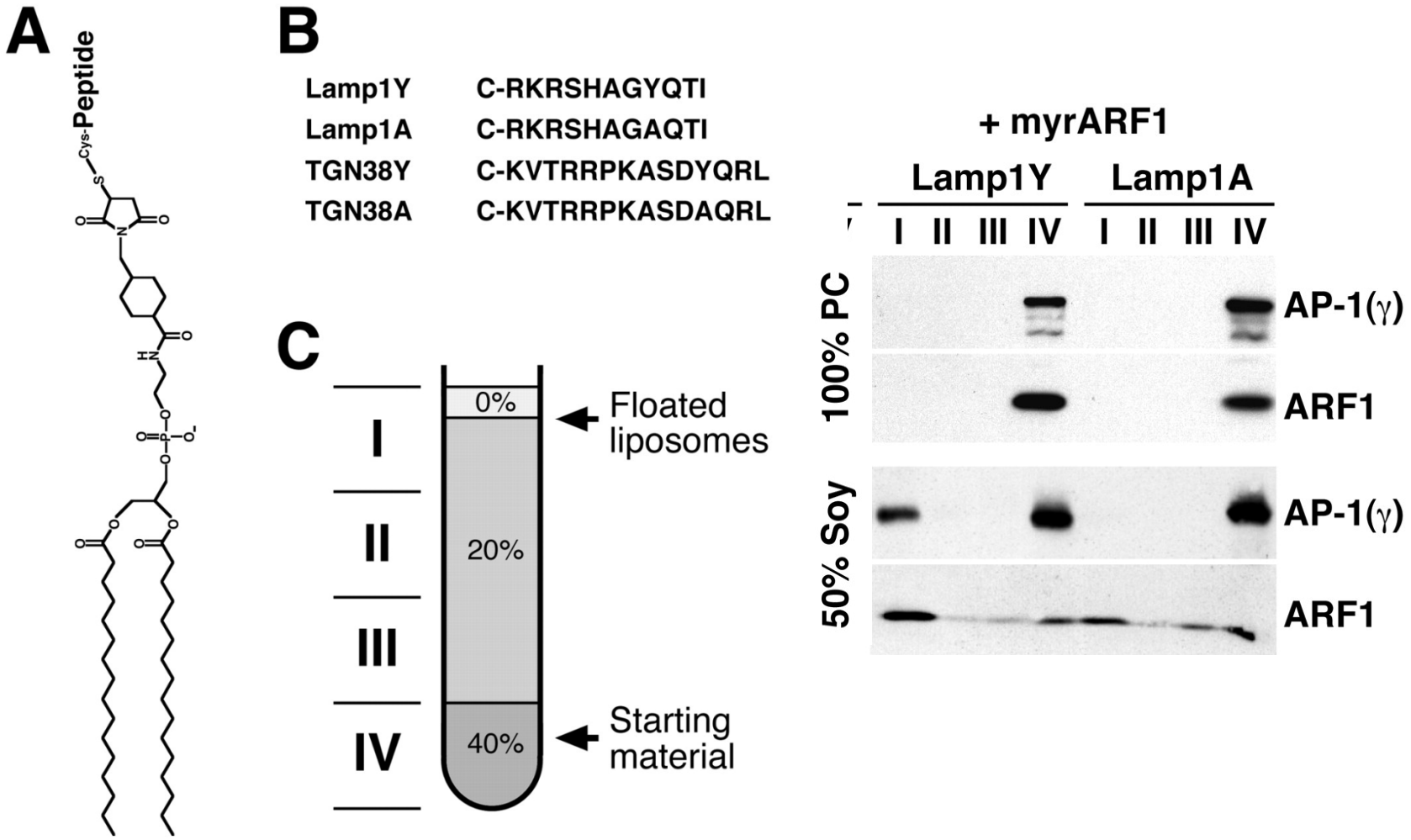
Pause to consider



Trans-membrane proteins (sec12 & cargo) that act as initiators of coat assembly, end up in the target organelle. Why don't they recruit coats at the target organelle?

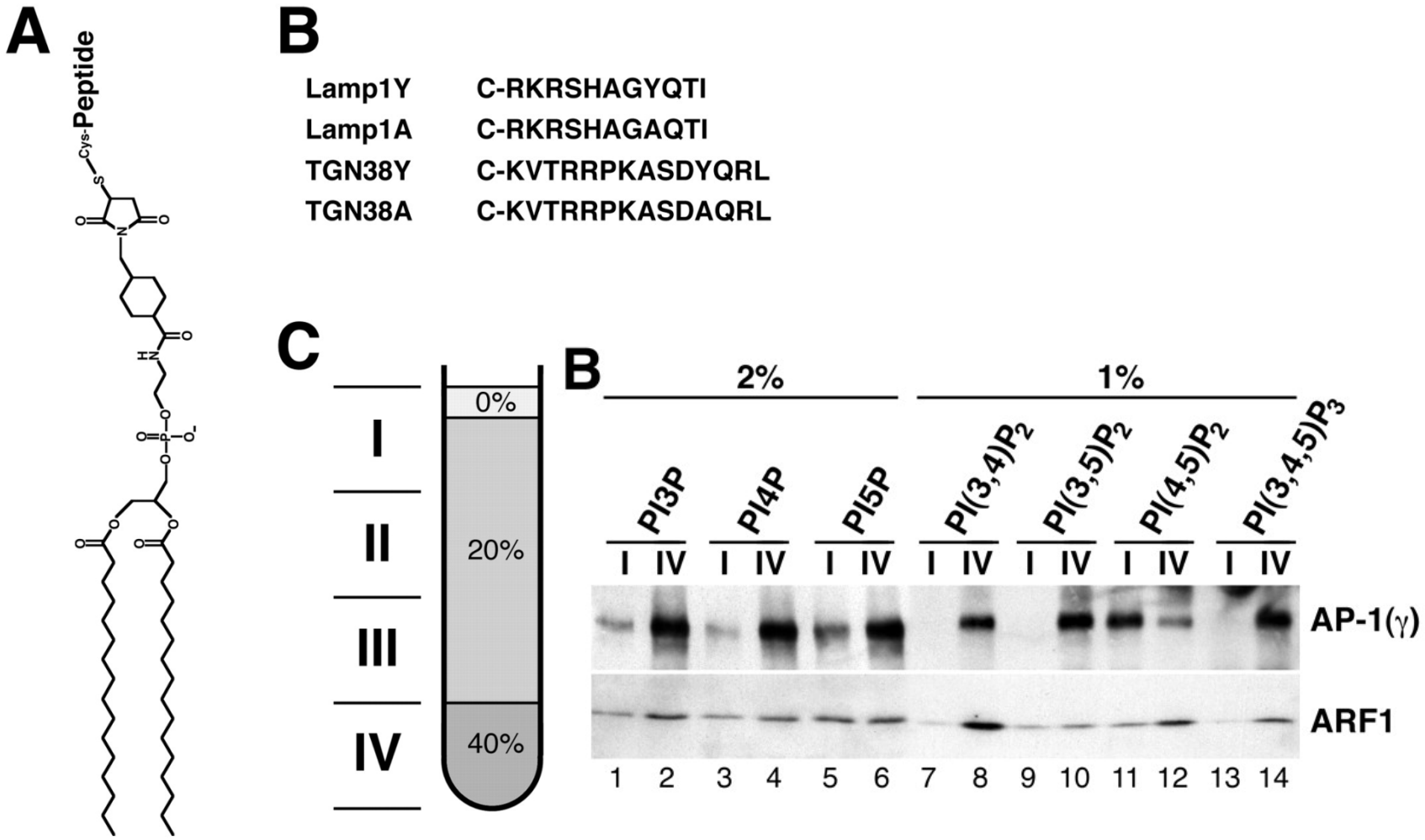
Budnik & Stevens 2009

Cooperative recruitment



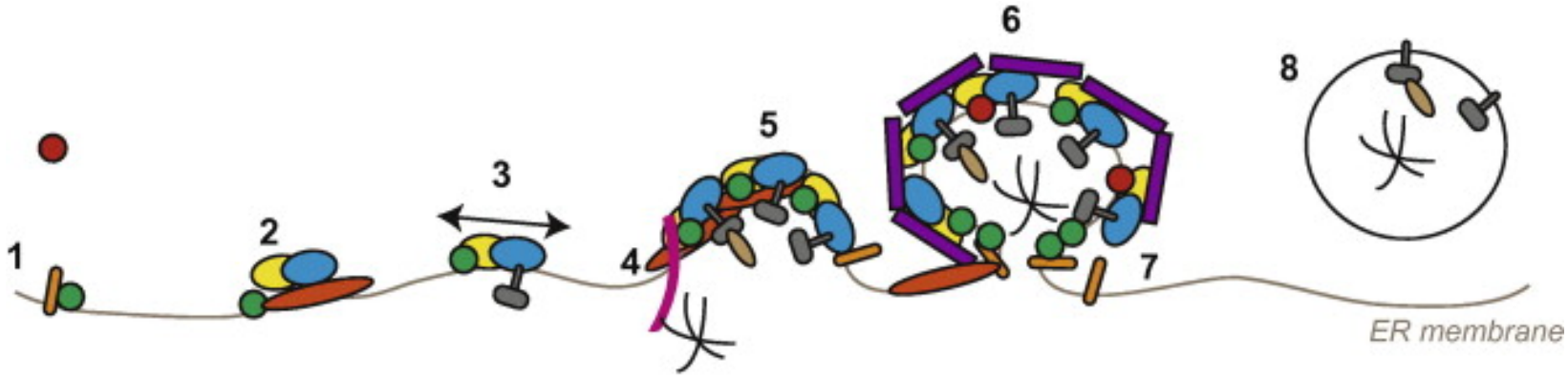
Crottet et al 2002

Cooperative recruitment



Crottet et al 2002

Pause to consider



Does knowing how coats are recruited suggest mechanisms for uncoating?

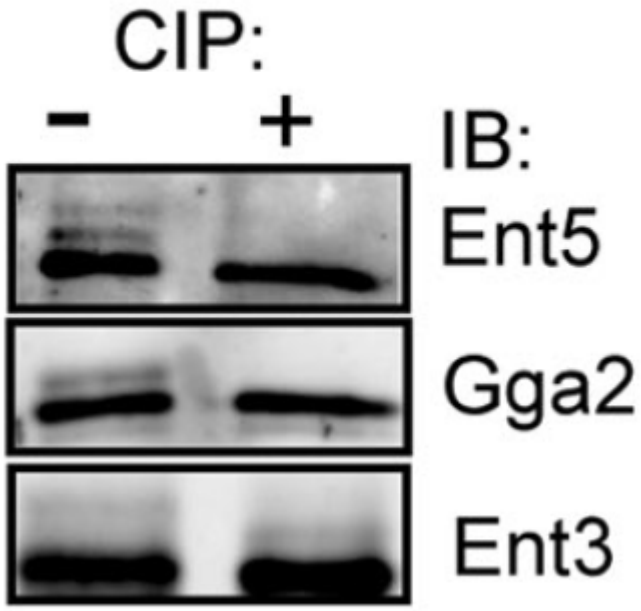
Budnik & Stevens 2009

Common mechanisms

- GTP hydrolysis
- Uncoating enzymes
- Lipid modifying enzymes
- Kinases/phosphatases

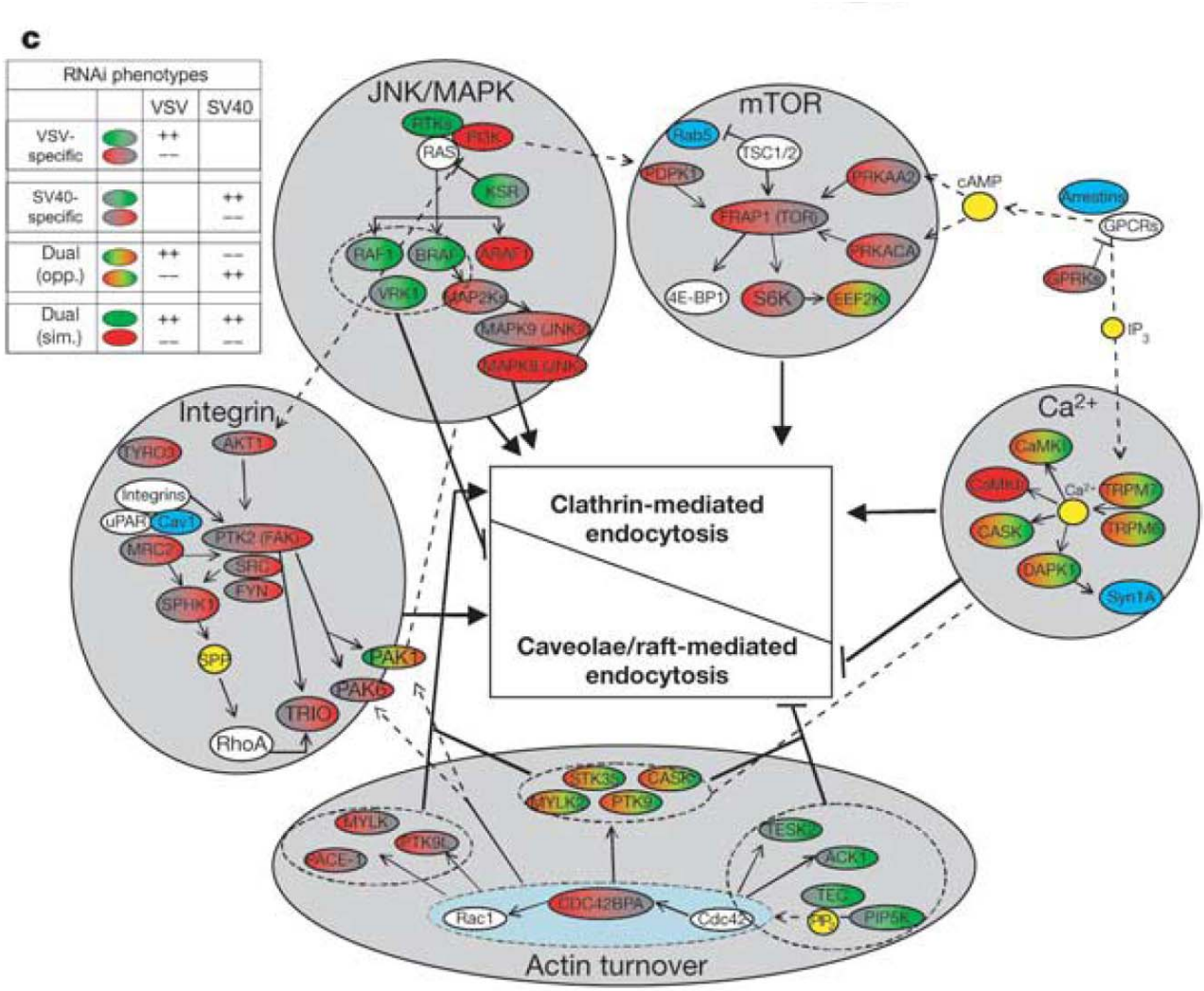
Phosphoregulation of coats

Pause to consider



Numerous proteins involved in membrane traffic are phosphorylated; how might this modulate function?

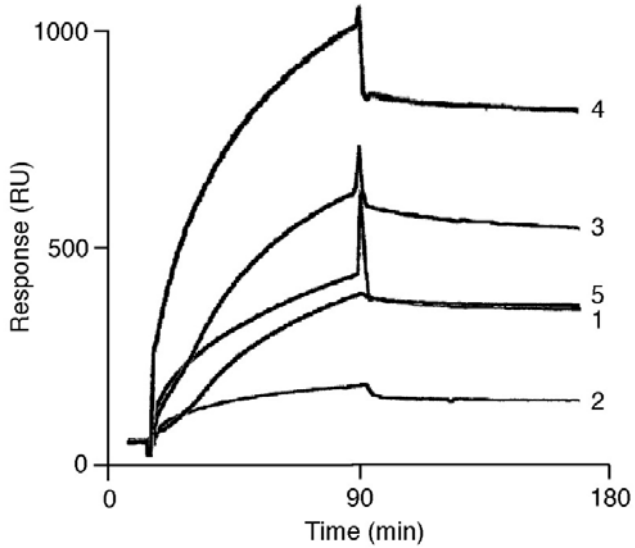
Multiple kinases regulate single traffic events



Common mechanisms

- Phosphorylation of tail promotes/inhibits coat binding
- Promote/inhibit autoregulation
- Promote/inhibit protein-protein interactions
- Activate/inhibit enzymatic activity

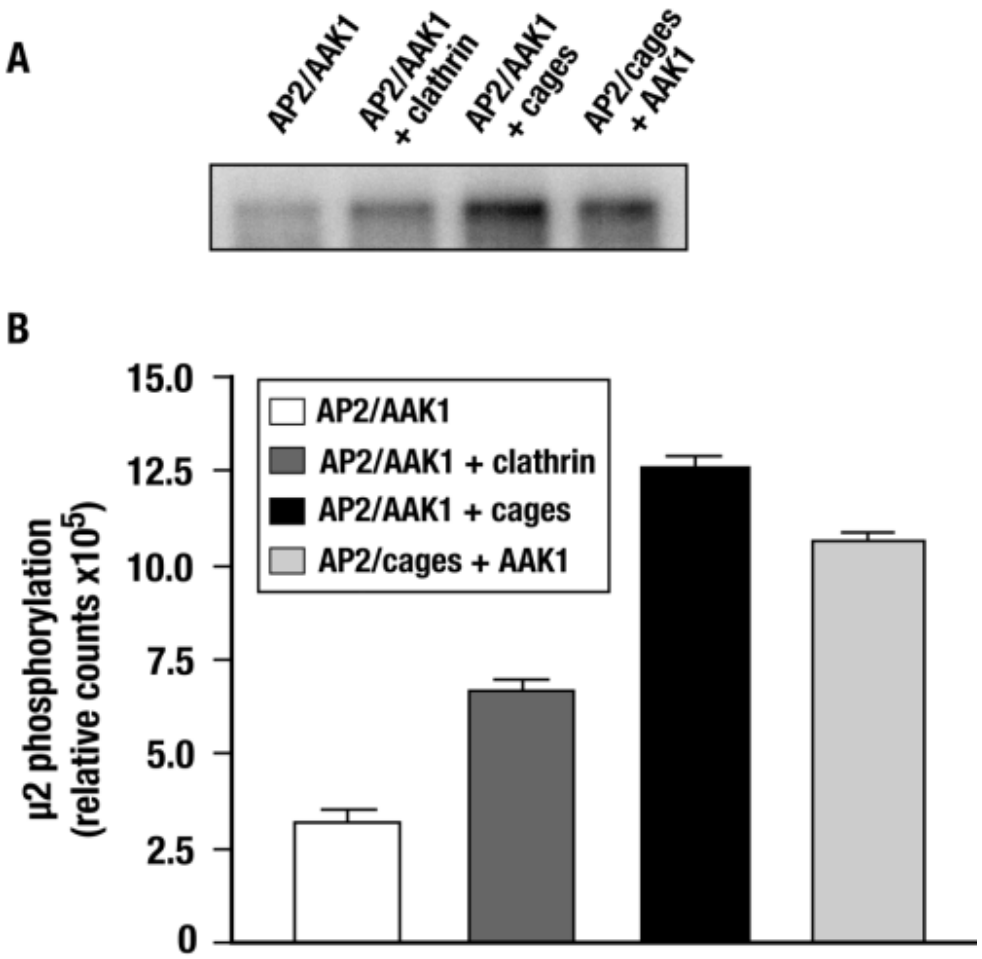
Phosphorylation of AP-2 enhances cargo binding



Treatment of AP2	k_a (1/Mxs)	k_d (1/s)	K_D k_d/k_a (nM)
1) –	$3.0x10^3$	$3.5x10^{-4}$	116
2) Phosphatase	$6.1x10^2$	$3.0x10^{-4}$	492
3) ATP	$6.6x10^3$	$3.8x10^{-4}$	58
4) ATP / AAK1	$1.5x10^4$	$2.9x10^{-4}$	19
5) ATP / Staurosporine	$2.4x10^3$	$3.0x10^{-4}$	125

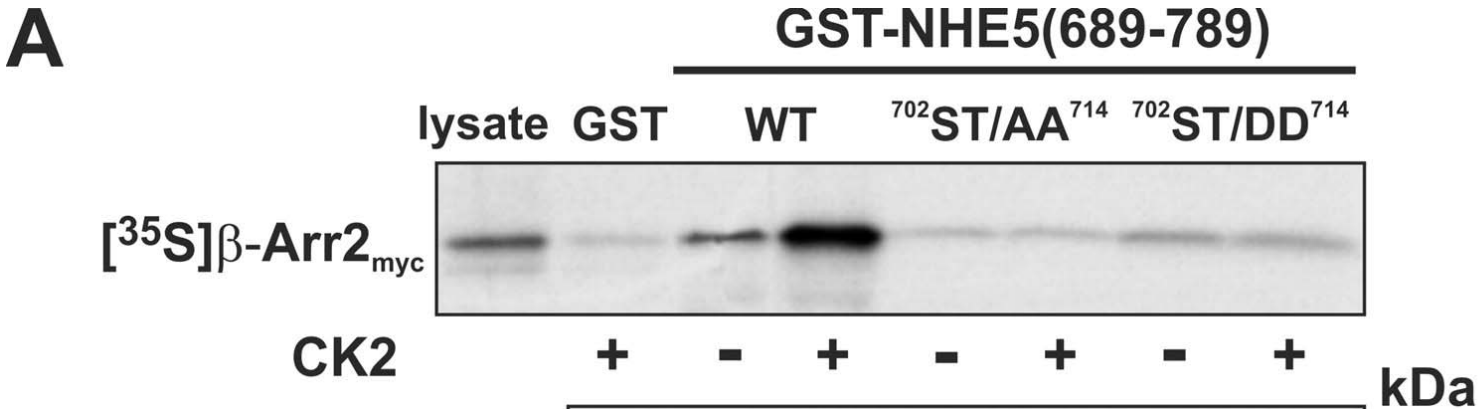
Ricotta et al. 2002

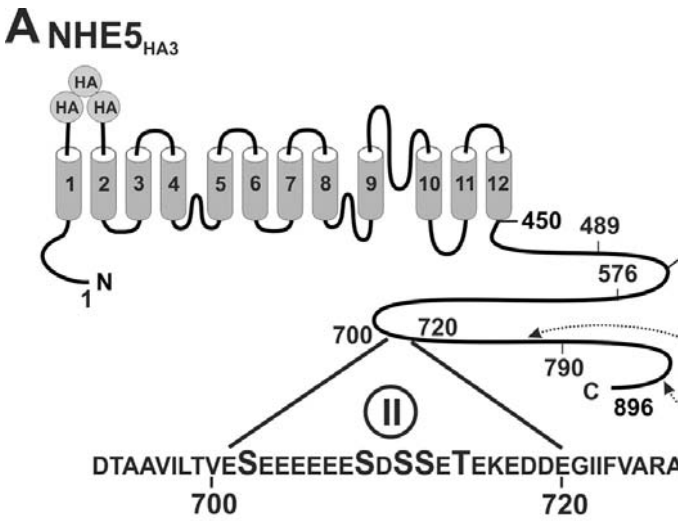
Clathrin stimulates AAK1 activity



Connor et al. 2003

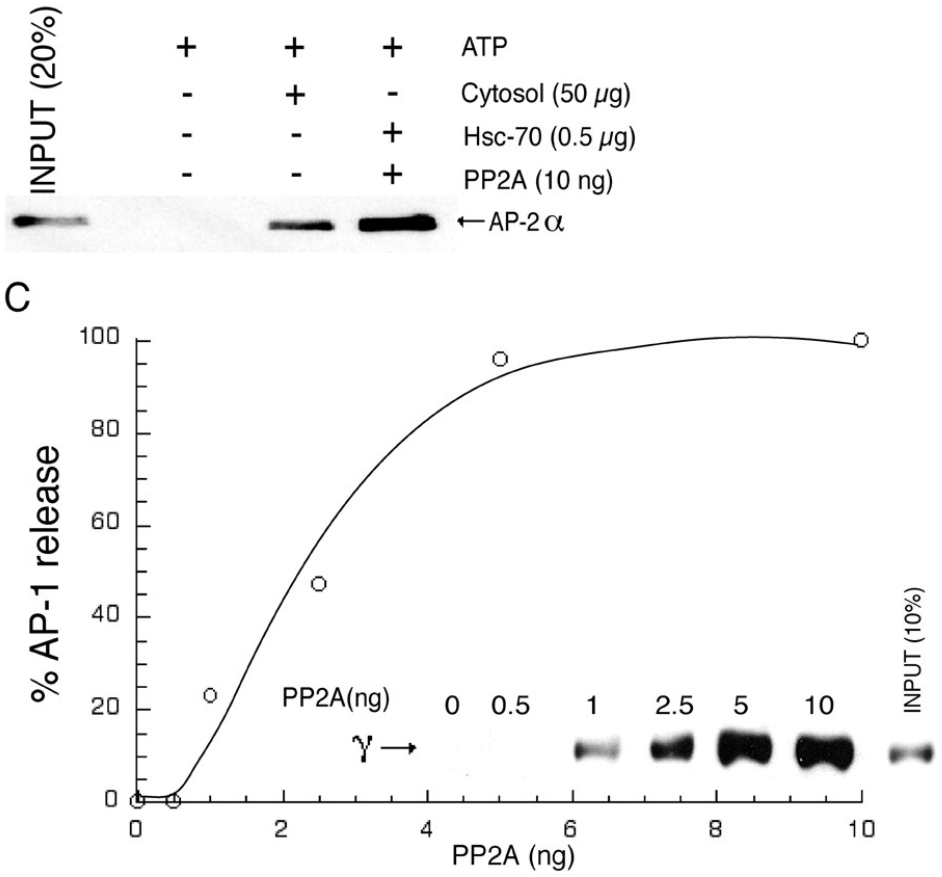
Phosphorylation of cargo enhances adaptor binding





Lukashova V et al. J. Biol. Chem. 2011;286:11456-11468

Dephosphorylation of an adaptor reduces membrane association



Ghosh P , Kornfeld S J Cell Biol 2003;160:699-708

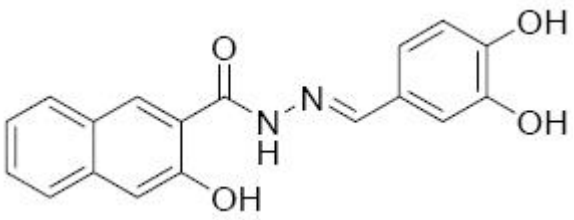
Phosphorylation of coat components promotes disassembly

Toret et al 2008

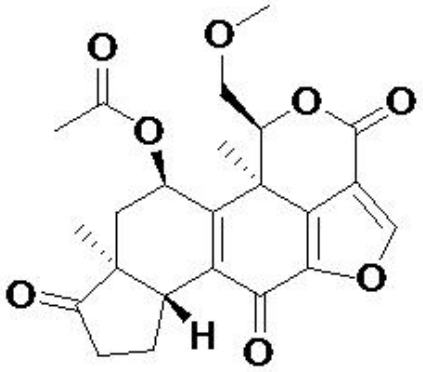
Chemical inhibitors of traffic

Chemicals that block coats

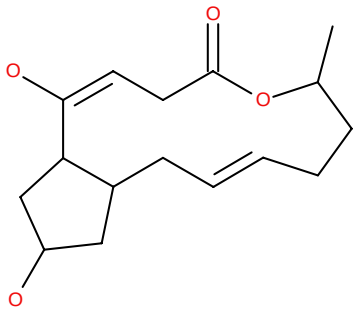
Dynasore



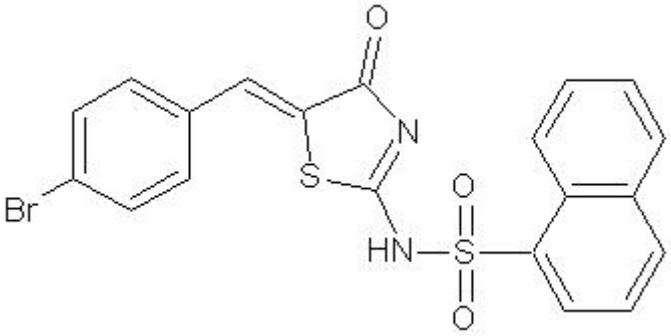
Wortmannin



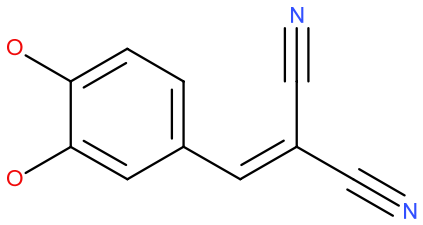
Brefeldin A



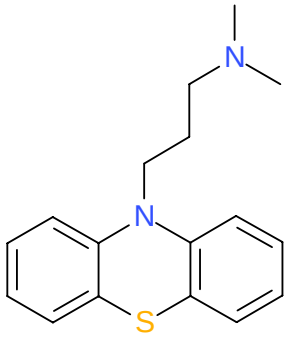
Pitstop



Tyrphostin A23

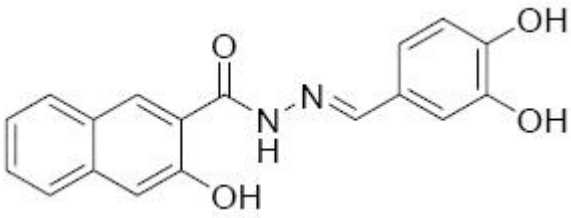


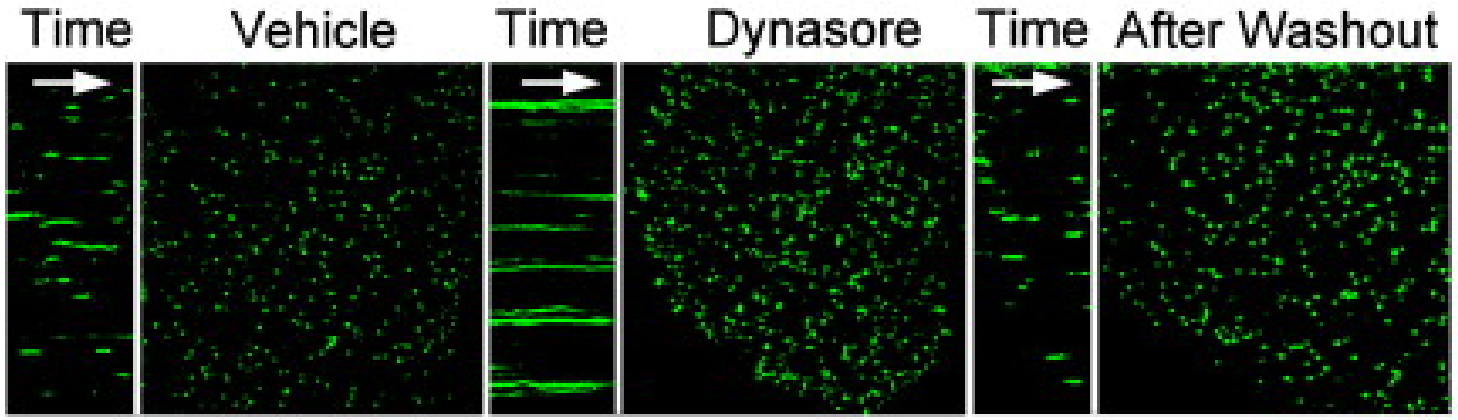
Chlorpromazine



Chemicals that block coats

Dynasore: Inhibitor of Dynamin GTPase activity



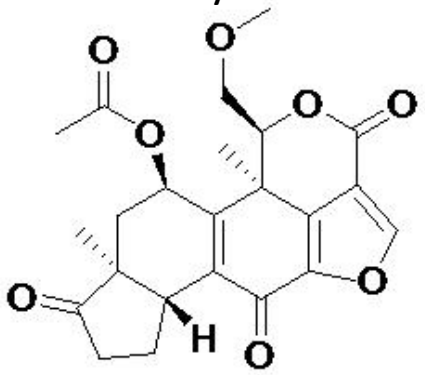


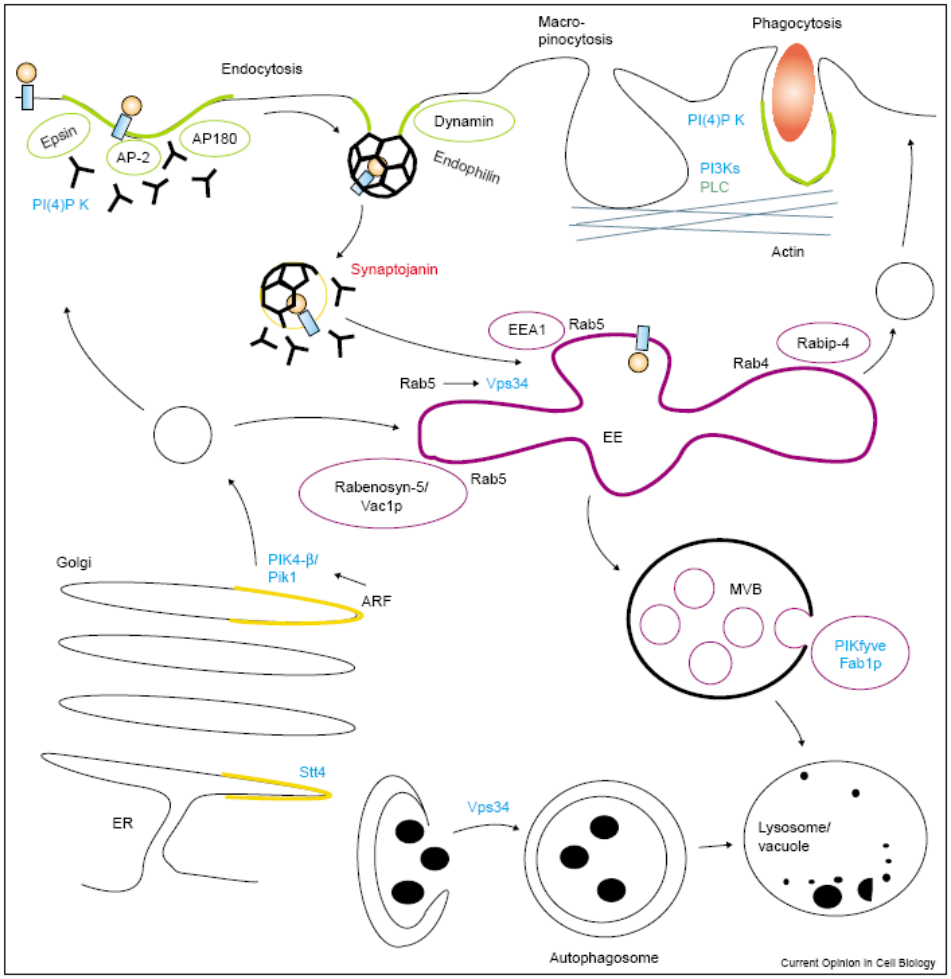
GFP-AP-2

Macia et al 2006

Chemicals that block coats

Wortmannin: Inhibitor of PI3 kinase activity

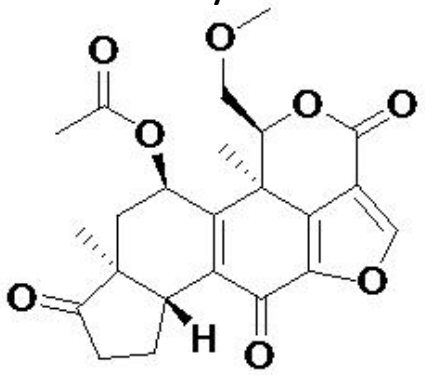


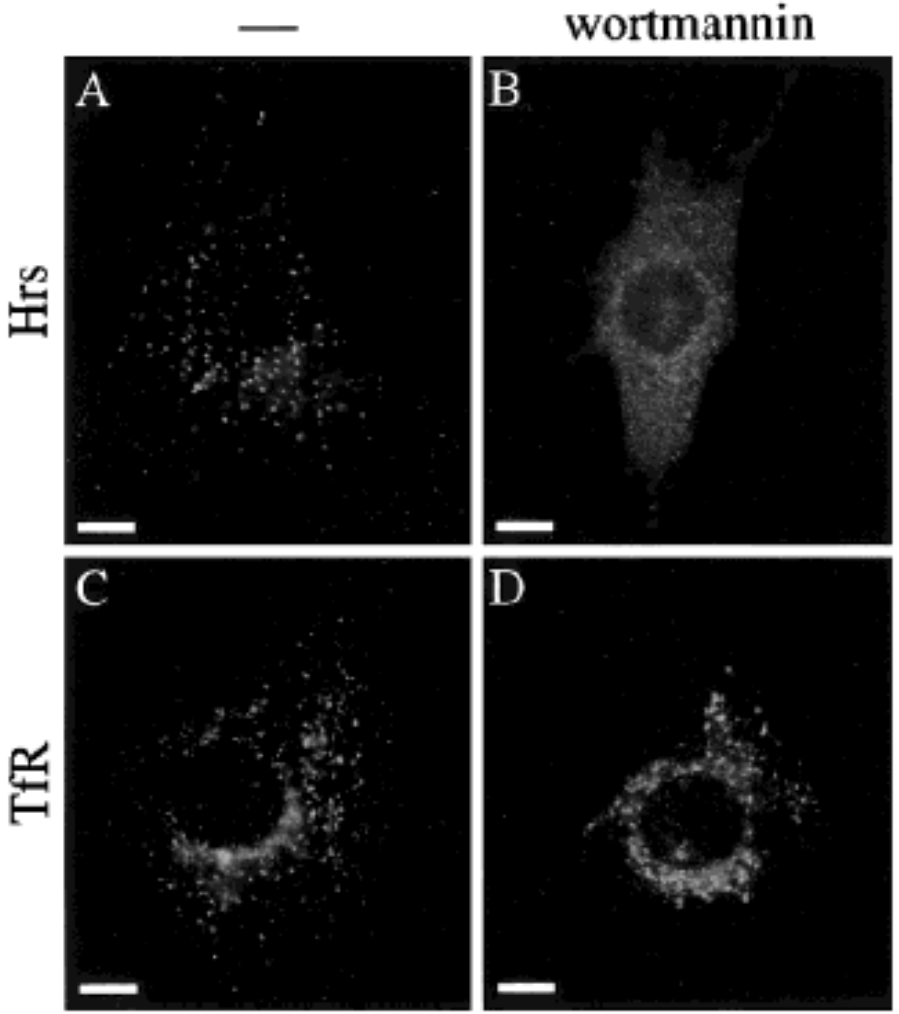


Komada M , Soriano P Genes Dev. 1999;13:1475-1485

Chemicals that block coats

Wortmannin: Inhibitor of PI3 kinase activity

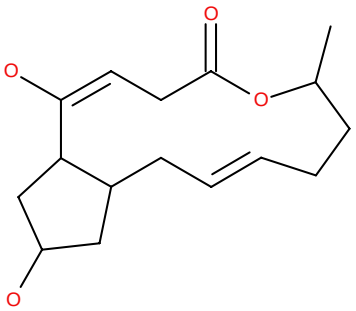


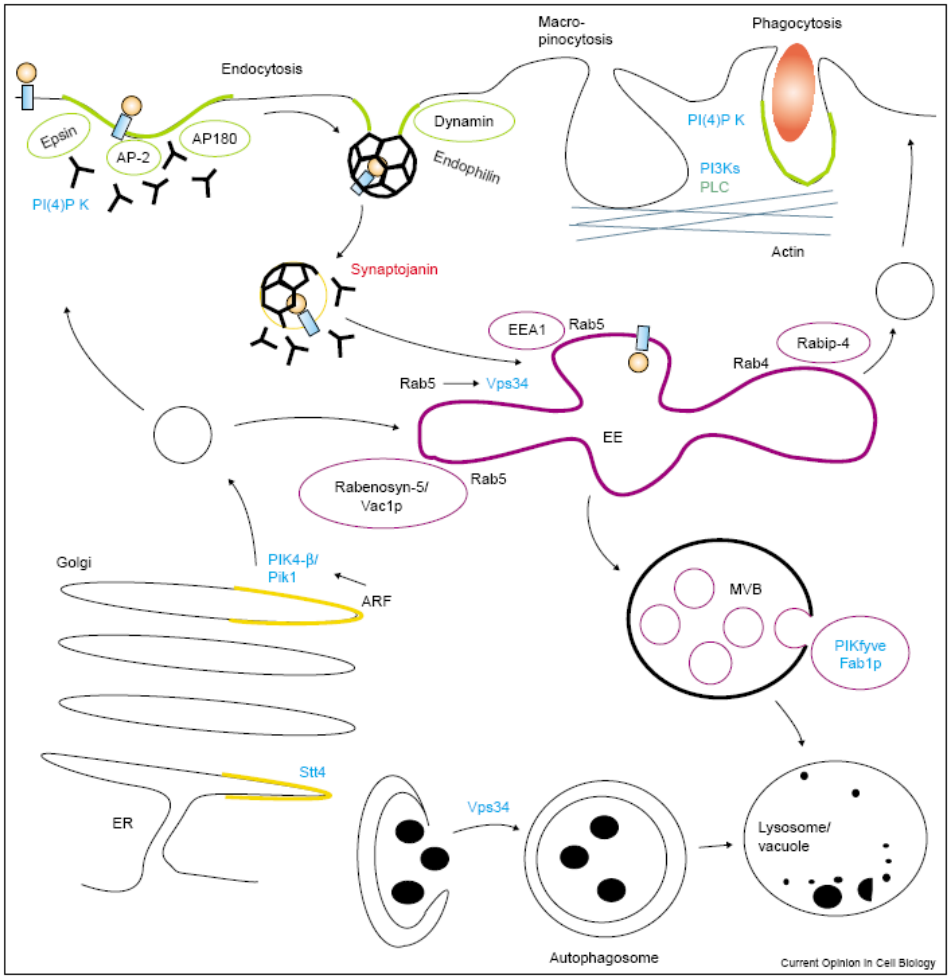


Komada M , Soriano P Genes Dev. 1999;13:1475-1485

Chemicals that block coats

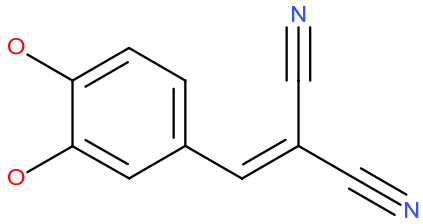
Brefeldin A: Inhibitor of many ARF GEFs

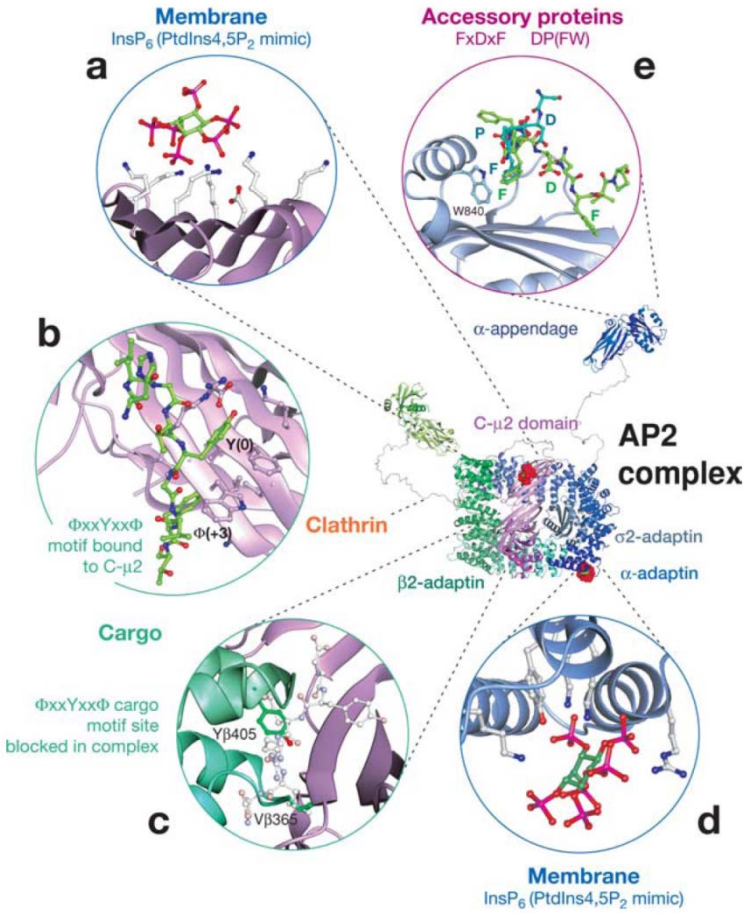




Chemicals that block coats

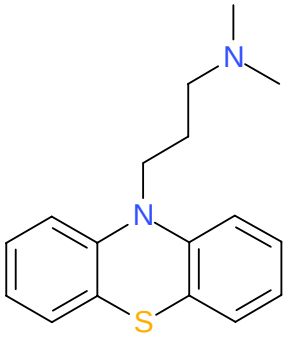
Tyrphostin A23-Mimics tyrosine sorting signal

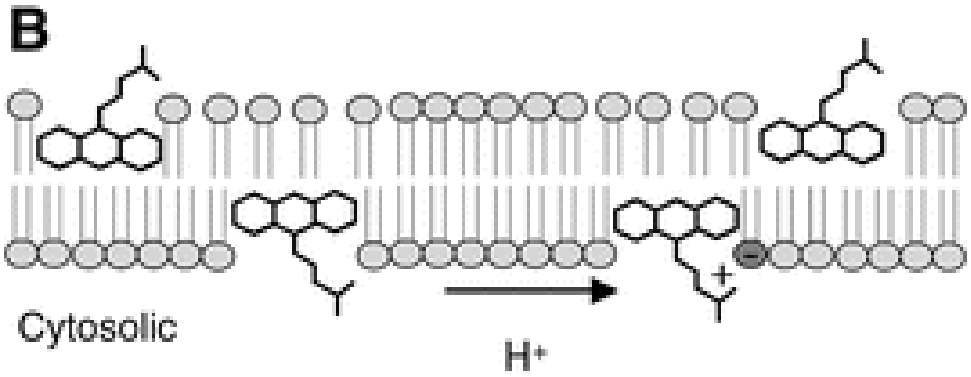




Chemicals that block coats

Chlorpromazine: Traps clathrin on membranes

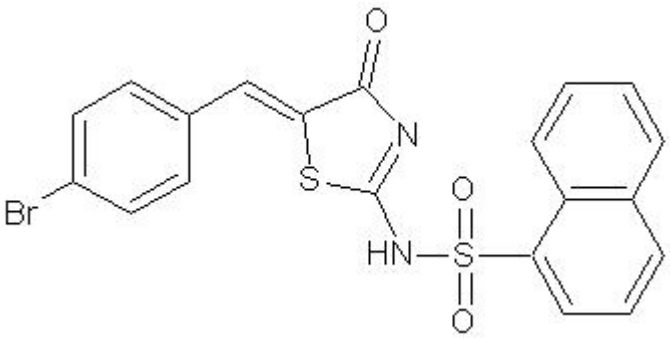


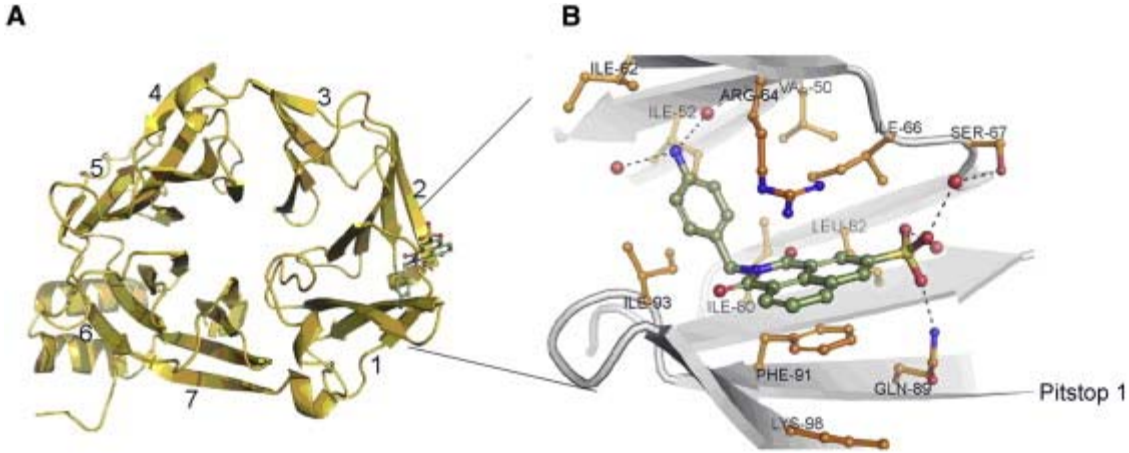


De Filippi L, et al . 2007

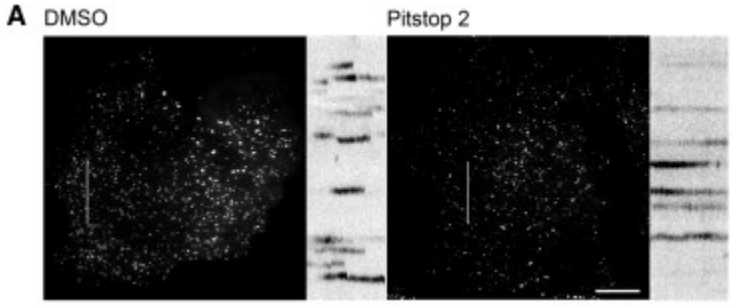
Chemicals that block coats

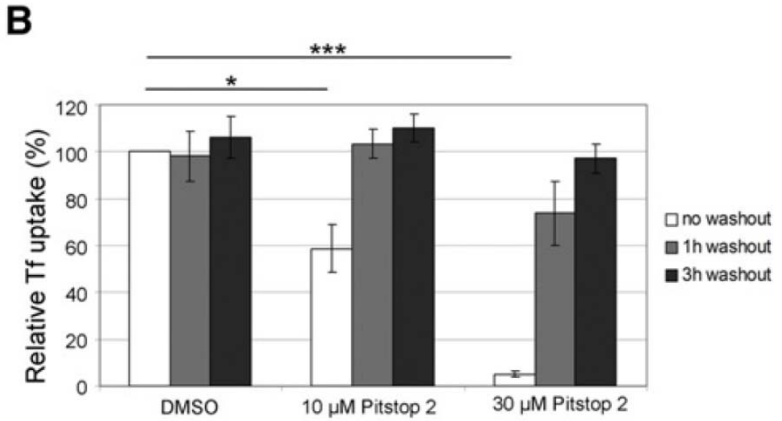
Pitstop-Blocks one adaptor binding site on the clathrin terminal domain





clc1EGFP

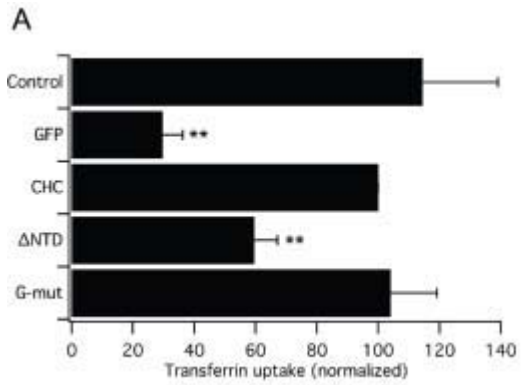


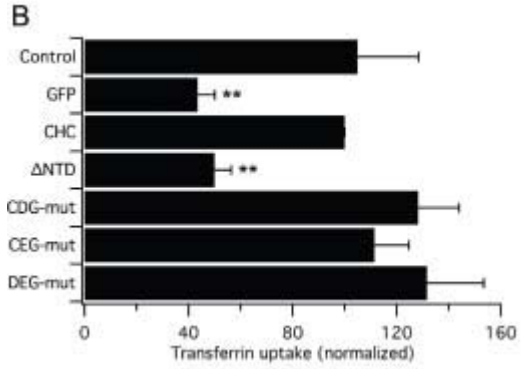


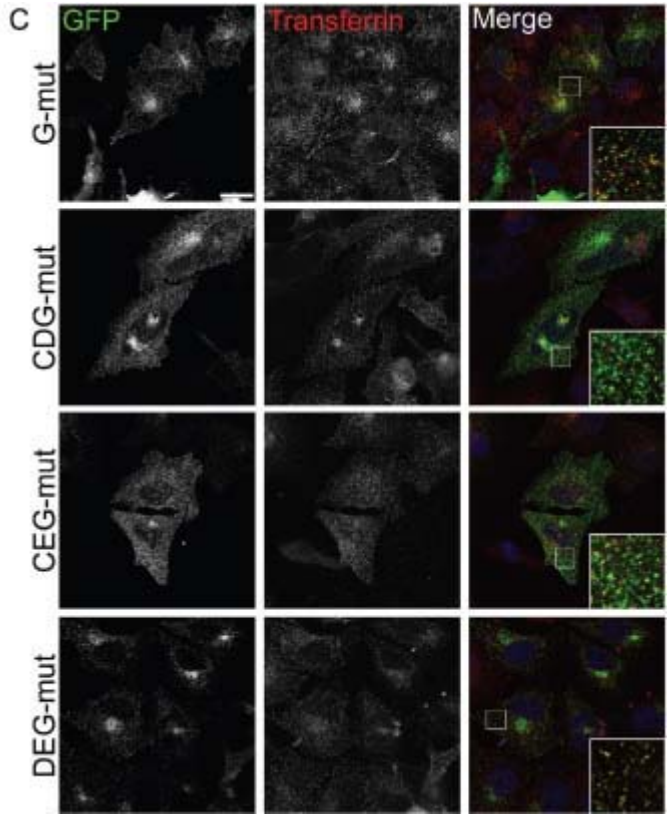
Von Kleist et al 2011

Controversies:

Pitstop arrests traffic but point mutations of its binding site do not? What are the possible explanations?



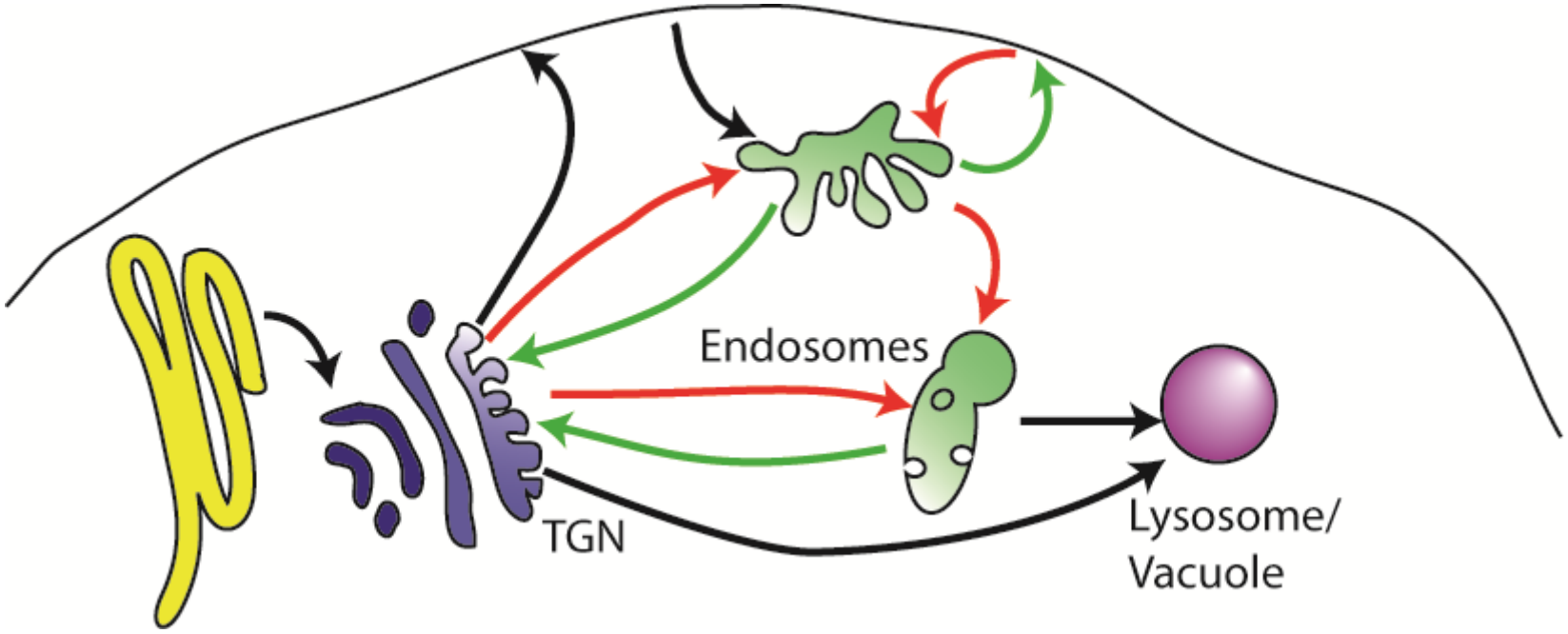




Wilcox and Royle 2011

Controversies

Why is it so hard to determine directionality?



Selected reading and references used

The retromer complex. Attar N, Cullen PJ. Adv Enzyme Regul. 2010;50(1):216-36

A suggested general review of retromer

Retromer. Bonifacino JS, Hurley JH. Curr Opin Cell Biol. 2008 Aug;20(4):427-36.

Coats of endosomal protein sorting: retromer and ESCRT. Schellmann S, Pimpl P. Curr Opin Plant Biol. 2009 Dec;12(6):670-6

The ESCRT pathway. Henne WM, Buchkovich NJ, Emr SD. Dev Cell. 2011 Jul 19;21(1):77-91.

A suggested general review of ESCRTs

Membrane budding and scission by the ESCRT machinery: it's all in the neck. Hurley JH, Hanson PI. Nat Rev Mol Cell Biol. 2010 Aug;11(8):556-66

A suggested general review of ESCRTs with a structural focus

ARF family G proteins and their regulators: roles in membrane transport, development and disease. Donaldson JG, Jackson CL. Nat Rev Mol Cell Biol. 2011 Jun;12(6):362-75.

A suggested general review of Arf and sar1

ER exit sites--localization and control of COPII vesicle formation. Budnik A, Stephens DJ. FEBS Lett. 2009 Dec 3;583(23):3796-803

The role of phosphoinositides in membrane transport. Simonsen A, Wurmser AE, Emr SD, Stenmark H. Curr Opin Cell Biol. 2001 Aug;13(4):485-92.

Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER. Lippincott-Schwartz J, Yuan LC, Bonifacino JS, Klausner RD. Cell. 1989 Mar 10;56(5):801-13.

Inhibition of the interaction between tyrosine-based motifs and the medium chain subunit of the AP-2 adaptor complex by specific tyrphostins. Crump CM, Williams JL, Stephens DJ, Banting G. J Biol Chem. 1998 Oct 23;273(43):28073-7.

Selected reading and references used

ENTH/ANTH domains expand to the Golgi. Duncan, M.C., Payne, G.S. Trends in Cell Biol. Vol.13 No.5 May 2003

Phosphoinositides: Regulators of membrane traffic and protein function, Kraub, M., Haucke, V. FEBS Letters 581 (2007) 2105–2111

A suggested general review of phosphoinositides

ARF1.GTP, tyrosine-based signals, and phosphatidylinositol 4,5-bisphosphate constitute a minimal machinery to recruit the AP-1 clathrin adaptor to membranes. Crottet P, Meyer DM, Rohrer J, Spiess M. Mol Biol Cell. 2002 Oct;13(10):3672-82.

Analysis of yeast endocytic site formation and maturation through a regulatory transition point. Carroll SY, Stimpson HE, Weinberg J, Toret CP, Sun Y, Drubin DG. Mol Biol Cell. 2011 Dec 21

Adaptors for clathrin coats: structure and function.Owen DJ, Collins BM, Evans PR. Annu Rev Cell Dev Biol. 2004;20:153-91.

A suggested general reciew of coats

Pelkmans L, Fava E, Grabner H, Hannus M, Habermann B, Krausz E, Zerial M. Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis. Nature 2005;436:78–86.

Small peptide recognition sequence for intracellular sorting. Pandey KN. Curr Opin Biotechnol. 2010 Oct;21(5):611-20.

Phosphorylation of the AP2 mu subunit by AAK1 mediates high affinity binding to membrane protein sorting signals. Ricotta D, Conner SD, Schmid SL, von Figura K, Honing S. J Cell Biol. 2002 Mar 4;156(5):791-5

AAK1-mediated micro2 phosphorylation is stimulated by assembled clathrin. Conner SD, Schröter T, Schmid SL. Traffic. 2003 Dec;4(12):885-9

Selected reading and references used

A PACS-1, GGA3 and CK2 complex regulates CI-MPR trafficking.Scott GK, Fei H, Thomas L, Medigeshi GR, Thomas G. EMBO J. 2006 Oct 4;25(19):4423-35.

AP-1 binding to sorting signals and release from clathrin-coated vesicles is regulated by phosphorylation. Ghosh P, Kornfeld S. J Cell Biol. 2003 Mar 3;160(5):699-708.

Multiple pathways regulate endocytic coat disassembly in Saccharomyces cerevisiae for optimal downstream trafficking. Toret CP, Lee L, Sekiya-Kawasaki M, Drubin DG.Traffic. 2008 May;9(5):848-59.

Role of the clathrin terminal domain in regulating coated pit dynamics revealed by small molecule inhibition. von Kleist L, Stahlschmidt W, Bulut H, Gromova K, Puchkov D, Robertson MJ, MacGregor KA, Tomilin N, Pechstein A, Chau N, Chircop M, Sakoff J, von Kries JP, Saenger W, Kräusslich HG, Shupliakov O, Robinson PJ, McCluskey A, Haucke V. Cell. 2011 Aug 5;146(3):471-84.

Dynasore, a cell-permeable inhibitor of dynamin. Macia E, Ehrlich M, Massol R, Boucrot E, Brunner C, Kirchhausen T. Dev Cell. 2006 Jun;10(6):839-50.

Functional analysis of interaction sites on the N-terminal domain of clathrin heavy chain. Willox AK, Royle SJ. Traffic. 2012 Jan;13(1):70-81

Membrane stress is coupled to a rapid translational control of gene expression in chlorpromazine-treated cells. De Filippi L, Fournier M, Cameroni E, Linder P, De Virgilio C, Foti M, Deloche O. Curr Genet. 2007 Sep;52(3-4):171-85.

Synaptic vesicle recycling at CNS snapses without AP-2. Kim SH, Ryan TA. J Neurosci. 2009 Mar 25;29(12):3865-74.