Cell-cycle-dependent trafficking in the endocytic pathway

Thesis for the degree of Philosophiae Doctor

Trygve Bergeland

Centre for Immune Regulation
Department of Molecular Biosciences
Faculty of Mathematics and Natural Sciences
University of Oslo
2008

Series of dissertations submitted to the
Faculty of Mathematics and Natural Sciences, University of Oslo
Nr. 806

ISSN 1501-7710

Cover: Inger Sandved Anfinsen.
Printed in Norway: AiT e-dit AS, Oslo, 2008.

Produced in co-operation with Unipub AS.
The thesis is produced by Unipub AS merely in connection with the
thesis defence. Kindly direct all inquiries regarding the thesis to the copyright
holder or the unit which grants the doctorate.

Unipub AS is owned by
The University Foundation for Student Life (SiO)

Kjære Anders,
nå skal vi leke
masse!

Table of contents

Acknowledgements

The work presented in this thesis was carried out at the Department of Molecular Biosciences, University of Oslo, from January 1999 to May 2001 and from May 2004 to October 2008. The Norwegian Cancer Society financially supported the work.

I want to thank professor Oddmund Bakke for giving me the opportunity to carry out the work presented in this thesis. I also want to thank him for his sharing of knowledge, great enthusiasm, confidence, and for teaching me to become a scientist. It has been a pleasure to be a part of Bakke-Lab.

I want to thank all my colleagues and friends at the Department of Molecular Biosciences. Tone Gregers and my co-supervisor Dmitrii Rodionov have given me some excellent advise in the lab, and specially in the process of writing this thesis. Frode Skjeldal has shared his great understanding and passion for live cell microscopy. Ole Landsverk has given many good advises and also been outstanding by helping me in the lab. Many thanks to my previous master students, Linda Haugen and Siril Skaret Bakke, for your good work.

My parents Anders and Kari have always given me confidence and support, and for that I am grateful. A special thank goes to my wife, Anne Hilde, and my son, Anders. Thank you for all the good times we share, for your support, laughs, smile, and love.

List of papers

Paper I: ***Mitotic partitioning of endosomes and lysosomes***
Trygve Bergeland, Jannicke Widerberg, Oddmund Bakke, and Tommy W. Nordeng
Curr Biol, 11, 644-651 (2001)

Paper II: ***Cell-cycle-dependent binding kinetics for the early endosomal tethering factor EEA1***
Trygve Bergeland, Linda Haugen, Ole J.B. Landsverk, Harald Stenmark, and Oddmund Bakke
EMBO Rep, 9, 171-178 (2008)

Paper III: ***Early Endosomal fusion stimulates tubular fission***
Frode M. Skjeldal, Trygve Bergeland, Even Walseng, and Oddmund Bakke
Manuscript

Abbreviations

AP	Adaptor protein
ADP	Adenosine-diphosphase
ATP	Adenosine-triphosphate
Cdk	Cyclin dependent kinase
CME	Clathrin mediated endocytosis
EEA1	Early endosomal antigen 1
EGFR	Epidermal growth factor receptor
EM	Electron microscopy
ER	Endoplasmatic Reticulum
FKBP	FK506 binding protein
FKRAP	FKBP-rapamycin associated protein
FLIP	Fluorescent loss in photobleaching
FRAP	Fluorescent recovery after photobleaching
FYVE	Fab1, YOTB, Vac1 and EEA1
GAG	Glycosaminoglycans
GAP	GTPase activating protein
GDF	GDI displacement factor
GDI	GDP dissociation inhibitor
GEF	Guanine nucleotide exchange factor
GGA	Golgi-associated, γ-adaptin homologuos, ARF-interacting protein
GDP	Guanosine-diphosphate
GFP	Green fluorescent protein
GTP	Guanosine-triphosphate
IF	Immobile fraction
LAMP	Lysosome associated membrane protein
LDLR	Low-density lipoprotein receptor
LIMP	Lysosome integrated membrane protein
M6PR	Mannose-6-phosphate receptor
MF	Mobile fraction
MHC	Major histocompatibility complex
MTOC	Microtubule Organizing Centre
NE	Nuclear envelope
NPC	Nuclear pore complex
NSF	N-etylmaleimide-sensitive factor
PAGFP	Photoactivable GFP
PDGFR	Platelete-derived growth factor receptor
PI	Phosphoinositide
PX	PhoX homology
PtdIns3P	Phosphatidyinositol-3-phosphate
Sara	Smad anchor for receptor activation
SNAP	Soluble N-etylmaleimide-sensitive factor attachment protein
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein receptor
Syn	Syntaxin
TGF-β	transforming growth factor-β
TGN	*trans*-Golgi-network
TfR	Transferrin receptor
Ub	Ubiquitin

Introduction

The membrane-bound organelles of eukaryotic cells facilitate a diverse range of microenvironments within the cell, which has an enormous implication on the range of tasks that can be carried out. New proteins are produced in cytosol and on the membrane of the Endoplasmatic Reticulum (ER), glycosylated in the ER and the Golgi complex, and at the same time proteins are degraded in the acidic environment of lysosomes. Maintaining the endomembrane system is fundamental and failure to do so causes a number of human diseases like Alzheimer´s disease, cancer and Huntington´s disease (briefly reviewed in (Howell et al., 2006; Olkkonen and Ikonen, 2006)). It is an enormous task for a cell to operate a healthy endomembrane system, and that is reflected through evolution where the transcendence from prokaryote to eukaryote cells is estimated to have taken twice as long as the transition from dead matter to life (Lopez-Garcia and Moreira, 1999).

The eukaryotic cell comprises internal membranes that divide the cell into structural and functional compartments, called organelles. There are many organelles within the cell, and all perform their specialized tasks. Much of the internal membranes are organized into the endomembrane system, and important organelles within this system are the Nuclear envelope, ER, Golgi, endosomes and lysosomes. Another important member of the endomembrane system is the plasma membrane, which surrounds the cell, and defines the cell as a functional unit within the organism, or as a single-cell structure like a protozoan.

Between the endomembrane organelles there is a dynamic flux of membrane, and it is generally divided into the secretory and endocytic pathway. The secretory pathway involves the transport from the ER to the Golgi, further through the cisternea of the Golgi, and from the Golgi to the plasma membrane. Much of the components that follow this route are newly synthesized proteins and lipids, and therefore it is often referred to as the biosynthetic pathway. The endocytic pathway involves the transport from the plasma membrane to endosomes, and further to lysosomes.

The endocytic pathway

The endosomal system contains a dynamic network of organelles that includes early endosomes, recycling endosomes, late endosomes and lysosomes. They are multi copy organelles with a copy number ranging from a few hundred and up to thousand copies (see PAPER I and (Steinman et al., 1976)). These organelles differ in biochemical composition, cellular location and morphology (Perret et al., 2005). They interact with the cytoskeleton, and both microtubule motors, dynein and kinesin, are involved (Soldati and Schliwa, 2006).

Compartments

An internalized molecule will first appear in the early endosomes that are mostly located at the cell periphery. Reaching the early endosomes, the molecule can be sorted into a pathway of recycling back to the cell surface, or it can be directed toward later endocytic compartments for degradation (Gru-

enberg and Maxfield, 1995; Lemmon and Traub, 2000; Miaczynska and Zerial, 2002). Recycling endosomes have a pH around 6.5 and contain proteins that are recycled to the cell surface, for example the transferrin receptor, and are positive for small GTP binding proteins Rab4 and Rab11 (Sonnichsen et al., 2000; Ullrich et al., 1996; Yamashiro et al., 1984). Early endosomes are positive for Rab5 (Chavrier et al., 1990) and early endosomal antigen 1 (EEA1) (Mu et al., 1995). Both Rab5 and EEA1 are cytosolic proteins that predominantly bind early endosomes, and they play an important role in the tethering of adjacent endosomes prior to fusion. Early endosomes have a pH around 6.0 and contain material directed for late endosomes, for example internalized epidermal growth factor receptor (Renfrew and Hubbard, 1991). In late endosomes, the concentration of membrane bound ATP-driven proton pump is higher than in early endosomes giving even more acidic environment (pH 5.0-6.0) (Tycko et al., 1983). Lysosomes are the end point for many internalized components where they are finally degraded. Late endosomes and lysosomes are similar in many ways. They both have lysosomal glycoproteins on the membrane like, lysosome associated membrane proteins (Lamp´s) and lysosome integral membrane proteins (Limp´s), and are concentrated in the perinuclear region (Eskelinen et al., 2003). However, there are differences that make late endosomes and lysosomes distinguishable. Late endosomes have rab7 and rab9 on the membrane (Feng et al., 1995; Lombardi et al., 1993), and have a high concentration of the mannose-6-phosphate receptor (M6PR) (Griffiths et al., 1988). Lysosomes do not contain rab7, rab9 and M6PR, and are also slightly more acidic (pH 5.0-5.5) (for reviews see (Lemmon and Traub, 2000; Miaczynska and Zerial, 2002; Perret et al., 2005)).

Trafficking

There are several mechanisms for uptake from the plasma membrane and delivery to the endosomal system like, phagocytosis, pinocytosis, caveolae mediated endocytosis, and clathrin mediated endocytosis (CME) (for reviews see (Jones, 2007; Lajoie and Nabi, 2007; Rottner et al., 2005; Ungewickell and Hinrichsen, 2007)). The major pathway to selective internalization from the plasma membrane occurs through CME. The general concept for CME is that subunits of the adaptor protein 2 (AP-2) complex binds to Tyr-based and di-Leu motifs in the cytoplasmic tails of the plasma membrane receptor and recruit clathrin (Rapoport et al., 1997; Rodionov and Bakke, 1998). Further, by the help of many accessory proteins, a basket-like structure is formed that pinches off the membrane to form a vesicle in cytosol (Ungewickell and Hinrichsen, 2007). Lately there has been a discussion about the role of AP-2 in this process. The uptake of the transferrin receptor requires both AP-2 and clathrin (Ohno et al., 1995; Owen and Evans, 1998). The uptake of the low-density lipoprotein receptor (LDLR) requires clathrin but not AP-2, and this is also suggested to be the case for the epidermal growth factor receptor (EGFR) (Motley et al., 2003). This open up the possibility for more specialized and tailored mechanisms for each given receptor (discussed in (Perret et al., 2005)).

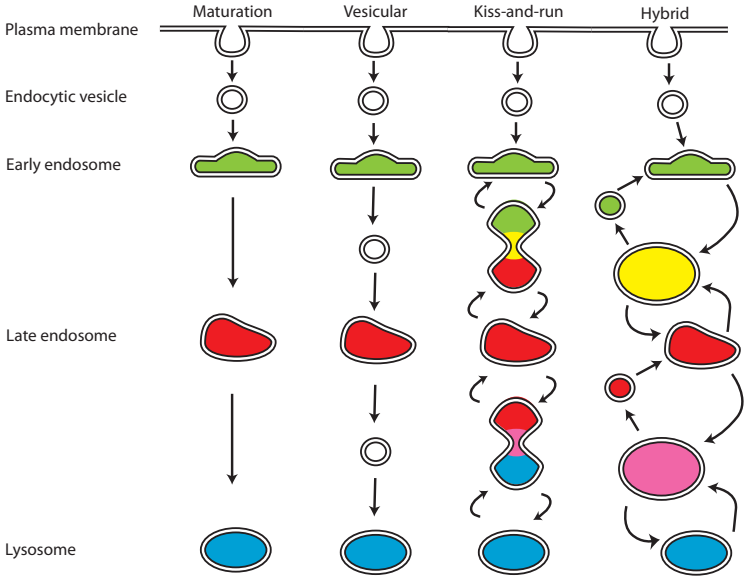

Figure 1. Models of the endocytic pathway
It is not entirely clear how the trafficking is organized in the endocytic pathway. In the vesicular model, the organelles are stable compartments in balance with incoming and departing membrane, and the trafficking occur by vesicles pinching off the departing organelle and fuse with destination organelle. In the maturation model the early endosome gradually transforms into a late endosome and thereafter into a lysosome. In the "kiss-and-run" model the organelles transiently fuse and exchange components before they separate. One other alternative is the hybrid model where intermediate organelles are formed. The organelle identity is thereafter restored by selective retrieval of the organelle specific components. The idea of this figure is taken from (Luzio et al., 2007).

Some newly produced proteins are directly transported from the biosynthetic pathway, from the *trans*-Golgi-network (TGN) to the endosomes. Acidic hydrolases are escorted to late endosomes and lysosomes by the M6PR, and in the late endosomes they dissociate from the receptor due to the acidic environment (Kornfeld, 1992; Kornfeld and Mellman, 1989). The transport out of the TGN for M6PR requires Clathrin, AP-1, GGA and ADP ribosylation factor binding protein (Bonifacino, 2004; Doray et al., 2002). Another adaptor protein, AP-3, is also active in the transport from TGN to endosomes, and both Lamp-I and Limp-II interact with AP-3 on the way out from TGN (Chapuy et al., 2008; Honing et al., 1998; Le Borgne et al., 1998).

How exactly the trafficking along the endocytic pathway is organized has been the point of discussions for many years, and the picture is still not clear (Figure 1) (for discussions see (Gruenberg and Maxfield, 1995; Luzio et al., 2007; Murphy, 1991; Rink et al., 2005; Vonderheit and Helenius, 2005)). In the vesicle shuttle model, the early endosomes are stable organelles that receive endocytosed vesicles or vesicles from the TGN by fusion. Transport vesicles are pinched off for the delivery of endosomal content to late endosomes. In this model, the endocytic organelles is proposed to be a pre-existing and stable organelle, in balance between incoming and departing material. On the contrary,

the maturation model suggests that early endosomes are formed by constant fusion of plasma membrane derived vesicles that gradually acquire new properties resulting in the formation of early endosomes. The further transport to late endosomes and lysosomes is not mediated by vesicles pinching off the organelle, but rather like the whole volume of an early endosomes is transformed into a late endosome. One alternative to the traditional carrier vesicle is the "kiss-and-run" model where organelles transiently fuse (kiss), allowing exchange of content, before they separate (run) (Luzio et al., 2003; Luzio et al., 2000; Mullins and Bonifacino, 2001; Storrie and Desjardins, 1996). Another alternative is the formation of hybrid organelles where, for instance, a late endosome fuses with a lysosome to form an intermediate organelle. The lysosome is then reformed by a selective retrieval of the late endosomal and/or lysosomal components (Bright et al., 2005; Pryor et al., 2000).

In early endosomes, the proteins directed for degradation are often concentrated in areas where there are invaginations of membrane into the lumen (for reviews see (Gruenberg and Stenmark, 2004; Piper and Luzio, 2007)). These invaginations can pinch off from the membrane and form free intralumenal vesicles. This is seen more extensively in late endosomes, where the number of internal structures is higher than in early endosomes. This trend continues along the endocytic pathway, and lysosomes are completely packed with internal membrane structures. One important signal that sends proteins to lysosomes for degradation is ubiquitin (Ub). The covalent attachment of Ub to proteins helps the cell to destroy damaged proteins. It also helps the cell to accurately regulate the amount of surface receptors at the plasma membrane. EGFR, platelete-derived growth factor receptor (PDGFR), and interleukin 1 receptor are examples of receptors that are ubiquitinylated and sorted into luminal structures to attenuate their signal potential from their cytosolic tails.

There is a retrograde transport to the TGN from both early and late endosomes, and this pathway is important specially for the recycling of M6PR (Bonifacino and Hurley, 2008; Bonifacino and Rojas, 2006). One important complex in this process is the retromer, which contain cytosolic components that are recruited to the endosomal membranes, and interacts with cargo and sorts it toward TGN (Seaman, 2007). This pathway is also used by protein toxins secreted by bacteria (for example shiga toxin, cholera toxin) and plants (for example ricin) to enter the cell (Sandvig and van Deurs, 2005).

Some cell types have late endocytic compartments that can be secreted. In dendritic cells, late endocytic compartments are transported to, and fuse with the plasma membrane to present loaded MHC class II for activation of the adaptive immune response (Chow et al., 2002). In cytotoxic T lymphocytes the lysosomes/secretory granules are secreted into the immunologic synapse that is formed between the lymphocyte and the target cell. (Feldmann et al., 2003; Stinchcombe et al., 2001).

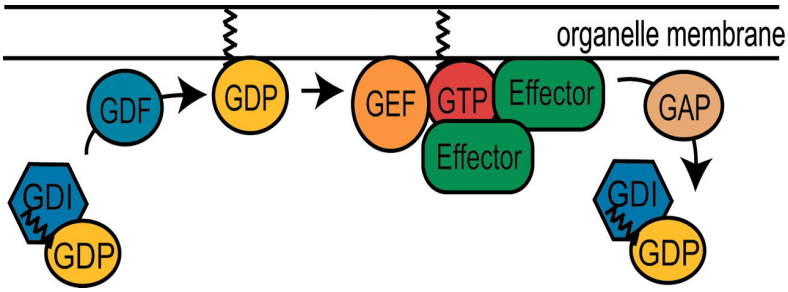

Figure 2. Rab protein cycle, membrane recruitment and activation
GDP-bound and inactive Rab GTPases are associated to GDI, which mask the hydrophobic prenyl group of Rab and makes the complex cytosolic. Membrane attachment occurs through exposure of the prenyl group, a process the facilitated by GDF, which dissociated the Rab-GDI complex. GEF exchange GDP to GTP and activates Rab. This activation facilitates interaction with various effectors that are important for membrane trafficking. GAP stimulates the hydrolysis of GTP to GDP, one process that inactivates Rab. Inactive and GDP bound Rab is escorted and recycled to cytosol by formation of the Rab-GDI complex. GDI, GDP-dissociation inhibitor; GDF, GDI-displacement factor; GEF, guanine nucleotide exchange factor; GAP, GTPase activating protein. Figure from (Grosshans et al., 2006).

Regulation of Rab proteins

Rab proteins are a family of small monomeric GTPases that are involved in vesicular trafficking (briefly reviewed in (Goody et al., 2005; Grosshans et al., 2006; Pfeffer and Aivazian, 2004; Seabra and Wasmeier, 2004)). Their regulatory principle is the ability to cycle back and forth between GTP and GDP bound confirmations (Figure 2). The Rab proteins are biological active when bound to GTP, and inactive when bound to GDP. This switch is controlled by guanine nucleotide exchange factors (GEFs), that trigger the binding of GTP, and GTPase activating proteins (GAPs), that stimulate to hydrolysis of the bound GTP to GDP. In addition to the nucleotide cycling, Rab proteins also cycles between a membrane bound and a cytosolic state. The membrane insertion requires a modification of two C-terminal cystines with isoprenyl lipid (geranylgeranyl) moieties (Kinsella and Maltese, 1992), and these are highly hydrophobic and are inserted into the membrane. When the protein is released from the membrane a protein called GDP dissociation inhibitor (GDI) binds to the Rab protein and mask the hydrophobic part and makes it soluble (Garrett et al., 1994; Rak et al., 2003; Shapiro and Pfeffer, 1995; Shisheva et al., 1999). The membrane attachment requires GDI displacement factor (GDF), and at the same time the Rab protein is displaced from the GDI it is available for GEF stimulation and activation through GTP binding. When active, the protein can bind to effector proteins that can perform their function.

Rab5 is present and has its function on early endosomes and endocytic vesicles derived from the plasma membrane (Gorvel et al., 1991; Li et al., 1994; Rubino et al., 2000; Sonnichsen et al., 2000; Trischler et al., 1999). The protein is activated by Rabex5, which acts as a Rab5-GEF (Horiuchi et al., 1997). The GTP-bound Rab5 is then capable of binding various effectors, and one of them is Rabaptin5 (Rubino et al., 2000). Further, Rabaptin5 binds to Rabex5 and sta-

bilizes the protein on the membrane, and this functions as a positive feedback where the GDP/GTP-exchange on Rab5 is increased (Zerial and McBride, 2001). One other Rab5-effector is the phosphatidyinositol (PtdIns) kinase, VPS34/p150, which catalyze the phosphorylation of phosphoinositide (PI) to phosphatidyinositol-3-phosphate (PtdIns3P) (Christoforidis et al., 1999; Siddhanta et al., 1998). The recruitment of this kinase gives an enrichment of PtdIns3P on early endosomes (Gillooly et al., 2000).

The trafficking in the endocytic pathway is a highly dynamic event where the early endosome is a place for sorting. In this compartment, lipids, membrane proteins and cargo are sorted to destinations like the Golgi, recycling of components to the plasma membrane, and for degradation toward late endosomes and lysosomes. Despite this continuous flux, the early endosome itself maintain its characteristics and do not mix with other organelles. In the step from early to late endosomes it is shown that one early endosome gradually looses its Rab5-GFP tagged coat and coincident with this loss, Rab7-RFP is recruited to this same endosome. This maturation from an early to late endosome seems to be a result of a Rab cascade (Rink et al., 2005). One member of the VPS/HOPS complex, Vps11, is a Rab5 effector (Caplan et al., 2001; Christoforidis et al., 1999; Kim et al., 2001; Richardson et al., 2004; Rieder and Emr, 1997; Rink et al., 2005; Seals et al., 2000). One other member of this complex, Vps39, is a Rab7-GEF (Rink et al., 2005; Wurmser et al., 2000). It is also suggested that the complex also contain one Rab5-GAP, however, that is only so far speculations (Rink et al., 2005). There are two described proteins that show Rab5-GAP activity, RabGAP-5 and RN-tre. Both proteins act as important regulators in membrane trafficking by deactivating Rab5; however, they act in different locations in the cell. RN-tre is suggested to be important for macropinocytosis and act on macropinosomes, whereas RabGAP-5 has its activity in the traditional degradative pathway (Haas et al., 2005; Pfeffer, 2005; Pfeffer and Aivazian, 2004).

Tethering by EEA1

EEA1 is a cytosolic 162 kD protein, which is predominantly localized to early endosomes. It displays homology to myosin heavy chain in most of its length, which is predicted to be α-helical and form a coiled-coil structure (Figure 3) (Mu et al., 1995). In addition, EEA1 has a calmodulin binding IQ motif at the C-terminus and zinc fingers at both ends. The N-terminal zinc finger is of a C2H2 type and has been found in many nucleic acid binding proteins. The C-terminus contains two zinc fingers that contain eight cystines which is called a FYVE domain, named after four proteins (Fab1, YOTB, Vac1 and EEA1) that all contain this domain (Stenmark et al., 1996). The FYVE domain binds specifically to the lipid, phosphatidyinositol-3-phosphate (PtdIns3P) (Gaullier et al., 1998). EEA1 also contains two binding sites for Rab5, one at the N-terminus and one at the C-terminus, close to the FYVE-domain (Callaghan et al., 1999a; Lawe et al., 2002; Simonsen et al., 1998). EEA1 is found to operate as an anti-parallel dimer and the important area for the dimerization are interactions through the coiled-coil region. This

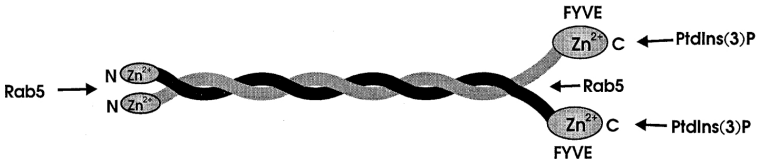

Figure 3. Bipolar organization of EEA1
Model presenting EEA1 organization as a parallel dimer. Figure from (Callaghan et al., 1999b).

rod-like molecule allows interactions with Rab5 and PtdIns3P from one end of the dimer and Rab5 interactions only at the other end (Callaghan et al., 1999b). EEA1 is important for endosome fusion, and it is suggested to facilitate tethering between early endosomes and cause homotypic fusion where both of the endosomes contain Rab5 and PtdIns3P. The bipolar organization of EEA1 also allows tethering with one membrane that contain both Rab5 and PtdIns3P, and another membrane that contain Rab5 and no PtdIns3P, and this situation is relevant when small vesicles from the plasma membrane are fusing with early endosomes (Callaghan et al., 1999a; Callaghan et al., 1999b; Gaullier et al., 1998; Lawe et al., 2002; Mills et al., 2001; Simonsen et al., 1998).

Docking by SNAREs

The downstream event after tethering and before fusion is the docking, a process that is facilitated by the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins (Hu et al., 2003; McNew et al., 2000; Sollner et al., 1993). They can be classified into v-SNAREs that are associated with vesicle and t-SNAREs that are associated with the target compartment. The v-SNAREs usually consist of a transmembrane domain and a single SNARE motif. The t-SNAREs are more complex and consist usually of two or three polypeptides (Fukuda et al., 2000). They contain normally a member of the syntaxin (Syn) subfamily that are transmembrane and has one SNARE motif. Syn can form a complex with SNAP-25, which is a cytosolic protein that contains two SNARE domains. Instead of SNAP-25, Syn can interact with two smaller proteins, where one share homology to the C-terminal part and the other share homology to the N-terminal part of SNAP-25. All together, both in the dimer and the trimer there are three SNARE domains (Antonin et al., 2002; Sutton et al., 1998; Weber et al., 1998) (and for a review see (Hong, 2005)).

The mode of action for SNARE proteins is outlined in Figure 4, and also thoroughly reviewed in (Hong, 2005). The v-SNARE is first sorted together with cargo proteins from the donor compartment into a vesicle and destined toward the target compartment. The tethering event between the vesicle and target compartment allows the v-SNAREs to be positioned in a region where the corresponding t-SNAREs are located. For early endosomes the tethering distance between two membranes is found by electron microscopy (EM) to be around 75-150 nm (Orci et al., 1998), and this is consistent with the measured length for EEA1 found by

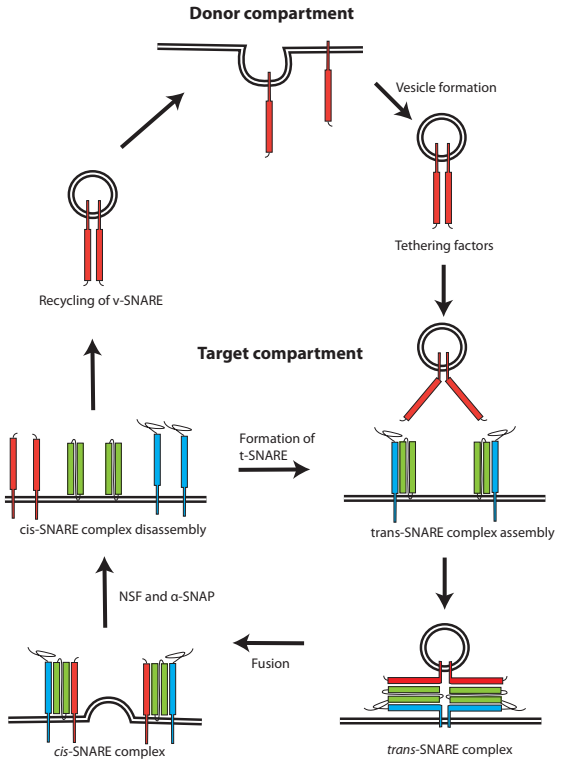

Figure 4. Action of SNARE proteins
The v-SNARE follows the vesicle from donor compartment and interacts with t-SNARE at the target compartment. A *trans*-SNARE complex is formed, which brings the opposing membranes to fusion proximity. A *cis*-SNARE complex is then formed where both t- and v-SNAREs are in the same membrane. The dissociation of the complex is catalyzed by α-SNAP and NSF, and thereafter the v-SNARE recycles back to donor compartment. The idea of this figure is taken from (Hong, 2005).

ultrastructual analysis (Dumas et al., 2001; Wilson et al., 2000). In addition it is also suggested that EEA1 can bring the membranes even closer through a change in conformation by binding of calmodulin in the IQ domain (Colombo et al., 1997; Mills et al., 2001; Zerial and McBride, 2001). This close distance allows short range docking where the v-SNARE interacts with the t-SNARE and they make a *trans*-SNARE complex (Chen and Scheller, 2001; Jahn et al., 2003; Rothman, 2002; Sudhof, 2004). The interactive forces between the two SNAREs are so strong that energy barrier made by the negatively charged lipids in the membrane is overcome, and the *trans*-SNARE complex thus catalyze the fusion of the two adjacent membranes. After fusion, the complex becomes a *cis*-SNARE complex in the target membrane. To be ready for the next round of transport, the *cis*-SNARE must disassemble and this is catalyzed by the combined action of the ATPase α-SNAP (soluble N-etylmaleimide-sensitive factor attachment protein) and NSF (N-etylmaleimide-sensitive factor). After disassembly the v-SNARE is recycled

back to the donor compartment (Hong, 2005; Zhao et al., 2007).

EEA1 interacts with Syn6 and 13, and both these SNAREs are found on early endosomes (Hong, 2005; McBride et al., 1999; Simonsen et al., 1999). The interaction between Syn6 and EEA1 is not clear, however the interaction with Syn13 is required for fusion (Hong, 2005; McBride et al., 1999; Zerial and McBride, 2001). In summary, EEA1 act as a fusogenic molecule in several aspects, by bringing the endosomal membranes in so close proximity to allow the formation of the *trans*-SNARE complex and also by recruiting members of the Syn-family.

Interactions with the cytoskeleton

To maintain an operative and functional endocytic pathway, it is necessary to interact with the actin and tubulin cytoskeleton. The formation of vesicles and delivery to their target membrane are dependent on forces generated by actin and microtubule-dependent motors (for reviews see (Girao et al., 2008; Soldati and Schliwa, 2006)). One example is the Myosin VI motor that traffics the clathrin-coated vesicles through the actin network underneath the plasma membrane (Aschenbrenner et al., 2004). Other examples are motor proteins that do cargo sorting through membrane tubulation, fission, and intracellular positioning (Goldstein, 2001; Goodson et al., 1997). Endosomes and endosomal vesicles exhibit both short and long-range movements with a wide range of velocities (Gasman et al., 2003; Nielsen et al., 1999; Valetti et al., 1999). In model cells, like MDCK, early endosomes are typically spread out in the cytoplasm, whereas the late endosomes and lysosomes are located more close to the nucleus (Gruenberg and Maxfield, 1995) (also observed in PAPER I, II, III).

There are two known families of microtubule-interacting motor proteins acting on endosomes, dyneins and kinesins (for reviews (Hirokawa, 1998; Soldati and Schliwa, 2006; Vale, 2003)). Most of the kinesin motors move along microtubules in a plus-end direction toward the cell periphery. They consist of a light chain and a heavy chain, where the heavy chain in most occasions has three sub domains: the globular motor head, a stalk, and a tail domain. The stalk and the tail domains show selectivity to which cargo they bind, and they also have a regulatory role on the motor unit (Lee et al., 2004; Seiler et al., 2000; Verhey et al., 1998). Dynein motors transport cargo in a minus-end direction toward the Microtubule Organizing Centre (MTOC) (Paschal and Vallee, 1987; Schnapp and Reese, 1989; Schroer et al., 1989). They have a quite complex composition and consist of two heavy chains, and several subunits like intermediate chains and light chains. The heavy chains form the motor unit while the accessory proteins display the cargo selectivity (Vallee et al., 2004). The Dynactin is one important activator protein that interacts with the intermediate chains and can link up with several types of cargo (Karki and Holzbaur, 1995; Schroer, 2004; Vaughan and Vallee, 1995). Both plus-end and minus-end directed motors can be found on the same cargo (Gross et al., 2002; Kural et al., 2005; Rogers et al., 1997), and it is shown that endosomes and lysosomes can

move in a bidirectional manner (Blocker et al., 1997; Wubbolts et al., 1999).

In the endocytic pathway, Rab proteins are important regulators of both kinesins and dyneins (for a review see (Jordens et al., 2005)). Rab4 binds to the kinesin KIF3M, and this interaction is important for exocytosis in the recycling process from early endosomes to the plasma membrane (Imamura et al., 2003; Reilein et al., 1998). Rab5 is shown to be an important upstream regulator of the kinesin KIF16B, through its effector, the PI-kinase, VPS34/p150. This kinesin contain PtdIns3P specific PhoX homology (PX) domain that has shown to be important for the recruitment of this motor protein to early endosomes (Hoepfner et al., 2005).

The cell cycle

Between formation and division a cell normally undergoes four phases, called the cell cycle: G1 phase (growth phase), S phase (DNA replication), G2 phase (growth phase) and M phase (mitosis) (for reviews see (Fukasawa, 2007; Schmit and Ahmad, 2007)). A cell proceeding through cell cycle is a well-regulated event, and it has to fulfill many qualifications on its way. The cell will not enter S phase and start the DNA replication unless the environment has the required growth factors. In G2 the cell will stop proceeding if the DNA replication is unsuccessful, if the cell size is to small, or if the environment is absent of the right growth factors. In this way the cell controls itself to check if it is competent of fulfilling mitosis. The cyclin dependent kinase complexes (Cdk-cyclins) have an important role in this process. They are responding on varieties of changes a cell undergoes, and put it into cellular action. In most eukaryotic cells there are four classes of cyclins and they are defined by the stage in the cell cycle where they bind Cdks and have their function. Cyclin D binds Cdk4 or Cdk6 at the start of G1 and helps to promote the passage through start or the restriction point in late G1. At the end of G1 phase, cyclin E binds to Cdk2 and assign the cell to DNA replication. During S-phase cyclin A interacts with Cdk2 and this is required for the initiation of DNA replication. For entry and completion of mitosis activation of the complex cyclin B-Cdk1 is required (in vertebrates Cdk1 is also called cdc2).

In most vertebrate cells, mitosis is divided into six stages that follow each other in a more or less strict order. The stages are defined out of events that are possible to study in a light microscope, and this has fascinated scientists for more than a century (Wilson, 1896). When leaving G2 and entering mitosis, the cell first enters prophase. The chromatin, which is diffuse in interphase, starts to condense into well-defined and distinct chromosomes. In prophase, the interphase microtubules start to disassemble, the centrosomes separate and are positioned on opposite sides of the nucleus. Mitotic spindle starts to form. Entry into prometaphase is accompanied by nuclear envelope breakdown. The mitotic spindle is able to enter the previous nucleoplasmic area and link up with the kinetochores. With the movement of the chromosomes into the metaphase plate, the cell enters metaphase. The entry into anaphase is accompanied by a synchronous separation of the sister chromatides that are

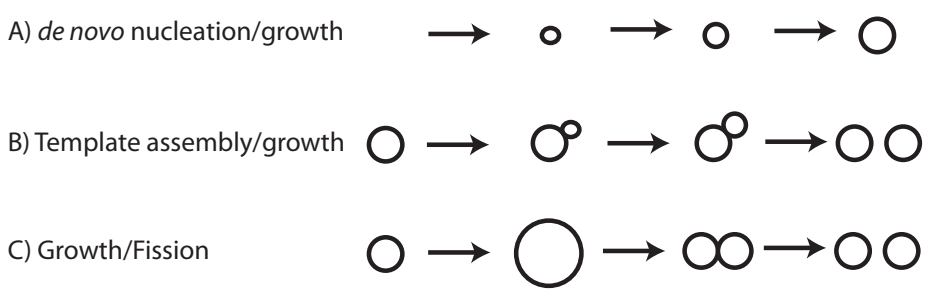

Figure 5. Models for the biogenesis of organelles
A cell can replicate its organelles through a de novo synthesis where there is no information in for of a template or a copy of the organelle (a). Another strategy is replication of organelles through growth from one already existing organelle, either through template growth (b), or growth and fission (c). Figure taken from (Lowe and Barr, 2007).

pulled toward the spindle pole they are facing. When the nuclear envelopes start to form, the cell enters telophase. The cytokinesis is the mechanism that physically divides the mother cell into the two daughter cells. This process starts during anaphase and ends with the completion of mitosis (Blow and Tanaka, 2005; Gadde and Heald, 2004; Walczak and Heald, 2008).

Inheritance and biogenesis of organelles

The eukaryotic life expands through growth and division. During division, the daughter cell receives cellular components that are required for proper function, and before the next division the daughter cell needs to grow and double its biomass to maintain the cellular size through generations. The inheritance of DNA is taken care of in S-phase of the cell cycle, where it is replicated, and further during mitosis where the daughter cells receive an exact copy of the mother cell´s chromosomes. Failure in this process might be fatal and cause death, uncontrolled cell division or cancer. Therefore, the segregation of chromosomes is a strictly controlled process (Fukasawa, 2007). A daughter cell must, however, receive more than chromosomes to start up its biological tasks. Only minutes after the cell division, proteins are synthesized in the ER, modified in the Golgi complex and transported to their right destinations. As for DNA, organelles grow and replicate through cell cycles (biogenesis), and are partitioned to the daughter cells during mitosis (Lowe and Barr, 2007; Warren and Wickner, 1996).

There are some ways one organelle can be replicated. One solution is *de novo* synthesis, which means that the cell is capable of producing one new organelle with no information in form of a template or a copy of the organelle (Figure 5A). It has been demonstrated that a daughter cell can build a functional Golgi without having received one from the mother cell (Zorn et al., 1979). However, the rebuilding process in this case was very slow, and *de novo* biogenesis of organelles alone is not typical. In most known cases, the organelles grow by proliferation

from preexisting organelles, either by template growth where the new organelle is formed alongside the mother organelle and buds off (Figure 5B), or the organelle can grow in size be divided by fission (Figure 5C) (Lowe, 2002; Lowe and Barr, 2007; Nunnari and Walter, 1996; Shorter and Warren, 2002).

Distribution of organelles from mother cell to daughter cells

The distribution of organelles and other cellular components into the two daughter cells can be divided into two inheritance strategies, either by a stochastic or by an ordered manner (Birky, 1983; Lowe and Barr, 2007; Warren and Wickner, 1996). An organelle or cellular component is stochastically distributed if it is freely dispersed throughout the mitotic cytoplasm. The outcome of such a distribution cannot be predicted with any certainty. However, by observing a large number of cell divisions, it may be possible to find a partitioning pattern, and mathematically calculate the probability of distribution (Figure 6A-C). Examples of stochastic distribution are the partitioning of chloroplasts and mitochondria. In most cells they are multiple copy organelles with a random dispersion in cytosol (Birky, 1983; Hennis and Birky, 1984; Warren and Wickner, 1996).

The second partitioning strategy is when organelles and cellular components are distributed to the daughter cell in an ordered manner. An ordered manner means a way of partitioning that is not stochastic (Figure 6D). This way of partitioning is caused by elements avoiding a random dispersion throughout mitotic cytoplasm. One example of ordered partitioning is the distribution of sister chromatides.

Two factors seem to have a restrictive influence on movements of organelles and cellular components in mitotic cytoplasm (Birky, 1983). First, the size and volume of the organelle may influence the way an organelle moves and is positioned. For small organelles this effect is negligible. Space limitation may, however, come into play for big organelles determining how many organelles that can be present at one side of a future division plane. For example, in *Saccharomyces cerevisiae* there is 1-50 mitochondria that occupies 3-14% of the cytoplasmic volume, and it is possible that organelles are inhibited from moving freely around (Birky, 1983; Grimes et al., 1974). Second, direct protein interaction between organelles/cellular components and the cytoskeleton prevent a free dispersion in mitotic cytoplasm. Protein motors (myosin, dynein, kinesin) provide in addition movement along the cytoskeletal structures, and allows organelles or cellular structures to be pulled or pushed around in the cytoplasm.

The segregation of chromosomes shows a distribution that diverges with high degree from a binomial partitioning giving an ordered pattern (Hirano, 2000). Another example is the partitioning of vacuoles in budding yeast where the vacuoles are more or less dragged into the new budding cell (Weisman et al., 1987). One inheritance strategy does not necessarily exclude the other. Both strategies can be used on the same organelle. The average number of mitochondria in scorpion spermatocyte is 24.

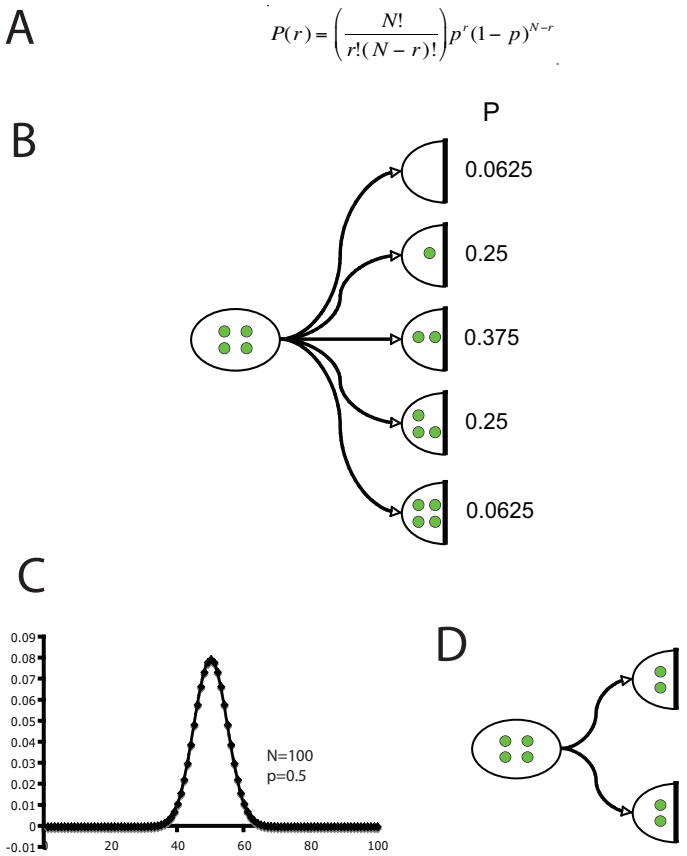

Figure 6. Ordered and stochastic partitioning
If a distribution is stochastic, and the size of the two daughter cells is equal, it is reasonable to expect a probability (p) of 0.5 of going to each cell. Based on this assumption, the partitioning will follow a binomial distribution. The probability (P) that a daughter cell receives r organelles is described in (Birky, 1983) and presented in (a), where the mother cell has N organelles, and the two daughter cells receive r and N-r organelles. If a mother cell has four organelles that are stochastically distributed, one can calculate the probability (P) of receiving a given number of organelles (b). The number of organelles in a cell can sometimes be more than four and this is exemplified in (c) where the mother cell has 100 copies of one cellular component and P (y-axis) is plotted against r (x-axis). If organelles or cellular components undergo an ordered partitioning this is a way of partitioning that deviates from the described binomial distribution. One example is when a mother cell has four organelles and the daughter cells always receive two (d).

After completion of two meiotic divisions, 70% of the cells contain 6 mitocondria, and 30% contain 5 or 7 mitochondria. This is too accurate to be stochastic and too imprecise to be ordered (Birky, 1983).

The endomembrane system in mitosis

Disassembly and reassembly of the Nuclear Envelope and the Endoplasmatic Reticulum

ER is a multifunctional organelle that contains a membrane-enclosed network that

is spread out in cytosol (For a comprehensive review see (Hetzer et al., 2005)). This network consists of areas with ribosomes (rough ER), and areas without ribosomes (smooth ER). In ER, proteins are translocated across the membrane and they are folded and modified before they enter the secretory pathway. In addition ER also plays an important role in regulation of the cytosolic level of Ca^{2+}, signaling and lipid synthesis. The nuclear envelope (NE) is continuous with ER and is often considered as one single organelle. The NE consists of three distinct but interconnected domains: outer, pore and inner membrane. The inner membrane is associated with nuclear lamina and chromatin. The pore membranes connect the inner and outer membrane domains at numerous points and are associated with the nuclear pore complexes (NPC). The outer membrane is continuous with ER.

In higher eukaryotes, during prophase, the nuclear lamina depolymerise, NPC components disperse into subunits, and the inner membrane no longer associates with the chromosomes (reviewed in (Hetzer et al., 2005)). The fate of ER and NE seem to vary among cell types and experimental approach. Based on partly contradictory results from different research groups, two models about what is actually happening during this process has been proposed (reviewed in (Du et al., 2004)). In the first model both ER and NE undergoes a progressive fragmentation, and small vesicles scatter throughout cytoplasm (Robbins and Gonatas, 1964; Tamaki and Yamashina, 1991; Zeligs and Wollman, 1979). This fragmentation allows a free diffusion in mitotic cytosol, and a stochastic partitioning of the organelle has been suggested (Warren, 1993; Warren and Wickner, 1996). In the second model, all membrane proteins become freely diffusible after the breakdown of NPC and loss of chromosome association. The specialized sub domains of the organelle fade and NE/ER resident proteins are homogenized in a mitotic network prior to division (Ellenberg et al., 1997; Koch et al., 1987a; Pecot and Malhotra, 2004; Puhka et al., 2007; Yang et al., 1997). Several groups have reported an accumulation of ER at the mitotic poles (Bobinnec et al., 2003; Henson et al., 1989; Terasaki, 2000), and there are some reports showing that interaction with microtubule is involved in the partitioning in mammalian cells (Beaudouin et al., 2002; Salina et al., 2002). For both models, during telophase, membranes containing inner nuclear components connect with the chromosomes, and a new nucleus is formed in the two daughter cells, and this is further continued with the rebuilding of the NPC. Short time after the cytokinesis the characteristic interphase NE and ER is reformed (Burke and Ellenberg, 2002; Ellenberg et al., 1997; Hetzer et al., 2005; Yang et al., 1997).

The inheritance of the NE and ER vary among spices. Budding yeasts have a closed mitosis where the NE does not break down. Mitotic spindle is formed inside the nucleus and the outcome is that both NE and ER are distributed to the new budding cell through interaction with the astral microtubules (Huffaker et al., 1988; Jacobs et al., 1988; Preuss et al., 1991). Both ER and NE are dragged into the new cell, and represent an ordered inheritance strategy (reviewed in (Lowe and Barr, 2007; Warren and Wickner,

1996)).

Periodic fragmentation of the Golgi Complex

In higher eukaryotes, the Golgi Complex is a single cellular organelle consisting of flatten polarized cisternae compactly arranged in a parallel formed stack from *cis* to *trans* (for a review see (Marsh and Howell, 2002)). It has a juxta-nuclear orientation and it plays an essential role in secretory traffic, lipid biosynthesis, protein modification, sorting and transport of proteins. In mammalian cells, the stacks are connected laterally by tubules, and it is reported that up to 100 stacks can be connected by tubular bridges that is called the Golgi ribbon. The organelle has a dynamic structure with high degree of transport between the stacks.

During G2-phase the Golgi ribbon fragments and give rise to isolated Golgi stacks (Colanzi et al., 2007; Feinstein and Linstedt, 2007). Further, in prophase, the organelle loses its organization and fragments into large units that gradually are sectioned into smaller units, which during metaphase is called the Golgi Haze (Hiller and Weber, 1982; Maul and Brinkley, 1970) and reviewed in (Colanzi and Corda, 2007; Lowe and Barr, 2007). The degree of fragmentation seems to vary between different types of cells and what kind of Golgi markers that is in use. In HeLa cells, Zaal et al., (1999) have shown a complete breakdown of the Golgi vesicles, followed by a total cytosolic dispersion of the Golgi markers (Zaal et al., 1999). However, another group used different Golgi markers in HeLa cells to show some degree of dispersion within cytosol, but most of the Golgi vesicles remained intact throughout cell division (Shima et al., 1998). The units do not move freely around in cytosol, and there are interactions with microtubule, dynein and other microtubule dependent motors (Allan et al., 2002; Barr and Egerer, 2005). An ordered inheritance strategy has been suggested (Shima et al., 1998; Shima et al., 1997). During telophase, the Golgi vesicles start to reassemble. The Golgi vesicles fuse and shortly after cytokinesis, a single copy cisternal organelle is formed in each daughter cell (Lucocq et al., 1989).

Endosomes and lysosomes

Since lysosomes are multicopy organelles, Warren et. al (1996) proposed that the partitioning of lysosomes could be due to a stochastic mechanism (Warren and Wickner, 1996). However, that was not based on empiric data and only the fact that lysosomes are present in the cell in multiple copies (Steinman et al., 1976). The organelle equivalent to a lysosome in fungi is the vacuole, and in budding yeast it is shown that vacuoles are inherited by an ordered mechanism (Warren and Wickner, 1996; Weisman et al., 1987). Nonetheless, vacuoles are present in yeast from one to five copies, and therefore not directly comparable to partitioning of lysosomes in mammalian cells.

In some cells the distribution of endosomes are highly regulated processes. One example is a subpopulation of apical endosomes in epithelial cells in the developing wing in *Drosophila* that associate with the mitotic spindle and concentrate at the midzone in mitosis. These early endosomes are positive for the adaptor protein Sara

(Smad anchor for receptor activation) that is a FYVE domain protein important in the signal transduction from the transforming growth factor-β (TGF-β) (Tsukazaki et al., 1998). This ensures that the highly polarized Sara positive compartments are partitioned to each daughter cell in an equal manner (Bokel et al., 2006). One other example is the first mitotic division of *Caenorhabditis elegans*. This first division gives the larger anterior cell and the smaller posterior cell, and is asymmetric. Prior to division, the early endosomes are evenly distributed in the cytosol, and during mitosis the early endosomes are enriched in the anterior cell. The significance of this asymmetry is not known (Andrews and Ahringer, 2007).

Membrane trafficking in mitosis

The secretory transport is inhibited during mitosis, and newly synthesized proteins do not reach the cell surface (Lucocq and Warren, 1987; Warren et al., 1983). The Golgi complex enzymes no longer process glycoproteins, and transport is arrested out of the ER (Figure 7A) (Featherstone et al., 1985). In interphase, the transport vesicles out of the ER are produced by budding mechanisms driven by the coat proteins COPII out from ER exit sites (for a review see (Kirk and Ward, 2007)). Upon entry in mitosis, the COPII components Sec23, Sec24, Sec13 and Sec31 changes localization. From being concentrated at the ER exit sites in interphase they are more widely distributed during cell division (Farmaki et al., 1999; Hammond and Glick, 2000; Prescott et al., 2001). Sec24 is also show to be de-O-glycosylated and phosphorylated in mitosis which can partly explain the arrest in ER to Golgi transport (Dudognon et al., 2004).

The mechanism of the retrograde transport from the Golgi to the ER during mitosis is currently under debate, so far the picture is not clear and there are two models (Figure 7B) (discussed in (Colanzi and Corda, 2007)). In the first model, it is proposed that the retrograde transport is continuous throughout mitosis. In 1999, Lippincott-Schwartz and colleagues showed a continuous Golgi to ER transport by using a Golgi resident protein GalTase-GFP and FRAP (Zaal et al., 1999). More recently, the same group has used an ER trapping assay based on the temperature sensitive mutant ts045-VSVG protein, and this mutant unfolds and gets trapped in ER at 40°C. In interphase, the protein is located in the Golgi; however, at the end of mitosis and followed by a shift to 40°C, the protein relocates to ER. Since the misfolding occurs in ER, these observations suggest an ongoing Golgi to ER transport in mitosis (Altan-Bonnet et al., 2006). Since there is an arrest in the ER to Golgi transport it is further suggested that Golgi actually is redistributed into ER (Altan-Bonnet et al., 2006; Zaal et al., 1999). The redistribution is seen when markers of Golgi are dispersed throughout the mitotic cytoplasm, and a Golgi resident protein is also shown by EM to colocalize with mitotic ER (Thyberg and Moskalewski, 1992). In the second model, a different conclusion has come from another ER trapping assay (Pecot and Malhotra, 2004; Pecot and Malhotra, 2006). Rapamycin, a small membrane permeant molecule, binds to the FK506 binding protein (FKBP)

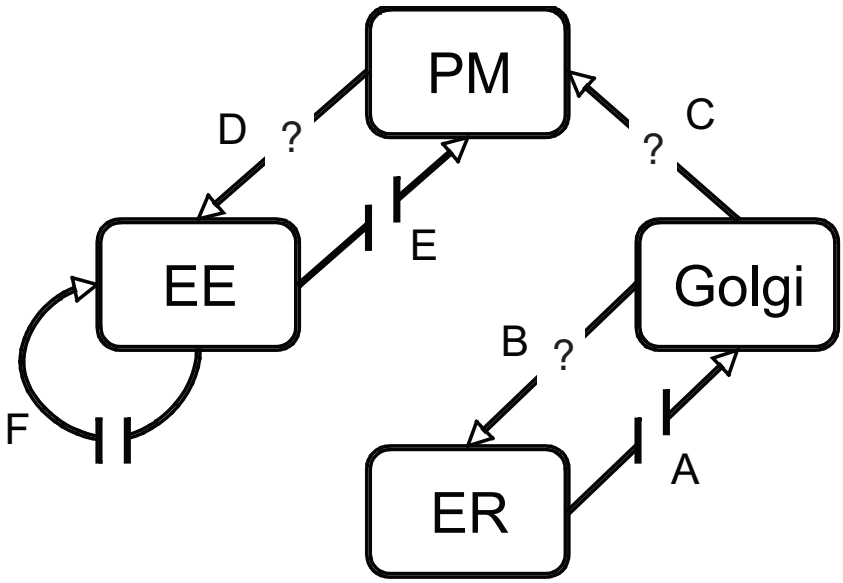

Figure 7. Mitotic changes of membrane trafficking
The broken arrows show interphase trafficking routes that are inhibited in mitosis. The arrows with question mark show pathways where it is unclear what happens in mitosis, and published data is contradictory, both supporting an arrest and an unaffected trafficking. PM, plasma membrane; EE, early endosomes; ER, endoplasmatic reticulum.

(Wiederrecht et al., 1991). The FKBP-rapamycin associated protein (FKRAP) binds the FKBP-rapamycin complex (Brown et al., 1994; Sabatini et al., 1994). By fusing FKBP to a Golgi resident motif and FKRAP to an ER resident motif, FKBP will be trapped in ER by a retrograde transport in the presence of rapamycin, which happens in interphase. This was not found to be the case in mitosis leading to the conclusion that the Golgi membranes remain distinct and separate from the ER during mitosis (Pecot and Malhotra, 2004). This is also supported in some earlier studies where the Golgi is shown to be separate from the ER during mitosis (Jesch and Linstedt, 1998; Jokitalo et al., 2001; Lucocq and Warren, 1987). The contradictory results are based on varying cell types (all mammalian) and expression systems, which can at least partly explain the conflicting conclusions. However, more experiments need to be done before the biogenesis of the Golgi is ruled out.

At the entry of mitosis there is clearly an arrest in the secretion, and this has been shown in several papers (Hesketh et al., 1984; Lucocq and Warren, 1987; Warren et al., 1983). However, it is not very clear if this is due to the arrested exit out of ER alone, or if there also is an inhibition in the trafficking out of TGN (Figure 7C). In *Xenopus laevis*, proteins are still glycosylated and processed as far as to the TGN, in mitosis, with an inhibition further out of TGN (Kanki and Newport, 1991). However, in mammalian cells, exogenously added substrates for

construction of glycosaminoglycans (GAGs) are transported to the Golgi complex where they are processed into GAG products and finally GAGs are secreted. This secretion is only minimally affected in mitosis, and almost at the same levels as the control cells that were in interphase (Kreiner and Moore, 1990).

For several decades scientists have suggested and shown by various means that endocytosis is down regulated or arrested in mitosis. In 1965, Fawcett studied binding of ferritin to cells by EM, and suggested that the invagination and separation from the plasma membrane to be temporarily suppressed in mitosis (Fawcett, 1965). Some years later the properties of phagocytosis, pinocytosis and endocytosis was investigated in a macrophage cell line (J774) and in Chinese hamster ovary cells. Receptor mediated endocytosis of concavalin A and phagocytosis of IgG opsonized erythrocytes were markedly reduced, and also fluid phase uptake of horseradish peroxidase declined (Berlin and Oliver, 1980; Berlin et al., 1978). The uptake of fluorescent dextran has also under some occasions shown to decline sharply during mitosis (Berlin and Oliver, 1980; Warren et al., 1984). More recently it is shown that both clathrin and dynamin change their location and also function during mitosis. Clathrin is, in mitosis, found on kinetochore fibers that are bundles of microtubules that connect the spindle pole to the kinetochore of chromosomes (Maro et al., 1985; Sutherland et al., 2001). Knocking down clathrin heavy chain causes mitotic defects which includes misaligned chromosomes in metaphase and destabilization of the kinetochore fibers (Royle et al., 2005). One isoform of dynamin, dynamin-2, which in interphase is involved in vesicle scission and actin reorganization shifts its location to the midzone during telophase and is suggested to play a role in the cytokinesis (Thompson et al., 2002). Dynamin-2 is also found at the centrosomes and contributes to centromer cohesion (Thompson et al., 2004). It is suggested that an arrest of clathrin mediated endocytosis during mitosis might be the result of clathrin and dynamin being absorbed in a new task (discussed in (Royle, 2006)). EM studies have shown, however, that coated pits at all stages exists on mitotic cells and when compared to interphase cells there are more coated pits with a flattened appearance which partly can explain the down regulated endocytosis during cell division (Pypaert et al., 1987). A candidate for the down-regulated endocytosis is cdc2, which is shown to inhibit coated pit formation in permeabilized HeLa cells (Pypaert et al., 1991). A debate about the extent of endocytosis is currently going on, and Kirchhausen and colleagues have studied σ2, a subunit in the AP-2 complex (Boucrot and Kirchhausen, 2007). When fused to GFP they show that σ2-GFP assembles and disassembles on punctuate spots at the plasma membrane at all stages of the cell cycle, including mitosis. Contradictory to the earlier studies (Berlin and Oliver, 1980; Warren et al., 1984) they show that fluorescent dextran is internalized, and they suggest that endocytosis is ongoing through mitosis (Boucrot and Kirchhausen, 2007). The contribution from Kirchhausen shows that there might be differences between cell lines and experimental setup (Figure 7D).

In interphase, a eukaryotic cell is spread out on a substrate, and when it enters mitosis, it rounds up. The surface area per volume ratio is decreased with a minimal value in metaphase where the cell is almost spherical. During cytokinesis and formation of the cleavage furrow the membrane area increases once again (Ohnuma et al., 2006). There are some different ways a cell can accommodate with these changes in cell area. EM studies have revealed an increase in the number of microvilli and ruffles on rounded up mitotic cells when compared to interphase cells, and it is suggested that this can explain the rounded up organization (Erickson and Trinkaus, 1976; Porter et al., 1973). Another way to accommodate with the reduced surface to volume ratio in mitosis is to regulate the ratio between secretion and endocytosis. There seem to be an agreement among most scientists that the secretion is arrested in mitosis (Kanki and Newport, 1991; Kreiner and Moore, 1990; Lowe and Barr, 2007; Lucocq and Warren, 1987; Warren et al., 1983). If endocytosis is also arrested, the surface to volume ratio will not be reduced, so if this is a way the cell handles the reduced area, the described arrest of endocytosis must occur after the blocked secretion, or it can be that endocytosis is continuous throughout mitosis as some suggests (Boucrot and Kirchhausen, 2007). The plasma membrane has also been shown to be laterally flexible, and it has also been shown that the plasma membrane in mitosis is stretched. By use of optical tweezers it is shown that the plasma membrane tension is higher when the cell is rounded up in mitosis, and this increased tension directly inhibits invagination and coated pit budding at the plasma membrane (Raucher and Sheetz, 1999).

The transferrin receptor (TfR) is internalized together with bound transferrin via coated pits, and delivered to endosomes. The change in pH causes the iron to be released from transferrin. Apotransferrin bound to TfR is recycled back to the cell surface (Klausner et al., 1983). During cell division, the amount of TfR on the plasma membrane is decreased, and it accumulates in early endosomes. There is a block in the endosomal recycling and it is activated prior to the suggested inhibition of endocytosis (Figure 7E) (Sager et al., 1984; Warren et al., 1984), and the block is recovered in anaphase (Schweitzer et al., 2005). Quite recently it was further shown that this inhibited recycling gives less TfR on the plasma membrane, and the arrested uptake of TfR in mitosis was not the result of an arrested endocytosis but due to the lack of TfR in the plasma membrane (Boucrot and Kirchhausen, 2007). A candidate for this inhibition of recycling is Rab4, which is phosphorylated by the mitotic kinase cdc2, and due to phosphorylated Rab4, changes its membrane to cytosol cycling and accumulates in cytosol (Ayad et al., 1997; Mohrmann et al., 2002; van der Sluijs et al., 1992). The recycling from early endosomes to the plasma membrane is reactivated during cytokinesis and is probably an important contributor to the delivery of membrane in the cleavage furrow (Albertson et al., 2005; Baluska et al., 2006; Schweitzer et al., 2005).

An arrest in the early stages of the endocytic pathway is also shown *in vitro* where fusion of early endosomes is down

regulated in the presences of mitotic extracts from mammalian cells, Xenopus eggs and starfish (Figure 7F). A candidate for the inhibited fusion is the mitotic kinase cdc2 that *in vitro* inhibits fusion of early endosomes (Tuomikoski et al., 1989; Woodman et al., 1993; Woodman et al., 1992).

Photobleaching and photoactivation

The diversity of genetically encoded fluorophores has increased since GFP was first cloned in 1992 (Prasher et al., 1992). There are now a variety of GFP mutants spanning much of the visual spectrum (for a review see (Chudakov et al., 2005)). Using standard molecular biology techniques, a protein of interest can be linked to a fluorophore. Depending on fusion partner, this approach can be used to label subcellular organelles, cells of interest and specific tissue regions. This has opened up the possibility of *in vivo* studies of, for example, organelle dynamics and function, proteins expression and turnover, protein interaction, and cell motility. Chimeric fluorescent proteins allow studies of dynamic events that range in duration from less than a second up to several days.

Fluorescent recovery after photobleaching

Fluorescent recovery after photobleaching (FRAP) is a technique where fluorescent molecules are irreversibly photobleached in a defined area in the cell by a high power focused laser beam (Figure 8A) (for comprehensive reviews see (Carrero et al., 2003; Lippincott-Schwartz and Patterson, 2003; Lippincott-Schwartz et al., 2001; Reits and Neefjes, 2001; Sprague and McNally, 2005)). The movement of unbleached molecules from the surrounding areas and into the bleached area is measured by time-lapse microscopy. By plotting the intensity in the bleached area over time one can get a FRAP recovery curve, and this is a way of measuring the kinetic properties of a molecule, for example, whether a proteins is immobilized, free to diffuse, or undergoes constant exchange between organelles and compartments.

To compare various experiments and various sets of data, the empirical collected values must be normalized and also corrected for general photobleaching that might occur during image acquisition. If the whole time lapse of images undergoes a general bleaching that will have an effect on the recovery curve, and have to be corrected by using the ratio between the bleached region and one reference area (Pelkmans et al., 2001). The corrected and normalized values are given by

$$F(t)_{FRAP} = \frac{I_{ref(0)}}{I_{bleach(0)}} \times \frac{I_{bleach(t)}}{I_{ref(t)}} \qquad (1)$$

where $I_{ref(0)}$ is the total intensity in the reference region prior to bleaching, $I_{bleached(0)}$ is the total intensity of the bleached area before bleaching, $I_{bleach(t)}$ is the intensity of the bleached area over time, and $I_{ref(t)}$ is the intensity of the reference area over time.

Two main parameters can be quantified by FRAP: degree of recovery and speed of recovery (Reits and Neefjes, 2001). The mobile fraction gives the degree of recovery and can be calculated by comparing the

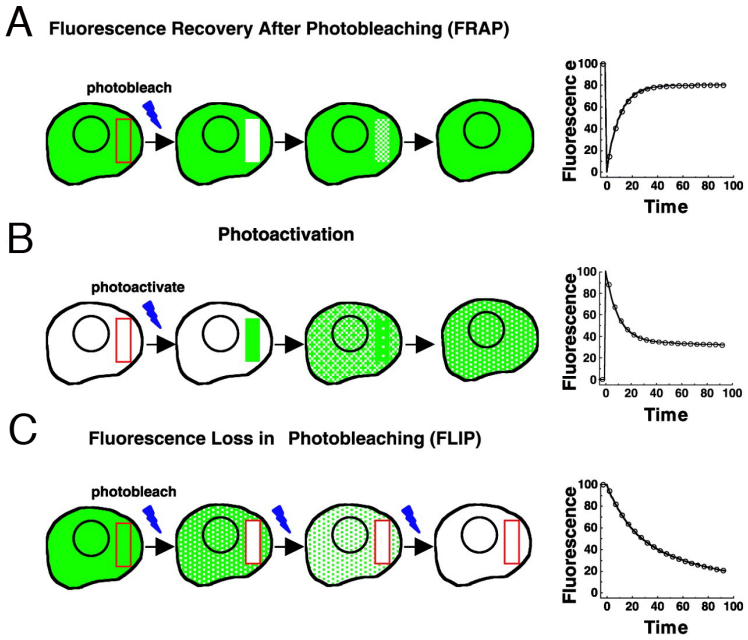

Figure 8. Techniques for photobleaching and photoactivation
One region of the cell is bleached by FRAP, and fluorescence measured before and after bleaching by time-lapse microscopy and plotted over time. Measuring how fast and how many unbleached molecules that move from the surrounding area into the bleached region can give answers of the molecules kinetics, the IF, MF and $T_{1/2}$ recovered intensity (a). Some fluorochromes have the potential to be photoactivated, and by studying such activation within the cell one can find how molecules move out from the activated region by calculating IF, MF and $T_{1/2}$ of lost intensity (b). By FLIP one region of the cell is continuously bleached during time-lapse microscopy. The loss of fluorescent intensity is measured in one region outside the bleached area, and can be plotted over time (c). IF, immobile fraction; MF, mobile fraction. Figure taken from (Lippincott-Schwartz and Patterson, 2003).

fluorescence in the bleached region after recovery (F_∞) with the fluorescence before bleaching (F_i) and just after bleaching (F_0). The mobile fraction (MF_{FRAP}) is given by

$$MF_{FRAP} = (F_\infty - F_0)/(F_i - F_0) \qquad (2)$$

If the fluorescent intensity in the bleached region does not reach the initial intensity, there will also be an immobile fraction (IF_{FRAP}). The size of the immobile fraction will depend on the mobile fraction and is defined as

$$IF_{FRAP} = (F_i - F_\infty)/(F_i - F_0) \qquad (3)$$

The sizes of the mobile and the immobile fractions can change in different settings, for example when the fluorescent protein are interacting with other molecules or membranes, and this can prevent or restrict the movement of the molecule. These restrictions can also have an effect of the other kinetic parameter, which is the speed of the recovery. This is a measurement on how fast the recovery of the mobile fraction occurs, and can be given as the diffusion coefficient, D, (um^2/second) (Cole et al., 1996; Nehls et al., 2000; Swaminathan et al., 1997), or the time where half of the recovered intensity

has been reached ($T_{1/2}$) (second) (Pelkmans et al., 2004; Raiborg et al., 2006). Computational methods can be used to calculate values both for D and $T_{1/2}$. For calculations of $T_{1/2}$ on single organelles the experimental data can be fitted by non-linear regression to

$$F(t) = \frac{F_0 + F_\infty(t/t_{1/2})}{1 + (t/t_{1/2})} \qquad (4)$$

, which assumes one diffusion coefficient (Yguerabide et al., 1982). By such an approach, a mathematic model is constructed that describes the biophysical parameters as binding and release from a substrate, residence time on an organelle, turnover and mobility. Many important properties in the cell have been discovered by this method, like the dynamics of large molecules in cytosol (Nikonov et al., 2002) and nucleus (McNally et al., 2000; Phair and Misteli, 2000), the exchange rate of cytosolic coat proteins (Pelkmans et al., 2004; Presley et al., 2002; Raiborg et al., 2006; Wu et al., 2001), the diffusion coefficient and residence time of proteins in the Golgi, organelle lumen, cytosol, and plasma membrane (Cole et al., 1996; Dayel et al., 1999; Hirschberg et al., 1998; Nehls et al., 2000; Partikian et al., 1998).

Photoactivation

Some GFP variants allow photoactivation, such that they increase their fluorescence intensity after exposure of specific wavelengths of light (Chudakov et al., 2005; Lippincott-Schwartz and Patterson, 2008). The photoactivable version of GFP (PAGFP) gains a 100-fold increase in fluorescence (with emission maximum at 517 nm) after exposure to light in the ultraviolet-to violet range (350-420 nm) (Patterson and Lippincott-Schwartz, 2002). The movement of activated molecules from one activated area and to the surroundings can be measured by time-lapse microscopy and plotted over time (Figure 8B).

The kinetic properties of molecules are measured both by FRAP and photoactivation. By FRAP we measure the movement of unbleached molecules into a bleached region, and by photoactivation we can measure the movement of activated molecules out from an activated region (Lippincott-Schwartz and Patterson, 2003). The kinetic parameters like IF, MF, and $T_{1/2}$ can also be calculated from photoactivation. The empiric data are normalized and corrected for general photobleaching similar to formula 1, and given by

$$F(t)_{PA} = \frac{I_{ref(0)}}{I_{PA(0)}} \times \frac{I_{PA(t)}}{I_{ref(t)}} \qquad (5)$$

where $I_{ref(0)}$ is the total intensity in the reference region, $I_{PA(0)}$ is the total intensity in the photoactivated area after activation, $I_{PA(t)}$ is the intensity in the activated area over time, and $I_{ref(t)}$ is the intensity in the reference region over time.

By photoactivation, the mobile fraction (MF_{PA}) gives the degree of lost fluorescent intensity in the activated region and is calculated by comparing the fluorescence in the activated area before activation (F_i) and right after activation (F_0) to the fluorescent level where no further molecules leaves the area (F_∞). MF_{PA} is given by:

$$MF_{PA} = (F_0 - F_\infty)/(F_0 - F_i) \qquad (6)$$

If the value for F_∞ is higher than F_i there will be an immobile fraction (IF_{PA}), which is given by

$$IF_{PA} = (F_\infty - F_i)/(F_0 - F_i) \quad (7)$$

The $t_{1/2}$ of lost intensity can, as for FRAP, be calculated by non-linear regression by equation 4.

Fluorescent loss in photobleaching

Fluorescent loss in photobleaching (FLIP) is another method where loss of fluorescent signal is measured (Figure 8C). By FLIP, fluorescence in one area of the cell is continuously bleached, and the fluorescent intensity measured in a region of interest. If fluorescent molecules can diffuse into the area that is bleached, loss off fluorescence will occur. FLIP can be used for many purposes, like to rule out continuity of membrane systems like ER (Cole et al., 1996; White and Stelzer, 1999), or diffusion and transport of both soluble and membrane bound proteins (Cole et al., 1996; Nehls et al., 2000; Subramanian and Meyer, 1997; Zaal et al., 1999). The measured loss of fluorescence will much depend on the intensity of the bleaching laser, and therefore it is a non-quantitative method, however, it can be a powerful tool to investigate transport or diffusion of proteins, and also if proteins are restricted to areas and do not diffuse (Lippincott-Schwartz et al., 2001).

Aims of the present study

In the endocytic pathway, there is a fast dynamic of lipids, cargo, membrane proteins and coat proteins. The whole pathway is in a fine tuned equilibrium of interactive forces provided by these highly mobile components. A single change, such as a phosphorylation or ubiquitinylation event, down- or up-regulation of a kinase, might have the potential to shift this complex interplay, resulting in changes in membrane trafficking, fission, fusion, sorting, and maturation.
During mitosis, the distribution of organelles into the two daughter cells is a process that is well studied for some organelles, but for others, like endosomes we do not know much. By the entry of mitosis the membrane trafficking undergoes major reorganizations. The secretion is known to be inhibited, and there is also a cessation in the endocytic pathway. Recycling of components from endosomes and to the plasma membrane is inhibited, and cell free studies show that fusion of endocytic vesicles are arrested.
We have studied the process of endocytosis in living cells by utilizing confocal microscopy, FRAP, FLIP, photoactivation and the use of both endogenous (GFP fusion proteins) and exogenous markers (uptake of fluorescent probes). Techniques and conditions had to be developed to study in particular dividing cells, fluorescent intensities of single organelles over time, and fusing endosomes, in order to reach the aims stated below:

Paper I: Study the stability of endosomes during cell division, how the copy number is maintained, and further determine the inheritance strategy of endosomes and lysosomes during mitosis, in particular whether this is a stochastic or an ordered process. Finally, study how the number of endosomes increases in daughter cells, if this is by fission of inherited endosomes.

Paper II: Investigate the membrane-to-cytosol cycling of EEA1, and further describe the kinetic properties of the molecule in a fusion active state (interphase), and in a situation where fusion is down regulated (mitosis).

Paper III: Investigate in detail the fusion process of early endosomes, how the actual merge of membrane occurs, and further how the newly fused organelle operates in the cell.

Synopsis of included papers

Paper I. Mitotic partitioning of endosomes and lysosomes

Since little was known about the fate endosomes and lysosomes in dividing cells this project started with morphological investigations of these organelles prior to, during and after mitosis. We were able to study both early and late endosomes/lysosomes during cell division by video and confocal microscopy. Prior to mitosis, both early and late endosomes had a dispersed distribution throughout cytoplasm; however, right after cytokinesis both organelles accumulated at a juxtanuclear position. Judging from single confocal plane images, there seemed to be an extensive colocalization, and to study this in detail we decided to take 3D images of dividing cells. By this approach, we found no colocalization and we concluded that early and late endosomes are distinct entities in mitosis as they are in interphase.

Further, by tracking single endosomes we were able to show that both early and late endosomes had, right after cytokinesis, coordinated movements toward the juxtanuclear position. This observation prompted us to investigate if there might be an order in the distribution of early and late endosomes into the newly formed daughter cells. We started by counting the number of endosomes in the mother cell and studied how these ended up in the two daughter cells. This partitioning was compared to a random binomial distribution, and we found that there were no strict mechanisms in the partitioning process. Further, since other members of the endomembrane system, like the ER and the Golgi, are reported to fragment and vesiculate during mitosis our final experiment in this project was to investigate if this also happens for endosomes. Endosomes are small in size and a possible fragmentation would not be visible in a light microscope. We decided therefore to enlarge the endosomes by overexpression of CD74 (see Methodological Considerations). This approach allowed us to study single endosomes in more details, and we found no fragmentation during division.

In summary, early and late endosomes are stable structures in mitosis. The coordinated movements of endosomes suggest an order in the distribution of endosomes, the partitioning is however imprecise. Further, an interesting observation during this project was the shift of EEA1-GFP from membrane bound locations to a more cytosolic distribution. This led us to the suggestion that there might be a mitotic shift in the membrane to cytosol cycling of EEA1, and this we have addressed in PAPER II.

Paper II. Cell cycle dependent binding kinetics for the early endosomal tethering factor EEA1

To study the membrane to cytosol cycling of EEA1 we used FRAP and photoactivation of EEA1 fused to GFP or PAGFP. We wished to examine single organelles, and normal size organelles are too small to be studied in detail by light microscopy. Therefore, we transfected our cells with CD74, which induce the formation of enlarged endosomes. By FRAP we found that EEA1 rapidly cycles on/off the membrane, and by photoactivation we found that EEA1 rapidly dissociates from the membrane and back to

cytosol. We were actually able to determine that it is the dissociation of EEA1 that is the regulating step in the on/off cycling.

Further, we found that there were two fractions of EEA1 on the membrane of early endosomes, one rapidly cycling on/off membranes and another fraction that was more resident. The speed of dissociation and the size of the resident fraction were not constant. In mitosis where fusion is down regulated, we found an accelerated dissociation and a reduced resident fraction of EEA1 when compared to cells in interphase. On the contrary, when we promoted fusion by overexpression of Rab5 we found a retarded dissociation and an increased resident fraction. EEA1 is present on a fusion active and a fusion inactive state. The capacity for tethering seems to be regulated by the dissociation kinetics for EEA1.

Paper III. Endosomal fusion stimulates tubular fission

Our major goal was to study fusion of early endosomes in detail, and further investigate how the early endosomes handled the access of membrane following a homotypic fusion. By studying docking zones of adjacent enlarged early endosomes with rapid 3D time-lapse microscopy, we found that the fusion pore originated from one single spot in the centre of the docking zone, and it expanded by a radial growth. Further within the docking zone we found a shift in the on/off cycling of EEA1. The residence time of EEA1-GFP increased within the contact zone when compared to a control area outside this zone. We speculate if this could be a result of EEA1 acting as a tethering molecule.

Right after the fusion we found extensive tabulations and fission, and this was regulated both by interactions with microtubule and actin. When we incubated the cells with the microtubule-depolymerizing agent, nocodazole, we found no tubulation after fusion. Instead we found inward budding and internal membrane structures. We also found an increase of fusion and a growth in size of the already enlarged early endosomes as well as normal size endosomes. The results were quite distinct when we inhibited actin polymerization in the cell by adding latrunculin A. Now we found tubulations following fusion; however, the emerging speed of the tubules was twice the speed when compared to cells with actin.

We conclude that the membrane area/volume ratio may be preserved after fusion by tubulation and fission under strict control of the cytoskeleton. We further suggest that fusion itself might be an important regulator for membrane fission.

Discussion

In this thesis we have investigated the fate of endosomes through fusion and the fate of the endocytic pathway through cell cycle. We have specially focused how the temporal distribution of EEA1 changes in various settings. This discussion will involve how EEA1 is recruited to early endosomes and how it functions as a tethering molecule prior to SNARE function and fusion. The discussion will also include how cytoskeletal elements play a role on the positioning and morphology of endosomes and lysosomes.

The membrane to cytosol cycling of EEA1 is not constant

In all the papers presented in this the-

sis (PAPER I, II and III) we show that the fraction of EEA1 that is interacting with the membrane of early endosomes is not constant. The equilibrium between the cytosolic pool and the membrane bound pool shifts, during cell cycle. In PAPER I we show relocation toward cytosol of EEA1-GFP in mitosis. This relocation was further investigated in PAPER II where we discovered two changes in the distribution. First, we found that there were two fractions of EEA1-GFP on the endosomal membranes, one mobile fraction and another fraction that was more residently bound to the membrane. When we investigated this resident fraction in detail we discovered that it actually had some degree of exchange. We named the fraction immobile, which can be misleading, however, the exchange half-life for the immobile fraction was 20 times longer as for the mobile fraction, and immobile is also the term used by others (Lippincott-Schwartz and Patterson, 2003; Lippincott-Schwartz et al., 2001).

In metaphase the immobile fractions were significantly reduced in all our experiments. We found also that speed of exchange for EEA1-GFP increased in mitosis. Both these changes depend on the ability of EEA1 to interact with Rab5 and PtdIns3P (PAPER II). Further, in interphase, we showed that the on/off kinetics of EEA1 also depends on interacting endosomes (PAPER III). The on/off kinetics of EEA1 is very sensitive to the number of interacting partners that are available. This we suggest to be mechanisms to control the action of this protein (see below, discussed in PAPER II and III).

Membrane-to-cytosol cycling of EEA1 is regulated through interactions with Rab5 and PtdIns3P

Both Rab5 and PtdIns3P must be present on the endosome membrane to recruit EEA1, and in PAPER II we show that Rab5 and PtdIns3P also have an effect on the variations in kinetics for EEA1. By using one PtdIns3P specific probe 2xFYVE-GFP and FRAP on single enlarged organelles we show that an elevated level of the GTPase defective $Rab5_{Q79L}$ (Rab5-GTP) causes a shift in the on/off cycling of 2xFYVE-GFP, where both the immobile fractions and the t1/2 intensities increase upon Rab5-GTP expression. We explain this observation to be the consequence of more PtdIns3P present. These data also suggest that Rab5 regulates the on/off kinetics of EEA1 through its FYVE domain by controlling the level of PtdIns3P. In addition of being a direct effector of Rab5, EEA1 is also recruited to the membrane indirectly by kinase activity of the Rab5 effector VPS34/p150 and production of PtdIns3P. However, the differences in kinetics are more pronounce for EEA1-GFP than for 2xFYVE-GFP upon expression of Rab5-GTP. Therefore we conclude in PAPER II that the direct interactions between the Rab5 and EEA1 are dominant, whereas interactions both by Rab5 and PtdIns3P are required, as others also have reported (Mills et al., 1998; Mills et al., 2001; Patki et al., 1997; Simonsen et al., 1998).

The machinery that controls the level of Rab5 and PtdIns3P on the membrane of endosomes is highly complex. Therefore, the observed mitotic shift toward cytosol (PAPER I) and the measured mitotic shift in on/off kinetics (PAPER II) for EEA1-GFP/

PAGFP can be the result of many events. In PAPER II, we show a reduction in the level of PtdIns3P in metaphase cells, and this we also show to be the result of the mitotic shift we observe in the on/off kinetic for 2xFYVE-GFP. However, the mitotic shift in kinetics for EEA1-GFP is more distinct than for 2xFYVE-GFP, therefore other factors must be involved, and there are many possible scenarios. It is reported that Rab5 is mitotically phosphorylated (Chiariello et al., 1999), and a phosphorylated Rab5 might interfere with the Rab5-EEA1 interaction. It is also reported that EEA1 is phosphorylated (thr-1392) by a MAP kinase (Mace et al., 2005), and there might be a possibility that the mitotic changes in kinetics for EEA1 are caused by levels of phosphorylation of EEA1 itself. Finally, a mitotic shift in kinetics for EEA1 could also be due to changes in the activity for the Rab5-GEFs, -GAPs, -GDIs, or -GDFs. A shift in activity level for these proteins might cause a change in the amount of active Rab5 on the membrane of early endosomes. The membrane-to-cytosol cycling of EEA1 seem to be the result of a fine tuned interaction of many factors, and the mitotic shift could be due to one or several changes in this network.

EEA1 and fusion

The SNAREs facilitate a crucial step for fusion. This was elegantly proved some years ago, where flipped SNAREs caused cell fusion (Hu et al., 2003). By flipping the SNAREs, Rothman and colleagues showed that fusion of membranes was possible without the upstream factors like the tethering proteins. However, in a normal cell without flipped SNAREs, EEA1 plays an important role for the specificity of which membranes that are brought in close proximity to potentiate the formation of *trans*-SNARE complexes (Reviewed in (Zerial and McBride, 2001)).

In this thesis we have investigated kinetics of EEA1 in systems where we from literature know fusion is up- or down-regulated (PAPER II), and shown that between these systems the EEA1 kinetics is highly variable. On the one hand, when fusion is promoted by Rab5 expression, the immobile fraction is high and the half-life of the mobile fraction is slow. On the other hand, when fusion is down regulated in mitosis, the immobile fraction is low and the half-life of the mobile fraction is fast. These shifts, both for the stable fraction and also the exchange half-life, we suggest to be important for recruitment and activation of the downstream proteins as Syn13 and formation of *trans*-SNARE complex (McBride et al., 1999). In metaphase, where most of EEA1 is rapidly cycling on/off membranes we suggest few molecules to facilitate tethering and the result is little formation of *trans*-SNARE complexes. In interphase, upon Rab5wt or Rab5-GTP expression, where we find a large immobile fraction and a more slow exchange of the mobile fraction, more *trans*-SNARE complexes may be formed and here the outcomes are more fusion (PAPER II), as also reported by others (Gorvel et al., 1991; Li et al., 1995; Simonsen et al., 1998; Stenmark et al., 1994). Therefore, the on/off kinetics for EEA1 we suggest to control fusion of early endosomes.

In PAPER II the kinetics of EEA1 was investigated on single organelles. In PAPER III we decided to study EEA1 in more detail in the contact zone between two vesicles, which is the place where EEA1 carry out its

action. To investigate the contact zone we used two versions of EEA1, one short version that contains the C-terminal FYVE domains and Rab5 interactions sites (CtEEA1), and the full-length version (EEA1). CtEEA1 does not contain the coiled-coil domain, which is important for dimerization, and therefore most likely present as a monomer in the cell. It does not contain the N-terminal Rab5 interaction motif, and therefore it does not provide any tethering itself, and will primarily function as a reporter for membranes positive of Rab5 and PtdIns3P. The full-length version contains both the coiled-coil region and the N-terminal interaction motif for Rab5. Our data from PAPER II support that EEA1 operates as a parallel dimer also when fused to GFP.

The kinetic for both CtEEA1 and EEA1 varies in PAPER II and III, and this is due to the experimental set-ups. In PAPER II we measure the kinetics on one whole endosome, and in PAPER III we measure the kinetics on one region on one endosome. In PAPER II we measure on/off cycling only, whereas in PAPER III we measure on/off cycling together with lateral diffusion. Both CtEEA1 and EEA1 were highly mobile outside the contact region, and in PAPER III both molecules experienced a complete recovery most likely due to lateral diffusion. The situations were different within the contact region where EEA1 was more strictly bound compared to CtEEA1 (PAPER III). Most of EEA1 was also exchanged with the cytosolic pool also within the contact region, however, one fraction was stable, and this could be due to the bipolar interactions for EEA1, where the C-terminus interacts with one vesicle and the N-terminus with the other adjacent vesicle. We speculate if this is the actual tethering provided by EEA1 that promote formation of the *trans*-SNARE complex that leads to fusion.

Cytoskeletal interactions on endosomes

The positioning of endocytic organelles seems to be regulated by the sum of forces provided by the variety of interactions with the microtubule (Soldati and Schliwa, 2006). The pulling strength can be decided by the number of motor proteins, but also on their level of activation, like phosphorylated dynein which is shown to negatively regulate the capacity of microtubule binding (Vaughan et al., 2002). In PAPER I, we show coordinated movements toward a juxtanuclear position of both early endosomes and late endocytic compartments at late stages of mitosis, in line with earlier reports (Dunster et al., 2002; Kaplan et al., 1992; Zeligs and Wollman, 1979) where a clustering of endosomes at the mitotic spindle and poles was shown. Since we see a movement toward MTOC, it is possible that we have an activation of dynein, or a down regulation of minus-end directed kinesins. It is shown, upon insulin treatment of cell, that the activity level of dynein is controlled by its level of phosphorylation, however, this was not investigated in mitosis (Huang et al., 2001). It is also possible that relocation is caused by mitotic changes in the activity of Rab proteins. The mitotic phosphorylation and distribution to cytosol for Rab4 (Ayad et al., 1997; van der Sluijs et al., 1992) can explain the loss of exocytic activity through KIF3B. The recruitment of KIF16B to the membrane depends on Rab5 activity through VPS34/p150. The decrease in the amount of PtdIns3P in mitosis (PAPER II) could cause less

KIF16B to be recruited, and this, with an unchanged dynein/dynactin activity, could explain the relocation of the early endosomes toward MTOC. However, this picture is not completely clear, since Rab5 and VPS34/p150 also regulate minus end directed movements of endosomes, and both dynein and kinesin are found on these organelles (Loubery et al., 2008; Nielsen et al., 1999).

We show in PAPER III that tubule formation after early endosome fusion is dependent on interaction with both actin and microtubule. The formation of tubules was completely reliant on microtubules since no tubules were present after nocodazole treatment. Actin had a regulatory role on the emerging speed of the tubules, since the speed increased after lantruncullin A treatment. In mammalian cells, most of the long range directed movements out of early endosomes are reported to occur through interactions with the microtubules, and both dyneins and kinesins are present on early endosomes (Soldati and Schliwa, 2006). Both classes of microtubule motors could form the tubules we study in PAPER III. The tubules were positive of EEA1 and therefore also likely to be positive of both Rab5 and PtdIns3P. The kinesin KIF16B also binds PtdIns3P and therefore is a potent candidate responsible for the tubulation. However, there are also other candidates: for example, Rab5 was reported to interact directly with the dynein/dynactin complex, and the level of active GTP bound Rab5 is a regulator both for cargo interaction and also for minus-end direction (Huang et al., 2001).

One general view is that actin mediated transport is important in the earliest stages of the endocytic pathway, span from the plasma membrane and down through the actin network underneath the plasma membrane. However, there is now growing evidence that actin might play several roles in vesicular motility many places in the endocytic pathway, on early endosomes, late endosomes and lysosomes (for a recent review see (Girao et al., 2008)). Both myosins and also components important for actin nucleation, like the Apr2/3 complex, are found on early endosomes (Llado et al., 2008). The contributions from Myosin-I and VI are reported to play a role in the recycling from early endosomes back to the plasma membrane (Chibalina et al., 2007; Raposo et al., 1999). We do not know if the observed tubules we observe in PAPER III contain elements that are interacting with actin; however it is likely to be the case, since we observe an increased emerging speed on the tubules in the presence of latrunculin A.

Methodological considerations

Enlarged endocytic structures

In this thesis I have enlarged early and late endosomes in three different ways, by overexpression of the CD74 (isoform p33), Rab5 wild type (wt) or Rab5-GTP, and the mechanisms behind these enlargements differ. CD74 is a type II transmembrane glycoprotein that has several important functions in antigen presentation. In ER, CD74 trimers associate with MHC class II molecules, and the complex is sorted to the endocytic pathway where they meet endocytosed antigens (for reviews see (Germain, 1994; Nordeng et al., 1998)). CD74 gradually degrades from the luminal side, however, the transmem-

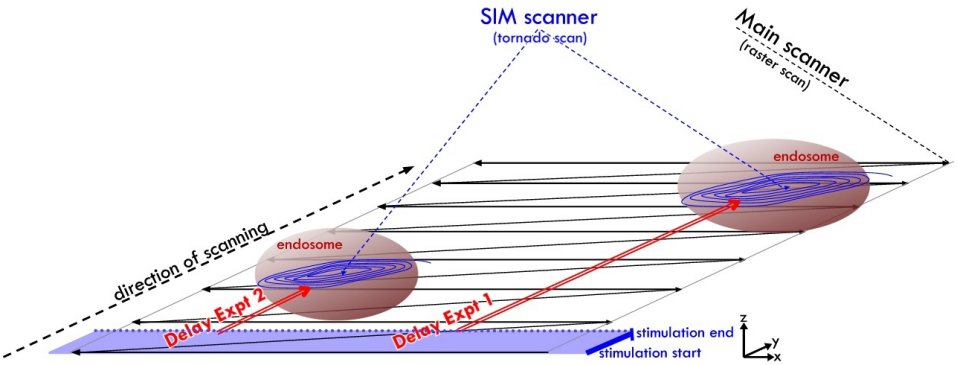

Figure 9. Simultaneous light stimulation and imaging
Illustration of how varying locations of a stimulated area can result in different delays between stimulation and scanning of the stimulated area. Blue area indicates start and end of stimulation. The circular enclosed areas indicate two examples where the stimulating light can be exposed. Figure taken from (Bergeland et al., 2006).

brane and cytosolic part of the molecule is more stable (Amigorena et al., 1995). Upon expression at high levels, CD74 is reported to accumulate in enlarge endosomal compartments and also cause a delayed endocytic transport (Gorvel et al., 1995; Pieters et al., 1993; Romagnoli et al., 1993). Morphological studies on these enlarged structures have revealed similarities to both early and late endosomes (Stang and Bakke, 1997). To make enlarged endocytic structures, CD74 has to trimerize and contain the 30 amino acid N-terminal cytosolic tail (Claesson and Peterson, 1983; Gedde-Dahl et al., 1997; Koch et al., 1987b; Lipp et al., 1987). It has been shown *in vitro* that the cytosolic tail of CD74 can cause fusion, and it is suggested that the tails of CD74 on adjacent endosomes can interact and dock the endosomes (Nordeng et al., 2002).

Overexpression of both the Rab5-GTP, and Rab5wt has been found to increase the recruitment of EEA1 on early endosomes. This is further suggested to give an increase in the tethering provided by EEA1 and consequently an enhanced fusion of early endosomes (Gorvel et al., 1991; Li et al., 1995; Simonsen et al., 1998; Stenmark et al., 1994) (See discussion and PAPER II). Some years ago it was thoroughly investigated if the enlargement from CD74 expression was independent from Rab5 and visa versa (Nordeng et al., 2002). First, cells have been double-transfected cells with CD74 and a dominant negative Rab5 mutant ($Rab5_{S34N}$) that has been found to inhibit endosome fusion and to result in smaller endosomes. When co-expressed with CD74, enlarged endosomes were still formed. Second, cells have been double-transfected with Rab5-GTP and $CD74_{D6R}$, a mutant not capable of forming enlarged endosomes. The expression of $CD74_{D6R}$ did not inhibit formation of enlarged endosomes. Third, the formation of large endosomes by CD74 was not sensitive to wortmannin, which indicates that the CD74 mediated fusion, was not dependent in PI3-kinase activity. Thus, we can be quite confident that the enlargement of endosomes through Rab5 and CD74 are independent processes

Live microscopy

Most of the microscopy in this thesis was done on living cells, and it is very important that the conditions on the microscope table are as good as possible for the cells. There are several aspects to have in mind, and these are important to take into consideration before every experiment since we do not want to study stressed or dying cells. The growth medium for microscopy does not contain a pH-indicator, therefore it is important to check the pH in the microscopy medium to verify that it is at a neutral pH. It is always important to minimize the amount of light into the microscope to prevent phototoxicity. The optimal growth temperature for MDCK II used in this study is 37°C. To obtain a stable temperature is important for the intracellular events that we study, especially rapid on/off cycling of EEA1, where control FRAP experiments showed statistical significant differences in the on/off cycling of EEA1 at 22°C when compared to 37°C (unpublished data (T.Bergeland)).

Simultaneous light stimulation and imaging

We used a confocal system with a proprietary SIM-scanner (simultaneous light stimulation and imaging) to simultaneously stimulate (bleach/photoactivate) and image (FRAP, photoactivation, and FLIP). Using this setup, one can use an independent laser for light stimulation while recording images with the main scanner (Figure 9). Structures of interest can be selected and stimulated during scanning, something that facilitate accurate measurements immediately after scanning (Bergeland et al., 2006). There are some important aspects to have in mind when measuring rapid kinetics after stimulation. First, it is important to have no pixel saturation. In the FRAP experiments, this is especially important and relevant when finding the initial pre-bleach intensity of the structures that will become bleached. In the photoctivating experiments, it is important not to get saturation in the activated area. The calculations of the kinetic parameters are all based on fluorescent signals and saturations might cause incorrect immobile fractions and $t_{1/2}$ intensities. Second, it is essential to know the exact interval between stimulation and measurement. If a stimulated area appears late in the frame, it will be a longer delay compared to an area early in the frame. The location of the stimulated region within the image will usually vary for each experiment, resulting in different delays between stimulation and start of measurement. The exposure of the stimulating laser can be controlled on a millisecond level, and the images acquired using the main scanner also have a millisecond precision. Therefore, it is possible to make repetitive setup in one line of experiments.

Conclusions and future perspectives

Many scientists are fascinated about the dynamics of membranes and proteins in the endomembrane system. As a researcher I would say it is satisfying to observe how vesicles move within the cell, how organelles fuse, how they develop, and how they divide. By just observing these multitudes of events by my own eyes, it reminds me that one single cell is very complex, and the proteins I have studied so far will only give fractions of answers in how membrane trafficking is regulated. The work presented in

this thesis gives some important insights into the biogenesis in the endocytic pathway, and how the flux of membrane within this system is regulated. It also leaves some interesting unanswered and open questions.

The definition of organelles has, in general, been based on the presence of specific organelle markers. Rab5 and EEA1 are widely accepted as early endosomal markers. Our work, however, shows that the simple presence of a specific marker on an organelle does not necessarily indicate its function as the binding kinetics may also be determining such function. This is an important finding, and one interesting question is whether this is a general mechanism. Several promising proteins emerge as candidates for further testing. Rab proteins are key regulators of trafficking, and in the endocytic pathway the most studied proteins are Rab4, Rab5, Rab7 and Rab11. It will be interesting to test if these proteins have a similar behavior pattern as EEA1. Other potentially attractive candidates are the lipid binding PX- and FYVE-domain proteins.

Many infectious bacteria have evolved into niches within organelles where they can control the cell in various ways to make a suitable environment for replication. On the one hand, *Salmonella enterica* have their replication niche in vacuoles similar to late endosomes that contain Rab7, Vacuolar ATPase, Lamp and Limp proteins but little M6PR or acidic hydrolases like Cathepsin D (Haraga et al., 2008). On the other hand, *Brucella* has their replication niche in vacuoles similar to ER (Starr et al., 2008). At early stages of infection both *Salmonella*- and *Brucella*- containing vacuoles are positive for EEA1 (Bujny et al., 2008; Pei et al., 2008), and it would be very interesting to investigate if these different ways of sorting have any effect on the kinetics on the many coat proteins in the endocytic pathway.

One cell can undergo many different states of activity that cause changes in membrane trafficking. For example, when a cell differentiates from a non-polarized to a polarized state, there is a segregation of the endocytic system that involves an apical and basolateral sorting as well as transcytosis (Perret et al., 2005). One other example is stimulation of the endocytic pathway through EGF treatment where endocytosis is increased and also the sorting into the endosomal internal structures (Gaborik and Hunyady, 2004). We have here shown that the kinetic properties of EEA1 depend on the cells stage in the cell cycle, mitosis. However, the changes in trafficking that are reported both in polarized cells and during EGF treatment could have effects on the membrane-to-cytosol cycling of many cytosolic coat proteins. There are many potential interesting candidates and at the plasma membrane it would be interesting to test the many components involved in CME like subunits of the AP-2 complex. On endosomes it would be interesting to measure the kinetics for Rab4, Rab5, Rab7, Rab11. It would also be interesting to investigate the machinery responsible for sorting components into internal luminal structures.

In this thesis we have shown that interactions with the cytoskeleton are important for regulation of the size of endosomes, where the presence of microtubule is crucial in exit of membrane after fusion through formation of tubules that lead to fission. The next step in this project is to identify the specific motor proteins that are involved in this process. One approach is to knock out specific motor proteins that have a function in

the endocytic pathway, like KAP-3 which in a subunit in Kinesin-2 (Young et al., 2005). Another approach is to overexpress dynamitin (p50) subunit of the dynactin complex. This overexpression inhibits the action of the dynein/dynactin complex (Burkhardt et al., 1997).

Interaction between the cytoskeleton and endocytic organelles we suggest also to be important when the organelles are clustered by directional movements toward the newly formed daughter cells at late stages of mitosis. Approximately half of the endocytic organelles from the mother cell are delivered to each daughter cell, and we conclude that the partitioning is ordered, however, imprecise. One interesting aspect to further investigate in this project would be to study the newly divided endocytic structures longer in the daughter cells, until the next cell division. The concept of using a GFP fused marker that is biosynthetically produced together with a chased fluorescent probe will be powerful. On the one hand, the fluorescent probe will give the portion of the maternal, inherited structures. On the other hand, the GFP fused markers will give answers on the biosynthesis of newly formed organelles. This approach has the potential to give new reflections and answers on the biogenesis of endocytic structures.

The knowledge of endocytic trafficking and mitosis is important for understanding many events in the cell, and further, this knowledge is vital for understanding the human body, infections and also sometimes diseases. When one cell divides into two daughter cells many processes are activated, but many other processes are arrested or down regulated. The switches that occur in dividing cells can give important clues on how proteins operate and function. We have investigated the endocytic pathway both in interphase and mitosis, and we have specifically focused on EEA1-positive compartments. We have found that the function of EEA1 depend on the membrane to cytosol cycling, and we speculate if this is a general mechanism the cell uses to regulate the action of cytosolic proteins. Many more proteins have to be tested both through interphase and mitosis, and that can give clues in how the trafficking and organelle biogenesis occur in the endocytic pathway.

References

Albertson, R., Riggs, B. and Sullivan, W. (2005) Membrane traffic: a driving force in cytokinesis. Trends Cell Biol, 15, 92-101.

Allan, V.J., Thompson, H.M. and McNiven, M.A. (2002) Motoring around the Golgi. Nat Cell Biol, 4, E236-242.

Altan-Bonnet, N., Sougrat, R., Liu, W., Snapp, E.L., Ward, T. and Lippincott-Schwartz, J. (2006) Golgi inheritance in mammalian cells is mediated through endoplasmic reticulum export activities. Mol Biol Cell, 17, 990-1005.

Amigorena, S., Webster, P., Drake, J., Newcomb, J., Cresswell, P. and Mellman, I. (1995) Invariant chain cleavage and peptide loading in major histocompatibility complex class II vesicles. J Exp Med, 181, 1729-1741.

Andrews, R. and Ahringer, J. (2007) Asymmetry of early endosome distribution in C. elegans embryos. PLoS ONE, 2, e493.

Antonin, W., Fasshauer, D., Becker, S., Jahn, R. and Schneider, T.R. (2002) Crystal structure of the endosomal SNARE complex reveals common structural principles of all SNAREs. Nat Struct Biol, 9, 107-111.

Aschenbrenner, L., Naccache, S.N. and Hasson, T. (2004) Uncoated endocytic vesicles require the unconventional myosin, Myo6, for rapid transport through actin barriers. Mol Biol Cell, 15, 2253-2263.

Ayad, N., Hull, M. and Mellman, I. (1997) Mitotic phosphorylation of rab4 prevents binding to a specific receptor on endosome membranes. Embo J, 16, 4497-4507.

Baluska, F., Menzel, D. and Barlow, P.W. (2006) Cytokinesis in plant and animal cells: endosomes 'shut the door'. Dev Biol, 294, 1-10.

Barr, F.A. and Egerer, J. (2005) Golgi positioning: are we looking at the right MAP? J Cell Biol, 168, 993-998.

Beaudouin, J., Gerlich, D., Daigle, N., Eils, R. and Ellenberg, J. (2002) Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina. Cell, 108, 83-96.

Bergeland, T., Tewinkel, M. and Bakke, O. (2006) Precise calculations of photoactivation kinetics. Nat Methods, 3, iii-iv.

Berlin, R.D. and Oliver, J.M. (1980) Surface functions during mitosis. II. Quantitation of pinocytosis and kinetic characterization of the mitotic cycle with a new fluorescence technique. J Cell Biol, 85, 660-671.

Berlin, R.D., Oliver, J.M. and Walter, R.J. (1978) Surface functions during Mitosis I: phagocytosis, pinocytosis and mobility of surface-bound Con A. Cell, 15, 327-341.

Birky, C.W., Jr. (1983) The partitioning of cytoplasmic organelles at cell division. Int Rev Cytol Suppl, 15, 49-89.

Blocker, A., Severin, F.F., Burkhardt, J.K., Bingham, J.B., Yu, H., Olivo, J.C., Schroer, T.A., Hyman, A.A. and Griffiths, G. (1997) Molecular requirements for bidirectional movement of phagosomes along microtubules. J Cell Biol, 137, 113-129.

Blow, J.J. and Tanaka, T.U. (2005) The chromosome cycle: coordinating replication and segregation. Second in the cycles review series. EMBO Rep, 6, 1028-1034.

Bobinnec, Y., Marcaillou, C., Morin, X. and Debec, A. (2003) Dynamics of the endoplasmic reticulum during early development of Drosophila melanogaster. Cell Motil Cytoskeleton, 54, 217-225.

Bokel, C., Schwabedissen, A., Entchev, E., Renaud, O. and Gonzalez-Gaitan, M. (2006) Sara endosomes and the maintenance of Dpp signaling levels across mitosis. Science, 314, 1135-1139.

Bonifacino, J.S. (2004) The GGA proteins: adaptors on the move. Nat Rev Mol Cell Biol, 5, 23-32.

Bonifacino, J.S. and Hurley, J.H. (2008) Retromer. Curr Opin Cell Biol, 20, 427-436.

Bonifacino, J.S. and Rojas, R. (2006) Retrograde transport from endosomes to the trans-Golgi network. Nat Rev Mol Cell Biol, 7, 568-579.

Boucrot, E. and Kirchhausen, T. (2007) Endosomal recycling controls plasma membrane area during mitosis. Proc Natl Acad Sci U S A, 104, 7939-7944.

Bright, N.A., Gratian, M.J. and Luzio, J.P. (2005) Endocytic delivery to lysosomes mediated by concurrent fusion and kissing events in living cells. Curr Biol, 15, 360-365.

Brown, E.J., Albers, M.W., Shin, T.B., Ichikawa, K., Keith, C.T., Lane, W.S. and Schreiber, S.L. (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature, 369, 756-758.

Bujny, M.V., Ewels, P.A., Humphrey,

S., Attar, N., Jepson, M.A. and Cullen, P.J. (2008) Sorting nexin-1 defines an early phase of Salmonella-containing vacuole-remodeling during Salmonella infection. J Cell Sci, 121, 2027-2036.

Burke, B. and Ellenberg, J. (2002) Remodelling the walls of the nucleus. Nat Rev Mol Cell Biol, 3, 487-497.

Burkhardt, J.K., Echeverri, C.J., Nilsson, T. and Vallee, R.B. (1997) Overexpression of the dynamitin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution. J Cell Biol, 139, 469-484.

Callaghan, J., Nixon, S., Bucci, C., Toh, B.H. and Stenmark, H. (1999a) Direct interaction of EEA1 with Rab5b. Eur J Biochem, 265, 361-366.

Callaghan, J., Simonsen, A., Gaullier, J.M., Toh, B.H. and Stenmark, H. (1999b) The endosome fusion regulator early-endosomal autoantigen 1 (EEA1) is a dimer. Biochem J, 338 (Pt 2), 539-543.

Caplan, S., Hartnell, L.M., Aguilar, R.C., Naslavsky, N. and Bonifacino, J.S. (2001) Human Vam6p promotes lysosome clustering and fusion in vivo. J Cell Biol, 154, 109-122.

Carrero, G., McDonald, D., Crawford, E., de Vries, G. and Hendzel, M.J. (2003) Using FRAP and mathematical modeling to determine the in vivo kinetics of nuclear proteins. Methods, 29, 14-28.

Chapuy, B., Tikkanen, R., Muhlhausen, C., Wenzel, D., von Figura, K. and Honing, S. (2008) AP-1 and AP-3 mediate sorting of melanosomal and lysosomal membrane proteins into distinct post-Golgi trafficking pathways. Traffic.

Chavrier, P., Parton, R.G., Hauri, H.P., Simons, K. and Zerial, M. (1990) Localization of low molecular weight GTP binding proteins to exocytic and endocytic compartments. Cell, 62, 317-329.

Chen, Y.A. and Scheller, R.H. (2001) SNARE-mediated membrane fusion. Nat Rev Mol Cell Biol, 2, 98-106.

Chiariello, M., Bruni, C.B. and Bucci, C. (1999) The small GTPases Rab5a, Rab5b and Rab5c are differentially phosphorylated in vitro. FEBS Lett, 453, 20-24.

Chibalina, M.V., Seaman, M.N., Miller, C.C., Kendrick-Jones, J. and Buss, F. (2007) Myosin VI and its interacting protein LMTK2 regulate tubule formation and transport to the endocytic recycling compartment. J Cell Sci, 120, 4278-4288.

Chow, A., Toomre, D., Garrett, W. and Mellman, I. (2002) Dendritic cell maturation triggers retrograde MHC class II transport from lysosomes to the plasma membrane. Nature, 418, 988-994.

Christoforidis, S., Miaczynska, M., Ashman, K., Wilm, M., Zhao, L., Yip, S.C., Waterfield, M.D., Backer, J.M. and Zerial, M. (1999) Phosphatidylinositol-3-OH kinases are Rab5 effectors. Nat Cell Biol, 1, 249-252.

Chudakov, D.M., Lukyanov, S. and Lukyanov, K.A. (2005) Fluorescent proteins as a toolkit for in vivo imaging. Trends Biotechnol, 23, 605-613.

Claesson, L. and Peterson, P.A. (1983) Association of human gamma chain with class II transplantation antigens during intracellular transport. Biochemistry, 22, 3206-3213.

Colanzi, A. and Corda, D. (2007) Mitosis controls the Golgi and the Golgi controls mitosis. Curr Opin Cell Biol, 19, 386-393.

Colanzi, A., Hidalgo Carcedo, C.,

Persico, A., Cericola, C., Turacchio, G., Bonazzi, M., Luini, A. and Corda, D. (2007) The Golgi mitotic checkpoint is controlled by BARS-dependent fission of the Golgi ribbon into separate stacks in G2. Embo J, 26, 2465-2476.

Cole, N.B., Smith, C.L., Sciaky, N., Terasaki, M., Edidin, M. and Lippincott-Schwartz, J. (1996) Diffusional mobility of Golgi proteins in membranes of living cells. Science, 273, 797-801.

Colombo, M.I., Beron, W. and Stahl, P.D. (1997) Calmodulin regulates endosome fusion. J Biol Chem, 272, 7707-7712.

Dayel, M.J., Hom, E.F. and Verkman, A.S. (1999) Diffusion of green fluorescent protein in the aqueous-phase lumen of endoplasmic reticulum. Biophys J, 76, 2843-2851.

Doray, B., Ghosh, P., Griffith, J., Geuze, H.J. and Kornfeld, S. (2002) Cooperation of GGAs and AP-1 in packaging MPRs at the trans-Golgi network. Science, 297, 1700-1703.

Du, Y., Ferro-Novick, S. and Novick, P. (2004) Dynamics and inheritance of the endoplasmic reticulum. J Cell Sci, 117, 2871-2878.

Dudognon, P., Maeder-Garavaglia, C., Carpentier, J.L. and Paccaud, J.P. (2004) Regulation of a COPII component by cytosolic O-glycosylation during mitosis. FEBS Lett, 561, 44-50.

Dumas, J.J., Merithew, E., Sudharshan, E., Rajamani, D., Hayes, S., Lawe, D., Corvera, S. and Lambright, D.G. (2001) Multivalent endosome targeting by homodimeric EEA1. Mol Cell, 8, 947-958.

Dunster, K., Toh, B.H. and Sentry, J.W. (2002) Early endosomes, late endosomes, and lysosomes display distinct partitioning strategies of inheritance with similarities to Golgi-derived membranes. Eur J Cell Biol, 81, 117-124.

Ellenberg, J., Siggia, E.D., Moreira, J.E., Smith, C.L., Presley, J.F., Worman, H.J. and Lippincott-Schwartz, J. (1997) Nuclear membrane dynamics and reassembly in living cells: targeting of an inner nuclear membrane protein in interphase and mitosis. J Cell Biol, 138, 1193-1206.

Erickson, C.A. and Trinkaus, J.P. (1976) Microvilli and blebs as sources of reserve surface membrane during cell spreading. Exp Cell Res, 99, 375-384.

Eskelinen, E.L., Tanaka, Y. and Saftig, P. (2003) At the acidic edge: emerging functions for lysosomal membrane proteins. Trends Cell Biol, 13, 137-145.

Farmaki, T., Ponnambalam, S., Prescott, A.R., Clausen, H., Tang, B.L., Hong, W. and Lucocq, J.M. (1999) Forward and retrograde trafficking in mitotic animal cells. ER-Golgi transport arrest restricts protein export from the ER into COPII-coated structures. J Cell Sci, 112 (Pt 5), 589-600.

Fawcett, D.W. (1965) Surface Specializations of Absorbing Cells. J Histochem Cytochem, 13, 75-91.

Featherstone, C., Griffiths, G. and Warren, G. (1985) Newly synthesized G protein of vesicular stomatitis virus is not transported to the Golgi complex in mitotic cells. J Cell Biol, 101, 2036-2046.

Feinstein, T.N. and Linstedt, A.D. (2007) Mitogen-activated protein kinase kinase 1-dependent Golgi unlinking occurs in G2 phase and promotes the G2/M cell cycle transition. Mol Biol Cell, 18, 594-604.

Feldmann, J., Callebaut, I., Raposo, G., Certain, S., Bacq, D., Dumont, C., Lambert, N., Ouachee-Chardin, M., Chedeville,

G., Tamary, H., Minard-Colin, V., Vilmer, E., Blanche, S., Le Deist, F., Fischer, A. and de Saint Basile, G. (2003) Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell, 115, 461-473.

Feng, Y., Press, B. and Wandinger-Ness, A. (1995) Rab 7: an important regulator of late endocytic membrane traffic. J Cell Biol, 131, 1435-1452.

Fukasawa, K. (2007) Oncogenes and tumour suppressors take on centrosomes. Nat Rev Cancer, 7, 911-924.

Fukuda, R., McNew, J.A., Weber, T., Parlati, F., Engel, T., Nickel, W., Rothman, J.E. and Sollner, T.H. (2000) Functional architecture of an intracellular membrane t-SNARE. Nature, 407, 198-202.

Gaborik, Z. and Hunyady, L. (2004) Intracellular trafficking of hormone receptors. Trends Endocrinol Metab, 15, 286-293.
Gadde, S. and Heald, R. (2004) Mechanisms and molecules of the mitotic spindle. Curr Biol, 14, R797-805.

Garrett, M.D., Zahner, J.E., Cheney, C.M. and Novick, P.J. (1994) GDI1 encodes a GDP dissociation inhibitor that plays an essential role in the yeast secretory pathway. Embo J, 13, 1718-1728.

Gasman, S., Kalaidzidis, Y. and Zerial, M. (2003) RhoD regulates endosome dynamics through Diaphanous-related Formin and Src tyrosine kinase. Nat Cell Biol, 5, 195-204.

Gaullier, J.M., Simonsen, A., D'Arrigo, A., Bremnes, B., Stenmark, H. and Aasland, R. (1998) FYVE fingers bind PtdIns(3)P. Nature, 394, 432-433.

Gedde-Dahl, M., Freisewinkel, I., Staschewski, M., Schenck, K., Koch, N. and Bakke, O. (1997) Exon 6 is essential for invariant chain trimerization and induction of large endosomal structures. J Biol Chem, 272, 8281-8287.

Germain, R.N. (1994) MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell, 76, 287-299.

Gillooly, D.J., Morrow, I.C., Lindsay, M., Gould, R., Bryant, N.J., Gaullier, J.M., Parton, R.G. and Stenmark, H. (2000) Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells. Embo J, 19, 4577-4588.

Girao, H., Geli, M.I. and Idrissi, F.Z. (2008) Actin in the endocytic pathway: From yeast to mammals. FEBS Lett, 582, 2112-2119.

Goldstein, L.S. (2001) Molecular motors: from one motor many tails to one motor many tales. Trends Cell Biol, 11, 477-482.

Goodson, H.V., Valetti, C. and Kreis, T.E. (1997) Motors and membrane traffic. Curr Opin Cell Biol, 9, 18-28.

Goody, R.S., Rak, A. and Alexandrov, K. (2005) The structural and mechanistic basis for recycling of Rab proteins between membrane compartments. Cell Mol Life Sci, 62, 1657-1670.

Gorvel, J.P., Chavrier, P., Zerial, M. and Gruenberg, J. (1991) rab5 controls early endosome fusion in vitro. Cell, 64, 915-925.

Gorvel, J.P., Escola, J.M., Stang, E. and Bakke, O. (1995) Invariant chain induces a delayed transport from early to late endosomes. J Biol Chem, 270, 2741-2746.

Griffiths, G., Hoflack, B., Simons, K., Mellman, I. and Kornfeld, S. (1988) The mannose 6-phosphate receptor and the biogenesis of lysosomes. Cell, 52, 329-341.

Grimes, G.W., Mahler, H.R. and Perlman, R.S. (1974) Nuclear gene dosage effects on mitochondrial mass and DNA. J Cell Biol, 61, 565-574.

Gross, S.P., Tuma, M.C., Deacon, S.W., Serpinskaya, A.S., Reilein, A.R. and Gelfand, V.I. (2002) Interactions and regulation of molecular motors in Xenopus melanophores. J Cell Biol, 156, 855-865.

Grosshans, B.L., Ortiz, D. and Novick, P. (2006) Rabs and their effectors: achieving specificity in membrane traffic. Proc Natl Acad Sci U S A, 103, 11821-11827.

Gruenberg, J. and Maxfield, F.R. (1995) Membrane transport in the endocytic pathway. Curr Opin Cell Biol, 7, 552-563.

Gruenberg, J. and Stenmark, H. (2004) The biogenesis of multivesicular endosomes. Nat Rev Mol Cell Biol, 5, 317-323.

Haas, A.K., Fuchs, E., Kopajtich, R. and Barr, F.A. (2005) A GTPase-activating protein controls Rab5 function in endocytic trafficking. Nat Cell Biol, 7, 887-893.

Hammond, A.T. and Glick, B.S. (2000) Dynamics of transitional endoplasmic reticulum sites in vertebrate cells. Mol Biol Cell, 11, 3013-3030.

Haraga, A., Ohlson, M.B. and Miller, S.I. (2008) Salmonellae interplay with host cells. Nat Rev Microbiol, 6, 53-66.

Hennis, A.S. and Birky, C.W., Jr. (1984) Stochastic partitioning of chloroplasts at cell division in the alga Olisthodiscus, and compensating control of chloroplast replication. J Cell Sci, 70, 1-15.

Henson, J.H., Begg, D.A., Beaulieu, S.M., Fishkind, D.J., Bonder, E.M., Terasaki, M., Lebeche, D. and Kaminer, B. (1989) A calsequestrin-like protein in the endoplasmic reticulum of the sea urchin: localization and dynamics in the egg and first cell cycle embryo. J Cell Biol, 109, 149-161.

Hesketh, T.R., Beaven, M.A., Rogers, J., Burke, B. and Warren, G.B. (1984) Stimulated release of histamine by a rat mast cell line is inhibited during mitosis. J Cell Biol, 98, 2250-2254.

Hetzer, M.W., Walther, T.C. and Mattaj, I.W. (2005) Pushing the envelope: structure, function, and dynamics of the nuclear periphery. Annu Rev Cell Dev Biol, 21, 347-380.

Hiller, G. and Weber, K. (1982) Golgi detection in mitotic and interphase cells by antibodies to secreted galactosyltransferase. Exp Cell Res, 142, 85-94.

Hirano, T. (2000) Chromosome cohesion, condensation, and separation. Annu Rev Biochem, 69, 115-144.

Hirokawa, N. (1998) Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science, 279, 519-526.

Hirschberg, K., Miller, C.M., Ellenberg, J., Presley, J.F., Siggia, E.D., Phair, R.D. and Lippincott-Schwartz, J. (1998) Kinetic analysis of secretory protein traffic and characterization of golgi to plasma membrane transport intermediates in living cells. J Cell Biol, 143, 1485-1503.

Hoepfner, S., Severin, F., Cabezas, A., Habermann, B., Runge, A., Gillooly, D., Stenmark, H. and Zerial, M. (2005) Modulation of receptor recycling and degradation by the endosomal kinesin KIF16B. Cell, 121, 437-450.

Hong, W. (2005) SNAREs and traffic. Biochim Biophys Acta, 1744, 120-144.

Honing, S., Sandoval, I.V. and von Figura, K. (1998) A di-leucine-based motif in the cytoplasmic tail of LIMP-II and tyrosinase

mediates selective binding of AP-3. Embo J, 17, 1304-1314.

Horiuchi, H., Lippe, R., McBride, H.M., Rubino, M., Woodman, P., Stenmark, H., Rybin, V., Wilm, M., Ashman, K., Mann, M. and Zerial, M. (1997) A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. Cell, 90, 1149-1159.

Howell, G.J., Holloway, Z.G., Cobbold, C., Monaco, A.P. and Ponnambalam, S. (2006) Cell biology of membrane trafficking in human disease. Int Rev Cytol, 252, 1-69.

Hu, C., Ahmed, M., Melia, T.J., Sollner, T.H., Mayer, T. and Rothman, J.E. (2003) Fusion of cells by flipped SNAREs. Science, 300, 1745-1749.

Huang, J., Imamura, T. and Olefsky, J.M. (2001) Insulin can regulate GLUT4 internalization by signaling to Rab5 and the motor protein dynein. Proc Natl Acad Sci U S A, 98, 13084-13089.

Huffaker, T.C., Thomas, J.H. and Botstein, D. (1988) Diverse effects of beta-tubulin mutations on microtubule formation and function. J Cell Biol, 106, 1997-2010.

Imamura, T., Huang, J., Usui, I., Satoh, H., Bever, J. and Olefsky, J.M. (2003) Insulin-induced GLUT4 translocation involves protein kinase C-lambda-mediated functional coupling between Rab4 and the motor protein kinesin. Mol Cell Biol, 23, 4892-4900.

Jacobs, C.W., Adams, A.E., Szaniszlo, P.J. and Pringle, J.R. (1988) Functions of microtubules in the Saccharomyces cerevisiae cell cycle. J Cell Biol, 107, 1409-1426.
Jahn, R., Lang, T. and Sudhof, T.C. (2003) Membrane fusion. Cell, 112, 519-533.

Jesch, S.A. and Linstedt, A.D. (1998) The Golgi and endoplasmic reticulum remain independent during mitosis in HeLa cells. Mol Biol Cell, 9, 623-635.

Jokitalo, E., Cabrera-Poch, N., Warren, G. and Shima, D.T. (2001) Golgi clusters and vesicles mediate mitotic inheritance independently of the endoplasmic reticulum. J Cell Biol, 154, 317-330.

Jones, A.T. (2007) Macropinocytosis: searching for an endocytic identity and role in the uptake of cell penetrating peptides. J Cell Mol Med, 11, 670-684.

Jordens, I., Marsman, M., Kuijl, C. and Neefjes, J. (2005) Rab proteins, connecting transport and vesicle fusion. Traffic, 6, 1070-1077.

Kanki, J.P. and Newport, J.W. (1991) The cell cycle dependence of the secretory pathway in developing Xenopus laevis. Dev Biol, 146, 214-227.

Kaplan, K.B., Swedlow, J.R., Varmus, H.E. and Morgan, D.O. (1992) Association of p60c-src with endosomal membranes in mammalian fibroblasts. J Cell Biol, 118, 321-333.

Karki, S. and Holzbaur, E.L. (1995) Affinity chromatography demonstrates a direct binding between cytoplasmic dynein and the dynactin complex. J Biol Chem, 270, 28806-28811.

Kim, B.Y., Kramer, H., Yamamoto, A., Kominami, E., Kohsaka, S. and Akazawa, C. (2001) Molecular characterization of mammalian homologues of class C Vps proteins that interact with syntaxin-7. J Biol Chem, 276, 29393-29402.

Kinsella, B.T. and Maltese, W.A. (1992) rab GTP-binding proteins with three different carboxyl-terminal cysteine motifs are modified in vivo by 20-carbon isoprenoids. J Biol Chem, 267, 3940-3945.

Kirk, S.J. and Ward, T.H. (2007) COPII under the microscope. Semin Cell Dev Biol, 18, 435-447.

Klausner, R.D., Ashwell, G., van Renswoude, J., Harford, J.B. and Bridges, K.R. (1983) Binding of apotransferrin to K562 cells: explanation of the transferrin cycle. Proc Natl Acad Sci U S A, 80, 2263-2266.

Koch, G.L., Macer, D.R. and Smith, M.J. (1987a) Visualization of the intact endoplasmic reticulum by immunofluorescence with antibodies to the major ER glycoprotein, endoplasmin. J Cell Sci, 87 (Pt 4), 535-542.

Koch, N., Lauer, W., Habicht, J. and Dobberstein, B. (1987b) Primary structure of the gene for the murine Ia antigen-associated invariant chains (Ii). An alternatively spliced exon encodes a cysteine-rich domain highly homologous to a repetitive sequence of thyroglobulin. Embo J, 6, 1677-1683.

Kornfeld, S. (1992) Structure and function of the mannose 6-phosphate/insulinlike growth factor II receptors. Annu Rev Biochem, 61, 307-330.

Kornfeld, S. and Mellman, I. (1989) The biogenesis of lysosomes. Annu Rev Cell Biol, 5, 483-525.

Kreiner, T. and Moore, H.P. (1990) Membrane traffic between secretory compartments is differentially affected during mitosis. Cell Regul, 1, 415-424.

Kural, C., Kim, H., Syed, S., Goshima, G., Gelfand, V.I. and Selvin, P.R. (2005) Kinesin and dynein move a peroxisome in vivo: a tug-of-war or coordinated movement? Science, 308, 1469-1472.

Lajoie, P. and Nabi, I.R. (2007) Regulation of raft-dependent endocytosis. J Cell Mol Med, 11, 644-653.

Lawe, D.C., Chawla, A., Merithew, E., Dumas, J., Carrington, W., Fogarty, K., Lifshitz, L., Tuft, R., Lambright, D. and Corvera, S. (2002) Sequential roles for phosphatidylinositol 3-phosphate and Rab5 in tethering and fusion of early endosomes via their interaction with EEA1. J Biol Chem, 277, 8611-8617.

Le Borgne, R., Alconada, A., Bauer, U. and Hoflack, B. (1998) The mammalian AP-3 adaptor-like complex mediates the intracellular transport of lysosomal membrane glycoproteins. J Biol Chem, 273, 29451-29461.

Lee, J.R., Shin, H., Choi, J., Ko, J., Kim, S., Lee, H.W., Kim, K., Rho, S.H., Lee, J.H., Song, H.E., Eom, S.H. and Kim, E. (2004) An intramolecular interaction between the FHA domain and a coiled coil negatively regulates the kinesin motor KIF1A. Embo J, 23, 1506-1515.

Lemmon, S.K. and Traub, L.M. (2000) Sorting in the endosomal system in yeast and animal cells. Curr Opin Cell Biol, 12, 457-466.

Li, G., Barbieri, M.A., Colombo, M.I. and Stahl, P.D. (1994) Structural features of the GTP-binding defective Rab5 mutants required for their inhibitory activity on endocytosis. J Biol Chem, 269, 14631-14635.

Li, G., D'Souza-Schorey, C., Barbieri, M.A., Roberts, R.L., Klippel, A., Williams, L.T. and Stahl, P.D. (1995) Evidence for phosphatidylinositol 3-kinase as a regulator of endocytosis via activation of Rab5. Proc Natl Acad Sci U S A, 92, 10207-10211.

Lipp, J., Dobberstein, B. and Haeuptle, M.T. (1987) Signal recognition particle arrests elongation of nascent secretory and membrane proteins at multiple sites in a transient manner. J Biol Chem, 262, 1680-1684.

Lippincott-Schwartz, J. and Patterson, G.H. (2003) Development and use of fluorescent protein markers in living cells. Science, 300, 87-91.

Lippincott-Schwartz, J. and Patterson, G.H. (2008) Fluorescent proteins for photoactivation experiments. Methods Cell Biol, 85, 45-61.

Lippincott-Schwartz, J., Snapp, E. and Kenworthy, A. (2001) Studying protein dynamics in living cells. Nat Rev Mol Cell Biol, 2, 444-456.

Llado, A., Timpson, P., Vila de Muga, S., Moreto, J., Pol, A., Grewal, T., Daly, R.J., Enrich, C. and Tebar, F. (2008) Protein Kinase C{delta} and Calmodulin Regulate Epidermal Growth Factor Receptor Recycling from Early Endosomes through Arp2/3 Complex and Cortactin. Mol Biol Cell, 19, 17-29.

Lombardi, D., Soldati, T., Riederer, M.A., Goda, Y., Zerial, M. and Pfeffer, S.R. (1993) Rab9 functions in transport between late endosomes and the trans Golgi network. Embo J, 12, 677-682.

Lopez-Garcia, P. and Moreira, D. (1999) Metabolic symbiosis at the origin of eukaryotes. Trends Biochem Sci, 24, 88-93.

Loubery, S., Wilhelm, C., Hurbain, I., Neveu, S., Louvard, D. and Coudrier, E. (2008) Different microtubule motors move early and late endocytic compartments. Traffic, 9, 492-509.

Lowe, M. (2002) Golgi complex: biogenesis de novo? Curr Biol, 12, R166-167.

Lowe, M. and Barr, F.A. (2007) Inheritance and biogenesis of organelles in the secretory pathway. Nat Rev Mol Cell Biol, 8, 429-439.

Lucocq, J.M., Berger, E.G. and Warren, G. (1989) Mitotic Golgi fragments in HeLa cells and their role in the reassembly pathway. J Cell Biol, 109, 463-474.

Lucocq, J.M. and Warren, G. (1987) Fragmentation and partitioning of the Golgi apparatus during mitosis in HeLa cells. Embo J, 6, 3239-3246.

Luzio, J.P., Poupon, V., Lindsay, M.R., Mullock, B.M., Piper, R.C. and Pryor, P.R. (2003) Membrane dynamics and the biogenesis of lysosomes. Mol Membr Biol, 20, 141-154.

Luzio, J.P., Pryor, P.R. and Bright, N.A. (2007) Lysosomes: fusion and function. Nat Rev Mol Cell Biol, 8, 622-632.

Luzio, J.P., Rous, B.A., Bright, N.A., Pryor, P.R., Mullock, B.M. and Piper, R.C. (2000) Lysosome-endosome fusion and lysosome biogenesis. J Cell Sci, 113 (Pt 9), 1515-1524.

Mace, G., Miaczynska, M., Zerial, M. and Nebreda, A.R. (2005) Phosphorylation of EEA1 by p38 MAP kinase regulates mu opioid receptor endocytosis. Embo J, 24, 3235-3246.

Maro, B., Johnson, M.H., Pickering, S.J. and Louvard, D. (1985) Changes in the distribution of membranous organelles during mouse early development. J Embryol Exp Morphol, 90, 287-309.

Marsh, B.J. and Howell, K.E. (2002) The mammalian Golgi--complex debates. Nat Rev Mol Cell Biol, 3, 789-795.

Maul, G.G. and Brinkley, B.R. (1970) The golgi apparatus during mitosis in human melanoma cells in vitro. Cancer Res, 30, 2326-2335.

McBride, H.M., Rybin, V., Murphy, C., Giner, A., Teasdale, R. and Zerial, M. (1999) Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin

13. Cell, 98, 377-386.

McNally, J.G., Muller, W.G., Walker, D., Wolford, R. and Hager, G.L. (2000) The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. Science, 287, 1262-1265.

McNew, J.A., Parlati, F., Fukuda, R., Johnston, R.J., Paz, K., Paumet, F., Sollner, T.H. and Rothman, J.E. (2000) Compartmental specificity of cellular membrane fusion encoded in SNARE proteins. Nature, 407, 153-159.

Miaczynska, M. and Zerial, M. (2002) Mosaic organization of the endocytic pathway. Exp Cell Res, 272, 8-14.

Mills, I.G., Jones, A.T. and Clague, M.J. (1998) Involvement of the endosomal autoantigen EEA1 in homotypic fusion of early endosomes. Curr Biol, 8, 881-884.

Mills, I.G., Urbe, S. and Clague, M.J. (2001) Relationships between EEA1 binding partners and their role in endosome fusion. J Cell Sci, 114, 1959-1965.

Mohrmann, K., Gerez, L., Oorschot, V., Klumperman, J. and van der Sluijs, P. (2002) Rab4 function in membrane recycling from early endosomes depends on a membrane to cytoplasm cycle. J Biol Chem, 277, 32029-32035.

Motley, A., Bright, N.A., Seaman, M.N. and Robinson, M.S. (2003) Clathrin-mediated endocytosis in AP-2-depleted cells. J Cell Biol, 162, 909-918.

Mu, F.T., Callaghan, J.M., Steele-Mortimer, O., Stenmark, H., Parton, R.G., Campbell, P.L., McCluskey, J., Yeo, J.P., Tock, E.P. and Toh, B.H. (1995) EEA1, an early endosome-associated protein. EEA1 is a conserved alpha-helical peripheral membrane protein flanked by cysteine “fingers” and contains a calmodulin-binding IQ motif. J Biol Chem, 270, 13503-13511.

Mullins, C. and Bonifacino, J.S. (2001) The molecular machinery for lysosome biogenesis. Bioessays, 23, 333-343.

Murphy, R.F. (1991) Maturation models for endosome and lysosome biogenesis. Trends Cell Biol, 1, 77-82.

Nehls, S., Snapp, E.L., Cole, N.B., Zaal, K.J., Kenworthy, A.K., Roberts, T.H., Ellenberg, J., Presley, J.F., Siggia, E. and Lippincott-Schwartz, J. (2000) Dynamics and retention of misfolded proteins in native ER membranes. Nat Cell Biol, 2, 288-295.

Nielsen, E., Severin, F., Backer, J.M., Hyman, A.A. and Zerial, M. (1999) Rab5 regulates motility of early endosomes on microtubules. Nat Cell Biol, 1, 376-382.

Nikonov, A.V., Snapp, E., Lippincott-Schwartz, J. and Kreibich, G. (2002) Active translocon complexes labeled with GFP-Dad1 diffuse slowly as large polysome arrays in the endoplasmic reticulum. J Cell Biol, 158, 497-506.

Nordeng, T.W., Gorvel, J.P. and Bakke, O. (1998) Intracellular transport of molecules engaged in the presentation of exogenous antigens. Curr Top Microbiol Immunol, 232, 179-215.

Nordeng, T.W., Gregers, T.F., Kongsvik, T.L., Meresse, S., Gorvel, J.P., Jourdan, F., Motta, A. and Bakke, O. (2002) The cytoplasmic tail of invariant chain regulates endosome fusion and morphology. Mol Biol Cell, 13, 1846-1856.

Nunnari, J. and Walter, P. (1996) Regulation of organelle biogenesis. Cell, 84, 389-394.

Ohno, H., Stewart, J., Fournier, M.C., Bosshart, H., Rhee, I., Miyatake, S., Saito, T., Gallusser, A., Kirchhausen, T. and Bonifacino, J.S. (1995) Interaction of tyrosine-based

sorting signals with clathrin-associated proteins. Science, 269, 1872-1875.

Ohnuma, K., Yomo, T., Asashima, M. and Kaneko, K. (2006) Sorting of cells of the same size, shape, and cell cycle stage for a single cell level assay without staining. BMC Cell Biol, 7, 25.

Olkkonen, V.M. and Ikonen, E. (2006) When intracellular logistics fails--genetic defects in membrane trafficking. J Cell Sci, 119, 5031-5045.

Orci, L., Perrelet, A. and Rothman, J.E. (1998) Vesicles on strings: morphological evidence for processive transport within the Golgi stack. Proc Natl Acad Sci U S A, 95, 2279-2283.

Owen, D.J. and Evans, P.R. (1998) A structural explanation for the recognition of tyrosine-based endocytotic signals. Science, 282, 1327-1332.

Partikian, A., Olveczky, B., Swaminathan, R., Li, Y. and Verkman, A.S. (1998) Rapid diffusion of green fluorescent protein in the mitochondrial matrix. J Cell Biol, 140, 821-829.

Paschal, B.M. and Vallee, R.B. (1987) Retrograde transport by the microtubule-associated protein MAP 1C. Nature, 330, 181-183.

Patki, V., Virbasius, J., Lane, W.S., Toh, B.H., Shpetner, H.S. and Corvera, S. (1997) Identification of an early endosomal protein regulated by phosphatidylinositol 3-kinase. Proc Natl Acad Sci U S A, 94, 7326-7330.

Patterson, G.H. and Lippincott-Schwartz, J. (2002) A photoactivatable GFP for selective photolabeling of proteins and cells. Science, 297, 1873-1877.

Pecot, M.Y. and Malhotra, V. (2004) Golgi membranes remain segregated from the endoplasmic reticulum during mitosis in mammalian cells. Cell, 116, 99-107.

Pecot, M.Y. and Malhotra, V. (2006) The Golgi apparatus maintains its organization independent of the endoplasmic reticulum. Mol Biol Cell, 17, 5372-5380.

Pei, J., Turse, J.E. and Ficht, T.A. (2008) Evidence of Brucella abortus OPS dictating uptake and restricting NF-kappaB activation in murine macrophages. Microbes Infect, 10, 582-590.

Pelkmans, L., Burli, T., Zerial, M. and Helenius, A. (2004) Caveolin-stabilized membrane domains as multifunctional transport and sorting devices in endocytic membrane traffic. Cell, 118, 767-780.

Pelkmans, L., Kartenbeck, J. and Helenius, A. (2001) Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. Nat Cell Biol, 3, 473-483.

Perret, E., Lakkaraju, A., Deborde, S., Schreiner, R. and Rodriguez-Boulan, E. (2005) Evolving endosomes: how many varieties and why? Curr Opin Cell Biol, 17, 423-434.

Pfeffer, S. (2005) Filling the Rab GAP. Nat Cell Biol, 7, 856-857.

Pfeffer, S. and Aivazian, D. (2004) Targeting Rab GTPases to distinct membrane compartments. Nat Rev Mol Cell Biol, 5, 886-896.

Phair, R.D. and Misteli, T. (2000) High mobility of proteins in the mammalian cell nucleus. Nature, 404, 604-609.

Pieters, J., Bakke, O. and Dobberstein, B. (1993) The MHC class II-associated invariant chain contains two endosomal targeting signals within its cytoplasmic tail. J Cell Sci, 106 (Pt 3), 831-846.

Piper, R.C. and Luzio, J.P. (2007)

Ubiquitin-dependent sorting of integral membrane proteins for degradation in lysosomes. Curr Opin Cell Biol, 19, 459-465.

Porter, K., Prescott, D. and Frye, J. (1973) Changes in surface morphology of Chinese hamster ovary cells during the cell cycle. J Cell Biol, 57, 815-836.

Prasher, D.C., Eckenrode, V.K., Ward, W.W., Prendergast, F.G. and Cormier, M.J. (1992) Primary structure of the Aequorea victoria green-fluorescent protein. Gene, 111, 229-233.

Prescott, A.R., Farmaki, T., Thomson, C., James, J., Paccaud, J.P., Tang, B.L., Hong, W., Quinn, M., Ponnambalam, S. and Lucocq, J. (2001) Evidence for prebudding arrest of ER export in animal cell mitosis and its role in generating Golgi partitioning intermediates. Traffic, 2, 321-335.

Presley, J.F., Ward, T.H., Pfeifer, A.C., Siggia, E.D., Phair, R.D. and Lippincott-Schwartz, J. (2002) Dissection of COPI and Arf1 dynamics in vivo and role in Golgi membrane transport. Nature, 417, 187-193.

Preuss, D., Mulholland, J., Kaiser, C.A., Orlean, P., Albright, C., Rose, M.D., Robbins, P.W. and Botstein, D. (1991) Structure of the yeast endoplasmic reticulum: localization of ER proteins using immunofluorescence and immunoelectron microscopy. Yeast, 7, 891-911.

Pryor, P.R., Mullock, B.M., Bright, N.A., Gray, S.R. and Luzio, J.P. (2000) The role of intraorganellar Ca(2+) in late endosome-lysosome heterotypic fusion and in the reformation of lysosomes from hybrid organelles. J Cell Biol, 149, 1053-1062.

Puhka, M., Vihinen, H., Joensuu, M. and Jokitalo, E. (2007) Endoplasmic reticulum remains continuous and undergoes sheet-to-tubule transformation during cell division in mammalian cells. J Cell Biol, 179, 895-909.

Pypaert, M., Lucocq, J.M. and Warren, G. (1987) Coated pits in interphase and mitotic A431 cells. Eur J Cell Biol, 45, 23-29.

Pypaert, M., Mundy, D., Souter, E., Labbe, J.C. and Warren, G. (1991) Mitotic cytosol inhibits invagination of coated pits in broken mitotic cells. J Cell Biol, 114, 1159-1166.

Raiborg, C., Wesche, J., Malerod, L. and Stenmark, H. (2006) Flat clathrin coats on endosomes mediate degradative protein sorting by scaffolding Hrs in dynamic microdomains. J Cell Sci, 119, 2414-2424.

Rak, A., Pylypenko, O., Durek, T., Watzke, A., Kushnir, S., Brunsveld, L., Waldmann, H., Goody, R.S. and Alexandrov, K. (2003) Structure of Rab GDP-dissociation inhibitor in complex with prenylated YPT1 GTPase. Science, 302, 646-650.

Rapoport, I., Miyazaki, M., Boll, W., Duckworth, B., Cantley, L.C., Shoelson, S. and Kirchhausen, T. (1997) Regulatory interactions in the recognition of endocytic sorting signals by AP-2 complexes. Embo J, 16, 2240-2250.

Raposo, G., Cordonnier, M.N., Tenza, D., Menichi, B., Durrbach, A., Louvard, D. and Coudrier, E. (1999) Association of myosin I alpha with endosomes and lysosomes in mammalian cells. Mol Biol Cell, 10, 1477-1494.

Raucher, D. and Sheetz, M.P. (1999) Membrane expansion increases endocytosis rate during mitosis. J Cell Biol, 144, 497-506.

Reilein, A.R., Tint, I.S., Peunova, N.I., Enikolopov, G.N. and Gelfand, V.I. (1998) Regulation of organelle movement

in melanophores by protein kinase A (PKA), protein kinase C (PKC), and protein phosphatase 2A (PP2A). J Cell Biol, 142, 803-813.

Reits, E.A. and Neefjes, J.J. (2001) From fixed to FRAP: measuring protein mobility and activity in living cells. Nat Cell Biol, 3, E145-147.

Renfrew, C.A. and Hubbard, A.L. (1991) Sequential processing of epidermal growth factor in early and late endosomes of rat liver. J Biol Chem, 266, 4348-4356.

Richardson, S.C., Winistorfer, S.C., Poupon, V., Luzio, J.P. and Piper, R.C. (2004) Mammalian late vacuole protein sorting orthologues participate in early endosomal fusion and interact with the cytoskeleton. Mol Biol Cell, 15, 1197-1210.

Rieder, S.E. and Emr, S.D. (1997) A novel RING finger protein complex essential for a late step in protein transport to the yeast vacuole. Mol Biol Cell, 8, 2307-2327.

Rink, J., Ghigo, E., Kalaidzidis, Y. and Zerial, M. (2005) Rab conversion as a mechanism of progression from early to late endosomes. Cell, 122, 735-749.

Robbins, E. and Gonatas, N.K. (1964) The Ultrastructure of a Mammalian Cell During the Mitotic Cycle. J Cell Biol, 21, 429-463.

Rodionov, D.G. and Bakke, O. (1998) Medium chains of adaptor complexes AP-1 and AP-2 recognize leucine-based sorting signals from the invariant chain. J Biol Chem, 273, 6005-6008.

Rogers, S.L., Tint, I.S., Fanapour, P.C. and Gelfand, V.I. (1997) Regulated bidirectional motility of melanophore pigment granules along microtubules in vitro. Proc Natl Acad Sci U S A, 94, 3720-3725.

Romagnoli, P., Layet, C., Yewdell, J., Bakke, O. and Germain, R.N. (1993) Relationship between invariant chain expression and major histocompatibility complex class II transport into early and late endocytic compartments. J Exp Med, 177, 583-596.

Rothman, J.E. (2002) Lasker Basic Medical Research Award. The machinery and principles of vesicle transport in the cell. Nat Med, 8, 1059-1062.

Rottner, K., Stradal, T.E. and Wehland, J. (2005) Bacteria-host-cell interactions at the plasma membrane: stories on actin cytoskeleton subversion. Dev Cell, 9, 3-17.

Royle, S.J. (2006) The cellular functions of clathrin. Cell Mol Life Sci, 63, 1823-1832.

Royle, S.J., Bright, N.A. and Lagnado, L. (2005) Clathrin is required for the function of the mitotic spindle. Nature, 434, 1152-1157.

Rubino, M., Miaczynska, M., Lippe, R. and Zerial, M. (2000) Selective membrane recruitment of EEA1 suggests a role in directional transport of clathrin-coated vesicles to early endosomes. J Biol Chem, 275, 3745-3748.

Sabatini, D.M., Erdjument-Bromage, H., Lui, M., Tempst, P. and Snyder, S.H. (1994) RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell, 78, 35-43.

Sager, P.R., Brown, P.A. and Berlin, R.D. (1984) Analysis of transferrin recycling in mitotic and interphase HeLa cells by quantitative fluorescence microscopy. Cell, 39, 275-282.

Salina, D., Bodoor, K., Eckley, D.M., Schroer, T.A., Rattner, J.B. and Burke, B. (2002) Cytoplasmic dynein as a facilitator of nuclear envelope breakdown. Cell, 108, 97-

107.

Sandvig, K. and van Deurs, B. (2005) Delivery into cells: lessons learned from plant and bacterial toxins. Gene Ther, 12, 865-872.

Schmit, T.L. and Ahmad, N. (2007) Regulation of mitosis via mitotic kinases: new opportunities for cancer management. Mol Cancer Ther, 6, 1920-1931.

Schnapp, B.J. and Reese, T.S. (1989) Dynein is the motor for retrograde axonal transport of organelles. Proc Natl Acad Sci U S A, 86, 1548-1552.

Schroer, T.A. (2004) Dynactin. Annu Rev Cell Dev Biol, 20, 759-779.

Schroer, T.A., Steuer, E.R. and Sheetz, M.P. (1989) Cytoplasmic dynein is a minus end-directed motor for membranous organelles. Cell, 56, 937-946.

Schweitzer, J.K., Burke, E.E., Goodson, H.V. and D'Souza-Schorey, C. (2005) Endocytosis resumes during late mitosis and is required for cytokinesis. J Biol Chem, 280, 41628-41635.

Seabra, M.C. and Wasmeier, C. (2004) Controlling the location and activation of Rab GTPases. Curr Opin Cell Biol, 16, 451-457.

Seals, D.F., Eitzen, G., Margolis, N., Wickner, W.T. and Price, A. (2000) A Ypt/Rab effector complex containing the Sec1 homolog Vps33p is required for homotypic vacuole fusion. Proc Natl Acad Sci U S A, 97, 9402-9407.

Seaman, M.N. (2007) Identification of a novel conserved sorting motif required for retromer-mediated endosome-to-TGN retrieval. J Cell Sci, 120, 2378-2389.

Seiler, S., Kirchner, J., Horn, C., Kallipolitou, A., Woehlke, G. and Schliwa, M. (2000) Cargo binding and regulatory sites in the tail of fungal conventional kinesin. Nat Cell Biol, 2, 333-338.

Shapiro, A.D. and Pfeffer, S.R. (1995) Quantitative analysis of the interactions between prenyl Rab9, GDP dissociation inhibitor-alpha, and guanine nucleotides. J Biol Chem, 270, 11085-11090.

Shima, D.T., Cabrera-Poch, N., Pepperkok, R. and Warren, G. (1998) An ordered inheritance strategy for the Golgi apparatus: visualization of mitotic disassembly reveals a role for the mitotic spindle. J Cell Biol, 141, 955-966.

Shima, D.T., Haldar, K., Pepperkok, R., Watson, R. and Warren, G. (1997) Partitioning of the Golgi apparatus during mitosis in living HeLa cells. J Cell Biol, 137, 1211-1228.

Shisheva, A., Chinni, S.R. and DeMarco, C. (1999) General role of GDP dissociation inhibitor 2 in membrane release of Rab proteins: modulations of its functional interactions by in vitro and in vivo structural modifications. Biochemistry, 38, 11711-11721.

Shorter, J. and Warren, G. (2002) Golgi architecture and inheritance. Annu Rev Cell Dev Biol, 18, 379-420.

Siddhanta, U., McIlroy, J., Shah, A., Zhang, Y. and Backer, J.M. (1998) Distinct roles for the p110alpha and hVPS34 phosphatidylinositol 3'-kinases in vesicular trafficking, regulation of the actin cytoskeleton, and mitogenesis. J Cell Biol, 143, 1647-1659.

Simonsen, A., Gaullier, J.M., D'Arrigo, A. and Stenmark, H. (1999) The Rab5 effector EEA1 interacts directly with syntaxin-6. J Biol Chem, 274, 28857-28860.

Simonsen, A., Lippe, R., Christoforidis, S., Gaullier, J.M., Brech, A., Cal-

laghan, J., Toh, B.H., Murphy, C., Zerial, M. and Stenmark, H. (1998) EEA1 links PI(3) K function to Rab5 regulation of endosome fusion. Nature, 394, 494-498.

Soldati, T. and Schliwa, M. (2006) Powering membrane traffic in endocytosis and recycling. Nat Rev Mol Cell Biol, 7, 897-908.

Sollner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. and Rothman, J.E. (1993) SNAP receptors implicated in vesicle targeting and fusion. Nature, 362, 318-324.

Sonnichsen, B., De Renzis, S., Nielsen, E., Rietdorf, J. and Zerial, M. (2000) Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of Rab4, Rab5, and Rab11. J Cell Biol, 149, 901-914.

Sprague, B.L. and McNally, J.G. (2005) FRAP analysis of binding: proper and fitting. Trends Cell Biol, 15, 84-91.

Stang, E. and Bakke, O. (1997) MHC class II-associated invariant chain-induced enlarged endosomal structures: a morphological study. Exp Cell Res, 235, 79-92.

Starr, T., Ng, T.W., Wehrly, T.D., Knodler, L.A. and Celli, J. (2008) Brucella intracellular replication requires trafficking through the late endosomal/lysosomal compartment. Traffic, 9, 678-694.

Steinman, R.M., Brodie, S.E. and Cohn, Z.A. (1976) Membrane flow during pinocytosis. A stereologic analysis. J Cell Biol, 68, 665-687.

Stenmark, H., Aasland, R., Toh, B.H. and D'Arrigo, A. (1996) Endosomal localization of the autoantigen EEA1 is mediated by a zinc-binding FYVE finger. J Biol Chem, 271, 24048-24054.

Stenmark, H., Parton, R.G., Steele-Mortimer, O., Lutcke, A., Gruenberg, J. and Zerial, M. (1994) Inhibition of rab5 GTPase activity stimulates membrane fusion in endocytosis. Embo J, 13, 1287-1296.

Stinchcombe, J.C., Barral, D.C., Mules, E.H., Booth, S., Hume, A.N., Machesky, L.M., Seabra, M.C. and Griffiths, G.M. (2001) Rab27a is required for regulated secretion in cytotoxic T lymphocytes. J Cell Biol, 152, 825-834.

Storrie, B. and Desjardins, M. (1996) The biogenesis of lysosomes: is it a kiss and run, continuous fusion and fission process? Bioessays, 18, 895-903.

Subramanian, K. and Meyer, T. (1997) Calcium-induced restructuring of nuclear envelope and endoplasmic reticulum calcium stores. Cell, 89, 963-971.

Sudhof, T.C. (2004) The synaptic vesicle cycle. Annu Rev Neurosci, 27, 509-547.

Sutherland, H.G., Mumford, G.K., Newton, K., Ford, L.V., Farrall, R., Dellaire, G., Caceres, J.F. and Bickmore, W.A. (2001) Large-scale identification of mammalian proteins localized to nuclear sub-compartments. Hum Mol Genet, 10, 1995-2011.

Sutton, R.B., Fasshauer, D., Jahn, R. and Brunger, A.T. (1998) Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 A resolution. Nature, 395, 347-353.

Swaminathan, R., Hoang, C.P. and Verkman, A.S. (1997) Photobleaching recovery and anisotropy decay of green fluorescent protein GFP-S65T in solution and cells: cytoplasmic viscosity probed by green fluorescent protein translational and rotational diffusion. Biophys J, 72, 1900-1907.

Tamaki, H. and Yamashina, S. (1991) Changes in cell polarity during mitosis in rat

parotid acinar cells. J Histochem Cytochem, 39, 1077-1087.

Terasaki, M. (2000) Dynamics of the endoplasmic reticulum and golgi apparatus during early sea urchin development. Mol Biol Cell, 11, 897-914.

Thompson, H.M., Cao, H., Chen, J., Euteneuer, U. and McNiven, M.A. (2004) Dynamin 2 binds gamma-tubulin and participates in centrosome cohesion. Nat Cell Biol, 6, 335-342.

Thompson, H.M., Skop, A.R., Euteneuer, U., Meyer, B.J. and McNiven, M.A. (2002) The large GTPase dynamin associates with the spindle midzone and is required for cytokinesis. Curr Biol, 12, 2111-2117.

Thyberg, J. and Moskalewski, S. (1992) Reorganization of the Golgi complex in association with mitosis: redistribution of mannosidase II to the endoplasmic reticulum and effects of brefeldin A. J Submicrosc Cytol Pathol, 24, 495-508.

Trischler, M., Stoorvogel, W. and Ullrich, O. (1999) Biochemical analysis of distinct Rab5- and Rab11-positive endosomes along the transferrin pathway. J Cell Sci, 112 (Pt 24), 4773-4783.

Tsukazaki, T., Chiang, T.A., Davison, A.F., Attisano, L. and Wrana, J.L. (1998) SARA, a FYVE domain protein that recruits Smad2 to the TGFbeta receptor. Cell, 95, 779-791.

Tuomikoski, T., Felix, M.A., Doree, M. and Gruenberg, J. (1989) Inhibition of endocytic vesicle fusion in vitro by the cell-cycle control protein kinase cdc2. Nature, 342, 942-945.

Tycko, B., Keith, C.H. and Maxfield, F.R. (1983) Rapid acidification of endocytic vesicles containing asialoglycoprotein in cells of a human hepatoma line. J Cell Biol, 97, 1762-1776.

Ullrich, O., Reinsch, S., Urbe, S., Zerial, M. and Parton, R.G. (1996) Rab11 regulates recycling through the pericentriolar recycling endosome. J Cell Biol, 135, 913-924.

Ungewickell, E.J. and Hinrichsen, L. (2007) Endocytosis: clathrin-mediated membrane budding. Curr Opin Cell Biol, 19, 417-425.

Vale, R.D. (2003) The molecular motor toolbox for intracellular transport. Cell, 112, 467-480.

Valetti, C., Wetzel, D.M., Schrader, M., Hasbani, M.J., Gill, S.R., Kreis, T.E. and Schroer, T.A. (1999) Role of dynactin in endocytic traffic: effects of dynamitin overexpression and colocalization with CLIP-170. Mol Biol Cell, 10, 4107-4120.

Vallee, R.B., Williams, J.C., Varma, D. and Barnhart, L.E. (2004) Dynein: An ancient motor protein involved in multiple modes of transport. J Neurobiol, 58, 189-200.

van der Sluijs, P., Hull, M., Huber, L.A., Male, P., Goud, B. and Mellman, I. (1992) Reversible phosphorylation--dephosphorylation determines the localization of rab4 during the cell cycle. Embo J, 11, 4379-4389.

Vaughan, K.T. and Vallee, R.B. (1995) Cytoplasmic dynein binds dynactin through a direct interaction between the intermediate chains and p150Glued. J Cell Biol, 131, 1507-1516.

Vaughan, P.S., Miura, P., Henderson, M., Byrne, B. and Vaughan, K.T. (2002) A role for regulated binding of p150(Glued) to microtubule plus ends in organelle transport. J Cell Biol, 158, 305-319.

Verhey, K.J., Lizotte, D.L., Abram-

son, T., Barenboim, L., Schnapp, B.J. and Rapoport, T.A. (1998) Light chain-dependent regulation of Kinesin's interaction with microtubules. J Cell Biol, 143, 1053-1066.

Vonderheit, A. and Helenius, A. (2005) Rab7 associates with early endosomes to mediate sorting and transport of Semliki forest virus to late endosomes. PLoS Biol, 3, e233.

Walczak, C.E. and Heald, R. (2008) Mechanisms of mitotic spindle assembly and function. Int Rev Cytol, 265, 111-158.

Warren, G. (1993) Membrane partitioning during cell division. Annu Rev Biochem, 62, 323-348.

Warren, G., Davoust, J. and Cockcroft, A. (1984) Recycling of transferrin receptors in A431 cells is inhibited during mitosis. Embo J, 3, 2217-2225.

Warren, G., Featherstone, C., Griffiths, G. and Burke, B. (1983) Newly synthesized G protein of vesicular stomatitis virus is not transported to the cell surface during mitosis. J Cell Biol, 97, 1623-1628.

Warren, G. and Wickner, W. (1996) Organelle inheritance. Cell, 84, 395-400.

Weber, T., Zemelman, B.V., McNew, J.A., Westermann, B., Gmachl, M., Parlati, F., Sollner, T.H. and Rothman, J.E. (1998) SNAREpins: minimal machinery for membrane fusion. Cell, 92, 759-772.

Weisman, L.S., Bacallao, R. and Wickner, W. (1987) Multiple methods of visualizing the yeast vacuole permit evaluation of its morphology and inheritance during the cell cycle. J Cell Biol, 105, 1539-1547.

White, J. and Stelzer, E. (1999) Photobleaching GFP reveals protein dynamics inside live cells. Trends Cell Biol, 9, 61-65.

Wiederrecht, G., Brizuela, L., Elliston, K., Sigal, N.H. and Siekierka, J.J. (1991) FKB1 encodes a nonessential FK 506-binding protein in Saccharomyces cerevisiae and contains regions suggesting homology to the cyclophilins. Proc Natl Acad Sci U S A, 88, 1029-1033.

Wilson, E.B. (1896) The cell in development and inheritance. The MacMillan Company & Co Ltd. New York.

Wilson, J.M., de Hoop, M., Zorzi, N., Toh, B.H., Dotti, C.G. and Parton, R.G. (2000) EEA1, a tethering protein of the early sorting endosome, shows a polarized distribution in hippocampal neurons, epithelial cells, and fibroblasts. Mol Biol Cell, 11, 2657-2671.

Woodman, P.G., Adamczewski, J.P., Hunt, T. and Warren, G. (1993) In vitro fusion of endocytic vesicles is inhibited by cyclin A-cdc2 kinase. Mol Biol Cell, 4, 541-553.

Woodman, P.G., Mundy, D.I., Cohen, P. and Warren, G. (1992) Cell-free fusion of endocytic vesicles is regulated by phosphorylation. J Cell Biol, 116, 331-338.

Wu, X., Zhao, X., Baylor, L., Kaushal, S., Eisenberg, E. and Greene, L.E. (2001) Clathrin exchange during clathrin-mediated endocytosis. J Cell Biol, 155, 291-300.

Wubbolts, R., Fernandez-Borja, M., Jordens, I., Reits, E., Dusseljee, S., Echeverri, C., Vallee, R.B. and Neefjes, J. (1999) Opposing motor activities of dynein and kinesin determine retention and transport of MHC class II-containing compartments. J Cell Sci, 112 (Pt 6), 785-795.

Wurmser, A.E., Sato, T.K. and Emr, S.D. (2000) New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7 GTPase to SNARE-dependent docking and fusion. J Cell Biol, 151, 551-562.

Yamashiro, D.J., Tycko, B., Fluss, S.R. and Maxfield, F.R. (1984) Segregation of transferrin to a mildly acidic (pH 6.5) para-Golgi compartment in the recycling pathway. Cell, 37, 789-800.

Yang, L., Guan, T. and Gerace, L. (1997) Integral membrane proteins of the nuclear envelope are dispersed throughout the endoplasmic reticulum during mitosis. J Cell Biol, 137, 1199-1210.

Yguerabide, J., Schmidt, J.A. and Yguerabide, E.E. (1982) Lateral mobility in membranes as detected by fluorescence recovery after photobleaching. Biophys J, 40, 69-75.

Young, J., Stauber, T., del Nery, E., Vernos, I., Pepperkok, R. and Nilsson, T. (2005) Regulation of microtubule-dependent recycling at the trans-Golgi network by Rab6A and Rab6A'. Mol Biol Cell, 16, 162-177.

Zaal, K.J., Smith, C.L., Polishchuk, R.S., Altan, N., Cole, N.B., Ellenberg, J., Hirschberg, K., Presley, J.F., Roberts, T.H., Siggia, E., Phair, R.D. and Lippincott-Schwartz, J. (1999) Golgi membranes are absorbed into and reemerge from the ER during mitosis. Cell, 99, 589-601.

Zeligs, J.D. and Wollman, S.H. (1979) Mitosis in rat thyroid epithelial cells in vivo. I. Ultrastructural changes in cytoplasmic organelles during the mitotic cycle. J Ultrastruct Res, 66, 53-77.

Zerial, M. and McBride, H. (2001) Rab proteins as membrane organizers. Nat Rev Mol Cell Biol, 2, 107-117.

Zhao, C., Slevin, J.T. and Whiteheart, S.W. (2007) Cellular functions of NSF: not just SNAPs and SNAREs. FEBS Lett, 581, 2140-2149.

Zorn, G.A., Lucas, J.J. and Kates, J.R. (1979) Purification and characterization of regenerating mouse L929 karyoplasts. Cell, 18, 659-672.