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Endocytosis and Exocytosis in Signal Transduction and in Cell Migration

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1. Introduction

Endocytosis is a complex process that is used by eukaryotic cells to internalize fragments of plasma membrane, cell-surface receptors, and various soluble molecules. Many different mechanisms have been developed to achieve internalization of membrane-bound receptors and their ligands and they can be distinguished in clathrin-mediated endocytosis and non-clathrin internalization routes. In the clathrin-mediated endocytosis, receptors bind to the adaptor protein AP2 that, in turn, recruits clathrin to coat the invaginating pits at the plasma membrane. Coated pits are pinched off by the large GTPase dynamin to generate vesicles that traffic from the plasma membrane, undergo uncoating and fuse to the early endosomal compartment. Of note, dynamin is also required in non-clathrin-mediated endocytosis [for detailed recent reviews see (Doherty & McMahon, 2009; Loerke et al, 2009; Mettlen et al, 2009; Traub, 2009)].

From early endosomes vesicles can be re-delivered to the plasma membrane through the exocytic pathway (Grant & Donaldson, 2009). Vesicle budding, uncoating, motility and fusion are controlled by the large family of Rab small GTPases. Rab proteins, in their active GTP-bound form, recruit downstream effectors that, in turn, are responsible for distinct aspects of endosomes function from signal transduction to selection and transport of cargoes. Furthermore, they control vesicular movements on microtubules thus supporting polarized distribution of internalized receptors and signalling molecules [reviewed in (Stenmark, 2009; Zerial & McBride, 2001)]. In this regards, the endo-exocytic processes are profoundly linked with the ability of the cell to elicit receptor-mediated signaling cascades.

Endocytosis has long been considered as an attenuator of signaling as it downregulates receptors at the plasma membrane. However, the ability of internalized receptors to signal from the endosomal compartment and to be recycled to specific regions of the plasma membrane allows signal modulation both in time and in space. Indeed, endocytosis-mediated recycling of receptors is also a major mechanism in the execution of spatially restricted functions, such as cell motility. Moreover, the endo-exocytic cycle of adhesive receptors back and forth from the plasma membrane represents another crucial regulatory

aspect played by traffic in the dynamic control of cell-to-cell and cell-to-extracellular matrix contacts.

We, therefore, propose to illustrate the state of the art together with most recent discoveries on the following issues:

1. The signaling endosome: a modality to finely tune persistence of extracellular stimuli inside the cell and to control their re-distribution and compartmentalization. The latter aspect is of extreme relevance for the role of endo-exo membrane trafficking in the execution of cell polarity programs.
2. Involvement of endocytosis and exocytosis in the formation and turnover of cell-to-cell and cell-to-extracellular matrix adhesion. We will review the major findings showing the relevance of membrane trafficking of adhesive receptors, namely cadherins and integrins, and describing the molecular machinery involved that has been identified so far. We will also address recent work indicating that distinct molecular machineries are required for trafficking integrins in active and inactive conformation.
3. Unconventional function of membrane trafficking proteins in mitosis. Trafficking molecules also participates to cell cycle progression and to the correct execution of mitosis. We will review the knowledge raised on this issue and discuss how the function of these molecules is related to their established role in membrane trafficking.

2. Endocytosis and signalling

Through endocytosis, active, signalling receptors - such as receptor tyrosine kinases (RTKs) - are removed from the plasma membrane (PM) and destined for degradation and this is crucial to achieve signal extinction and long-term attenuation. Endocytosis is able to remodel the composition of the plasma membrane (PM), thus allowing plasticity in the cellular responses to the microenvironment. Recent evidence, however, has demonstrated that endocytosis has a broader impact on signalling than simply signal extinction (Scita & Di Fiore, 2010; Sorkin & von Zastrow, 2009). Indeed, internalized receptors (and sometimes their ligands) are not only routed to the lysosome for degradation, but, in some cases, can be recycled to specific regions of the PM where polarized signalling is needed for events such as cell migration. Furthermore, signalling might not only occur from the PM, but also could persist along the endocytic pathway as, in the endosomal compartments, signalling receptors are often still bound to their ligands, and continue to be active. More interestingly, signalling receptors in the endosomal compartment could potentially interact with substrates that are not present at the PM. Under this scenario, endocytosis would be a mechanism to sustain signalling and to achieve signal diversification and specificity.

2.1 Signalling elicited by the endocytic compartments

The concept that signalling continues along the endocytic pathway was shown in the case of several signalling receptors, including RTKs and the TGF β R (tumor growth factor β receptor) (Sorkin & von Zastrow, 2009). In all cases, receptors remain bound to their ligand and active once internalized within endosomes, thus sustaining signalling from the intracellular compartments (Burke et al, 2001; Di Guglielmo et al, 1994; Grimes et al, 1996; Haugh et al, 1999; Hayes et al, 2002; Howe et al, 2001; Lai et al, 1989; Pennock & Wang, 2003;

Wada et al, 1992; Wang et al, 2004; Wang et al, 1996). In agreement with this, all the components of the MAPK (mitogen-activated protein kinase) activation cascade can be found in endosomes (Pol et al, 1998; Roy et al, 2002), showing that RTKs signalling persist also after internalization. In this way, sufficient duration and amplitude to signalling is allowed. Furthermore, endosomal-specific proteins have been identified and shown to be required to sustain signalling. One example is represented by P18, which works at the endosomal membrane as an anchor for an ERK-activating scaffold and is required to achieve maximal activation of ERK1/2 (Nada et al, 2009). A similar mechanism occurs in the case of GPCR (G protein-coupled receptor) signalling, where β -arrestin, similarly to P18, acts as a specific scaffold to anchor ERK1/2 to the endosome (DeWire et al, 2007) thus allowing proper signal duration.

A series of genetic evidence support a role for endocytosis in the sustaining of the signalling. Historically, the first proof was provided by the use of a dominant-negative mutant of dynamin that blocks EGF internalization and causes the inhibition of EGF-induced activation of PI3K and ERKs (extracellular signal-regulated kinases) (Vieira et al, 1996). This initial evidence was then reinforced by experiments with siRNAs (small interfering RNAs) targeting proteins involved in internalization, which show that endocytosis is required for ERK activation by several receptor kinases [reviewed in (Sorkin & von Zastrow, 2009)]. Not only endocytosis is crucial to sustain signalling, but it is also required to determine signal specificity and diversification. Indeed, endosomes can support signalling cascades that cannot happen at the PM. The existence of endosome-specific signalling cascades has been shown for different receptor systems, including RTKs, GPCRs and Notch (reviewed in (Scita & Di Fiore, 2010; Sorkin & von Zastrow, 2009)). In the TGF β R pathway, specific signalling proteins are recruited to endosomes through their binding to PI3P (phosphatidylinositol 3-phosphate, which is enriched in endosomal membrane compared to PM) and this allows intracellular-specific signalling. Indeed, the activated TGF β R receptor interacts with the adaptor protein SARA (smad anchor for receptor activation) in early endosomes. SARA is associated with the receptor target SMAD2, and this allows the efficient phosphorylation of SMAD2 by TGF β R in endosomes (Chen et al, 2007; Hayes et al, 2002; Tsukazaki et al, 1998). Once phosphorylated, SMAD2 forms a complex with SMAD4, which translocates to the nucleus to regulate gene transcription.

Importantly, early endosomes are a morphologically and functionally heterogeneous population, characterized by the presence of biochemically distinct membrane subdomains (Lakadamyali et al, 2006; Miaczynska et al, 2004; Sonnichsen et al, 2000; Zoncu et al, 2009).

At the molecular level, small GTPases play a crucial role in determining the different sorting fates of cargoes at these stations, which ultimately impact on the final signalling response [reviewed in (Stenmark, 2009)]. For instance, APPL1-containing endosomes are precursors of early endosomes specifically enriched in Rab5 but lacking EEA1. It has been proposed that the progressive accumulation of PI3P species (through association and activity of phosphatidylinositol 3-kinase, PI3KC3/Vps34) causes the recruitment of EEA1, which competes with APPL1 for Rab5 binding, displacing it from the maturing early endosomes (Zoncu et al, 2009). Importantly, APPL1- but not EEA1-positive endosomes are competent for AKT signalling (Zoncu et al, 2009). This “phosphoinositide switch” is responsible for the maturation of endosomes and it is involved in signalling specification.

A non-canonical example of endosome-specific signalling is provided by the TNFR (tumor necrosis factor receptor) signalling cascade (Schutze et al, 2008) that promotes pro-apoptotic signalling. The components of this pathway are recruited to the ligand-bound TNFR at the plasma membrane (Micheau & Tschopp, 2003). In order for apoptosis to be achieved, the cysteine protease caspase-8 has to be activated by its proteolytic cleavage and this occurs on endosomes (Schneider-Brachert et al, 2004). Although, the mechanisms that prevent caspase-8 recruitment and activation at the PM are not yet known, this represents another example of how endocytosis contributes to signal specificity.

2.2 Regulation of signalling by endosome sorting

Once internalized and sorted to the early endosomes, cargoes can be routed to degradative pathways, terminating signalling, or recycled back to PM, allowing further rounds of activation. Both these mechanisms contribute to regulate signalling in space and time [reviewed in (Marchese et al, 2008; Sorokin & von Zastrow, 2009)].

Transfer of activated receptors to late endosomes/multivesicular bodies (MVB) terminates signalling, either by sequestering receptors in intraluminal vesicles, thus preventing their interaction with signal transducers, or by promoting their lysosomal degradation. Receptor ubiquitination plays a critical role in this process. Indeed, several protein complexes harbouring ubiquitin (Ub)-binding domains recognize ubiquitinated cargoes and escort them along the degradative route to the lysosome (Dikic et al, 2009). These complexes called ESCRT (endosomal sorting complex required for transport) act sequentially at various stations of the degradative route and are involved in MVB inward vesicles budding and cargo sequestration in the intraluminal vesicles of MVBs [for reviews see (Hurley & Hanson, 2010; Raiborg & Stenmark, 2009)].

On the other hand, recycling of internalized receptors to the PM allows the recovery of unoccupied/free receptors to the cell surface and restores receptor sensitivity to extracellular ligands, as is the case for GPCRs. One classical example is represented by β 2AR (β 2 adrenergic receptor). This class of receptors signals through interaction with PM-resident trimeric G proteins, which transduce signalling from the PM. Upon agonist stimulation, coupling of β 2AR trimeric G proteins is inhibited by receptor phosphorylation events [see, for instance, (Benovic et al, 1985; Benovic et al, 1986; Pitcher et al, 1992), reviewed in (Kelly et al, 2008)], which cause functional desensitization of signalling in the absence of endocytosis. However, β -arrestins are recruited to the phosphorylated receptors, triggering their internalization and sorting into a rapid recycling pathway. This step promotes receptor dephosphorylation by an endosome-associated PP2A protein phosphatase, thus ensuring the return of intact receptor for successive rounds of signalling (Pitcher et al, 1995; Vasudevan et al 2011; Yang et al, 1988), a process called "resensitization".

A related example, where the differential trafficking fate determines the duration of the signal, is represented by the EGFR system. When stimulated with TGF α or EGF, EGFR is rapidly internalized. However, while EGF binding to EGFR remains stable at the pH of endosomes, TGF α rapidly dissociates from the receptor. This results in different signalling outputs: EGFR/EGF complex remains stable and active at the endosomal station and is then transported to lysosomes for degradation, allowing signal termination; in contrast, in the case of TGF α , the receptor detaches from ligand at the endosomal station and it is recycled

back to the PM, ready to undergo an additional round of activation (Decker, 1990; Ebner & Derynck, 1991; French et al, 1995; Longva et al, 2002). In agreement with this, TGF α is a more potent mitogen than EGF (Waterman et al, 1998). The idea that endosome sorting regulates signalling output as a function of ligand type was shown also in the case of KGFR (keratinocyte growth factor receptor). Indeed, stimulation with two different ligands, KGF or FGF10, targets the receptor to two distinct trafficking routes, degradation vs. recycling, respectively, and this correlates with the higher mitogenic activity exerted by FGF10 on epithelial cells (Belleudi et al, 2007).

The central role of endocytosis in cellular signalling raises the possibility that alteration of this process might contribute to pathological phenotypes in which aberrant signalling is central, such as development and progression of cancer. Several lines of indirect evidence support a role of endocytosis in cancer [reviewed in (Lanzetti & Di Fiore, 2008; Mosesson et al, 2008)]. However, solid proof for a causative role of endocytosis in tumourigenesis is missing. A recent advance in this direction came from a study by Kermorgant's group (Joffre et al, 2011), who investigated the mechanism leading to tumourigenesis of two oncogenic Met mutants (M1268T and D1246N). These mutations cause constitutive Met kinase activity that was originally considered at the basis of their oncogenic potential. By using a combination of *in vitro* and *vivo* approaches, Kermorgant's group showed that endocytosis and intracellular trafficking of these mutants play a crucial role in determining their tumorigenic activity, besides their basal kinase activation. Indeed, these mutants are constitutively internalized and recycled back to the PM at a higher rate compared to WT receptor, and they also show impaired degradation. Importantly, inhibition of internalization with different genetic and pharmaceuticals tools is able to significantly reduce the ability of these mutants of induce transformation *in vitro* and to generate tumours in *ex vivo xenograft* experiments, without altering their activation status. Although the endocytic mechanism used by these mutant receptors is far to be clear (they seem to enter a constitutive pathway that depends on Cbl, Grb2, Clathrin and dynamin and that is independent from receptor kinase activity and ubiquitination), this is the first evidence for a direct involvement of endocytosis and endosome sorting in cancer development.

2.3 Different trafficking routes determine signalling outputs

Different internalization pathways are often associated to distinct intracellular fates. Several signalling receptors, including RTKs, GPCRs, TGF β R, NOTCH and WNT undergo both clathrin-mediated endocytosis (CME) and non-clathrin endocytosis (NCE) and this influences the final signalling output (Le Roy & Wrana, 2005). A mechanism of this kind takes place during internalization and signalling of the EGFR (Sigismund et al, 2005). At low doses of EGF, the EGFR is almost exclusively internalized through CME, which leads to recycling of the receptor and sustains signalling, with only a minor fraction of EGFRs targeted to degradation (Sigismund et al, 2008). At higher doses, about half of the ligand-engaged receptors are then internalized through NCE, a pathway that targets EGFRs to lysosomal degradation causing rapid signal extinction (Sigismund et al, 2008). This dual mechanism perfectly couples with the different EGF concentrations found in body fluids [ranging from 1 to hundreds of ng/ml, reviewed in (Sigismund et al, 2005)]. Indeed, under scarce ligand availability, endocytosis (through CME) preserves the activated receptors from degradation, maximizing the signalling response; at high EGF, the NCE pathway destines

the excess of activated EGFR/EGF complex to degradation, protecting cells from overstimulation. This concept has been challenged in other studies, where EGFR was reported to be internalized exclusively through CME at all concentrations of EGF (Kazazic et al, 2006; Rappoport & Simon, 2009). The discrepancy may be due to the different cellular systems used in these studies. It still remains to establish the nature of the NCE pathway used by the EGFR and the molecular mechanism involved [which is still poorly characterized, although it has been shown to be caveolin-independent and to require receptor ubiquitination (Sigismund et al, 2008; Sigismund et al, 2005)].

A similar scenario was previously reported in the case of TGF β R. This receptor is internalized both by CME and NCE and this has profound impact on the final signalling output (Di Guglielmo et al, 2003). Proteins of the TGF β superfamily signal through the transmembrane Ser-Thr kinase TGF β R type I and type II heteromeric complex (T β RI and T β RII). Ligand-induced assembly of the heteromeric receptor complex activates T β RI, which initiates Smad signalling by phosphorylating the receptor-regulated Smads. The Smad adaptor protein SARA plays a crucial role at this step. Indeed, SARA binds the receptor and contains a FYVE (Fab1p, YOTB, Vac1p and EEA1) domain, which also binds to membranes through specific interactions with phosphatidyl inositol 3' phosphate (PI3P). Receptor internalization through the clathrin pathway is essential for signalling and SARA has been found in the PI(3)P-enriched EEA1-positive endosomes that are downstream of this route (Di Guglielmo et al, 2003). Conversely, receptors that enter cells through NCE are associated with Smad7 and the E3 Ub ligase SMURF; they are ubiquitinated and subjected to degradation (Di Guglielmo et al, 2003).

It is important to note that CME is not always associated to signalling and NCE to degradation, but the opposite is also true, as it was shown in the case of LRP6 [WNT3a-activated low-density receptor-related protein 6, (Yamamoto et al, 2008)]. In the presence of Wnt3a, LRP6 is phosphorylated and internalized into caveolin-positive vesicles, where it can stabilize β -catenin and activates signalling via the CK1g kinase. If LRP6 binds the Wnt3a antagonist Dkk (Dickkopf), it is targeted to the clathrin pathway, which is not competent for signalling but rather directs LRP6 to degradation.

Other examples on how the route of internalization influences the final signalling output have been recently provided in the case of IGF-1R (Martins et al, 2011; Sehat et al, 2008) and PDGFR (De Donatis et al, 2008). In both cases, it has been proposed that they can enter through both clathrin-dependent and -independent pathways depending on the amount of ligand used to stimulate cells, similarly to what has been shown for the EGFR system. This again impacts on the final biological response. For instance, in the case of PDGFR, cells switch from a migrating to a proliferating phenotype in response to an increasing PDGF gradient. It was proposed that the decision to proliferate or migrate relies on the distinct endocytic route followed by the receptor in response to ligand concentration (De Donatis et al, 2008). Although these studies remain at the phenomenological level with no mechanistic insights, they confirm the idea that integration of different internalization pathways is crucial to decode signal information and to specify the signalling response.

2.4 Role of endo-exo membrane trafficking in the execution of cell polarity programs

Endo and exocytosis not only control the persistence and the nature of signals as highlighted above, but also the restricted compartmentalization of the signals. This has profound

implications in particular in the establishment of cell polarity, a process that largely relies on the correct localization of protein complexes and signalling platforms at cell-to-cell and cell-to-extracellular matrix contacts. In this regards, a key role in the controlled distribution of signal transducers in restricted areas of the plasma membrane, in response to extracellular cues, is played by small GTPases of the Rab family like Rab5, Rab8 and Rab11.

Rab5 is a master regulator of endocytosis and actin remodelling (Lanzetti et al, 2004; Lanzetti et al, 2000; Palamidessi et al, 2008; Zerial & McBride, 2001). It controls the internalization of a variety of distinct receptors, including the adhesive molecules integrins and cadherins (Palacios et al, 2005; Pellinen et al, 2006), as detailed in paragraph 3, thus participating to the processes of cell-to-cell and cell-to-extracellular matrix adhesion. Importantly, in *Drosophila melanogaster* deletion of Rab5 or disruption of the endocytic protein Syntaxin/Avalanche affects the polarized, restricted apical distribution of the fate-decision receptor Notch and of the polarity determinant Crumbs (Lu & Bilder, 2005). Failure in internalization of Notch and Crumbs causes their accumulation and results in the expansion of the apical membrane domain. Impaired Notch internalization severely impacts on its degradation and signalling and, in turn, this leads to overgrowth of imaginal epithelial tissues (Lu & Bilder, 2005) indicating that endocytosis may also control epithelial tissue proliferation.

Rab8 participates in polarized transport of molecules to the basolateral membrane (Huber et al, 1993) and also in cilia (Nachury et al, 2007). Genetic deletion of Rab8 in mice has been found to affect the distribution of apical proteins to the surface of intestinal epithelial cells resulting in accumulation of vacuoles containing apical hydrolases and microvilli with the final outcome of animal death by starvation (Sato et al, 2007). Thus, Rab8 has been proposed to play a crucial role in the biogenesis of the apical membrane, a process that is profoundly influenced also by another Rab protein involved in recycling routes: Rab11 [reviewed in (Hoekstra et al, 2004)]. Indeed trafficking *via* the recycling endosomes is required for the establishment or rearrangement of cell polarity in various settings including cellularization, cell-to-cell boundary rearrangement, asymmetric cell division, and cell migration (Assaker et al, 2010; Bryant et al, 2010; Emery et al, 2005; Xu et al, 2011). Furthermore, it provides a very efficient mechanism to reinforce polarity by feedback loops (Assaker et al, 2010).

In addition to these GTPases, the endocytic protein Numb has also been implicated in the establishment of apical-basolateral polarity. Numb participates to cadherin endocytosis by interacting with the E-cadherin/p120 complex and promotes E-cadherin endocytosis. Impairment of Numb induces mislocalization of E-cadherin from the lateral to the apical membrane. This function of Numb appears to rely on its phosphorylation by Atypical protein kinase C (aPKC), a member of the PAR complex, as it prevents association of phosphorylated Numb with p120 and α -adaptin thereby attenuating E-cadherin endocytosis (Sato et al, 2011).

Beside the involvement of endo-exocytosis in apical-basolateral polarity, these trafficking routes are also required in the establishment of planar cell polarity (PCP) [for a detailed reviews on membrane trafficking in cell polarity see (Nelson, 2009)]. Intracellular membrane trafficking has emerged as a crucial regulator of PCP in the *Drosophila* wing where inhibition of dynamin or Rab11 disrupts PCP-dependent hexagonal repacking (Classen et al, 2005). More recently, Rab5 has been found to bind to Go and to participates in PCP and in Wingless signal transduction, pathways initiated by G-protein coupled receptors of the

Frizzled (Fz) family. Additionally, Rab4 and Rab11 function in Fz- and Go-mediated signaling to favor PCP over canonical Wntless signaling (Purvanov et al, 2010). Furthermore, the Rab5-effector Rabenosyn-5 is required for the polarized distribution of PCP proteins at the apical cell boundaries aiding the establishment of planar polarity (Mottola et al, 2010).

The requirement for regulation of clathrin-mediated endocytosis in planar cell polarity also emerges from the study showing that the planar polarized RhoGEF2 controls the function of Dia and Myosin II which, in turn, are responsible for the initiation of E-cadherin endocytosis by regulating their lateral clustering (Levayer et al, 2011).

Another relevant instance of the involvement of endo/exocytosis in the execution of polarized function is directed cell migration. Also in this case important lessons come from the *Drosophila* model. In the fruit fly, endocytosis of motogenic receptors and their recycling to the plasma membrane serve to maintain their polarized distribution at the leading edge of migrating cells, thus promoting directional motility (Jekely et al, 2005; McDonald et al, 2006; McDonald et al, 2003; Montell, 2003; Wang et al, 2006). This is achieved *via* a tight control of endocytosis and recycling in restricted areas of the cell membrane through the regulation of a subset of molecules such as the endocytic E3 ligase Cbl, or the Rab5 GEF Sprint (Jekely et al, 2005).

Collectively, these observations provide genetic evidence that one physiological role of endocytosis is to ensure localized intracellular responses to extracellular cues, i.e. the spatial restriction of signalling. Similar circuitries are also exploited in mammalian cells to achieve and maintain cell polarity and also to execute polarized functions such as directed cell migration (Balasubramanian et al, 2007; Caswell & Norman, 2008; Jones et al, 2006; Palamidessi et al, 2008; Riley et al, 2003; Schlunck et al, 2004). Of note, directed cell migration in mammalian cells has been found to require Rab proteins like Rab25 and Rab5 (Caswell et al, 2007; Palamidessi et al, 2008). Rab25 promotes the extension of long pseudopodia in 3D matrices, by regulating the recycling of a pool of $\alpha 5 \beta 1$ (Caswell et al., 2007; detailed in paragraph 3). Instead, Rab5-dependent endocytosis allows for the activation of Rac, induced by motogenic stimuli, on early endosomes. Subsequent recycling of Rac to the plasma membrane ensures localized formation of actin-based migratory protrusions (Palamidessi et al, 2008).

3. Regulation of cell adhesion dynamics by trafficking adhesive receptors

The acquisition of key molecular strategies that support social cell functions, such as intercellular communication and adhesion either to other cells or to the surrounding environment, represented a tenet in the evolution from simple unicellular to complex multicellular organisms on the Earth (Rokas, 2008). Indeed, the appearance of genes encoding for adhesion receptors is likely to have represented a major driving force of the so called Cambrian explosion during which, around 500 million years ago, the appearance on our planet of multicellular organisms, aka metazoans, and an astonishingly wide exploration of most of their possible morphological organizations took place (Abedin & King, 2010). In mammals, the ability of dynamically regulating cell adhesion in space and time is crucial for several physiological and pathological phenomena, such as embryonic development (Hynes, 2007), tissue and organ morphogenesis and repair (Insall & Machesky,

2009), leukocyte extravasation (Hogg et al, 2011), platelet aggregation (Tao et al, 2010), and cancer cell metastatic dissemination throughout the body (Roussos et al, 2011). Cadherins (Takeichi, 2011) and integrins represent the main classes of transmembrane receptors respectively mediating cell-to-cell and cell-to-extracellular matrix (ECM) adhesion in mammals. A dynamic control of cell adhesion can be accomplished by regulation of either conformation or endo-exocytic traffic of adhesion receptors. Cadherin and integrin conformational activation can be triggered by the binding of either extracellular divalent cations, e.g. Ca^{2+} for cadherins (Takeichi, 2011) or Mg^{2+} for integrins (Tiwari et al, 2011), or cytosolic proteins, such as talin and kindlin in the case of integrins (Moser et al, 2009). The mechanisms that directly supersede to the control of cadherin (Gumbiner, 2005; Niessen et al, 2011; Takeichi, 2011) and integrin conformation (Moser et al, 2009; Shattil et al, 2010) have been extensively described elsewhere. Here, we will instead review the emerging evidence of how cell adhesion and migration critically depends on cadherin and integrin traffic.

3.1 Role of E-cadherin traffic in adherens junction maintenance and remodeling

Normal epithelial tissues are held together by adherens junctions (AJs), *i.e.* cell-to-cell adhesion sites that originate after the dimerization *in trans* of epithelial (E)-cadherin molecules (Gumbiner, 2005; Niessen et al, 2011; Takeichi, 2011). E-cadherin-dependent assembly of AJs is required to assemble and maintain the apico-basal polarity of functional epithelia (Rodriguez-Boulan et al, 2005).

In *Drosophila* and in mammals, the maintenance of both AJs and epithelial polarity depends on a complex formed by the small GTPase Cdc42 and its partner PAR6 that binds aPKC (Goldstein & Macara, 2007; Iden & Collard, 2008; McCaffrey & Macara, 2009).

Interestingly, Cdc42, PAR6, and aPKC are required for the activation of a signaling pathway responsible for the dynamin-driven pinch-off of vesicles during E-cadherin endocytosis from *Drosophila* AJs (Baum & Georgiou, 2011; Georgiou et al, 2008; Leibfried et al, 2008) and a genome wide siRNA screen in *C. elegans* also identified Cdc42, PAR6, and aPKC as key regulators of endocytosis (Balklava et al, 2007). In addition, pharmacological inhibition of dynamin coupled to two-photon FRAP microscopy demonstrated that in mammalian cells E-cadherin engaged at mature stationary AJs turns over by endocytosis and not by free diffusion through the PM (de Beco et al, 2009). *Drosophila* Cdc42 interacting protein 4 (Cip4), aka transducer of Cdc42-dependent actin assembly 1 (TOCA-1) in mammals, displays both an FCH-Bin-Amphiphysin-Rvs (F-BAR) and a Src homology 3 (SH3) domains that respectively bind curved membranes and dynamin (Fricke et al, 2009). Of note, Cip4 knockdown causes AJ and E-cadherin endocytosis defects identical to those caused by the lack of components of the Cdc42/PAR6/aPKC apical complex (Baum & Georgiou, 2011; Leibfried et al, 2008).

Once internalized, E-cadherin is first trafficked to Rab5 containing early endosomes and from there to a Rab11-positive recycling compartment (Emery & Knoblich, 2006; Harris & Tepass, 2010; Wirtz-Peitz & Zallen, 2009). Sec10 and Sec15 proteins then directly bind and interconnect the β -catenin-bound endosomal E-cadherin to the exocyst complex located at the PM, hence favoring the recycling of the adhesion receptor (Langevin et al, 2005).

There is now a mounting consensus that the maintenance of stable AJs requires the continuous and local traffic of E-cadherin back and forth from the PM (Baum & Georgiou,

2011; Emery & Knoblich, 2006; Harris & Tepass, 2010; Wirtz-Peitz & Zallen, 2009). Therefore, endless cycles of polarized endocytosis and recycling of E-cadherin are responsible for the existence in space and time of AJs that warrant an efficient intercellular adhesion in stable epithelia.

This would suggest that in living cells, because of the intrinsic physical and biochemical properties of its molecular components, what appears as a stable adhesion site is nothing but an almost continuous and swift spatio-temporal succession of short-lived adhesive events. In this framework, endocytosis could be required either to remove and then replenish *via* recycling the adhesive material or to provide a substantial fraction of the force required to maintain adhesion.

Moreover, the incessant turnover of E-cadherins would allow cells to rapidly adapt the structure of their AJs in response to extracellular signals during tissue reshaping. Indeed, during embryonic development, cancer cell metastatization, and tissue fibrosis epithelial cells activate the epithelial-mesenchymal transition (EMT) program during which they lose their AJs and become motile (Kalluri & Weinberg, 2009; Thiery & Sleeman, 2006). For example, in epithelial cells, hepatocyte growth factor (HGF), acting through the MET tyrosine kinase receptor, activates H-Ras that, by stimulating the Rab5 guanine exchange factor Ras and Rab interactor 2 (RIN2), induces E-cadherin endocytosis (Kimura et al, 2006). In addition, HGF signals *via* Src and generates a tyrosine phosphosite on E-cadherin where the E3-ubiquitin ligase Hakai docks to trigger the ubiquitination and lysosomal degradation of E-cadherin (Fujita et al, 2002; Palacios et al, 2005).

3.2 Combined regulation of integrin function by conformation and traffic

Integrin heterodimers can switch from low (inactive) to high affinity (active) conformation for their ECM ligands (Hynes, 2002). Conformational activation of integrins can be due to the interaction of their cytoplasmic tails with different proteins acting as positive (e.g. talin and kindlin) (Moser et al, 2009; Shattil et al, 2010) or negative (e.g. mammary-derived growth inhibitor, MDGI) modulators (Nevo et al, 2010). Due to their ability to mechanosense the surrounding ECM environment and mediate the interactions that support cell adhesion and migration (Parsons et al, 2011), active integrins are key regulators of several important adhesion dependent functions, such as assembly and morphogenetic movements of tissues and organs or migration of isolated/clustered cells through the body (e.g. immune or cancer cells). For example, the remodeling of immature vascular networks that occurs during embryonic, but not tumor angiogenesis, depends on the ability of endothelial cells (ECs) to instantaneously mechanotransduce variations in fluid shear stress (Hahn & Schwartz, 2009).

Integrin traffic is increasingly recognized as a key determinant in the dynamic control of cell adhesion to the ECM (Caswell et al, 2009; Pellinen et al, 2006). Integrins can be internalized in a clathrin-dependent as well as in a clathrin-independent way. For example, $\alpha 5\beta 1$ integrin, the major fibronectin (FN) receptor, can be endocytosed into clathrin-coated vesicles (CCVs) (Pellinen et al, 2008) or by a caveolin-mediated pathway (Shi & Sottile, 2008). It was initially hypothesized that in migrating cells integrins can be preferentially endocytosed in ECM-adhesion sites located at the trailing edge and then recycled back *en masse* toward the leading edge (Bretscher, 1989). More recently, such a theoretical long range model has been challenged by an experimental short range model that showed how in cells

migrating in 3D matrices a spatially restricted subpopulation of $\alpha 5\beta 1$ integrin is instead internalized from the PM of ECM-adhesions located at the cell front and quickly recycled back to the same or proximal adhesive structures (Caswell & Norman, 2008; Caswell et al, 2007; Caswell et al, 2009). The Rab11 subfamily member Rab25, which resides in a vesicular compartment located in close proximity of the tips of invading pseudopods, physically interacts with the $\beta 1$ subunit of the internalized integrins and promotes tumor cell invasion, likely by favoring the localized recycling of $\alpha 5\beta 1$ integrin (Caswell & Norman, 2008; Caswell et al, 2007; Caswell et al, 2009). Another key regulator of integrin traffic in motile cells is the Rab11 effector Rab-coupling protein (RCP), which binds with $\beta 3$ integrin and, when $\alpha v\beta 3$ integrin is inhibited, switches to the cytodomain of $\beta 1$ integrin, connecting $\alpha 5\beta 1$ integrin with Rab11 and thus favoring its recycling to the PM (Caswell et al, 2008; Caswell et al, 2009). Of note, RCP also associates with EGFR and, upon $\alpha v\beta 3$ inhibition, the recycling to the PM of endocytosed EGFR is enhanced in coordination with that of $\alpha 5\beta 1$, finally resulting in an increased EGFR auto-phosphorylation and downstream activation of AKT (Caswell et al, 2008; Caswell et al, 2009).

In the last couple of years, the new concept that endocytosis of active and inactive integrins could be mediated by different sorting machineries started emerging. Neuropilin 1 (Nrp1) is a transmembrane protein, initially identified in neurons, that is also expressed in ECs, where it works as a co-receptor for both pro- and anti-angiogenic factors, such as vascular endothelial growth factor (VEGF)-A165 and semaphorin 3A (SEMA3A) respectively (Bussolino et al, 2006; Neufeld & Kessler, 2008; Serini & Bussolino, 2004). The very C-terminal SEA motif of Nrp1 cytodomain binds the endocytic adaptor GAIIP interacting protein C terminus 1 (GIPC1)/synectin (Cai & Reed, 1999) that can also bind to the motor Myosin VI (Myo6) (Reed et al, 2005). Nrp1, *via* its cytodomain, controls EC adhesion to FN in a way that does not depend on its function as co-receptor for either VEGF-A or SEMA3A, but rather on its ability to promote the GIPC1/synectin- and Myo6-dependent endocytosis of the active, but not inactive conformation of $\alpha 5\beta 1$ integrin from ECM adhesions (Valdembri et al, 2009). Remarkably, Nrp1 silencing does not affect the ratio between active and inactive $\alpha 5\beta 1$ integrin, indicating that not only the conformational switch of integrins, but also the regulation of active integrin traffic and distribution constitutes an equally crucial parameter in the control of EC adhesion to the ECM (Valdembri et al, 2009). It has hence been proposed a model in which, upon FN binding, active $\alpha 5\beta 1$ integrin associates with Nrp1 at the PM. GIPC1/synectin and Myo6 then favor the rapid internalization of the active $\alpha 5\beta 1$ /Nrp1 complex into Rab5-positive early endosomes, from which (active) $\alpha 5\beta 1$ is then recycled back to the PM, likely in newly forming ECM-adhesion sites.

The described endo-exocytic cycle of active integrins back and forth from ECM adhesions is remarkably similar to the traffic dependent E-cadherin dynamics observed in AJs of epithelial cells (*see above*). It is therefore tempting to speculate that also an ECM adhesion site could result from a rapid sequence of localized and exceptionally brief adhesive events, during which traffic could be crucial either to endocytose and then immediately recycle active integrins or to generate the force that has to be applied on ECM-bound active integrins to allow cell adhesion. Likely because GIPC1/synectin also binds the C-terminal SDA motif of the $\alpha 5$ integrin subunit cytodomain (Tani & Mercurio, 2001), while in ECs Nrp1 and Myo6 are specifically dedicated to the endocytosis of active $\alpha 5\beta 1$ integrin, GIPC1 controls inactive $\alpha 5\beta 1$ internalization as well (Valdembri et al, 2009). The different molecular

composition of the machineries that control active *vs.* inactive integrin traffic could imply that higher amounts of endocytic proteins are required to effectively internalize ECM-bound integrins. Accordingly, the force-generating retrograde motor Myo6 (Spudich & Sivaramakrishnan, 2010) participates to endocytosis, transport of endosomal vesicles along F-actin (Hasson, 2003), and active integrin internalization (Valdembri et al, 2009) as well.

Clathrin coats exist either as classical curved clathrin-coated pits or as flat clathrin-coated plaques that depend on the presence of the actin cytoskeleton and occur only at ECM-adherent surfaces, indicating that integrin-mediated adhesion of cells to the ECM likely control the organization of the different clathrin-based endocytic structures (Kirchhausen, 2009; Saffarian et al, 2009). The potential role of cell-to-ECM adhesion in regulating clathrin-mediated endocytosis is further supported by the recent experimental observation that the closer clathrin-coated pits are to integrin-containing adhesion sites the slower are their internalization dynamics (Batchelder & Yarar, 2010). It is indeed possible that the binding of integrins to the ECM could give rise to forces that counteract the pulling forces required to deform and curve the PM to finally allow clathrin-based internalization. Such a hypothesis could also account for the requirement of different molecular complexes for active *vs.* inactive integrin internalization.

To date, only few proteins have been selectively involved in inactive, but not active, integrin traffic and the degree of specificity for the bent/inactive integrin conformation is still matter of debate. A prominent example is represented by the endocytic adaptor protein disabled 2 (DAB2), that is able to directly bind the cytodomain of integrin β subunits (Calderwood et al, 2003), and was recently found to selectively promote the internalization of inactive $\beta 1$ integrins (Teckchandani et al, 2009). However, during ECM-adhesion disassembly experiments, Chao and Kunz, by incubating living cells with the anti-active $\beta 1$ integrin monoclonal antibody 12G10, found that active $\beta 1$ integrins could be endocytosed in a DAB2-dependent manner as well (Chao & Kunz, 2009). However, since incubation of living cells with function activating or blocking antibodies represents a significant bias in the study of integrin activation physiology, further work is needed to better characterize the role of DAB2.

4. Unconventional function of membrane trafficking proteins in mitosis

Recent findings have shown that clathrin-mediated endocytosis is active throughout mitosis, while the recycling pathway slows down from prophase until the completion of anaphase (Boucrot & Kirchhausen, 2007). These data have been generated by monitoring the changes in plasma membrane area during mitosis in living cells with a membrane-impermeant dye that becomes fluorescent upon binding to the outer leaflet of the plasma membrane. Since the dye cannot flip to the inner leaflet, only endocytic vesicles generated by internalization of the plasma membrane can be visualized. At metaphase, these plasma membrane-derived vesicles are not delivered back at the surface resulting in a net decrease of the cell area. In turn, this translates in cell detachment and round up from prophase to anaphase. The recycling pathway recovers at telophase when the forming daughter cells start to spread again (Boucrot & Kirchhausen, 2007).

Interestingly, mitotic phosphorylation of Rab4, a GTPase required for recycling from early endosomes to the plasma membrane (van der Sluijs et al, 1992), prevents its localization at

endosomal membranes (Ayad et al, 1997). During mitosis, phosphorylated Rab4 is in the cytosol complexed with the peptidyl-prolyl isomerase Pin1 and it is no longer able to recruit downstream effectors on endosomes (Gerez et al, 2000). Thus an appealing possibility is that Rab4 phosphorylation might participate in the inhibition of the recycling pathway measured by Boucrot and Kirchhausen during the early steps of mitosis.

Of note, fusion of early endosomes in mitosis is blocked via cdc2-dependent phosphorylation events (Tuomikoski et al, 1989). This might represent an additional mechanism to inhibit vesicles recycling at the plasma membrane by altering the homeostasis of the endosomal compartment and affecting the generation of exocytic vesicles. Inhibition of homotypic fusion of early endosomes at mitosis is also caused by decreased residence time of the early endosome-tethering molecule EEA1 on endosomal membranes (Bergeland et al, 2008). It would be interesting to define how the acceleration of the EEA1 cycle between cytosol and membranes is achieved in mitotic cells.

Endocytic/trafficking proteins are also emerging as important factors required for the proper execution of cell division. Beside the involvement of trafficking molecules in membrane delivery to the cleavage furrow at cytokinesis [for recent reviews see (McKay & Burgess, 2011; Montagnac et al, 2008)], some of these proteins also display specific functions in mitosis. Here we will review knowledge rising on this issue.

One of the best-characterized endocytic molecules showing a distinct role in mitosis is the clathrin heavy chain. The clathrin complex is organized in a triskelion made of three heavy chains each with an associated light chain (ter Haar et al, 1998). At metaphase, clathrin also localizes to kinetochore fibers (spindle microtubules connecting kinetochores to spindle poles) of the spindle apparatus (Royle et al, 2005). Here it stabilizes spindle microtubules aiding congression of chromosomes on the metaphase plate. Depletion of clathrin heavy chain by RNA interference causes failure in the correct attachment of chromosomes to kinetochore fibers resulting in misaligned chromosomes and in persistent activation of the mitotic checkpoint thus prolonging mitosis (Royle et al, 2005). More recently, some advances in understanding clathrin function at the spindle have been made. Clathrin heavy chain has been found to bind to TACC3, phosphorylated on serine 558 by Aurora A, and to recruit it to the spindle. In turn, TACC3 is responsible for localization of ch-TOG, a protein that promotes microtubule assembly and spindle stability, to spindles (Lin et al, 2010). In agreement, functional ablation of clathrin heavy chain causes loss of ch-TOG from spindles and destabilizes kinetochore fibers affecting chromosome congression. Based on electron microscopy data, it has been proposed that TACC3/ch-TOG/clathrin heavy chain complex works as an inter-microtubules bridge that stabilizes kinetochore fibers by physical crosslinking reducing the rate of microtubule catastrophe (Booth et al, 2011).

Another important endocytic player is epsin, an adaptor molecule that binds and deforms membranes driving curvature of clathrin-coated pits (Ford et al, 2002). At mitosis, epsin participates in spindle morphogenesis indirectly through its ability to regulate mitotic membrane organization (Liu & Zheng, 2009). In cells depleted of epsin, by RNAi-mediated silencing, the membrane network that uniformly surrounds the chromosomes is distorted with uneven membrane distribution frequently showing layers of membrane whorls. This, in turn, alters spindle morphology resulting in splayed spindle poles and multipolar spindles (Liu & Zheng, 2009).

Huntingtin-interacting protein 1-related (HIP1r) functions in clathrin-mediated endocytosis and links endocytosis to the actin cytoskeleton (Engqvist-Goldstein et al, 2001). HIP1r also localizes to the spindle and its depletion by RNA interference causes chromosome misalignment and activation of the spindle checkpoint (Park, 2011).

In addition, it is worth to mention that Rab6A', a GTPase that regulates trafficking between the Golgi and post-Golgi membrane compartments, is also required for spindle stability (Mallard et al, 2002). At mitosis, depletion of Rab6A' arrests cells at metaphase (Miserey-Lenkei et al, 2006). Aligned chromosomes, in Rab6A'-depleted cells, show increased amount of p150^{Glued}, a subunit of the dynein/dynactin complex, and of Mad2 at kinetochores. p150^{Glued} takes part in the release of the checkpoint protein Mad2 from kinetochores thus switching off the mitotic checkpoint, an operation required for the transition of cells from metaphase to anaphase. The inability of Rab6A'-silenced cells to progress mitosis might be the consequence of defective p150^{Glued}-mediated transport of Mad2 out of kinetochores resulting in the failure to turn off the checkpoint. Thus Rab6A', by regulating the dynamics of the dynein/dynactin complex at the kinetochores, cooperates to the inactivation of the Mad2-spindle checkpoint.

Some trafficking proteins have also been found to act at the centrosome which is part of the mitotic machinery that ensure proper chromosome segregation. One of these proteins is dynamin. In addition to its membrane localization, dynamin is at the centrosome throughout the cell cycle and localizes to the spindle midzone and to the cleavage furrow during cytokinesis (Thompson et al, 2004; Thompson et al, 2002). Depletion of dynamin by RNA interference causes centrosome separation indicating a role for dynamin in the maintenance of centrosome cohesion (Thompson et al, 2004).

The Autosomal Recessive Hypercholesterolemia (ARH) protein provides another example. ARH is a cargo-specific adaptor that functions in clathrin-mediated endocytosis of receptors of the LDLR family (Shin et al, 2001). It displays a complex subcellular localization being on endocytic vesicles and at the centrosome in interphase. During mitosis, it also localizes to kinetochores, spindle poles and midbody. The suggested function for ARH is in centrosome assembly, as ARH-/- embryonic fibroblasts show smaller centrosomes. Since ARH binds to the dynein motor protein it could cooperate in the transport of components to the centrosome. Of note, functional ablation of ARH also strongly delays cytokinesis (Lehtonen et al, 2008).

In addition, the Rab-GAP protein RN-tre is phosphorylated at mitosis and dephosphorylated by the dual-specificity phosphatase Cdc14A (Lanzetti et al, 2007). Cdc14A controls key mitotic events and it is also implicated in centrosome function in human cells (Mailand et al, 2002). Mitotic phosphorylation on RN-tre modulates its GAP activity establishing an additional link between endocytosis and the machinery working at mitosis (Lanzetti et al, 2007).

Finally, Rab5 is required for nuclear membrane breakdown at mitosis, as depletion of this GTPase in *C. elegans* delays nuclear envelope disassembly and the release of nuclear envelope and lamina components (Audhya et al, 2007). The activity of Rab5 in nuclear envelope disassembly appears to result from its involvement in structuring the ER, of which the nuclear membrane represents a functional district (Audhya et al, 2007). Rab5 participates to mitotic ER clustering and to disassembly of the nuclear envelope also in mammalian cells

(Audhya et al, 2007; Serio et al, 2011). Although the molecular mechanisms are unclear, it has been proposed that Rab5 might act *in trans*, while localized on endosomes, by interacting with effectors on the ER membrane to induce their homotypic fusion. Furthermore, recent findings have shown that, at mitosis, Rab5 is required for proper chromosome alignment both in human cells and in the *Drosophila* system (Serio et al, 2011; Capalbo et al, 2011).

One relevant question is whether the modality of function for these molecules at mitosis is distinct from their role in membrane trafficking during interphase. A couple of observations argue in favor of this possibility.

First, some of these proteins display a subcellular localization in mitosis distinct from trafficking membranes. For instance, the globular N-terminal domain of the clathrin heavy chain is responsible for clathrin localization to kinetochore fibers and a number of assays, including labeling of intracellular membranes, electron microscopy analysis and mass spectrometry, revealed that it does not coat membranes at the spindle but it rather bind to microtubules or to microtubules-associated proteins (Royle et al, 2005). Localization of dynamin to centrosome, which is a non-membranous organelle, is dynamic and occurs through its middle domain in a microtubules-independent manner (Thompson et al, 2004).

Second, these molecules appear to interact with binding partners distinct from those involved in vesicular trafficking pathways and such interactions seem to be relevant during cell division. Indeed, clathrin has been reported to bind and stabilize spindle microtubules (Royle et al, 2005) while dynamin interacts with the centrosomal protein γ -tubulin (Thompson et al, 2004). In addition, the β 2-adaptin subunit of the clathrin adaptor AP2 associates, at least *in vitro*, with a component of the mitotic spindle checkpoint, the kinase BubR1. Although the physiological meaning of this interaction is unknown, it might provide a link between endocytic proteins and the mitotic checkpoint machinery (Cayrol et al, 2002). Of note, two accessory components of clathrin coated pits, epsin and Eps15 are phosphorylated at mitosis and such modification reduces their binding to the α -adaptin subunit of AP2 (Chen et al, 1999). Among the different hypothesis that can be envisioned, one appealing possibility is that mitotic phosphorylation of epsin and Eps15 alters their binding capabilities promoting formation of protein complexes working at mitosis and involving partners distinct from AP2. Importantly, epsin has been shown to facilitate spindle organization independently from its endocytic function by using cell-free spindle assembly assays. In these assays, *Xenopus* egg extracts, lacking the membrane cortex, have been depleted of epsin and reconstituted with purified epsin or with epsin lacking the membrane-bending domain. Only full length epsin was able to rescue the spindle defects demonstrating that the membrane curvature activity of epsin is required for the establishment of spindle morphology independently from endocytosis (Liu & Zheng, 2009). This study nicely extends the concept that endocytic proteins have a role in mitosis distinct from the one exerted during interphase.

Given that endocytosis is active throughout the cell cycle and that, at mitosis, some endocytic proteins are also involved in pathways different from internalization, these molecules might play two distinct functions simultaneously thus coordinating membrane traffic with the execution of mitotic events.

Genetic instability is a driving force in tumourigenesis and it is prompted by alteration in centrosome function and in spindle assembly (Lengauer et al, 1998; Lingle et al, 2002; Orr-Weaver & Weinberg, 1998). Since endocytic proteins participate in the regulation of mitotic events, this could represent a novel, previously unrecognized, link between endocytosis and cancer.

5. Conclusions

Endo and exocytosis are well-known mechanisms that regulate signal transduction and the execution of different cellular programs. The number of players, their crosstalk and the networks that they generate is continuously growing adding novel layers of complexity and definition to the current picture.

Intriguingly, increasing evidence shows that signalling itself can control and modulate endocytic pathways (Collinet et al, 2010). Activated receptors elicit a variety of signals that directly or indirectly control endocytosis by several means including phospho-modification of downstream effectors involved in endocytosis, control of protein synthesis and also modulation of actin cytoskeleton dynamics, a process that aids clathrin-mediated endocytosis. This is an emerging view in the trafficking field that will certainly disclose new areas of investigation.

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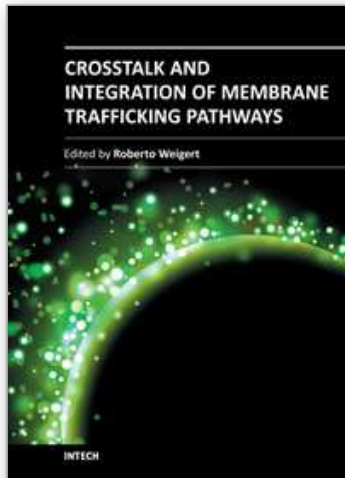
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Membrane traffic is a broad field that studies the complex exchange of membranes that occurs inside the cell. Protein, lipids and other molecules traffic among intracellular organelles, and are delivered to, or transported from the cell surface by virtue of membranous carriers generally referred as "transport intermediates". These carriers have different shapes and sizes, and their biogenesis, modality of transport, and delivery to the final destination are regulated by a multitude of very complex molecular machineries. A concept that has clearly emerged in the last decade is that each membrane pathway does not represent a close system, but is fully integrated with all the other trafficking pathways. The aim of this book is to provide a general overview of the extent of this crosstalk.

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