cAMP-dependent protein kinase A and the dynamics of epithelial cell surface domains: moving membranes to keep in shape

Wojtal, K A; Hoekstra, D; van IJzendoorn, S C D

cAMP-dependent protein kinase A and the dynamics of epithelial cell surface domains: moving membranes to keep in shape

Abstract

Cyclic adenosine monophosphate (cAMP) and cAMP-dependent protein kinase A (PKA) are evolutionary conserved molecules with a well-established position in the complex network of signal transduction pathways. cAMP/PKA-mediated signaling pathways are implicated in many biological processes that cooperate in organ development including the motility, survival, proliferation and differentiation of epithelial cells. Cell surface polarity, here defined as the anisotropic organisation of cellular membranes, is a critical parameter for most of these processes. Changes in the activity of cAMP/PKA elicit a variety of effects on intracellular membrane dynamics, including membrane sorting and trafficking. One of the most intriguing aspects of cAMP/PKA signaling is its evolutionary conserved abundance on the one hand and its precise spatial-temporal actions on the other. Here, we review recent developments with regard to the role of cAMP/PKA in the regulation of intracellular membrane trafficking in relation to the dynamics of epithelial surface domains.

cAMP-dependent protein kinase A and the dynamics of epithelial cell surface domains: moving membranes to keep in shape

Kacper A. Wojtal[1], Dick Hoekstra, Sven C.D. van IJzendoorn*

Department of Cell Biology/ Membrane Cell Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

* Corresponding author: Department of Cell Biology/ Membrane Cell Biology, University Medical Center Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands. Tel: +31 (0)50 3636603, Fax: +31 (0)50 3632728, e-mail: s.c.d.van.ijzendoorn@med.umcg.nl

Key words: protein kinase A, intracellular trafficking, cell polarity, apical, epithelial cell

[1] Current address: Division of Clinical Pharmacology and Toxicology, University Hospital Zurich, Zurich, Switzerland

Abstract

Cyclic adenosine monophosphate (cAMP) and cAMP-dependent protein kinase A (PKA) are evolutionary conserved molecules with a well-established position in the complex network of signal transduction pathways. cAMP/PKA-mediated signaling pathways are implicated in many biological processes that cooperate in organ development including the motility, survival, proliferation, and differentiation of epithelial cells. Cell surface polarity, here defined as the anisotropic organisation of cellular membranes, is a critical parameter for most of these processes. Changes in the activity of cAMP/PKA elicit a variety of effects on intracellular membrane dynamics, including membrane sorting and trafficking. One of the most intriguing aspects of cAMP/PKA signaling is its evolutionary conserved abundance on the one hand and its precise spatial-temporal actions on the other. Here, we review recent developments with regard to the role of cAMP/PKA in the regulation of intracellular membrane trafficking in relation to the dynamics of epithelial surface domains.

An introduction to epithelial cell surface polarity and cAMP-dependent protein kinase A

The ability of cells to establish a polarized phenotype, which includes an anisotropic organisation and asymmetry of their plasma membranes, is fundamental for organism development and functioning. While this includes many cell types including (migrating) fibroblasts, one of the best studied cases of cell surface asymmetry is that exhibited by the epithelium, one of the four primary body tissues. Epithelial cells line most body cavities and display structurally and functionally distinct cell surface domains [(1)]. These include apical and basolateral surface domains which face the body exterior and underlying tissue, respectively (figure 1A). Apical and basolateral cell surface domains differ in protein and lipid composition, a feature that is vital for these cells to perform domain-specific functions such as the selective uptake and excretion of molecules and protection of the body against pathogens and toxic compounds. Cell surface polarity is generated and maintained in spite of continuous exo-, endo-, and transcytotic membrane fluxes between these domains and intracellular organelles (figure 1A). The intracellular sorting of newly synthesized and recycling proteins and lipids is therefore crucial to generate and maintain such specific plasma membrane compositions, as well as to tailor these to meet changing physiological needs [(2,3)]. Predominant sorting stations for basolateral and apical plasma membrane components in epithelial cells are provided by the Golgi apparatus and/or the recently identified subapical compartment/ common recycling endosome [(4-6)] (Figure 1A).

Cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) have emerged as key signaling molecules that regulate the intracellular sorting and trafficking of basolateral and apical membrane components and, consequently, are involved in the functional dynamics of epithelial cell surface domains, in response to

extracellular cues. For example, in the renal collecting duct, the reabsorption of water is regulated by the antidiuretic hormone vasopressin which, upon binding to its receptor on the basolateral surface of kidney epithelial cells stimulates cAMP production and the PKA-mediated trafficking of water channels from the Golgi apparatus and/or endosomes to the apical membrane, thereby allowing water reabsorption from the pro-urine [7] (figure 1B). In the entero-hepatic circulation, peptide hormones that are secreted following a meal increase cAMP production in hepatocytes leading to increased PKA-mediated trafficking of ABC transporters from the Golgi apparatus and/or endosomes to the apical, bile canalicular domain. This stimulates the secretion of bile acids that, in turn, can help digest fats in the intestinal apical lumen [8] (figure 1B). One of the most important questions in this research field is how PKA activation, elicited by various extracellular stimuli, produces specific effects on the dynamics of distinct and spatially separated intracellular membrane systems. To address this question, insight in the structural and spatial-temporal dynamics of cAMP-PKA complexes is crucial.

In mammalian cells, PKA is a holoenzyme [9] which consists of two regulatory and two catalytic subunits. Four different regulatory subunits are distinguished, RIα, RIβ, RIIα, and RIIβ, the expression of which is tissue-dependent and developmentally regulated by a protein kinase A inhibitor called PKI. Regulatory subunits of PKA bind to free catalytic subunits (α, β, γ, or PRkX) and their primary function appears to keep the catalytic subunits in an inactive state. Two molecules of cAMP, which is produced by any of the nine known mammalian adenylyl cyclases in response to activation of G proteins coupled to different membrane receptors, bind to each regulatory subunit of PKA and causes the subsequent release and activation of the catalytic subunits [10,11]. These, in turn, can phosphorylate target proteins at serine and threonine residues.

cAMP phosphodiesterases (PDEs), which comprise eleven distinct families yielding a multitude of invariably expressed PDE isoforms [12], play an important part in regulating PKA activity by restricting local cAMP levels. In addition to the control of local cAMP concentrations by adenylyl cyclase and PDEs, a family of more than fifty A-kinase anchoring proteins (AKAPs) [13] control the spatial distribution of the different PKA holoenzymes and, thereby, focuss their access to substrate proteins. AKAPs interact with dimers of PKA regulatory subunits with a high but varying affinity [14] and anchor RII subunits at among others the nucleus, cytoskeleton, organelle membranes, and plasma membrane [15-17]. While most AKAPs anchor RII subunits, so-called dual AKAPS have recently been identified which also anchor RI subunits. These include D-AKAPs at the endoplasmic reticulum and mitochondria [18], and BIG1 and BIG2 at the Golgi apparatus and recycling endosomes [19]. Most AKAPs also contain regions for binding additional enzymes, e.g. PDEs, adenlyl cyclase, protein phosphatases, and other kinases [20-21]. As unique signaling platforms AKAPs thus can create regulatory networks that ensure the coordinated propagation of PKA signals through different locations in the cell. Such a coordinated signal propagation may be of particular importance in directing the sequential steps involved in the sorting and vesicular trafficking of proteins and lipids through the cells, often bridging many micrometers. Indeed, AKAPs and PKA isoforms are found at many organelles that mediate the intracellular sorting and trafficking of membrane proteins and lipids, e.g. the microtubule-organizing center (MTOC) or centrosome [22], cytoskeleton [23], the plasma membrane [24], Golgi apparatus [19,25] and endoplasmic reticulum [18] (figure 2A), sometimes complexed with cargo proteins (see below). In the following sections, we will review the literature with regard to the role of

cAMP/PKA signaling in intracellular membrane trafficking and the dynamics of cell surface domains.

The role of cAMP/PKA in exocytosis

The polarization of exocytosis is a fundamental mechanism for the targeted secretion of molecules and to localize plasma membrane components to specific regions of the cell surface in order to establish, maintain, and/or tailor functional cell surface domains. Elevation of the intracellular cAMP concentration enhances exocytic transport of proteins to the apical surface in among others pancreatic β-cells [26], kidney [27,28], intestine [29,30], hepatic [31,32], and principal [33-35] epithelial cells. A similar process is observed in neurons, where a rise in intracellular cAMP concentration enhances exocytic transport to and neurotransmitter release at the axonal synapse [36,37], the latter being considered the equivalent of the epithelial apical surface domain. Most of the effects of cAMP are mediated by PKA, although some are mediated by other cAMP effectors such as Epac, a guanine nucleotide exchange factor for the small GTPase Rap1, or calcium channels [38]. While the cAMP/PKA system in mammalian cells is highly redundant with multiple genes encoding several PKA regulatory and catalytic subunits (see paragraph above), Drosophila have a single or predominant gene encoding the PKA catalytic subunit, DC0, which is preferentially expressed in mushroom bodies in the brain [39]. Drosophila DC0 mutants which lack PKA catalytic activity show defects in neurotransmitter release in response to extracellular cues including cAMP [40,41], and display learning and memory deficits [39,42,43], underscoring the involvement of PKA in the dynamics of the axonal surface and synaptic plasticity. Excellent review articles have recently addressed the role of cAMP/PKA in regulating exocytosis in neurons in relation to synaptic plasticity, learning, and memory [44,45]. Here we will focus on epithelial cells.

Studies with cultured primary cells and epithelial cell lines have demonstrated that stimulation of apical surface-directed trafficking in response to an elevated

intracellular cAMP concentration can be linked to signaling molecules that circulate outside epithelial cells. For instance, the peptide hormone secretin stimulates apical exocytosis of membrane vesicles in bile duct epithelial cells through elevation of cAMP levels and subsequent activation of PKA [46]. A similar phenomenon is observed in hepatocytes stimulated by glucagon which activates adenylyl cyclase activity and cAMP production [47]. In accordance with those observations, H89, an inhibitor that also inhibits the activity of the catalytic subunit of PKA, perturbs intracellular trafficking of apical secretory proteins, as shown for instance in lacrimal cells [48]. It has been proposed that cAMP modulates the rate of 'constitutive' exocytosis [31], showing little specificity for the different apical membrane components. This may suggest that cAMP/PKA targets molecular machineries that control vesicular membrane flow in general. However, as will be discussed in the next paragraph, there are also examples in which PKA controls the intracellular flow of specific 'cargo' proteins and lipids.

The involvement of cAMP/PKA signaling in the apical exocytosis of specific proteins has mostly focused on polytopic membrane transporter proteins. These include aquaporins, a class of integral membrane proteins that form water channels in the plasma membrane to selectively conduct water molecules, and important for the functioning of all fluid-transporting epithelia. In Madin-Darby canine kidney (MDCK) epithelial cells, PKA activity is required for the apical trafficking of vasopressin-controlled aquaporin (AQP)2 [49]. AQP2 possesses a single consensus cAMP-dependent PKA phosphorylation site at Ser256. PKA phosphorylation modulates its distribution between plasma membrane and intracellular vesicular compartments [50,51]. The role of PKA-mediated phosphorylation of AQP2 at Ser256 is somewhat obscured by the notion that AQP2 transition in the Golgi apparatus is

associated with a PKA-independent increase in AQP2 phosphorylation at Ser256, probably mediated by Golgi-associated casein kinase 2 [52]. In renal collecting duct principal cells, cAMP/PKA-induced AQP2 translocation is sensitive to Ht31, a peptide that binds with high affinity to PKA type II regulatory subunits and in this way displaces the PKA-RII holoenzyme from its subcellular anchoring sites [37]. A similar sensitivity of exocytosis to HT31 was earlier demonstrated for the cAMP-responsive insulin secretion in clonal beta cells in response to the insulinotropic hormone glucagon-like peptide 1 [53]. Localized activity of PKA type II at AQP2-bearing vesicles thus appears responsible for the efficient trafficking of AQP2 to apical surface [37]. Interestingly, PKA-RIIα is part of a multi-protein signalling complex located at endosomal membranes in inner medullary collecting duct (IMCD) cells [54]. In this study, AQP2 is shown to be a substrate for protein phosphatase 2B which, in conjunction with PKA, is responsible for the phosphorylation status that controls AQP2 trafficking and steady state distribution. In addition, PDE4D interacts with PKA-RII on AQP2-bearing vesicles, and is activated in a PKA-dependent manner upon translocation of these vesicles to the apical cell surface to reduce osmotic water permeability [55]. The responsible AKAP that mediates PKA-RII-regulated AQP2 translocation in renal collecting duct principal cells is AKAP18delta [56,57]. Although the consensus PKA phosphorylation site at Ser256 in AQP2 is clearly important for the subcellular distribution of AQP2, the mechanism by which phosphorylation of AQP2 by PKA controls its intracellular trafficking remains unclear. For instance, it remains to be verified that PKA type II, anchored to AKAP18delta in endosomal membranes, is responsible for the phosphorylation of AQP2 at Ser256. It can be speculated that phosphorylation of AQP2 masks or unmasks a signal that is recognized by molecular machineries that prevent or promote

apical surface delivery, respectively, similar as proposed for the adhesion protein NgCAM [58].

In addition to controlling the apical exocytosis of aquaporins, PKA-RIIα anchoring controls the efficient trafficking of the polytopic multidrug resistance protein MDR-1 (or ABCB1) from the Golgi apparatus to the apical bile canaliuclar surface of hepatocytes, the prime epithelial cells of the liver [59]. At the apical surface of hepatocytes, MDR-1 is necessary for the formation of bile and to reduce the body load of potentially harmful compounds [60]. Wojtal et al. [59] displaced PKA-RIIα from Golgi-associated AKAPs in human hepatocytes using the small interfering AKAP-IS peptide designed by Scott and colleagues [61]. Displacement of PKA-RIIα causes a delay of MDR-1 trafficking to the apical surface of hepatic HepG2 cells. This effect is specific for MDR-1 as other apical resident proteins such as the multidrug resistance protein MRP2, dipeptidyl peptidase IV and 5'-nucleotidase are unaffected. This suggests that PKA-RIIα anchoring is not required for membrane traffic to the apical domain per se. In addition to the delay in trafficking of MDR1, which in contrast to AQP2 lacks a consensus PKA phosphorylation site, the displacement of PKA-RIIα from Golgi-associated AKAPs inhibits the Golgi to apical surface-directed transport of newly synthesized glycosphingolipid analogues and instead reroutes these to the basolateral surface. This suggests that the trafficking of MDR-1 and glycosphingolipids are mechanistically linked in a manner that depends on PKA-RIIα anchoring at the Golgi. This is supported by the observation that treatment of HepG2 cells with an inhibitor of glucosylceramide synthesis results in a delayed translocation of MDR-1, but not MRP2, to the apical surface, very similar as observed upon displacement of PKA-RIIα [59]. Because of the known interrelation between

glycosphingolipids and MDR1 [62], it is proposed that the mistargeting of glycosphingolipids may be responsible for the delay in MDR1 exocytosis [59].

As a final example, activation of PKA enhances the apical surface-directed transport of the cystic fibrosis transmembrane conductance regulator (CFTR) [63-64]. CFTR activity functionally correlates to the interaction of PKA-RII with unspecified but Ht31 peptide-sensitive AKAPs [65]. Also, the direct phosphorylation of CFTR by PKA affects its intracellular trafficking [66,67] and cAMP increases CFTR expression [67]. Taken together, the evidence indicates that PKA-RII anchoring and activity at endosomes and organelles of the secretory pathway control the proper trafficking and, consequently, the steady state distribution of select polytopic apical plasma membrane transporter proteins.

In addition to regulating the trafficking of specific 'cargo' proteins and lipids as described in the paragraph above, PKA targets molecular machineries that control vesicular membrane flow in general. For instance, PKA activity influences the rate of membrane vesiculation at the Golgi apparatus by stimulating the scission of membrane transport vesicles [68] (figure 2B). It has been proposed that PKA-RIIα controls this process and that the interaction of PKA-RIIα subunits with Golgi cisternae is modulated by trimeric G proteins [69]. Increased PKA activity (isoform not specified) triggers the redistribution of the ADP-ribosylating factor Arf1 from cytosol to trans-Golgi membranes in a cell-free assay, and this is abolished with PKA inhibitory peptides or when cytosol is depleted of PKA catalytic subunits [70]. Two Golgi-associated Arf-activating proteins, the Brefeldin A-inhibited guanine nucleotide-exchange proteins (GEPs) BIG1 and BIG2, are both AKAPs [19]. Elevation of cAMP caused PKA-catalyzed phosphorylation of the BIGs and, in an in vitro assay, recombinant PKA altered their GEP activity [71]. The involvement of PKA in

Golgi membrane dynamics was recently supported by the notion that the cell-wide downregulation of PKA-RIIα subunits by siRNA results in severe perturbation of Golgi morphology [72]. Protein phosphorylation mediated by PKA affects Golgi morphology in yeast via phosphorylation of the t-SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors) protein Sed-5, which controls membrane fusion [73]. Also in mammalian cells, several potential targets of PKA involved in vesicular trafficking act in membrane fusion. In polarized neurons for example, these include syntaphilin, a protein interacting with dynamin-1 and syntaxin-1 which, in turn, regulate the scission and fusion of secretory vesicles, respectively, at the axonal synapse (the neuronal equivalent of the epithelial apical plasma membrane domain [74]). The phosphorylation of syntaphilin by PKA on Ser43 in isolated rat brain synaptosomes or syntaphilin-transfected HEK293 cells inhibits its interaction with dynamin- and syntaxin-1 [75], and annuls its inhibitory effect on synaptic vesicle exocytosis (figure 2B). In cultured superior cervical ganglion neurons, PKA phosphorylates tomosyn which, like synthaphilin, is a member of SNARE regulatory protein family that limits synaptic transmission. Thus, PKA-mediated phosphorylation of tomosyn reduces its inhibitory interaction with syntaxin-1 and promotes SNARE assembly, exocytic vesicle fusion, and the release of neurotransmitters in response to a potent biological mediator, the pituitary adenylate cyclase-activating polypeptide [26]. Other molecular targets of PKA implicated in exocytosis include cystein string protein, rabphilin 3A, αSNAP (N-ethylmaleimide sensitive factor attachment protein), snapin, SNAP-25 and syntaxin 4 [38,76]. The PKA-mediated phosphorylation of these proteins changes their respective protein-protein interactions and, in this way, modulates the vesicle priming and/or fusion stages of exocytosis. PKA may thus control exocytosis at different steps of the pathway, e.g.

vesicle budding and scission from the donor membrane such as the Golgi apparatus or endosomes, and vesicle fusion with the target (plasma) membrane.

The cytoskeleton network, including actin filaments and microtubules, are instrumental in directing transport vesicles to defined subcellular sites [77]. Cytoskeleton-associated AKAPs have been reported [23]. PKA regulatory subunits form complexes with dynein, and with kinesin II and myosin V, and in this way control the spatial organisation of pigment granules in melanophores [78]. Also the epinephrine-induced clustering of secretory Weibel-Pallade bodies in endothelial cells involves a PKA-dependent regulation of the dynein-dynactin complex [79], and inhibition of PKA-RII anchoring in human hepatoma cells results in a (non-polar) repositioning of the centrosome and surrounding recycling endosomes [80]. In the latter study, surprisingly, inhibition of catalytic PKA activity did not alter the position of the centrosome and recycling endosomes. It thus appears that regulatory PKA subunits, through their interaction with cytoskeleton motor proteins, can control the spatial distribution of secretory organelles. Whether and how this may influence the movement of transport vesicles remains to be investigated.

The role of cAMP/PKA in endocytosis

Besides stimulating exocytosis, activation of the cAMP-dependent second messenger pathway causes a significant reduction in endocytosis in epithelial T84 cells as measured by uptake of fluid-phase markers [81]. Furthermore, cAMP stimulates the exocytosis of CFTR but at the same time inhibits its apical endocytosis [82,83]. In case of AQP2, PKA-mediated phosphorylation of Ser256 not only stimulates exocytosis of the water channel, but is also required for its subsequent reinternalization [84]. This and other data underscore that the process of exocytosis is closely correlated to the endocytosis of membrane components, which provides an efficient way of controlling the size and composition of plasma membrane domains and, therefore, functional cell surface polarity. Perhaps therefore not surprisingly, there are numerous reports implicating PKA as a regulator of the endocytic process. In some instances, PKA directly phosphorylates the 'cargo' protein. Indeed, the low density lipoprotein-related protein LRP is phosphorylated by PKA at Ser76, and mutations of Ser76 result in a decrease in the initial endocytosis rate of LRP and a lower efficiency in delivery of ligand for degradation [85]. While PKA activity may promote the endocytosis of LRP, the agonist-stimulated endocytosis of glutamate receptors is inhibited by PKA activation, which may reduce the interaction of glutamate receptors with G-coupled receptor kinase 2 and arrestins [87]. Interestingly, inhibition of basal PKA activity induces clathrin-mediated endocytosis of unoccupied, inactive epidermal growth factor receptors (EGFR) and its accumulation into early endosomes without affecting the endocytosis of transferrin and μ-opioid receptors. It is proposed that the predominant distribution of inactive EGFR at the plasma membrane involves a PKA-dependent restrictive condition resulting in receptor avoidance of endocytosis until it is bound and activated by a ligand [86]. PKA may control endocytosis by associating

with compositionally and biophysically distinct plasma membrane domains. For instance, the PKA catalytic subunit interacts with caveolin-1, a key component of caveolar membranes domains [88], and PKA-mediated phosphorylation triggers the agonist-induced internalization of G-protein coupled beta1-adrenergic receptors via a caveolar pathway in non-polarized cells [89]. PKA also controls protein recycling to the plasma membrane following endocytosis. For instance, AKAP79, which interacts with a PDZ domain in the beta1-adrenergic receptor, mediates the targeting of PKA-RII to these receptors, and their subsequent PKA-mediated phosphorylation promotes recycling of the receptors from endosomal membranes back to the plasma membrane and, in this way, the functional resensitization of the receptor [90]. Whether AKAPs, PKA, and receptors traffic as a complex and whether they can, in this way, direct their transit through the heterogeneous endosomal membrane system (as suggested by Stefan et al., [55]) remains to be investigated. In addition to the role of PKA in setting the trafficking itinerary of beta1-adrenergic receptors, AQP-4 in human gastric cells is phosphorylated by PKA subsequent to its endocytosis from the basolateral surface, and it is suggested that this phosphorylation is involved in retaining AQP4 in an endosomal recycling compartment [91]. Downstream in the endocytic pathway, PKA-RIIα regulates membrane traffic between endosomes and the Golgi apparatus and plays a pivotal role in endosome-to-Golgi transport of the plant toxin ricin upon stimulation with cAMP analogues [92]. A similar stimulating effect is observed in case of retrograde transport of these proteins from the Golgi apparatus to the endoplasmic reticulum, where the toxin is eventually translocated to the cytosol where it blocks protein synthesis. In concert, overexpression of PKA-RIIα sensitizes cells to ricin. Intriguingly, non-hydrolyzable cAMP analogues stimulate non-clathrin-mediated endocytosis of ricin from the apical but not basolateral surface to the Golgi apparatus

in MDCK cells [93], suggesting that PKA-RIIα discriminates between different, i.e. apical versus basolateral populations of endosomes, and/or affects different endocytic pathways in different cell types.

Also PKA-RI localizes to endosomes. The RIα subunit of PKA was found to localize on Rab7-positive late endosomes and on microtubule-associated protein light chain 3-positive autophagosomes in cultured cells. RIα was also shown to physically interact with the mTOR (mammalian target of rapamycin) kinase and affect its phosphorylation and activity [94]. While the regulation of autophagocytosis by cAMP levels is highly conditional [95], in RIα downregulated mouse embryonic fibroblasts the number of autophagosomes is significantly reduced compared with wild-type cells. This suggests that PKA type I in a complex with mTOR modulates the rate of autophagocytosis and, possibly, the various autophagocytosis-related developmental processes and diseases including cancer and neurodegeneration. Taken all together, PKA activity is involved in the endocytosis and endocytic recycling of several proteins from both basolateral and apical surfaces in different cell types, and distinct PKA holo-enzymes may participate in the different endocytic routes.

The role of cAMP/PKA in transcytosis

In epithelial cells, endocytic and exocytic membrane trafficking pathways converge to allow the transcellular trafficking (transcytosis) of proteins and lipids between basolateral and apical surfaces. Transcytosis is used by epithelia to move molecules across the cells in response to extracellular factors. It has been reported that Gαs stimulates transcytosis and apical secretion in MDCK cells through cAMP and PKA [(29)]. PKA is implicated in cholesterol and caveolae-controlled transcytosis of basolaterally localized high-density lipoprotein scavenger receptor class B type I (SR-BI), in MDCK cells [(30)]. In this study, a scenario is proposed in which cholesterol-based membrane microdomains, or rafts, promote internalization and basolateral recycling of internalized SR-BI whereas a PKA pool sensitive to cholesterol depletion mediates SR-BI transcytosis [(30)]. The exact intracellular location at which PKA promotes SR-BI transcytosis is not clear. However, a switch from basolateral recycling to apical transcytosis of membrane components typically occurs in the endosomal system. This has been clearly demonstrated in polarized hepatocytes. In these cells, cAMP/PKA activates an apical surface-directed pathway exiting from a subapical compartment/ common recycling endosome (SAC/CE), and changes the trafficking of the fluorescently labelled sphingolipid analogues C6-NBD-sphingomyelin and -galactosylceramide from a apical-to-SAC/CE-to-basolateral itinerary to a apical-to-SAC/CE-to-apical pathway [(96,97)]. The activated SAC/CE-to-apical pathway represents the final leg in the basolateral to apical transcytotic route [(90)]. Indeed, the SAC/CE connects basolateral and apical endocytic routes and thus takes a prominent position in the transcytotic pathway [(5,98,99)]. The PKA inhibitor H89 prevents the cAMP/PKA-induced apical flow of the fluorescent lipids from the SAC/CE as well as that of transcytosing proteins [(100)]. By contrast, the constitutive

apical recycling of C6-NBD-glucosylceramide from the SAC/CE is unaffected by PKA inhibition [100]. Importantly, the stimulatory effect of cAMP/PKA on the apical-directed flow of the sphingomyelin analogues from the SAC/CE strongly coincides with enhanced development of apical plasma membrane domains, suggesting that PKA-stimulated trafficking from the SAC/CE (i.e. the last step of the transcytotic pathway) and the biogenesis of apical plasma membrane domains are intimately linked.

The downstream targets of cAMP/PKA that regulate trafficking between the SAC/CE and the apical surface are not yet known. Elevated cAMP promotes the turnover of the sphingoid base dihydro-sphingosine (sphinganine) to dihydroceramide by stimulating the activity of dihydroceramide synthase [101], and reduced and elevated levels of sphinganine promote and inhibit apical surface development, respectively. As a part of the underlying mechanism, dihydroceramide synthase activity and ensuing low sphinganine levels are required for cAMP/PKA-mediated activation of the apical-surface-directed trafficking pathway from the SAC/CE [101]. Interestingly, a sphingosine kinase-interacting protein, SKIP, which mediates the phosphorylation of sphingoid bases, anchors PKA [102]; supporting the notion that sphingoid base metabolism may be regulated by PKA.

The correlation between PKA-stimulated trafficking from the SAC/CE and the biogenesis of apical plasma membrane domains is further corroborated by the observation that the interleukin 6 family cytokine oncostatin M (OSM), an important factor in fetal liver development, stimulates apical plasma membrane biogenesis in hepatocytes in a PKA-dependent manner [103]. Stimulation of hepatocytes with OSM does not elevate cAMP levels or stimulate overall PKA activity but enhances the association of PKA-RIIα with centrosomes in an ERK1-dependent manner [80,103].

Displacement of PKA-RIIα from the centrosome, by means of a small interfering peptide [80] or by forcing synchronised hepatocytes into the S-phase of the cell cycle [104], prevents the stimulatory effect of OSM on apical membrane biogenesis. Given that OSM stimulates PKA-dependent membrane transport exiting from the SAC/CE to promote apical membrane biogenesis [103], the recruitment of PKA-RIIα at the centrosome may be an important factor in the regulation of polarized, apical surface-directed membrane trafficking from the SAC/CE in response to extracellular cytokines. However, this remains to be investigated.

Conclusions and perspectives

PKA holoenzymes play an important role in the polarized trafficking of membrane proteins and lipids, including exocytosis, endocytosis, and transcytosis. In this way, cAMP/PKA signalling contributes to the compositional and functional dynamics of epithelial cell surface domains and, accordingly, developmental processes and organ function. The dynamics of membrane protein trafficking can be regulated by PKA-mediated phosphorylation of the cargo protein itself, such as in case of the aquaporins and other transporter proteins, or regulated by PKA-mediated phosphorylation of components of the molecular machineries that more generally control vesicular membrane trafficking. Furthermore, PKA can interfere with the morphology and, consequently, the functioning of organelles that control membrane trafficking, such as the Golgi apparatus or endosomes. One of the most intriguing developments may be the interaction of selected cargo with PKA scaffolds to create a multi-signal transduction module that controls its trafficking itinerary through the different organelles.

The specificity of cAMP/PKA signalling in the regulation of membrane trafficking in any given cell is dictated at multiple and interconnected levels. These include: i) the nature of the extracellular (ant)agonist and cellular receptor, ii) spatial-temporal cAMP gradients, carefully controlled by a large family of adenylyl cyclases and PDEs, iii) the composition and spatial distribution of the PKA holoenzyme, mediated by different regulatory and catalytic subunits and a large family of AKAPs, and iv) the nature of the catalytic subunit's substrate, which can be cargo itself or traffic regulatory proteins.

Because the increase of exocytosis by cAMP/PKA signalling is observed in a wide variety of secretory cell types, this is likely an important and fundamental

mechanism. However, although this phenomenon has been recognized for more than a decade, it remains largely unexplained at the molecular level. This is primarily due to the increasingly apparent complexity of cAMP/PKA signaling. For instance, to date there are at least five AKAPs reported just in the secretory pathway. Each of these AKAPs anchor RI or RII subunits or, in case of D-AKAP-1 and BIG1/2, both, sometimes via distinct and/or multiple binding sites. These AKAPs in addition can bind PDEs, adenylyl cyclases, and other signalling molecules, thereby tuning and propagating local cAMP/PKA responses. It is for that reason that the use of non-hydrolyzable cAMP analogues in combination with inhibitors of general PKA catalytic activity may be useful to reveal the general involvement of PKA activity in the exocytic process but, importantly, does not provide the necessary information with regard to the PKA isoenzymes involved and their precise subcellular location. This limitation also hampers the identification and analysis of relevant phosphorylation targets. The current development of novel and innovative tools, such as PKA holoenzyme-specific cAMP analogues [105], In vivo assays that measure the interaction of specific regulatory and catalytic subunits as a mesasure for PKA holoenzyme-specific activity [105], and peptide-based disruptors of specific PKA-AKAP interactions [61; 106-109], used in combination with established biochemical and (live) cell biological assays, is expected to boost our understanding of the role of PKA signaling in membrane dynamics and the plasticity of cell surface domains, and the life-facilitating processes that are so critically dependent thereof.

References

1. Yeaman C, Grindstaff KK, Nelson WJ. 1999. New perspectives on mechanisms involved in generating epithelial cell polarity. Physiol Rev. 79:73-98.
2. van der Wouden JM, Maier O, van IJzendoorn SC, Hoekstra D. 2003. Membrane dynamics and the regulation of epithelial cell polarity. Int Rev Cytol. 226:127-164.
3. Rodriguez-Boulan E, Musch A, Le Bivic A. 2004. Epithelial trafficking: new routes to familiar places. Curr Opin Cell Biol. 16:436-442.
4. van IJzendoorn SC, Hoekstra D. 1999. The subapical compartment: a novel sorting centre? Trends Cell Biol. 9:144-149.
5. Hoekstra D, Tyteca D, van IJzendoorn SC. 2004. The subapical compartment: a traffic center in membrane polarity development. J Cell Sci. 117:2183-2192.
6. Thompson A, Nessler R, Wisco D, Anderson E, Winckler B, et al.. 2007. Recycling Endosomes of Polarized Epithelial Cells Actively Sort Apical and Basolateral Cargos into Separate Subdomains. Mol Biol Cell. In press.
7. Brown D. 2003. The ins and outs of aquaporin-2 trafficking. Am J Physiol Renal Physiol. 284:F893-F901.
8. Wakabayashi Y, Kipp H, Arias IM. 2006. Transporters on demand: intracellular reservoirs and cycling of bile canalicular ABC transporters. J Biol Chem. 281:27669-27673.
9. Kim C, Vigil D, Anand G, Taylor SS. 2006. Structure and dynamics of PKA signaling proteins. Eur J Cell Biol. 85:651-654.
10. Taylor SS, Kim C, Vigil D, Haste NM, Yang J, et al. 2005. Dynamics of signaling by PKA. Biochim Biophys Acta. 1754:25-37.

11. Das R, Esposito V, Abu-Abed M, Anand GS, Taylor SS, et al. 2007. cAMP activation of PKA defines an ancient signaling mechanism. Proc Natl Acad Sci U S A. 104:93-98.
12. Houslay MD, Adams DR. 2007. PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. Biochem J. 370:1-18.
13. Smith FD, Scott JD. 2006. Anchored cAMP signaling: onward and upward - a short history of compartmentalized cAMP signal transduction. Eur J Cell Biol. 85:585-592.
14. Herberg FW, Maleszka A, Eide T, Vossebein L, Tasken K. 2000. Analysis of A-kinase anchoring protein (AKAP) interaction with protein kinase A (PKA) regulatory subunits: PKA isoform specificity in AKAP binding. J Mol Biol. 298:329-339.
15. Singh AK, Tasken K, Walker W, Frizzell RA, Watkins SC, et al. 1998. Characterization of PKA isoforms and kinase-dependent activation of chloride secretion in T84 cells. Am J Physiol. 275:C562-C570.
16. Constantinescu A, Diamond I, Gordon AS. 1999. Ethanol-induced translocation of cAMP-dependent protein kinase to the nucleus. Mechanism and functional consequences. J Biol Chem. 274:26985-26991.
17. Alto NM, Soderling J, Scott JD. 2002. Rab32 is an A-kinase anchoring protein and participates in mitochondrial dynamics. J Cell Biol. 158:659-668.
18. Huang LJ, Wang L, Ma Y, Durick K, Perkins G, et al. 1999. NH2-Terminal targeting motifs direct dual specificity A-kinase-anchoring protein 1 (D-AKAP1) to either mitochondria or endoplasmic reticulum. J Cell Biol. 145:951-959.

19. Li H, Adamik R, Pacheco-Rodriguez G, Moss J, Vaughan M. 2003. Protein kinase A-anchoring (AKAP) domains in brefeldin A-inhibited guanine nucleotide-exchange protein 2 (BIG2). Proc Natl Acad Sci U S A. 100:1627-1632.
20. Alto N, Carlisle Michel JJ, Dodge KL, Langeberg LK, Scott JD. 2002. Intracellular targeting of protein kinases and phosphatases. Diabetes. 51:S385-S358.
21. Michel JJ, Scott JD. 2002. AKAP mediated signal transduction. Annu Rev Pharmacol Toxicol. 42:235-257.
22. Witczak O, Skalhegg BS, Keryer G, Bornens M, Tasken K, et al. 1999. Cloning and characterization of a cDNA encoding an A-kinase anchoring protein located in the centrosome, AKAP450. EMBO J. 18:1858-1868.
23. Diviani D, Scott JD. 2001. AKAP signaling complexes at the cytoskeleton. J Cell Sci. 114:1431-1437.
24. Henn V, Edemir B, Stefan E, Wiesner B, Lorenz D, et al. 2004. Identification of a novel A-kinase anchoring protein 18 isoform and evidence for its role in the vasopressin-induced aquaporin-2 shuttle in renal principal cells. J Biol Chem. 279:26654-26665.
25. Shanks RA, Steadman BT, Schmidt PH, Goldenring JR. 2002. AKAP350 at the Golgi apparatus. I. Identification of a distinct Golgi apparatus targeting motif in AKAP350. J Biol Chem. 277:40967-40972.
26. Yang S, Fransson U, Fagerhus L, Holst LS, Hohmeier HE, et al. 2004. Enhanced cAMP protein kinase A signaling determines improved insulin secretion in a clonal insulin-producing beta-cell line (INS-1 832/13). Mol Endocrinol. 18:2312-2320.

27. Hansen SH, Casanova JE. 1994. Gs alpha stimulates transcytosis and apical secretion in MDCK cells through cAMP and protein kinase A. J Cell Biol. 126:677-687.

28. Burgos PV, Klattenhoff C, de la Fuente E, Rigotti A, Gonzalez A. 2004. Cholesterol depletion induces PKA-mediated basolateral-to-apical transcytosis of the scavenger receptor class B type I in MDCK cells. Proc Natl Acad Sci U S A. 101:3845-3850.

29. Brignoni M, Pignataro OP, Rodriguez ML, Alvarez A, Vega-Salas DE, et al. 1995. Cyclic AMP modulates the rate of 'constitutive' exocytosis of apical membrane proteins in Madin-Darby canine kidney cells. J Cell Sci. 108:1931-1943.

30. Ameen NA, Marino C, Salas PJ. 2003. cAMP-dependent exocytosis and vesicle traffic regulate CFTR and fluid transport in rat jejunum in vivo. Am J Physiol Cell Physiol. 284:C429-C438.

31. Zegers MM, Hoekstra D. 1997. Sphingolipid transport to the apical plasma membrane domain in human hepatoma cells is controlled by PKC and PKA activity: a correlation with cell polarity in HepG2 cells. J Cell Biol. 138:307-321.

32. Kipp H, Arias IM. 2000. Intracellular trafficking and regulation of canalicular ATP-binding cassette transporters. Semin Liver Dis. 20:339-351.

33. Mordasini D, Bustamante M, Rousselot M, Martin PY, Hasler U, et al. 2005. Stimulation of Na+ transport by AVP is independent of PKA phosphorylation of the Na-K-ATPase in collecting duct principal cells. Am J Physiol Renal Physiol. 289:F1031-F1039.

34. Vinciguerra M, Hasler U, Mordasini D, Roussel M, Capovilla M, et al. 2005. Cytokines and sodium induce protein kinase A-dependent cell-surface Na,K-ATPase recruitment via dissociation of NF-kappaB/IkappaB/protein kinase A catalytic subunit complex in collecting duct principal cells. J Am Soc Nephrol. 16:2576-2585.

35. Klussmann E, Maric K, Wiesner B, Beyermann M, Rosenthal W. 1999. Protein kinase A anchoring proteins are required for vasopressin-mediated translocation of aquaporin-2 into cell membranes of renal principal cells. J Biol Chem. 274:4934-4938.

36. Baba T, Sakisaka T, Mochida S, Takai Y. 2005. PKA-catalyzed phosphorylation of tomosyn and its implication in Ca2+-dependent exocytosis of neurotransmitter. J Cell Biol. 170:1113-1125.

37. Bouchard JF, Moore SW, Tritsch NX, Roux PP, Shekarabi M, et al. 2004. Protein kinase A activation promotes plasma membrane insertion of DCC from an intracellular pool: A novel mechanism regulating commissural axon extension. J Neurosci. 24:3040-3050.

38. Seino S, Shibasaki T. 2005. PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. Physiol Rev. 85:1303-1342.

39. Skoulakis EM, Kalderon D, Davis RL. 1993. Preferential expression in mushroom bodies of the catalytic subunit of protein kinase A and its role in learning and memory. Neuron. 11:197-208

40. Yoshihara M, Suzuki K, Kidokoro Y. 2000. Two independent pathways mediated by cAMP and protein kinase A enhance spontaneous transmitter release at Drosophila neuromuscular junctions. J Neurosci. 20:8315-8322.

41. Suzuki K, Grinnell AD, Kidokoro Y. 2002. Hypertonicity-induced transmitter release at Drosophila neuromuscular junctions is partly mediated by integrins and cAMP/protein kinase A. J Physiol. 538:103-119.
42. Davis RL, Cherry J, Dauwalder B, Han PL, Skoulakis E. 1995. The cyclic AMP system and Drosophila learning. Mol Cell Biochem. 149-150:271-278
43. Li W, Tully T, Kalderon D. 1996. Effects of a conditional Drosophila PKA mutant on olfactory learning and memory. Learn Mem. 2:320-333.
44. Bauman AL, Goehring AS, Scott JD. 2004 Orchestration of synaptic plasticity through AKAP signaling complexes. Neuropharmacology. 46:299-310.
45. Dell'Acqua ML, Smith KE, Gorski JA, Horne EA, Gibson ES, Gomez LL. 2006 Regulation of neuronal PKA signaling through AKAP targeting dynamics. Eur J Cell Biol. 85:627-633.
46. Kato A, Gores GJ, LaRusso NF. 1992. Secretin stimulates exocytosis in isolated bile duct epithelial cells by a cyclic AMP-mediated mechanism. J Biol Chem. 267:15523-15529.
47. Banales JM, Prieto J, Medina JF. 2006. Cholangiocyte anion exchange and biliary bicarbonate excretion. World J Gastroenterol. 12:3496-3511.
48. Robin P, Rossignol B, Raymond MN. 1998. PKA inhibitor, H-89, affects the intracellular transit of regulated secretory proteins in rat lacrimal glands. Am J Physiol. 274:C262-C271.
49. Nejsum LN, Zelenina M, Aperia A, Frokiaer J, Nielsen S. 2005. Bidirectional regulation of AQP2 trafficking and recycling: involvement of AQP2-S256 phosphorylation. Am J Physiol Renal Physiol. 288:F930-F938.

50. Lande MB, Jo I, Zeidel ML, Somers M, Harris HW Jr. 1996. Phosphorylation of aquaporin-2 does not alter the membrane water permeability of rat papillary water channel-containing vesicles. J Biol Chem. 271:5552-5557.
51. Kamsteeg EJ, Heijnen I, van Os CH, Deen PM. 2000. The subcellular localization of an aquaporin-2 tetramer depends on the stoichiometry of phosphorylated and nonphosphorylated monomers. J Cell Biol. 151:919-930.
52. Procino G, Carmosino M, Marin O, Brunati AM, Contri A, et al. 2003. Ser-256 phosphorylation dynamics of Aquaporin 2 during maturation from the ER to the vesicular compartment in renal cells. FASEB J. 17:1886-1888.
53. Lester LB, Langeberg LK, Scott JD. 1997. Anchoring of protein kinase A facilitates hormone-mediated insulin secretion. Proc Natl Acad Sci U S A. 94:14942-14947.
54. Jo I, Ward DT, Baum MA, Scott JD, Coghlan VM, Hammond TG, et al. 2001. AQP2 is a substrate for endogenous PP2B activity within an inner medullary AKAP-signaling complex. Am J Physiol Renal Physiol. 281:F958-F965.
55. Stefan E, Wiesner B, Baillie GS, Mollajew R, Henn V, et al. 2007. Compartmentalization of cAMP-dependent signaling by phosphodiesterase-4D is involved in the regulation of vasopressin-mediated water reabsorption in renal principal cells. J Am Soc Nephrol. 18:199-212.
56. McSorley T, Stefan E, Henn V, Wiesner B, Baillie GS, Houslay MD, et al. 2006. Spatial organisation of AKAP18 and PDE4 isoforms in renal collecting duct principal cells. Eur J Cell Biol. 85:673-678.
57. Klussmann E, Rosenthal W. 2001. Role and identification of protein kinase A anchoring proteins in vasopressin-mediated aquaporin-2 translocation. Kidney Int. 60:446-449.

58. Anderson E, Maday S, Sfakianos J, Hull M, Winckler B, et al. 2005. Transcytosis of NgCAM in epithelial cells reflects differential signal recognition on the endocytic and secretory pathways. J Cell Biol. 170:595-605.

59. Wojtal KA, de Vries E, Hoekstra D, van IJzendoorn SC. 2006. Efficient trafficking of MDR1/P-glycoprotein to apical canalicular plasma membranes in HepG2 cells requires PKA-RIIalpha anchoring and glucosylceramide. Mol Biol Cell. 17:3638-3650.

60. Dietrich CG, Geier A, Oude Elferink RP. 2003. ABC of oral bioavailability: transporters as gatekeepers in the gut. Gut. 52:1788-1795.

61. Alto NM, Soderling SH, Hoshi N, Langeberg LK, Fayos R, et al. 2003. Bioinformatic design of A-kinase anchoring protein-in silico: a potent and selective peptide antagonist of type II protein kinase A anchoring. Proc Natl Acad Sci U S A. 100:4445-4450.

62. Klappe K, Hinrichs JW, Kroesen BJ, Sietsma H, Kok JW. 2004. MRP1 and glucosylceramide are coordinately over expressed and enriched in rafts during multidrug resistance acquisition in colon cancer cells. Int J Cancer. 110:511-522.

63. Chang SY, Di A, Naren AP, Palfrey HC, Kirk KL, et al. 2002. Mechanisms of CFTR regulation by syntaxin 1A and PKA. J Cell Sci. 115:783-791.

64. Huang P, Gilmore E, Kultgen P, Barnes P, Milgram S, et al. 2004. Local regulation of cystic fibrosis transmembrane regulator and epithelial sodium channel in airway epithelium. Proc Am Thorac Soc. 1:33-37.

65. Seibert FS, Chang XB, Aleksandrov AA, Clarke DM, Hanrahan JW, et al. 1999. Influence of phosphorylation by protein kinase A on CFTR at the cell surface and endoplasmic reticulum. Biochim Biophys Acta. 1461:275-283.
66. Kleizen B, Braakman I, de Jonge HR. 2000. Regulated trafficking of the CFTR chloride channel. Eur J Cell Biol. 79:544-556.
67. Taouil K, Hinnrasky J, Hologne C, Corlieu P, Klossek JM, et al. 2003. Stimulation of beta 2-adrenergic receptor increases cystic fibrosis transmembrane conductance regulator expression in human airway epithelial cells through a cAMP/protein kinase A-independent pathway. J Biol Chem. 278:17320-17327.
68. Muniz M, Martin ME, Hidalgo J, Velasco A. 1997. Protein kinase A activity is required for the budding of constitutive transport vesicles from the trans-Golgi network. Proc Natl Acad Sci U S A. 94:14461-14466.
69. Martin ME, Hidalgo J, Vega FM, Velasco A. 1999. Trimeric G proteins modulate the dynamic interaction of PKAII with the Golgi complex. J Cell Sci. 112:3869-3878.
70. Martin ME, Hidalgo J, Rosa JL, Crottet P, Velasco A. 2000. Effect of protein kinase A activity on the association of ADP-ribosylation factor 1 to golgi membranes. J Biol Chem. 275:19050-19059.
71. Kuroda F, Moss J, Vaughan M. 2007. Regulation of brefeldin A-inhibited guanine nucleotide-exchange protein 1 (BIG1) and BIG2 activity via PKA and protein phosphatase 1gamma. Proc Natl Acad Sci U S A. 104:3201-3206.
72. Bejarano E, Cabrera M, Vega L, Hidalgo J, Velasco A. 2006. Golgi structural stability and biogenesis depend on associated PKA activity. J Cell Sci. 119:3764-3775.

73. Weinberger A, Kamena F, Kama R, Spang A, Gerst JE. 2005. Control of Golgi morphology and function by Sed5 t-SNARE phosphorylation. Mol Biol Cell. 16:4918-4930.

74. de Hoop MJ, Dotti CG. 1993. Membrane traffic in polarized neurons in culture. J Cell Sci 17:85-92.

75. Boczan J, Leenders AG, Sheng ZH. 2004. Phosphorylation of syntaphilin by cAMP-dependent protein kinase modulates its interaction with syntaxin-1 and annuls its inhibitory effect on vesicle exocytosis. J Biol Chem. 279:18911-18919.

76. Evans GJ, Morgan A. 2003. Regulation of the exocytotic machinery by cAMP-dependent protein kinase: implications for presynaptic plasticity. Biochem Soc Trans. 31:824-827.

77. Apodaca G. 2001. Endocytic traffic in polarized epithelial cells: role of the actin and microtubule cytoskeleton. Traffic 2:149-159.

78. Kashina AS, Semenova IV, Ivanov PA, Potekhina ES, Zaliapin I, et al. 2004. Protein kinase A, which regulates intracellular transport, forms complexes with molecular motors on organelles. Curr Biol. 14:1877-1881.

79. Rondaij MG, Bierings R, Kragt A, Gijzen KA, Sellink E, et al. 2006. Dynein-dynactin complex mediates protein kinase A-dependent clustering of Weibel-Palade bodies in endothelial cells. Arterioscler Thromb Vasc Biol. 26:49-55.

80. Wojtal KA, Hoekstra D, van IJzendoorn SC. 2007. Anchoring of PKA-RII{alpha} to subapically positioned centrosomes mediates apical bile canalicular lumen development in response to oncostatin M but not cAMP. Mol Biol Cell. In press.

81. Bradbury NA, Bridges RJ. 1992. Endocytosis is regulated by protein kinase A, but not protein kinase C in a secretory epithelial cell line. Biochem Biophys Res Commun. 184:1173-1180.

82. Lukacs GL, Segal G, Kartner N, Grinstein S, Zhang F. 1997. Constitutive internalization of cystic fibrosis transmembrane conductance regulator occurs via clathrin-dependent endocytosis and is regulated by protein phosphorylation. Biochem J. 328:353-361.

83. Prince LS, Workman RB Jr, Marchase RB. 1994. Rapid endocytosis of the cystic fibrosis transmembrane conductance regulator chloride channel. Proc Natl Acad Sci U S A. 91:5192-5196.

84. Katsura T, Gustafson CE, Ausiello DA, Brown D. 1997. Protein kinase A phosphorylation is involved in regulated exocytosis of aquaporin-2 in transfected LLC-PK1 cells. Am J Physiol. 272:F817-F822.

85. Li Y, van Kerkhof P, Marzolo MP, Strous GJ, Bu G. 2001. Identification of a major cyclic AMP-dependent protein kinase A phosphorylation site within the cytoplasmic tail of the low-density lipoprotein receptor-related protein: implication for receptor-mediated endocytosis. Mol Cell Biol. 21:1185-1195.

86. Salazar G, Gonzalez A. 2002. Novel mechanism for regulation of epidermal growth factor receptor endocytosis revealed by protein kinase A inhibition. Mol Biol Cell. 13:1677-1693.

87. Mundell SJ, Pula G, More JC, Jane DE, Roberts PJ, et al. 2004. Activation of cyclic AMP-dependent protein kinase inhibits the desensitization and internalization of metabotropic glutamate receptors 1a and 1b. Mol Pharmacol. 65:1507-1516.

88. Razani B, Rubin CS, Lisanti MP. 1999. Regulation of cAMP-mediated signal transduction via interaction of caveolins with the catalytic subunit of protein kinase A. J Biol Chem. 274:26353-26360

89. Rapacciuolo A, Suvarna S, Barki-Harrington L, Luttrell LM, Cong M, et al. 2003. Protein kinase A and G protein-coupled receptor kinase phosphorylation mediates beta-1 adrenergic receptor endocytosis through different pathways. J Biol Chem. 278:35403-35411.

90. Gardner LA, Naren AP, Bahouth SW. 2007. Downregulation of bone morphogenetic protein 4 expression in coronary arterial endothelial cells: role of shear stress and the cAMP/protein kinase A pathway. Arterioscler Thromb Vasc Biol. 27:776-782.

91. Carmosino M, Procino G, Tamma G, Mannucci R, Svelto M, et al. 2006. Trafficking and phosphorylation dynamics of AQP4 in histamine-treated human gastric cells. Biol Cell. 99:25-36.

92. Birkeli KA, Llorente A, Torgersen ML, Keryer G, Tasken K, et al. 2003. Endosome-to-Golgi transport is regulated by protein kinase A type II alpha. J Biol Chem. 278:1991-1997.

93. Eker P, Holm PK, van Deurs B, Sandvig K. 1994. Selective regulation of apical endocytosis in polarized Madin-Darby canine kidney cells by mastoparan and cAMP. J Biol Chem. 269:18607-18615.

94. Mavrakis M, Lippincott-Schwartz J, Stratakis CA, Bossis I. 2007. mTOR kinase and the regulatory subunit of protein kinase A (PRKAR1A) spatially and functionally interact during autophagosome maturation. Autophagy. 3:151-153.

95. Holen I, Gordon PB, Stromhaug PE, Seglen PO. 1996. Role of cAMP in the regulation of hepatocytic autophagy. Eur J Biochem. 236:163-170.

96. van IJzendoorn SC, Zegers MM, Kok JW, Hoekstra D. 1997. Segregation of glucosylceramide and sphingomyelin occurs in the apical to basolateral transcytotic route in HepG2 cells. J Cell Biol 137:347-357.

97. van IJzendoorn SC, Hoekstra D. 1998. (Glyco)sphingolipids are sorted in sub-apical compartments in HepG2 cells: a role for non-Golgi-related intracellular sites in the polarized distribution of (glyco)sphingolipids. J Cell Biol. 142:683-696.

98. van IJzendoorn SC, Hoekstra D. 1999. Polarized sphingolipid transport from the subapical compartment: evidence for distinct sphingolipid domains. Mol Biol Cell. 10:3449-3461.

99. Tuma PL, Hubbard AL. 2003. Transcytosis: crossing cellular barriers. Physiol Rev. 83:871-932.

100. van IJzendoorn SC, Hoekstra D. 2000. Polarized sphingolipid transport from the subapical compartment changes during cell polarity development. Mol Biol Cell. 11:1093-10101.

101. van IJzendoorn SC, van Der Wouden JM, Liebisch G, Schmitz G, Hoekstra D. 2004. Polarized membrane traffic and cell polarity development is dependent on dihydroceramide synthase-regulated sphinganine turnover. Mol Biol Cell. 15:4115-4124.

102. Lacana E, Maceyka M, Milstien S, Spiegel S. 2002. Cloning and characterization of a protein kinase A anchoring protein (AKAP)-related protein that interacts with and regulates sphingosine kinase 1 activity. J Biol Chem. 277:32947-32953.

103. van der Wouden JM, van IJzendoorn SC, Hoekstra D. 2002. Oncostatin M regulates membrane traffic and stimulates bile canalicular membrane biogenesis in HepG2 cells. EMBO J. 21:6409-6418.

104. van IJzendoorn SC, Théard D, van der Wouden JM, Visser W, Wojtal KA, et al. 2004. Oncostatin M-stimulated apical plasma membrane biogenesis requires p27(Kip1)-regulated cell cycle dynamics. Mol Biol Cell. 15:4105-4114.

105. Prinz A, Diskar M, Erlbruch A, Herberg FW. 2006. Novel, isotype-specific sensors for protein kinase A subunit interaction based on bioluminescence resonance energy transfer (BRET). Cell Signal. 18:1616-1625.

106. Burns-Hamuro LL, Ma Y, Kammerer S, Reineke U, Self C, et al. 2003. Designing isoform-specific peptide disruptors of protein kinase A localization. Proc Natl Acad Sci U S A. 100:4072-4077.

107. Carlson CR, Lygren B, Berge T, Hoshi N, Wong W, et al. 2006. Delineation of type I protein kinase A-selective signaling events using an RI anchoring disruptor. J Biol Chem. 281:21535-21545.

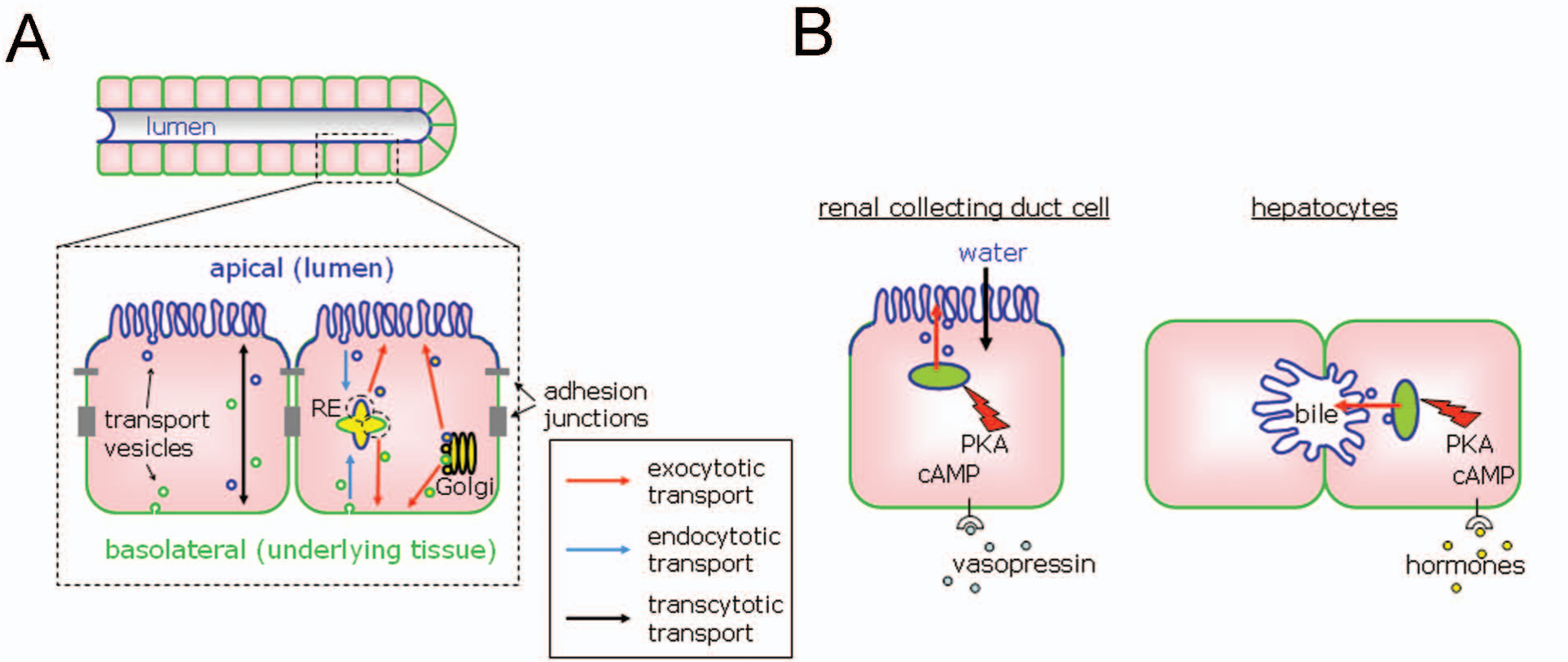
108. Hundsrucker C, Rosenthal W, Klussmann E. 2006. Peptides for disruption of PKA anchoring. Biochem Soc Trans. 34:472-473.

109. Stokka AJ, Gesellchen F, Carlson CR, Scott JD, Herberg FW, et al. 2006. Characterization of A-kinase-anchoring disruptors using a solution-based assay. Biochem J. 400:493-499.

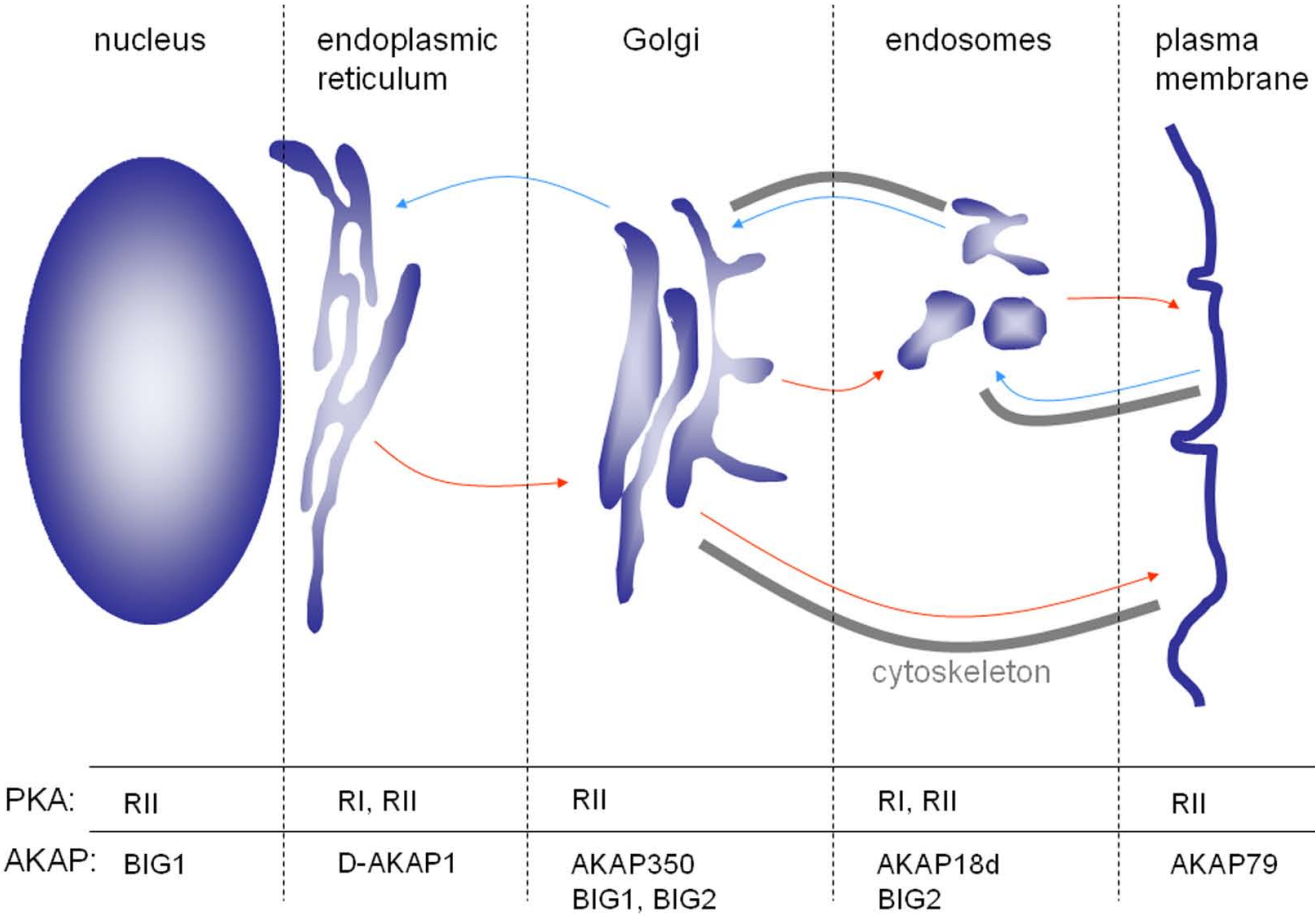
Legends

Figure 1. Organization of and trafficking pathways in epithelial cells. A. Illustration of trafficking pathways serving the apical (blue) and basolateral (green) surface domains in polarized epithelial cells. Red, blue and black arrows and circles indicate exocytic, endocytic, and transcytotic transport routes, respectively. RE: recycling endosomes. B. examples of stimulated apical exocytosis in renal collecting duct cells and hepatocytes in response to a cAMP/PKA-stimulating agonist/ligand.

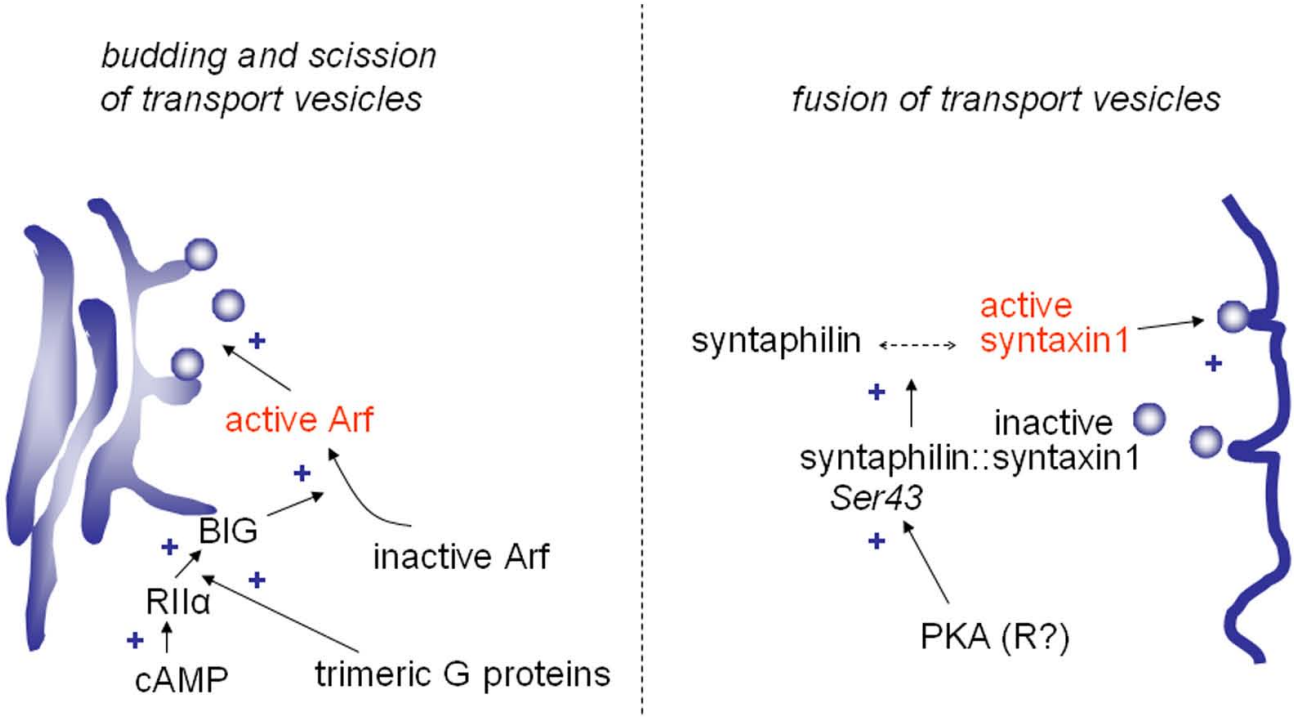
Figure 2. The involvement of PKA-AKAP in vesicular transport. A. PKA and AKAPs localize to the different organelles that make up the exocytotic pathaway including the endoplasmic reticulum, the Golgi apparatus, endosomes, and the plasma membrane. Thick grey lines represent cytoskeleton fibers. B. Possible molecular roles of PKA in the budding and scission of transport vesicles from a donor organelle, in this case the Golgi apparatus, and in the fusion of transport vesicles with the acceptor membrane, in this case the plamsa membrane. Note that the distinct contributions of each PKA isoenzyme (I and II) in the regulation of vesicular transport pathways by D-AKAP1 or BIG2 has not been experimentally addressed.


A
lumen
apical (lumen)
transport vesicles
RE
Golgi
basolateral (underlying tissue)
adhesion junctions
exocytotic transport
endocytotic transport
transcytotic transport
B
renal collecting duct cell
water
PKA
cAMP
vasopressin
hepatocytes
bile
PKA
cAMP
hormones

A


nucleus
endoplasmic reticulum
Golgi
endosomes
plasma membrane
cytoskeleton
PKA:
RII
RI, RII
RII
RI, RII
RII
AKAP:
BIG1
D-AKAP1
AKAP350
BIG1, BIG2
AKAP18d
BIG2
AKAP79

B


budding and scission of transport vesicles
active Arf
BIG
inactive Arf
RIIα
cAMP
trimeric G proteins
fusion of transport vesicles
syntaphilin
active syntaxin1
inactive
syntaphilin::syntaxin1
Ser43
PKA (R?)