Chapter 8

Perspective
The sub-apical compartment: a novel sorting center?

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Summary - Establishment of plasma membrane polarity involves numerous intracellular sorting events. In the past few years, it has become apparent that there is a sub-apical, non-Golgi compartment located in the hub of the sorting pathways involved. This 'sub-apical compartment' which probably consists of a heterogeneous subset of functionally distinct domains related to endosomes, contains some well-characterized components involved in polarity-dependent sorting and targeting of proteins and lipids. This article discusses the evidence supporting the existence of such a compartment, its biogenesis and its role in cell polarity.

Polarized cells have distinct plasma membrane (PM) domains, a basolateral and an apical domain, each characterized by a specific composition of proteins and lipids (Simons and Fuller, 1985). The underlying mechanisms by which polarized cells generate and maintain such specific compositions are still largely unknown. An important step towards understanding this requires a detailed characterization of the intracellular 'roadmap'. Such studies will include identification of 'traffic stations' along the different transport routes where molecules are sorted and targeted to their preferred PM domains. Highly relevant issues are whether novel sorting mechanisms are induced upon polarization, involving biosynthesis of new regulatory components, and whether these events occur in unique compartments typical of polarized cells. It is also possible that polarized sorting is achieved by taking advantage of existing cognate apical and basolateral membrane-directing machineries, known to be present in nonpolarized cells (Yoshimori et al., 1996), operating in a polarized fashion when required.

In recent years, one particular endosomal compartment in polarized cells has gained increasing attention. This compartment appears to connect the apical and basolateral-derived transport pathways and is involved in transcytosis and recycling of both proteins (Apodaca et al., 1994; Barosso and Sztul, 1994; Knight et al., 1995; Odorizzi et al., 1996; Futter et al., 1998; Gibson et al, 1998; Hughson and Hopkins, 1990) and, as very recently revealed, lipids (van IJzendoorn and Hoekstra, 1998; van IJzendoorn et al., 1997) as well. Gradually, a picture has emerged of a 'sorting station' in polarized cells, which differs from and operates next to the trans-Golgi network (TGN) to which such qualities have been primarily attributed thus far. It thus appears that in the presence of continuous transcellular transport, typical of polarized cells, this non-Golgi compartment might serve as an important intermediate in sorting and subsequent targeting of molecules derived from and destined for either the apical or basolateral PM domain. These features make this compartment, which we refer to as the sub-apical compartment (SAC), the focus of intense investigations into morphological and molecular aspects of the complex sorting machinery it contains. Moreover, its similarity to endosomal compartments in nonpolarized cells has implications for its biogenesis and the establishment of polarized sorting.

The first evidence for the existence of a specialized compartment at the intersection of the apical and basolateral endocytic pathways came from the transient colocalization of basolaterally-derived proteins with proteins that had been internalized from the apical surface (Apodaca et al., 1994; Barosso and Sztul, 1994; Knight et al., 1996; Hughson and Hopkins, 1990) (Fig. 1). One such basolaterally-derived protein found in the interconnecting compartments is the polymeric immunoglobulin receptor (pIgR). This well-characterized

transcytotic marker mediates basolateral-to-apical transcytosis of both IgA and IgM (Apodaca et al., 1994). Following endocytosis from the basolateral surface, pIgR-bound IgA (pIgR-IgA) first reaches peripheral early 'sorting' endosomes. Here, the pIgR-IgA complex is sorted from molecules that recycle or have destinations downstream in the late endosomal/lysosomal route, and is subsequently targeted to the SAC, prior to delivery to the apical PM domain (Apodaca et al., 1994; Barosso and Sztul, 1994; van IJzendoorn and Hoekstra, 1998). Sphingolipids (van IJzendoorn and Hoekstra, 1998), as well as transcytosing apical membrane-resident proteins (Barr et al., 1995; Hemery et al., 1996) appear to follow the same, SAC-mediated route. In the reverse apical-to-basolateral transcytotic pathway, the SAC also appears to serve as an intermediate compartment for transport of both proteins and lipids (Odorizzi et al., 1996; van IJzendoorn and Hoekstra, 1998), although Ihrke *et al.* (1998) failed to detect such a reverse pathway in WIF-B cells. Nevertheless, it is apparent that the SAC constitutes an intrinsic part of the transcytotic pathway, which is crucial to the functioning of polarized cells. Importantly, in addition to transcytosing distinct proteins and lipids, substantial fractions of molecules that recycle at either PM domain also appear to pass through the SAC (Apodaca et al., 1994; Futter et al., 1998; van IJzendoorn et al., 1997; Okamoto et al., 1998). Hence, the SAC, being centrally located in the complex web of intracellular trafficking pathways, can receive and deliver molecules from and to either PM domain.
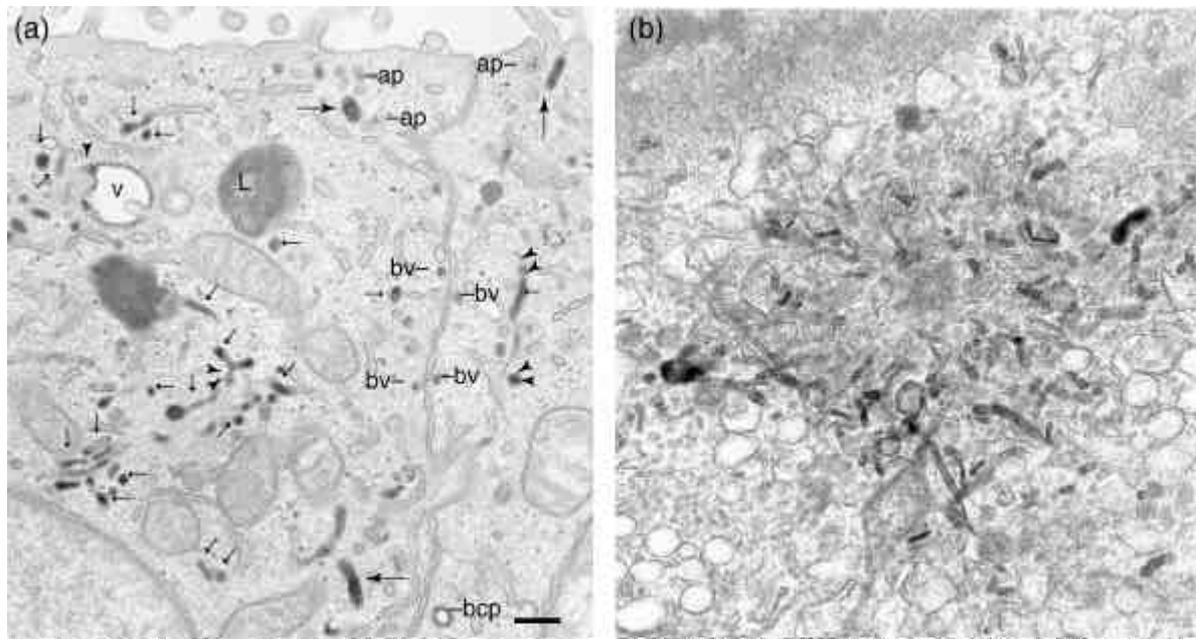

Figure 1. (a) codistribution of Tf-HRP and IgA-gold applied to the basolateral and apical surfaces of MDCK cells, respectively. For further details see Futter et al. (1998). (b) Thick section high voltage electron micrograph of an MDCK cell showing the pericentriolar localization of transcytosing IgA-HRP to the tubules of the SAC, which occurs before delivery to the apical surface.

The SAC is an endosomal compartment

To appreciate the hierarchical position of the SAC in the context of the endosomal system, and its distinct role as a sorting compartment in maintaining and establishing cell polarity, the following considerations are of interest. Vesicular transport of proteins from the PM to the peripheral, early 'sorting' endosomes is typically independent of microtubules (Gruenberg and Maxfield, 1995), whereas their subsequent vesicular transfer to the SAC is a mictotubule-dependent process (Apodaca et al, 1994; Mukherjee et al., 1997). Apically derived sphingolipids also reach the SAC in a microtubule-dependent manner, in contrast to apical endocytosis per se (van IJzendoorn and Hoekstra, 1998). Thus, in nocodazole-treated cells, apical endocytic transport of sphingolipids is blocked at the level of the apical early 'sorting' endosome (van IJzendoorn and Hoekstra, 1998) which, like its basolateral counterpart (Bomsel et al., 1989; Parton et al., 1989), might be involved in sorting membrane-bound receptors from fluid-phase markers, which do not gain access to the SAC (Barosso and Sztul, 1994). The SAC can also be discerned from early 'sorting' endosomes by a low-temperature block. At 17-18°C, when transport is slowed down, proteins transcytosing from the basolateral to the apical PM domain pass through the early basolateral 'sorting' endosome and accumulate in the SAC (Apodaca et al., 1994; Barosso and Sztul, 1994; Futter et al., 1998; van IJzendoorn and Hoekstra, 1998). An 18°C-induced accumulation in the SAC is also observed for apically internalized sphingolipids, traveling along the apical-to-basolateral pathway in HepG2 cells (van IJzendoorn and Hoekstra, 1998) (Fig. 2). Hence, the SAC appears to be located distal to early 'sorting' endosomes, originating from either basolateral or apical surface. In several studies, it has been shown that a substantial fraction of basolaterally internalized transferrin (Tf), a classical marker of early endosomes and typically absent in the late endosomal/lysosomal pathway (Mukherjee et al., 1997), recycles via the SAC (Apodaca et al., 1994; Odorizzi et al., 1996; Futter et al., 1998; Gibson et al., 1998). This implies that the compartment displays a functional analogy to the recycling endosome. Importantly, in this context, several pieces of evidence argue against the SAC being merely an extension of the TGN, through which the Tf receptor (TfR) has also been reported to cycle. Immunofluorescence microscopic studies of MDCK cells never revealed a colocalization of TfR and sialyltransferase, a trans-Golgi and TGN-specific marker (Futter et al., 1998). Furthermore, monensin, which destroys the integrity of the Golgi apparatus, perturbs the Golgi-mediated transport of newly synthesized sphingolipids, while leaving the recycling of apically internalized sphingolipids unaffected (van IJzendoorn et al., 1997).

The association of specific marker proteins has proven a valuable tool to more accurately define the boundaries of distinct compartments. Indeed, the presence and/or absence of distinct rab proteins further reveals SAC's modality. Whereas rab4 is exclusively located to the early 'sorting' endosomes (Daro et al., 1996), rab11 appears to associate with SAC (Casanova et al., 1999), although not exclusively, since this small GTPase also associates with the Golgi (Chen et al., 1998). However, two epithelium-specific rab proteins have recently been identified, i.e. rab17 (Zacchi et al., 1998; Hunziker and peters, 1998) and rab25 (Casanova et al., 1999), which are both located to the SAC. As will be further discussed

below, both these rab proteins are likely to play a role in regulating SAC-mediated trafficking in polarized cells.

Taken together, in polarized cells, the SAC derives its distinct identity from posing as a joint compartment of the basolateral and apical endocytic pathways in a route that connects the early apical and basolateral 'sorting' endosomes, and because of its separation from the classic late endosomal/lysosomal route. The involvement of the SAC in intracellular transport in polarized cells is depicted schematically in Fig. 3a.
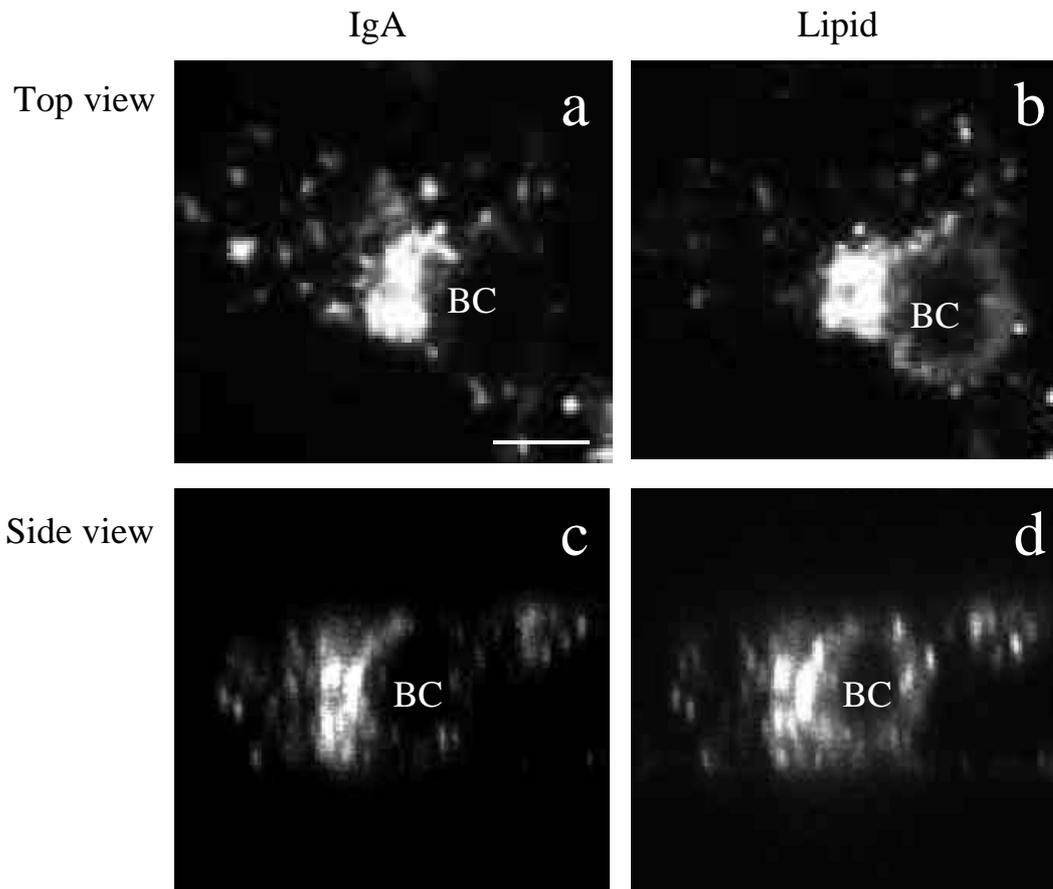

Figure 2. Colocalization of basolaterally-derived, texas-red-labeled IgA (a,c) with apically derived C_6-NBD-labeled sphingolipid (b,d) in SAC in polarized hepatic HepG2 cells. In these cells, the bile canalicular plasma membrane domain (BC) represents the apical plasma membrane domain in other polarized epithelial cells. Note the clearly overlapping staining patterns of labeled IgA and sphingolipid. Side view (x-z section) images c and d correspond to top view images (a and b), respectively of the same cells. Bar: 5 μM. For further details see ref. 10.

Molecular aspects of the sorting machinery in the SAC

The SAC appears to be equipped with highly accurate sorting machineries directing proteins and lipids to the different PM domains. For instance, transcytosing pIgR-IgA is transported from the SAC to the apical PM domain (Apodaca et al., 1994; Barosso and Sztul, 1994; van Ijzendoorn and Hoekstra, 1998; Hemery et al., 1996), whereas Tf is typically targeted from this compartment to the basolateral surface (Apodaca et al., 1994; Odorizzi et al., 1996; Futter

et al., 1998). Among others, a clathrin-based sorting mechanism may be operating, since clathrin-coated buds containing γ-adaptin have been observed on the SAC (Futter et al., 1998). In oxyntic cells, clathrin-AP1-γ-adaptin coat complexes have been implicated in the regulation of apical H^+-K^+-ATPase recycling via a tubulovesicular compartment, which may resemble the SAC in other polarized epithelial cells (Okamoto et al., 1998). Furthermore, brefeldin A (BFA), a fungal metabolite that dissociates functional coat protein complexes, disperses SAC-associated γ-adaptin and, concomitantly, abolishes polarized sorting of Tf, suggesting that targeting of Tf from the SAC to the basolateral PM domain is regulated by γ-adaptin-clathrin complex-coated domains on the SAC tubules (Futter et al., 1998). Indeed, sorting of the TfR no longer occurs when it lacks its cytoplasmic domain, which results in the protein's exiting from the SAC in a non-polarized manner (Odorizzi et al., 1996). BFA also inhibits transport of transcytosing pIgR-IgA from the SAC to the apical PM domain, but does not affect apical recycling of pIgR-IgA or ricin (Barosso and Sztul, 1994). This suggests that, apart from coat complexes, different sorting mechanisms operate in the SAC that can distinguish molecules that enter the apical recycling pathway from those that transcytose from the basolateral to the apical surface. In strong support of this view, protein kinases stimulate apical PM-directed transcytotic protein transport at the level of the SAC, whereas recycling of apical proteins from SAC is unaffected (Cardone et al, 1994; Hansen and Casanova, 1994). However, these observations might also indicate that the SAC represents a collection of heterogeneous, functionally distinct compartments.

Sorting of apical PM components could well involve glycosphingolipid-enriched domains. In the TGN, newly synthesized apical proteins, including glycosylphosphatidylinositol (GPI)-anchored proteins, supposedly associate with these glycosphingolipid domains, after which apically directed transport vesicles are produced (Simons and Ikonen, 1997). Recent data have shown that, in hepatic HepG2 cells, sphingolipids are segregated in the apical-to-basolateral transcytotic pathway, independent of Golgi apparatus involvement (van IJzendoorn et al., 1997). Detailed analysis revealed that sorting of these lipids occurred in the SAC (van IJzendoorn and Hoekstra, 1998). In these experiments, fluorescent analogues of sphingomyelin (SM), galactosyl- (GalCer) and glucosyl-ceramide (GlcCer) were first endocytosed from the apical surface and transported to SAC, where they accumulate at 18°C (see Fig. 2). From there, SM and GalCer were preferentially targeted to the basolateral surface, whereas GlcCer was recycled efficiently to the apical PM domain. Interestingly, the apical GPI-anchored protein 5'-nucleotidase passes through the SAC (Ihrke et al., 1998) via transcytosis, after initial delivery to the basolateral membrane. It is thus possible that a sphingolipid-domain-mediated sorting mechanism also operates in the SAC. Taken together, it is likely that multiple sorting machineries (γ-adaptin-clathrin coats, sphingolipid domains) operate in SAC and promote the formation of distinct sets of apical vesicles. Furthermore, γ-adaptin-clathrin-AP1-coated and sphingolipid-enriched domains on and in the SAC, respectively, might be involved in both apically and basolaterally directed transport. A challenging issue will be to unravel the subtle mechanisms that allow recognition of domains, including similarly coated ones, yet producing vesicles with different

subcellular destinations. Distinct sets of protein kinases may play a role in these events, analogous to their involvement in the production of transport vesicles from the TGN (Muniz et al., 1997). Instrumental to such a mechanism would be a highly dynamic transition of proteins between distinct subdomains/ subcompartments that comprise the SAC, resulting in structures enriched in, for example, either receptor-bound IgA or Tf receptor (Gibson et al., 1998). Such a transition could in fact be influenced (Gibson et al., 1998) by the presence of specific proteins, such as IgA, in the SAC. Indeed, Luton *et al.* (1998) showed that binding of IgA to its receptor pIgR at the basolateral surface enhances the transcytosis of pIgR by specific stimulation of its apical transport at the level of SAC.

As noted above, several rab proteins, i.e. rab11, rab17 and rab25, have been reported to associate with the SAC. In general, these proteins are known to regulate vesicular membrane trafficking, distinct steps in the endocytic and biosynthetic/exocytic pathways being controlled by individual members of this small GTPase family. Obviously, the exclusive expression of rab17 and rab25 in epithelial cells (Casanova et al., 1999; Zacchi et al., 1998) indicates that they must govern specialized functions that are restricted to polarized trafficking. Given the role of the SAC in these events, their association with this compartment is therefore anticipated. In fact, both rab proteins have been claimed to play a role in the regulation of transport through the SAC. Although its precise function remains to be determined (Zacchi et al., 1998; Hunziker and Peters, 1998), rab17 specifically interferes with transport between the SAC and the apical plasma membrane domain (Zacchi et al., 1998). Interestingly, overexpression of rab25 slows the rate of transcytotic transport and apical recycling, while leaving basolateral recycling via the SAC unaffected (Casanova et al., 1999). It thus appears that rab17, unlike rab25, interferes with polarized sorting. Thus far, no functional studies of rab11 in polarized cells have been reported. However, since rab11 regulates TfR recycling in non-polarized cells (Ren et al., 1998; Ullrich et al., 1996), a similar mode of action might be anticipated in polarized cells, implying a regulation of basolateral recycling via the SAC.

The intracellular distribution of these rab proteins was studied by the use of fluorescence microscopy (Casanova et al., 1999; Zacchi et al., 1998; Hunziker and Peters, 1998), and their localization was compared to that of fluorescently tagged Tf and IgA. Intriguingly, data thus obtained indicate that overlap in the colocalization with the transcytotic markers is often not complete, while the SAC-associated rab proteins also show distinct degrees of overlap. Such distinctions in localization became also apparent when the microtubule-dependent role in stabilizing the integrity of the SAC was examined by the treatment of the cells with the microtubule-disrupting drug nocodazole. Whereas SAC marked by the presence of rab11 and rab25 dispersed upon treatment with nocodazole (casanova et al., 1999), rab17-marked SAC did not (Zacchi et al., 1998). These data may underscore the aforementioned possibility of subcompartmentalization of SAC. Thus, it is tempting to suggest that different rab proteins act at specific SAC subcompartments, where they regulate the transfer of molecules to, from and/or between these compartments.

On a cautioning note, it should be kept in mind that not all molecules might pass

through the SAC with similar kinetics or at the same time, following initial PM internalization. Hence, not all molecules known to pass through the SAC necessarily colocalize, as colocalization of specific components in the SAC might be restricted to certain subcompartments of this sub-apical organelle. Thus, molecules might initially colocalize in a subcompartment of the SAC, but subsequently be redistributed within the SAC to a site where final inclusion into a specific vesicle population and budding occurs. This might also explain why Tf is not always detected in the SAC (Barosso and Sztul, 1994). In addition, trafficking of ligand-bound (vs 'empty') receptors through the SAC might affect transfer of other molecules between SAC subcompartments as a result of intracellular signaling pathways activated upon receptor-ligand interaction at the surface (Luton et al., 1998). Clearly, a further detailed characterization of those issues and SAC subdomains and/or subcompartments, their biogenesis and role in apical versus basolateral targeting is imperative. In summary, the SAC appears to be equipped with important elements of both basolateral and apical sorting machineries that had thus far exclusively been attributed to the TGN in the biosynthetic pathway.

The SAC is related to the pericentriolar recycling compartment

Why is the SAC so crucially involved in different trafficking pathways? A likely possibility is that the SAC, given its junctional features, functions as an intracellular site where apical and basolateral proteins and lipids can be readily exchanged, sorted and targeted. This would allow the cell to maintain its polarized phenotype, while simultaneously economizing on its trafficking and sorting machineries. In addition, such a centrally located compartment could adapt quickly to situations where a rapid, efficient targeting or redistribution of PM components is required.

Not only polarized epithelial cells, but also cells that do have distinct PM domains but no tight junctions that separate these domains, are in need of the SAC machinery. In migrating fibroblasts (Fig. 3b), recycling TfR are concentrated in narrow tubules in the pericentriolar area, probably representing the perinuclear recycling endosomes or pericentriolar recycling compartment (PCRC), via which they are subsequently routed to the PM of the leading lamella (Hopkins et al., 1994). Also in neurons, which have distinct axonal and somatodendritic PM domains, the polarized sorting of TfR in the endocytic system was proposed to be mediated in the PCRC (West et al., 1997). The sorting function of the PCRC, which can thus be inferred, is further supported by observations in a mutant CHO cell line, displaying the capacity to discriminate between the flux of recycling receptors and that of bulk membrane (Presley et al., 1993). Several lines of evidence suggest that the PCRC and SAC are closely related. In nonpolarized cells, the GPI-anchored folate receptor is sorted from the TfR in the PCRC. The efficiency of this sorting appears to depend on the presence of cholesterol (Mayor et al., 1998), which is also implicated in sphingolipid-domain-mediated sorting (Simons and Ikonen, 1997). Therefore, the mechanism of sphingolipid-domain-mediated sorting might also operate in the PCRC, similarly as proposed for the SAC (van IJzendoorn and Hoekstra, 1998). Analogous to the SAC, the PCRC displays a pleiomorphic

nature and, additionally, is also heterogeneous with respect to biochemical composition, ion transport properties and pH value (Teter et al., 1998). Furthermore, also the PCRC is located distal to the early 'sorting' endosome (Gruenberg and Maxfield, 1995; Mukherjee et al., 1997) and acquires early 'sorting' endosome-derived cargo in a microtubule-dependent manner (Mukherjee et al., 1997). Like the SAC in epithelial cells, the PCRC in nonpolarized cells (Fig. 3c) constitutes part of the TfR-recycling pathway, but is neither involved in sorting or transfer of molecules that follow the late endosomal/lysosomal pathway. Moreover, at 18°C, pre-endocytosed pIgR-IgA complex and sphingolipids accumulated in the SAC in fully polarized HepG2 cells, and in the PCRC in nonpolarized HepG2 cells (van IJzendoorn and Hoekstra, 1998). In full agreement with such a distribution in polarized and nonpolarized cells is the observation that an apical endosomal marker is targeted to the PCRC when expressed in nonpolarized cells (Wilson and Colton, 1997). Also the SAC-associated rab17 (Zacchi et al., 1998; Hunziker and Peters, 1998) localizes to the tubular PCRC when expressed in nonpolarized cells (Zacchi et al., 1998). As noted above, rab11 is associated with the PCRC in nonpolarized cells, where it regulates receptor recycling from the PCRC (Ren et al., 1998; Ullrich et al., 1996), while it associates with the SAC in polarized epithelial cells (Casanova et al. 1999). Although comparison of its localization in polarized and nonpolarized cells was mostly between different cell types, rab11 was recently found to localize to the SAC and the PCRC in polarized and nonpolarized HepG2 cells, respectively, present in the same cell culture (S. van IJzendoorn and D. Hoekstra, unpublished observations). These data suggest that membrane recycling via the SAC in polarized cells and via the PCRC in nonpolarized cells, at least in part, contain common elements of traffic regulation. Not only do these observations strongly suggest equivalence between both compartments, implying a closer similarity between the endocytic system in nonpolarized and polarized cells than previously appreciated, they also underscore that the SAC is not a unique compartment, restricted to polarized cells. Rather, the SAC appears to display its function in polarized trafficking by acquiring the specific sorting machinery when redistribution of PM components is required upon induction of cell polarity. Presumably, Rab17 and rab25 are part of these acquired machineries. Thus, the PCRC can be considered as the precursor compartment for the SAC.

Concluding remarks and perspectives
The evidence, currently available, strongly supports an involvement of the SAC in polarized sorting in the transcytotic route, redirecting apical and basolateral membrane constituents to the appropriate membrane domains. The molecular sorting devices that carry out these functions remain to be resolved, although it becomes apparent that 'polarity-specific' rab proteins are playing a role in regulating SAC-associated trafficking. Also kinase activity (protein kinase A and C) seems to act at the level of the SAC in governing sorting and membrane flow (Cardone et al., 1994; Hansen and Casanova, 1994). It has been shown that PKC activation leads to cell depolarization, whereas the opposite effect on the state of cell
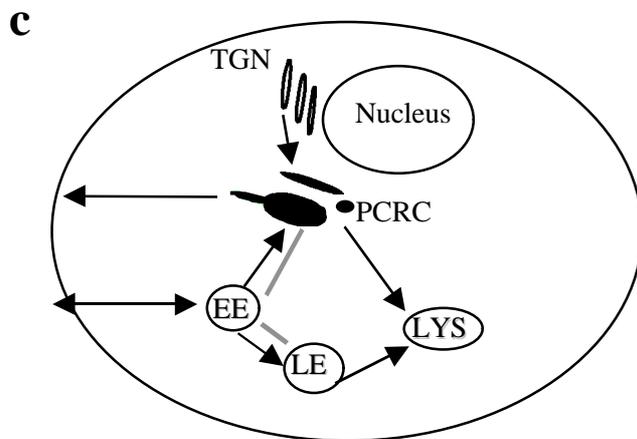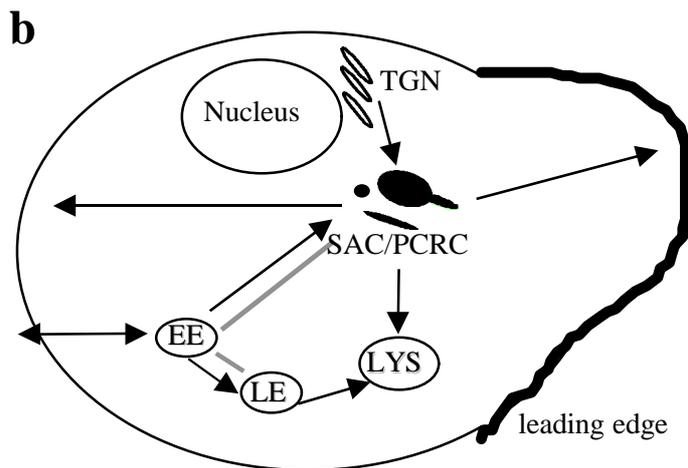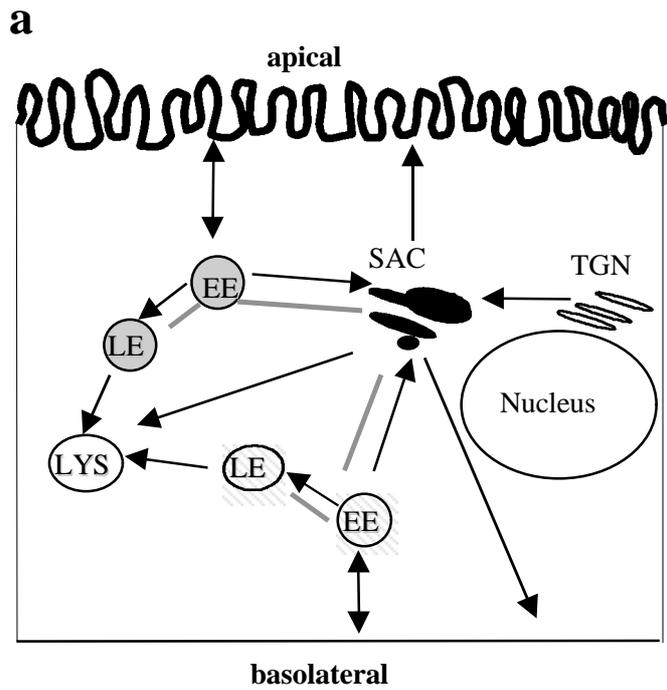
a

b

c

Figure 3.
Schematic representation of the involvement of SAC/PCRC in specific PM directed transport in fully polarized cells with two distinct PM domains (a), in semi-polarized cells, i.e. with a specific PM domain such as a leading edge in migrating fibroblast (b), and in semi-polarized cells (c). Molecules that are internalized from either the basolateral (light gray with hatched pattern) or apical (gray) PM domain in polarized cells initially reach the early sorting endosome (EE). Here, PM proteins such as receptors are sorted from fluid phase cargo and from molecules destined for the late endosomal (LE)/lysosomal (LYS) pathway. Subsequently, the remaining PM protein fraction is transported to the SAC (in polarized cells) or PCRC (in non- and semi-polarized cells). From SAC/PCRC (depicted in black to emphasize its distinct position in the different traffic routes), PM components are then selectively targeted to their preferred PM domain. In (semi-) polarized cells SAC/PCRC might govern targeting of specific molecules to distinct PM domains, such as a leading edge (b) or, in fully polarized cells, to either apical or basolateral PM domain (a).

polarity is seen upon activation of PKA (Zegers and Hoekstra, 1997). However, the molecular targets of these activities remain obscure.

An important issue that remains to be resolved is how the SAC relates to the previously described apical recycling endosome, also referred to as apical recycling compartment (ARC, Apodaca et al., 1994; Barosso and Sztul, 1994). As argued above, the SAC as defined here likely comprises a number of distinct compartments involved in different aspects of polarized targeting. It is possible, that the ARC might be considered as a specialized subcompartment of the SAC, mainly involved in apical disposition (Gibson et al., 1998), and 'activated' upon sorting during transition of molecules between SAC subcompartments. However, the availability of well-defined lipid and protein markers in terms of apical and basolateral destination, will aid in unraveling the complex net and the regulation of different pathways, that lead to and originate from the SAC. In conjunction with the emergence of specific SAC-associated proteins, and the distinct microtubule-dependent organization of the SAC, further insight into the compartmental organization of the SAC and its functional significance may soon be anticipated.

Another topic of interest will be as to whether a functional relationship exists between the SAC and TGN. Although separate compartments, as outlined above, both display the capacity of polarized sorting, the TGN also targeting newly synthesized proteins and lipids to their preferred plasma membrane domain. The question can thus be raised as to whether, and if so, why and to what extent, the SAC and TGN operate in separate routes to secure plasma membrane polarity. Indeed, depending on the (polarized) cell type, (some) newly synthesized apical membrane constituents are first transported to the basolateral membrane, prior to apical delivery via transcytosis (Mostov and Cardone, 1995). In this case the explicit involvement of the SAC is anticipated (Barr et al., 1995; Ihrke et al., 1998). Interestingly, the SAC has also been implicated in the direct recruitment of newly synthesized PM components from the biosynthetic route via a (probably) signal-regulated exocytic route (Futter et al., 1998). Thus, the SAC might also account for the polarized distribution of certain proteins that travel directly from the TGN to this compartment, where polarized sorting subsequently occurs (Futter et al., 1998; Mostov and Cardone, 1995; Leitinger et al., 1995; Futter et al., 1995). It will be a challenge to identify molecular mechanisms that determine as to why distinct proteins are processed along different pathways before they reach the same target site. Possibly, sorting into different lateral domains, thus giving rise to distinctly targeted transport vesicles, could be involved. Among others, kinases, which may trigger vesiculation (Muniz et al., 1997; Zegers and Hoekstra, 1997, and references therein), and specific rab proteins could act as traffic regulators.

Taken together, revealing morphological, mechanistic and molecular aspects of the SAC and its machineries will not only provide insight into the functioning of this particular compartment. Rather, its central localization literally harbors numerous aspects that are crucial towards understanding the biogenesis of organelles and their intracellular communication.

Acknowledgments