Summary and future prospects

The work described in this thesis has provided a novel insight into the process of sphingolipid transport and sorting in polarized cells. We have used HepG2 cells as a model system to study polarized traffic in hepatic cells. Under specific culture conditions, HepG2 cells acquire a polarized phenotype, displaying a sinusoidal, basolateral plasma membrane and a bile-canalicular, apical plasma membrane domain. By employing fluorescently tagged sphingolipids to visualize lipid traffic in life cells, we have revealed the existence of an apical to basolateral transcytotic pathway in these cells (chapter 2), allowing extensive transcellular membrane flow between the two membrane domains. Because different sphingolipids display a polarized distribution in polarized cells, which most likely reflects their diverse functions, the occurrence of polarized sorting during transcytosis was anticipated. However, rather unexpectedly, segregation of SM and GlcCer was apparent in the apical to basolateral transcytotic pathway (chapter 1). Taking advantage of the well-studied transcytotic marker pIgR-IgA, this reverse transcytotic route has been examined in detail and it was demonstrated that sphingolipid sorting is orchestrated in non-Golgi-related, sub-apical compartments (SAC; chapter 2), that constitute an integral part of transcellular flow in both directions (chapter 2 and 7) as well as in the biosynthetic route (chapter 7). Thus, from the SAC, SM and GalCer are directed to the basolateral domain whereas GlcCer is directed to the apical surface. Importantly, it was established that sphingolipid transport in the transcytotic route occurred by vesicular means, implying that segregation of the lipids occurred in the inner leaflet of the SAC. That SM and GlcCer were present in distinct pools or domains in the SAC is further supported by the observation that apical or basolateral trafficking of these domains could be distinctly regulated by known modulators of intracellular traffic, e.g. calmodulin antagonists and cAMP/protein kinase A (PKA; chapter 4) and the microtubule-disrupting compound nocodazole (chapter 6). Intriguingly, SM can be rerouted from the SAC to the apical surface under conditions of elevated intracellular cAMP levels, while its segregation from GlcCer is unaffected (chapter 1 and 4). Interestingly, increased cAMP levels also caused hyper-polarization of the cells. Furthermore, SAC-to-apical traffic of this lipid as well as the (hyper)polarization of the cells correlated with an enhanced PKA activity (chapter 4 and 5). These data revealed the existence of two distinct SAC-to-apical transport routes, marked by the trafficking of either SM or GlcCer. In particular the route as marked by SM, which may represent the final step of basolateral to apical transcytosis, appears to be directly involved in the biogenesis of the apical plasma membrane. This was strongly supported after investigation of polarized sphingolipid transport from the SAC in cells that were in the process of polarity development, suggesting that the (physiological) process of polarity development appears to be closely related to cAMP/ PKA-regulated polarized targeting from the SAC. The SAC may not represent a single compartment, but rather appears to display a subcompartmental nature. This was inferred from by experiments that suggested that cAMP/PKA-induced trafficking of

SM from the SAC to the apical PM domain occurred via separate rab11-positive compartments (chapter 6). In addition, SAC-associated sphingolipids and IgA dispersed after disruption of the microtubule network, giving rise to distinct structures that were either enriched in sphingolipid or IgA (chapter 7).

Although the existence of, likely, a subcompartment of the SAC, i.e. the apical recycling compartment, and its function in intracellular transport was first reported only a few years ago, the role of a more extensive network-like SAC in polarized cells is now rapidly gaining attention. Indeed, because of *i)* its harboring several sorting mechanisms that had previously been attributed solely to the Golgi/TGN, *ii)* its intersection with most known intracellular transport routes and its analogy to and, possibly, maturation from a compartment present in non-polarized cells (chapter 8), a central role of the SAC in cell polarity can be readily anticipated. The use of sphingolipid analogs and the HepG2 model cell system, of which polarity progression can be carefully monitored as described in the experiments in this work, has revealed some novel and important aspects of the involvement of the SAC in polarized traffic and cell polarity development. A major challenge will be to unravel its (heterogeneous) structure and its function in (signal-regulated) polarized traffic of proteins and lipids, and cell polarity in general. Future studies that are anticipated to reveal novel and detailed insight into the molecular aspects of (regulated) polarized lipid and protein traffic will include photoaffinity-labeled sphingolipids to cross-link ‘neighbor’ proteins. In this way, proteins that are associated with the different SM- and GlcCer-enriched domains in the SAC can be identified. Furthermore, electron-microscopical analysis of SAC-associated fluorophore-labeled sphingolipids (by photoconversion of diaminobenzidine), possibly in conjunction with gold-particle-labeled antibodies against proteins that reside in or traffic through the SAC, will be helpful to obtain insight into the way polarized sorting of sphingolipids is related to the subcompartmental organization of the SAC. Finally, (over)expression of (mutant) proteins that are known effectors of polarized transport, e.g. rab proteins, will be performed.