7 Summary

PKC is known to stimulate the formation of constitutive transport vesicles at the trans Golgi network [165]. In an attempt to understand the mechanism behind that process, two possible experimental approaches were followed:

In the first approach PKC-binding proteins at the Golgi apparatus (GA) were analyzed according to a PKC-overlay assay technique. β-Actin was identified as a major binding protein of activated PKCα by high resolution 2D-electrophoresis and microsequencing techniques.

In the second experimental setup isolated Golgi cisternae or permeabilized HepG2-cells were used to phosphorylate and separate Golgi proteins by high resolution 2D-electrophoresis according to Hartinger et al. [259] and Görg et al. [303] in the presence of PKCα [260]. Proteins not phosphorylated in the presence of Calphostin C, Ro 31-8220 and/or Gö 6976, were sequenced by mass spectroscopy (MALDI-MS and Q-TOF). For the identified in vitro PKC-substrates MARCKS, MacMARCKS, Myosin RLC, Cytokeratin 8 and Cytokeratin 18, it was possible to demonstrate their phosphorylation in permeabilized HepG2 cells too. Therefore, a biological relevance of this phosphorylation can be assumed.

Other known Golgi associated proteins like Rab-6, Rab-8 and VAMP-2 were also identified or were shown to be Golgi associated for the first time as in the case of Annexin-IV, Profilin-I, Rab-7, GRP-78 and Endobrevin.

Additionally Annexin IV and Profilin I were tested for their effect on in-vitro-vesicle biogenesis: Even though an Annexin IV-specific antibody did not influence the budding efficacy of HSPG in the cell free system at all [371], the addition of a Profilin I-specific antibody did actually reduce the budding efficiency [368]. Supporting the above result, Profilin I was also identified in post-Golgi-vesicle fractions biochemically.

Generally the identification of the mayor PKC substrates MARCKS, MacMARCKS and Myosin RLC is pointing towards a new signal transduction pathway, by which PKC might regulate the vesicle biogenesis at the TGN.