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Dissertation Title:

Alkyl-Acyl GPI anchor Synthesis Depends on the Peroxisomal Pathway of Ether Lipid Biosynthesis

Geboren am 06.06.1975 in Chicago, Illinois, USA.

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Promotionsfach: Biochemie
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Up to 20% of total membrane proteins are post-translationally modified at their C-terminus by glycosylphosphatidyl inositol (GPI). GPI-anchoring is a complex lipid modification requiring more than twenty gene products. GPIs can be modified by additional side groups and their lipid moieties can be remodeled as well. These modifications can be species-, protein-, or cell-specific. The phosphatidyl inositol (PI) moiety of GPI-anchored proteins (GPI-APs) is comprised either of two acyl chains (diacyl) or of alkyl-acyl chains. This study focusses on the significance of peroxisomal ether lipid synthesis on the alkyl chain formation in GPI-APs. To this end, Thy-1 and PLAP were used as model GPI-APs. Ether lipid deficient cells were derived from ether lipid knockout mice previously generated in this lab. The initial analysis in raft preparations indicated reduced levels of endogenous Thy-1. A quantitative estimation by FACS and immunofluorescence staining revealed that the surface expression of endogenous Thy-1 on T cells and fibroblasts was completely absent in about 25% of the knockouts. As the only defect present in these cells is the lack of the peroxisomal steps involved in ether lipid biosynthesis, one can conclude that this peroxisomal pathway is essential for the synthesis and/or assembly of the Thy-1 GPI anchor. This conclusion was

also supported by the ESI-MS characterization of the Thy-1 GPI anchor that demonstrated both anchor types; the diacyl and alkyl/acyl type to be found on Thy-1. Surprisingly, transfection of myc-tagged Thy-1 and VSVG-tagged PLAP into fibroblasts lacking endogenous Thy-1 surface expression revealed plasma membrane localization of these exogenous proteins. However, co-localization studies with fluorescence-labeled cholera toxin, a GM1 lipid raft marker, indicated that contrary to the endogenous Thy-1 the exogenous proteins do not correctly sort into lipid raft microdomains (LRMs). The partitioning of over expressed PLAP into LRMs was also affected in mutant cells. In summary these results show that, the over expressed proteins having the potentiality to accept an alkyl/acyl anchor, under ether lipid-deficient conditions might be forced to obtain an available diacyl GPI anchor and that the alkyl/acyl anchor is essential for sorting the corresponding proteins to their respective raft domains.