ABSTRACT OF THE DISSERTATION

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FUNCTIONAL ELUCIDATION OF PAG THROUGH THE GENERATION OF TRUNCATION AND POINT MUTANTS

PAG, the phosphoprotein associated with GEMs (glycosphingolipid-enriched microdomains) is a ubiquitously expressed transmembrane adaptor protein that negatively regulates Src-family kinases via the phosphotyrosine-dependent recruitment of Csk to the GEMs. Csk is a cytoplasmic protein tyrosine kinase that phosphorylates the C-terminal inhibitory tyrosine of Src kinases. Upon TCR engagement, PAG becomes dephosphorylated by a yet unknown phosphatase thus displacing Csk from the membrane and thereby enabling the activation of Src kinases (Fyn, Lck). Since Fyn is responsible for the majority of PAG phosphorylation, this provides a feed back loop, as the rephosphorylation of PAG recruits Csk to the membrane to inhibit the Src kinases.

The aim of my thesis was to gain a better understanding of the regulatory function of PAG by studying the role of various motifs in GEM localisation and Fyn binding.

Palmitoylation at the juxtamembrane CxxC motif is thought to target transmembrane adaptors into the lipid rafts. We have therefore studied the effect of a C→A mutation at this site within PAG in a Jurkat T-cell line, using both biochemical and functional assays. While the CxxC mutant is localized to the membrane, it does not target to the GEMs. However it becomes Tyr phosphorylated, binds Fyn, EBP-50 and recruits Csk to the membrane, similar to wt PAG. To monitor the effect of displacing PAG from the lipid rafts we studied the effects of the overexpressed palmitoylation mutant on CD3 and SDF-mediated signalling and as functional read outs we present proximal signalling studies, calcium flux measurements and migration assays. Migration assays show clear increase in migratory response of the cells overexpressing the CxxC mutant. Experiments obtained with siRNA further strengthen these data as suppression of PAG also leads to increased migrartion.

Fyn is the kinase responsible for phosphorylation of PAG, but unlike Csk and EBP-50, the site of interaction between Fyn and PAG is still unknown. To study the mechanism of Fyn binding, several truncation and site directed mutants needed to be generated. Identification of the Fyn binding site should help us to better understand how the kinase interacts with its target protein and by mutating the site of interaction, we can study whether direct association is necessary for PAG phosphorylation and whether Fyn has an additional adaptor function.