Is a putative tilted sequence in the transmembrane domain of the transmembrane adaptor proteins involved in their raft location and function?

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Transmembrane adaptor proteins (TRAPS) constituted a small family of 5 members: SIT (SHP2-interacting transmembrane adaptor protein), TRIM (T-cell receptor-interacting molecule), NTAL (Non T Activation Linker), LAT (Linker for T cell Activation) and CBP/PAG (Csk Binding Protein/ Phosphoprotein Associated with Glycosphingolipids) (Linquist JA et al, Immunol Rev. 2003, 191: 165-82). TRAPs share several common structural features. Starting from the N- to C-terminal parts, they possess a short extracellular domain, a transmembrane domain (TM), and a large cytoplasmic domain containing multiple sites of tyrosine phosphorylation, by which they act as scaffolds for recruiting cytosolic adapter and/or effector proteins. They are all located in membrane lipid rafts where they serve as activator of signalling except for CBP/PAG that is involved in the negative regulation of signalling. This unique property is due to its ability to bind Csk (C terminal Src Kinase), the main negative regulator of the Src family of tyrosine kinases. These kinases regulate multiple biological functions such as proliferation, differentiation and motility and are activated in various cancers.

In fibroblasts stimulated with PDGF (Platelet Derived Growth Factor), Src family kinases are activated and initiate a signalling cascade culminating in the induction of the transcription factor cMyc preceding DNA synthesis. We studied the function of CBP/PAG in this cellular response. We found that overexpressing CBP/PAG inhibit DNA synthesis, cMyc induction and Src family kinase activation under PDGF stimulation. However, we found that this occurs independently of Csk recruitment by CBP/PAG: the overexpression of a CBP/PAG mutant unable to bind Csk still inhibited PDGF-induced DNA synthesis, co-expression of CBP/PAG with a Csk dominant negative mutant did not restore mitogenesis, and finally the N-terminal part of CBP/PAG still inhibited the PDGF-induced DNA synthesis. In addition, we found that the N-terminal mutant was still located in lipid raft. In search for a specific pattern responsible for lipid raft addressing and PDGF signalling inhibition, we found that the TM of CBP/PAG, and of all TRAPS, contains a putative tilted sequence. Since these tilted sequences have membrane-perturbing effects, we tested the hypothesis that the tilted sequence in the TM of CBP/PAG is responsible for its inhibitory action. We generated wt and mutated peptides to verify their orientation in model membranes, CBP/PAG TM mutants and we started the knock-in of endogenous CBP/PAG in fibroblasts to study the reinsertion of CBP/PAG mutants in a context of endogenous CBP/PAG depleted fibroblasts. CBP/PAG depleted fibroblasts are hyper responsive to PDGF stimulation, confirming a function of CBP/PAG in regulating PDGF mitogenesis. In addition, we found that reintroducing wt CBP/PAG, and more interestingly the N-terminal part of CBP/PAG, restores PDGF response, confirming that the N-terminal part of CBP/PAG summarises wt CBP/PAG function.