# The scaffold protein CNK1 in Raf-dependent signalling

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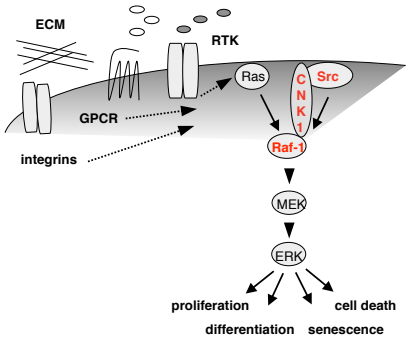


## INTRODUCTION

The Ras-Raf-MEK-ERK pathway is a ubiquitously expressed signalling module and activated by receptor tyrosine kinases, G-protein coupled receptors and integrins. This pathway can regulate different biological programs. Deregulation of this pathway is involved in development of human tumors.

The kinase activity of Raf-1 is tightly regulated. This includes phosphorylation and dephosphoryation by Src and PP2A, respectively, GTPases that recruit Raf to the plasma membrane e.g. Ras and scaffold proteins that are platforms for signalling complexes e.g. CNK1 as shown here.

## I. CNK1 regulates Src-mediated Raf-1 activation

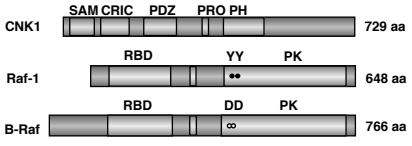


Fig. 1: (Top) Schematic representation of human CNK1. SAM: sterile alpha motif domain; CRIC: conserved region in CNK; PDZ: PSD-95/Dlg-1/ZO-1 domain; PRO: prolin-rich region; PH: pleckstrin homology region. (Bottom) Schematic representation of Raf-1 and B-Raf. RBD: Ras-binding domain; PK: protein kinase domain; YY: two neighboured tyrosine residues; DD: two neighboured aspartic acid residues.

## Physical interaction between CNK1 and Raf proteins

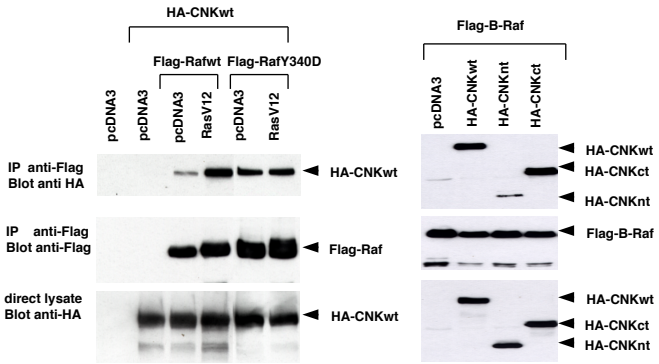


Fig. 2: HEK 293 cells were transfected with plasmids as indicated. Interaction between CNK1 and Raf proteins were analysed by immunoprecipitation with anti-Flag antibody followed by immunoblotting with anti-HA antibody. (Left) Interaction of Flag-Rafwt and the activated Raf-1 mutant Flag-RafY340D with HA-CNKwt in the absence or presence of activated RasV12. (Right) Interaction of Flag-B-Raf with HA-CNK proteins.

## CNK1 binds to Src and forms a trimeric complex with Raf-1 and Src

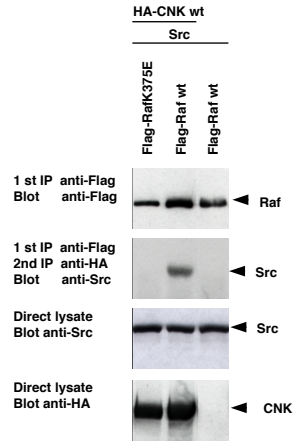


Fig.3: HEK 293T cells were cotransfected with HA-CNKwt, Flag-Rafwt and Src. Immunopurified Flag-Raf proteins (upper panel) were eluted with Flag peptide and eluates were subsequently immunoprecipitated with anti-HA antibody and immunoblotted with anti-Src antibody (second panel).

**References:** **A. Ziogas, K. Moelling, and G. Radziwill; *J. Biol. Chem.* 280, 24205-24211 (2005)**
**R. D. Fritz and G. Radziwill; *Biochem. Biophys. Res. Commun.*, in press (2005)**

## CNK1 protein level determines Src-mediated Raf-1 activation

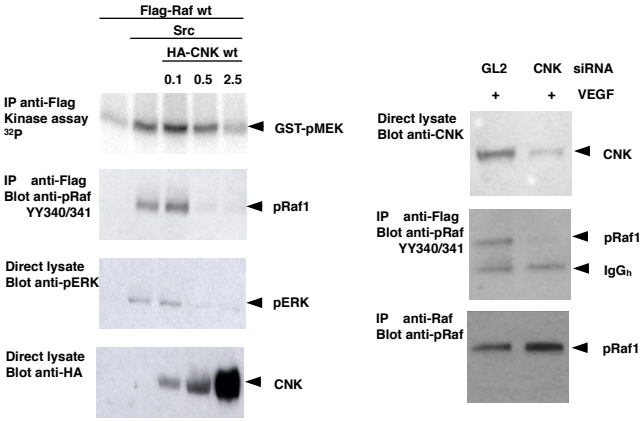


Fig. 4: (Left) Flag-Rafwt (1μg), Src (0.5 μg) and increasing amounts of HA-CNK wt (0.1; 0. 5, 2.5 μg) were expressed in HEK 293T cells. Immunopurified Raf-1 was analysed by in vitro kinase assays using GST-MEK as specific substrate for Raf. In addition phosphorylation of Raf and ERK were analysed by the antibodies anti- pYpY340/341 Raf and anti-pERK, respectively. (Right) HEp2 cells stably expressing siRNA directed against CNK or control siRNA (GL2) were serum-starved and treated with or without VEGF (100 ng/ml) for 15 min. Cells were lysed an immunoprecipitated with anti-Raf antibiody and immunoblotted with anti-pYY340/341 Raf and anti-Raf antibody. Downregulation of CNK1 was controlled by anti-CNK1 blot.

## II. CNK1 interacts with the angiotensin II type 2 receptor

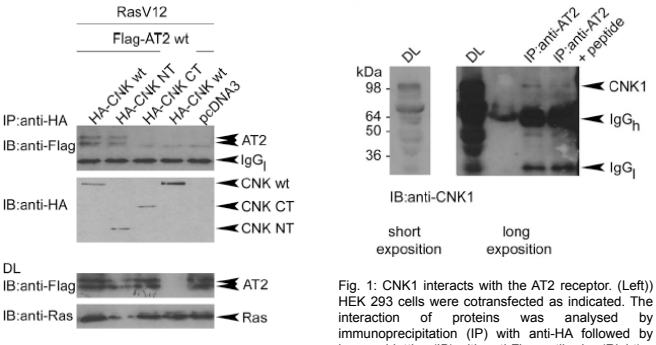


Fig. 1: CNK1 interacts with the AT2 receptor. (Left)) HEK 293 cells were cotransfected as indicated. The interaction of proteins was analysed by immunoprecipitation (IP) with anti-HA followed by immunoblotting (IB) with anti-Flag antibody. (Right) Mouse heart extract was immunoprecipitated with anti-AT2 antibody and copreciptiation of CNK1 was detected by immunoblotting with anti-CNK antibody. The specificity of the precipitation was confirmed by preincubation of the antibody with the peptide used as antigen.

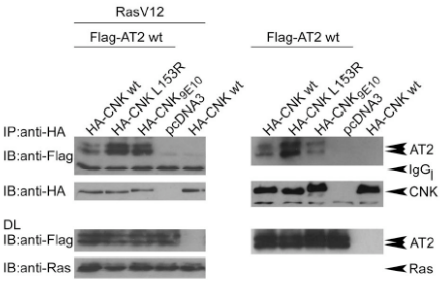


Fig. 2: Mutants of CNK showed an increased binding to the AT2 receptor. Coprecipitation was analysed as in figure 1. Note that the right panels are about ten times longer exposed than the left panels.

## Putative role of CNK in AT2-dependent signalling pathways

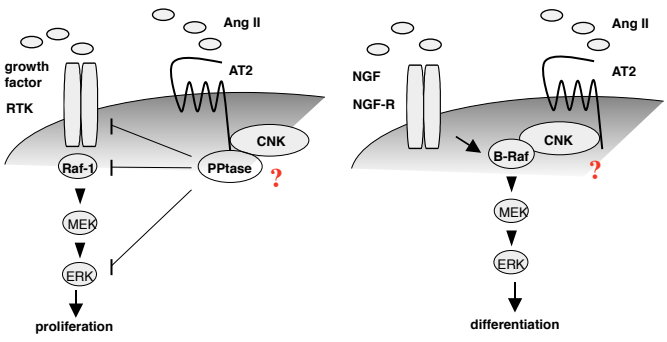


Fig.3: (Left) CNK may be involved in the AT2-dependent inhibition of proliferation via activation of protein tyrosine phosphatases. (Right) CNK proteins and the AT2 receptor act in differentiation of neuronal cells mediated by the Raf/MEK/ERK pathway.