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THE P21-ACTIVATED KINASE 3 (PAK3) REGULATES SYNAPTIC TRANSMISSION THROUGH ITS INTERACTION WITH THE NCK2/GRB4 ADAPTOR

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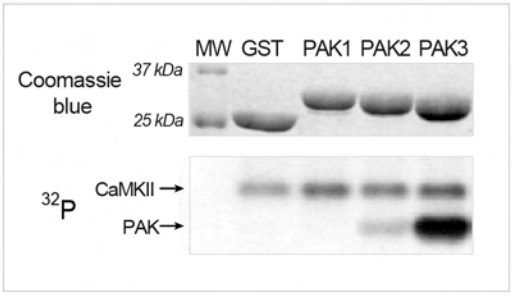
Supplementary data

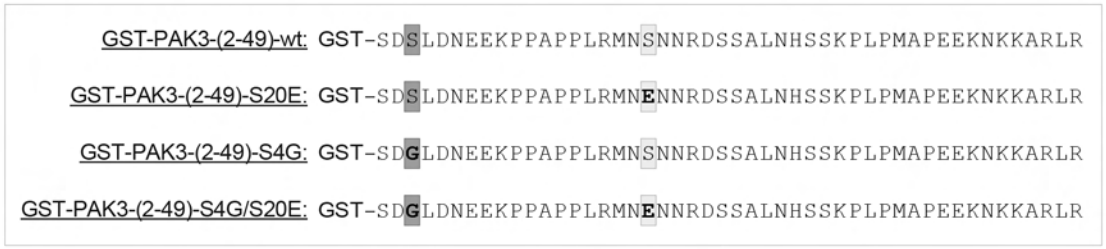
SD 1, CaMKII phosphorylation of the PAKs N-ter parts. PAK1-(2-56), PAK2-(2-52), and PAK3-(2-49) GST-fused proteins were purified (Coomassie Blue staining, upper), and tested on CaMKII kinase assay (lower). The high molecular weight phosphoprotein corresponds to autophosphorylated CaMKII. Since we identified CaMKII as a potential kinase for the serine 20 of PAK3, we compared the kinase efficiency of CaMKII to phosphorylate the N-ter part of the three PAK homologous. GST or GST-fused to the N-ter part of PAKs, corresponding to the mouse (2-56) N-ter part of PAK1, to the rat (2-52) N-ter part of PAK2, and to mouse (2-49) N-ter part of PAK3 were purified were tested in a kinase assay with a catalytically active recombinant CaMKII. The amino-terminal portion of mouse PAK3 was highly phosphorylated by CaMKII whereas the GST portion alone, or fused with homologous parts of PAK1 or PAK2 were not.

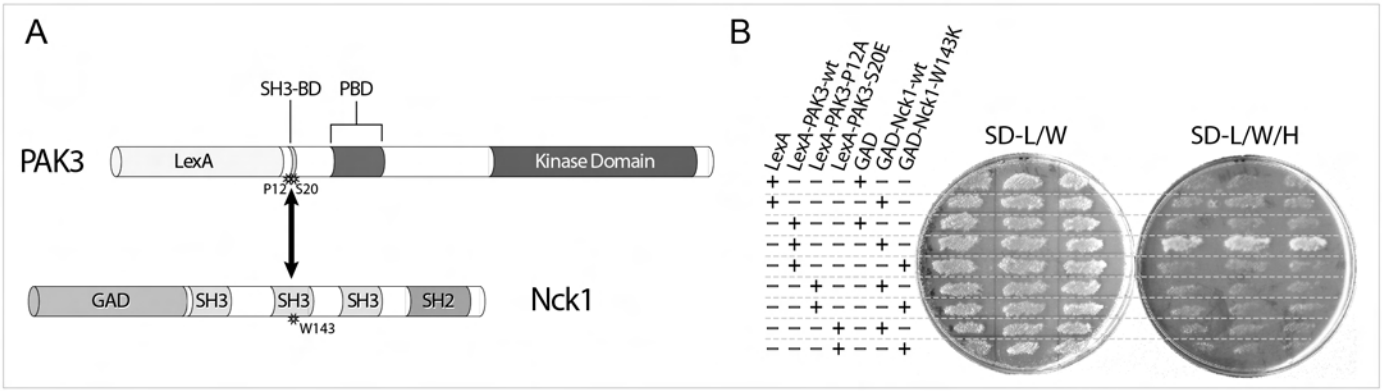
SD 2, sequence of the amino part of the different mutants used in the figure 4*D*. The serine 20 residue is a potential regulatory site whose homologous residue in PAK1 is autophophorylated and phosphorylated by Akt. This residue is crucial for Nck binding and in PAK1 its phosphorylation and its mutation totally inhibit Nck binding. In primates, the serine 4 residue found in rodents and other vertebrates is replaced by a glycine residue.

SD 3, two-hybrid analysis of PAK3-Nck1 interaction. *A,* schematic representation of PAK3 and Nck1 proteins expressed in yeast. The LexA binding domain was fused to the NH2 extremity of the full length PAK3 protein and the GAD domain was fused to the full length Nck1 protein. The P12A and S20E mutations in the first proline rich domain of PAK3 and the W143K mutation in the second SH3 domain of Nck1 are indicated. *B*, the interaction between PAK3 and Nck1 involves the first proline-rich domain of PAK3 and the second SH3 domain of Nck1. The two mutations P12A and S20E in PAK3 and the W143K mutation in Nck1 abolish the two-hybrid complex. Growth of colonies on SD-L/W media Growth confirms double transfection of the 2 plasmids. This experiment was performed on three independent colonies and repeated three times.

SD 4, table of the interactions analyzed between PAK3 and the Nck adaptors. T: method used, PD: pull down, 2H: two hybrid assay, CIP: co-immunprecipitation. R: result : +: interaction, -: no interaction.


MW GST PAK1 PAK2 PAK3
Coomassie blue
37 kDa
25 kDa
32P
CaMKII
PAK


GST-PAK3-(2-49)-wt: GST-SDSLDNEEKPPAPPLRMNSNNRDSSALNHSSKPLPMAPEEKNKKARLR
GST-PAK3-(2-49)-S20E: GST-SDSLDNEEKPPAPPLRMNENNRDSSALNHSSKPLPMAPEEKNKKARLR
GST-PAK3-(2-49)-S4G: GST-SDGLDNEEKPPAPPLRMNSNNRDSSALNHSSKPLPMAPEEKNKKARLR
GST-PAK3-(2-49)-S4G/S20E: GST-SDGLDNEEKPPAPPLRMNENNRDSSALNHSSKPLPMAPEEKNKKARLR


A
SH3-BD
PBD
PAK3
LexA
Kinase Domain
P12
S20
GAD
SH3
SH3
SH3
SH2
Nck1
W143
B
LexA
LexA-PAK3-wt
LexA-PAK3-P12A
LexA-PAK3-S20E
GAD
GAD-Nck1-wt
GAD-Nck1-W143K
SD-L/W
SD-L/W/H

Goal	Pak3	Nck	T	R	Figure
Characterization of the PAK3/Nck interaction domains	Flag-PAK3-FL-wt	HA-Nck1-FL-wt	CIP	+	2A
	Flag-PAK3-FL-wt	HA-Nck2-FL-wt	CIP	+	2A
	HA-PAK3-FL-wt	GST-Nck1-(SH3)2-wt	PD	+	3A
	HA-PAK3-FL-wt	GST-Nck2-(SH3)2-wt	PD	+	3A
	LexA-PAK3-FL-wt	GAD-Nck1-FL-wt	2H	+	SD3
	GST-PAK3-(2-49)-wt	Myc-Nck1-FL-wt	PD	+	not shown
	LexA-PAK3-(2-270)-wt	GAD-Nck1-(SH3)2-wt	2H	+	not shown
	PAK3-(2-270)-wt-LexA	GAD-Nck1-(SH3)2-wt	2H	+	not shown
	GST-PAK3-(2-49)-wt	Myc-NCK1-FL-W143K	PD	-	not shown
	LexA-PAK3-FL-wt	GAD-Nck1-FL-W143K	2H	-	SD3
	LexA-PAK3-(2-270)-wt	GAD-Nck1-(SH3)2-W143K	2H	-	not shown
	PAK3-(2-270)-wt-LexA	GAD-Nck1-(SH3)2-W143K	2H	-	not shown
	HA-PAK3-FL-wt	Myc-Nck1-FL-W143K	CIP	+	not shown
Mental retardation mutations	Flag-PAK3-FL-R67C	HA-Nck2FL-wt	CIP	+	2D
	Flag-PAK3-FL-A365E	HA-Nck2-FL-wt	CIP	+	2D
	Flag-PAK3-FL-R419Stop	HA-Nck2-FL-wt	CIP	+	2D
Role of the activity and activation in complex formation	Flag-PAK3-FL-ca	GST-Nck1-(SH3)2-wt	PD	+	3A
	Flag-PAK3-FL-kd	GST-Nck1-(SH3)2-wt	PD	+	3A
	Flag-PAK3-FL-ca	Myc-Nck1-FL-wt	CIP	+	not shown
	Flag-PAK3-FL-kd	Myc-Nck1-FL-wt	CIP	+	not shown
	Flag-PAK3-FL-wt + Cdc42-V12	GST-Nck1-(SH3)2-wt	PD	+	not shown
	Flag-PAK3-FL-ca + Cdc42-V12	GST-Nck1-(SH3)2-wt	PD	+	not shown
	Flag-PAK3-FL-kd + Cdc42-V12	GST-Nck1-(SH3)2-wt	PD	+	not shown
	Flag-PAK3-FL-wt + Cdc42-N17	GST-Nck1-(SH3)2-wt	PD	+	not shown
	Flag-PAK3-FL-ca + Cdc42-N17	GST-Nck1-(SH3)2-wt	PD	+	not shown
	Flag-PAK3-FL-kd + Cdc42-N17	GST-Nck1-(SH3)2-wt	PD	+	not shown
	Flag-PAK3-FL-wt + Cdc42-V12	HA-Nck1-FL-wt	CIP	+	3B
	Flag-PAK3-FL-wt + Cdc42-N17	HA-Nck1-FL-wt	CIP	+	3B
	Flag-PAK3-FL-kd + Cdc42-V12	HA-Nck1-FL-wt	CIP	+	3B
	Flag-PAK3-FL-wt + Cdc42-V12	HA-Nck2-FL-wt	CIP	+	3C
	Flag-PAK3-FL-wt + Cdc42-N17	HA-Nck2-FL-wt	CIP	+	3C
	Flag-PAK3-FL-kd + Cdc42-V12	HA-Nck2-FL-wt	CIP	+	3C
Characterization of the role of the Serine 20 residue	HA-PAK3-FL-S20E	GST-Nck1-(SH3)2-wt	PD	-	not shown
	HA-PAK3-FL-S20A	GST-Nck1-(SH3)2-wt	PD	-	not shown
	GST-PAK3-(2-49)-S20E	Myc-Nck1-FL-wt	PD	-	not shown
	LexA-PAK3-FL-S20E	GAD-Nck1-FL-wt	2H	-	SD3
Characterisation of the mimetic peptide P_{12}	Flag-PAK3-FL-wt + P_{12}	HA-Nck1-FL-wt	CIP	+	5C
	Flag-PAK3-FL-wt + A_{12}	HA-Nck1-FL-wt	CIP	+	5C
	Flag-PAK3-FL-wt + P_{12}	HA-Nck2-FL-wt	CIP	-	5C
	Flag-PAK3-FL-wt + A_{12}	HA-Nck2-FL-wt	CIP	+	5C
Characterization of the P12A mutation	HA-PAK3-FL-P12A	GST-Nck1-(SH3)2-wt	PD	-	6B
	HA-PAK3-FL-WT	GST-Nck1-(SH3)2-wt	PD	+	6B
	HA-PAK3-FL-P12A	GST-Nck2-(SH3)2-wt	PD	-	6B
	HA-PAK3-FL-wt	GST-Nck2-(SH3)2-wt	PD	+	6B
	Flag-PAK3-FL-P12A	HA-Nck1-FL-wt	CIP	+	6C
	Flag-PAK3-FL-Δnter	HA-Nck1-FL-wt	CIP	+	6C
	Flag-PAK3-FL-P12A	HA-Nck2-FL-wt	CIP	-	6E
	Flag-PAK3-FL-Δnter	HA-Nck2-FL-wt	CIP	-	6E
	PAK3-(2-270)-FL-P12A-LexA	GAD-Nck1-(SH3)2-wt	2H	-	not shown
	LexA-PAK3-P12A	GAD-Nck1-FL-wt	2H	-	SD3