

Brain Research Centre



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### Abstract

The catalytic domains of most eukaryotic protein kinases are highly conserved. Phosphorylation of the activation T-loop, a variable region between kinase catalytic subdomains VII and VIII, is a common mechanism for stimulation of catalytic activity. The MAP kinase Extracellular signal-regulated kinase 1 (ERK1) serves as a paradigm for regulation of protein kinases in signaling modules. Our group investigated the possible roles of three conserved phosphosites in the activation loop of ERK1 flanking the well-documented pTEpY activating site. In vitro kinase assays with myelin basic protein (MBP) using the purified ERK1 phosphosite mutants supported the functional importance of T207 and Y210, but not T198 in regulating ERK1 catalytic activity. Single substitution of the T207 to glutamic acid abolished the activity of ERK1 without affecting the phosphorylation at TEY by MEK1. The Y210 site could be important for proper folding of ERK1 in this regulatory region, since the mutation of this residue caused decreases in protein solubility, and the Y210F mutant was not recognized by MEK1 for phosphorylation in vitro. Our data also indicated that ERK1 autophosphorylated T207, while the phosphorylation of Y210 was enhanced in presence of MEK1. We hypothesize that following the activation of ERK, subsequent slower phosphorylation of the flanking sites may result in autoinhibition of the kinase. Hyperphosphorylation within the kinase activation Tloop may serve as a general mechanism for protein kinase down-regulation after initial activation by their upstream kinases.

#### Background

#### Figure 1. Distribution of phosphosites in human protein kinase catalytic domains



Y axis - Number of confirmed phosphosites (A, total = 1942) and activation sites (B, total = 303) at each position in the alignment of 496 typical human protein kinase catalytic domains.

#### Table 1. Phosphosites from activation T-loops of protein-serine/threonine kinases

	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	
CDK1	Ρ	Ι	R	Т	Y	Т	Н	Е	V	V	Т	L	W	Υ	R	S	Ρ	Ε	Gre
CDK2	Р	V	R	Т	Y	Т	н	Е	V	V	Т	L	W	Y	R	Α	Ρ	E	sites
GSK3	R	G	Е	Ρ	Ν	۷	S	Y	Т	С	S	R	Y	Y	R	Α	Ρ	E	Yello
ERK1	Н	Т	G	F	L	Т	Е	Y	V	А	Т	R	W	Y	R	Α	Ρ	E	with
p38 alpha	Т	D	D	Е	Μ	Т	G	Y	V	А	Т	R	W	Y	R	А	Ρ	E	Gre
JNK1	Т	S	F	Μ	Μ	Т	Ρ	Y	V	۷	Т	R	Υ	Y	R	Α	Ρ	E	hase
PKC alpha	D	G	V	Т	Т	R	Т	F	С	G	Т	Р	D	Y	Ι	А	Ρ	E	505
PKC delta	G	Е	S	R	А	S	Т	F	С	G	Т	Р	D	Y	Т	Α	Ρ	E	
RSK1	Н	Е	Κ	Κ	А	Y	S	F	С	G	Т	Т	Е	Y	Μ	Α	Ρ	E	
MAPKAPK2	S	Н	Ν	S	L	Т	Т	Р	С	Y	Т	Р	Y	Y	V	Α	Ρ	E	
CAMK1	Ρ	G	S	V	L	S	Т	А	С	G	Т	Ρ	G	Y	V	Α	Ρ	E	
PIM1	К	D	Т	۷	Y	Т	D	F	D	G	Т	R	V	Y	S	Ρ	Ρ	E	
AKT2	D	G	А	Т	Μ	Κ	Т	F	С	G	Т	Ρ	Е	Y	L	Α	Ρ	E	
PLK1	D	G	Е	R	Κ	Κ	Т	L	С	G	Т	Ρ	Ν	Y		Α	Ρ	E	

en, confirmed activation ow, confirmed phosphosites unknown function; y, potential phosphosites ed on similarities.

# **Regulatory Roles of Conserved Phosphorylation Sites** in the Activation T-Loop of the MAP Kinase ERK1

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#### **Results and Discussion**

Figure 2. Phosphorylation of GST-ERK1 by MEK1-∆N3EE in vitro



Table 2. Mutants of ERK1 on flanking phosphosites T198, T207 and Y210

ERK1		198				207 210												
WT	Н	Т	G	F	L	Т	Е	Υ	V	А	Т	R	W	Y	R	А	Ρ	Е
T198A	-	Α	-	-	-	Т	-	Υ	-	-	Т	-	-	Υ	-	-	-	-
T207A	-	Т	-	-	-	Т	-	Υ	-	-	Α	-	-	Υ	-	-	-	-
T207E	-	Т	-	-	-	Т	-	Υ	-	-	Е	-	-	Υ	-	-	-	-
Y210F	-	Т	-	-	-	Т	-	Υ	-	-	Т	-	-	F	-	-	-	-
Y210E	-	Т	-	-	-	Т	-	Υ	-	-	Т	-	-	Е	-	-	-	-
2AF	-	Α	-	-	-	Т	-	Υ	-	-	Α	-	-	F	-	-	-	-

#### Figure 3. Phosphorylation of ERK1 mutants and their kinase activity towards MBP





KD\* stands for the kinasedead mutant (K71A) of ERK1



GST-ERK1 and its mutants were activated by MEK1-ΔN3EE before incubated with kinase substrate peptide microarray at 30°C for 2 hours. MEK1 activity was suppressed by UO126. One field (CTRL) was incubated with MEK1- $\Delta$ N3EE/UO126 only.





Flag-tagged wild-type ERK1 and T207 mutants were overexpressed in HEK293 cells. Stimulation with 10% fetal bovine serum for 10 minutes led to activation of the MAPK cascade and phosphorylation of ERK1/2 at the TEY site (A). Flag-ERK1 was immunoprecipitated with Flag tag antibody and assayed for MBP phosphorylation *in vitro* (B).

# Conclusion

Based on our results, we propose the Y210 phosphosite of ERK1 becomes hyperphosphorylated after the phosphorylation of the "TEY" site by MEK1, which inhibits its catalytic activity. The T207 site also appears to be inhibitory, and arises from autophosphorylation. Our findings contribute to an improved understanding of the activation of MAPKs and protein kinases in general. Hyper-phosphorylation within the kinase activation T-loop following the initial activation by upstream kinases may serve as a general mechanism for protein kinase inhibition to prevent prolonged signaling.

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#### Figure 4. Substrate specificity of ERK1 T207 mutants on peptide microarrays

Figure 5. Phosphorylation and kinase activity of ERK1 T207 mutants in HEK293 cells