A comprehensive screen for protein kinases involved in histone phosphorylation using a biochemical in-vitro approach

Daniel Müller, Constance Ketterer, Christian Beisenherz-Huss, Frank Totzke, Carolin Heidemann-Dinger, Diane Krämer, and Michael H.G. Kubbutat

ProQinase GmbH, Tumor Biology Center, Freiburg, Germany

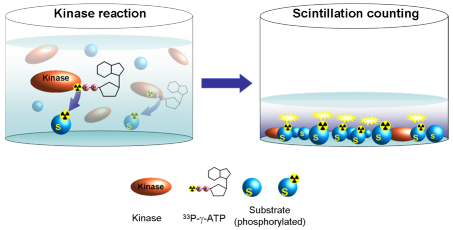
Introduction

Gene expression is regulated by multiple mechanisms. The structure of the chromatin has been identified as one major regulator of gene expression. Histone proteins constitute the core of the nucleosomal particles, which are essential for structuring the DNA into chromatin. Various post-translational modifications of histones mainly in the N-terminal region of these proteins are compacting or opening the chromatin which in turn changes the accessibility of the DNA.

Such histone modifications like methylation, acetylation and phosphorylation can therefore result either in activation or in repression of the transcription of the respective genes, depending on the specific amino acid residues modified. While histone-acetylation and -methylation are currently in the focus of epigenetic research, it is also well documented that histones are extensively modified and regulated by phosphorylation.

In this study we used the ProQinase *KinaseFinder* screening approach to identify kinases able to phosphorylate the N-termini of human core histone proteins under in-vitro conditions.

Principle of the assay

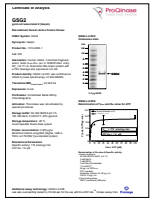


A radiometric, filter-plate based assay setup was used to determine the phosphorylation of Histone N-termini by 339 protein kinases. Kinase and potential substrate were incubated in presence of ATP containing ^{33}P-γ-ATP as tracer. After the kinase reaction was stopped, the proteins were immobilised on the filter membrane and the incorporated radioactivity measured by scintillation counting

Material

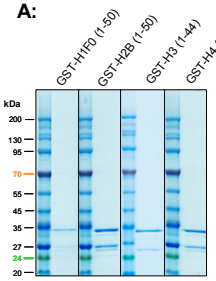
Recombinant human protein kinases

- expressed in insect cells or E.coli
- tag-free or N-terminally GST- or HIS-tagged
- full length or fragments
- in-vitro activity validated for all kinases with various substrates
- details for each kinase are available at **http://proqinase.com/content/view/26**

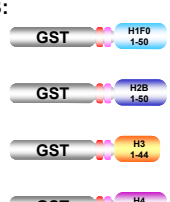


Histone substrate proteins

A:



B:



Human Histone H1F0 amino acids 1-50
NCBI/Protein accession number NP_005309.1

Human Histone H2B amino acids 1-50
NCBI/Protein accession number NP_003504.2

Human Histone H3 amino acids 1-44
NCBI/Protein accession number NP_003520.1

Human Histone H4 amino acids 1-50
NCBI/Protein accession number NP_003529.1

C:

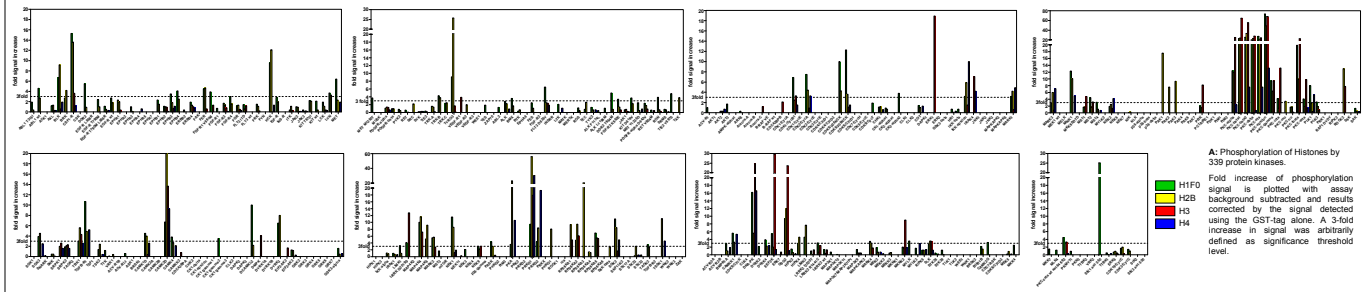
H1F0: MTENSTSAPA AKPKRAKASK KSTDHPKYSD MIVAAIQAEK NRAGSSRQSI
H2B: MPEPSKSAPA PKKGSKKAIT KAQKKDGKKR KRSRKESYSI YVYKVLKQVH
H3: MARTKQTARK STGGKAPRKQ LATKAARKSA PATGGVKKPH RYRPG
H4: MSGRGKGGKG LGKGGAKRHR KVLRDNIQGI TKPAIRRLAR RGGVKRISGL

A: Coomassie stained SDS-PA gel of GST-Histone N-termini purification from E.coli (1µg each)
B: Schematic depiction of the recombinant Histone constructs. The GST-tag is followed by a 6HIS-tag (red) and a 3C protease cleavage site (pink).
C: Expressed Histone amino acid sequences. Potential phosphorylation sites highlighted

Results

KinaseFinder in-vitro screen of Histone phosphorylation using 339 protein kinases

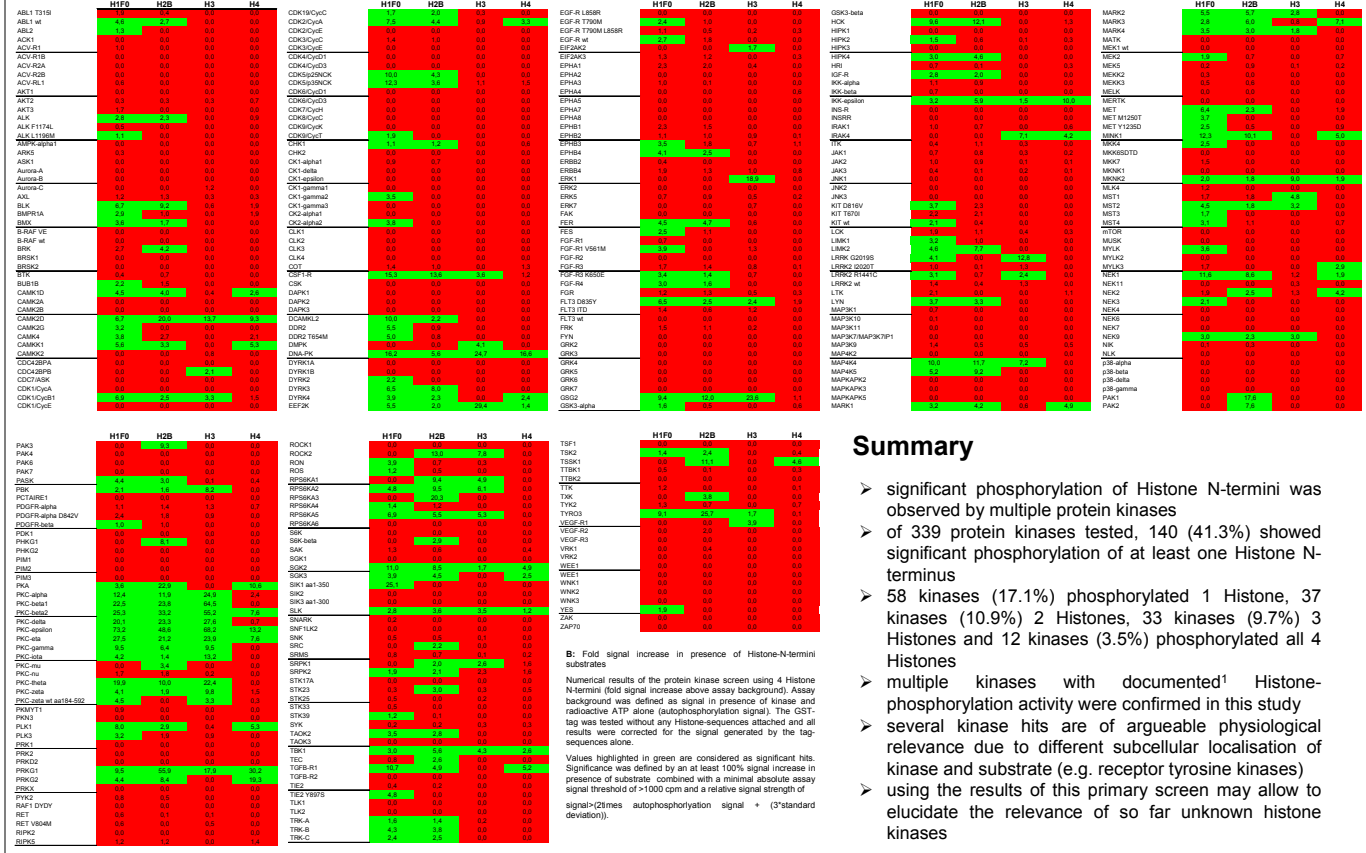
A:



A: Phosphorylation of Histones by 339 protein kinases.

Fold increase of phosphorylation signal is plotted with assay background subtracted and results corrected by the signal detected using the GST-tag alone. A 3-fold increase in signal was arbitrarily defined as significance threshold level.

B:



B: Fold signal increase in presence of Histone-N-termini substrates

Numerical results of the protein kinase screen using 4 Histone N-termini (fold signal increase above assay background). Assay background was defined as signal in presence of kinase and radioactive ATP alone (autophosphorylation signal). The GST-tag was tested without any Histone-sequences attached and all results were corrected for the signal generated by the tag-sequences alone.

Values highlighted in green are considered as significant hits. Significance was defined by an at least 100% signal increase in presence of substrate combined with a minimal absolute assay signal threshold of >1000 cpm and a relative signal strength of

signal>(2times autophosphorylation signal + (3*standard deviation)).

Summary

- significant phosphorylation of Histone N-termini was observed by multiple protein kinases
- of 339 protein kinases tested, 140 (41.3%) showed significant phosphorylation of at least one Histone N-terminus
- 58 kinases (17.1%) phosphorylated 1 Histone, 37 kinases (10.9%) 2 Histones, 33 kinases (9.7%) 3 Histones and 12 kinases (3.5%) phosphorylated all 4 Histones
- multiple kinases with documented[1] Histone-phosphorylation activity were confirmed in this study
- several kinase hits are of argueable physiological relevance due to different subcellular localisation of kinase and substrate (e.g. receptor tyrosine kinases)
- using the results of this primary screen may allow to elucidate the relevance of so far unknown histone kinases

1: Dorine Rossetto, Nikita Avvakumov & Jacques Côté (2012) Histone phosphorylation, Epigenetics, 7:10, 1098-1108