Histone H1 phosphorylation during mitosis

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Abstract

Histone H1 phosphorylation is important for the regulation of high order chromosome organisation during mitosis. One of these phosphorylation sites in the linker histone subtype H1.4 is shown here to be phosphorylated by Aurora B kinase, a master regulator of mitosis. Altered phosphorylation of H1.4 on this phosphorylation site at serine 27 illustrated the significance of the timing of this phosphorylation. When serine 27 of H1.4 is mutated to prevent this phosphorylation chromosome congression to the equatorial plate during metaphase is hindered. In contrast, in the presence of the constitutive H1.4 serine 27 phosphorylation mimic, bridging and lagging chromosomes occurred, leading to a corresponding increase in the proportion of cells with a micronucleus. These phenotypes could be brought about through disruption of the Heterochromatin protein 1 family members bound to the adjacent methylated lysine. Such aberrations during mitosis can lead to genetic instability and ultimately aneuploidy, a hallmark of cancer. With the frequently reported over-expression of Aurora B in cancer this shows another mechanism in which this kinase, via histone H1.4 phosphorylation, can push a cell toward malignancy.

Another important mitotic kinase, Cyclin dependent kinase 1 together with cyclin B, is responsible for the hyperphosphorylation of histone H1.4 during mitosis; which is required for condensing the cells genetic information into highly compact metaphase chromosomes. This vital mitotic event ensures the faithful transmission of the duplicated DNA into the dividing daughter cells. The mechanisms through which histone H1 hyperphosphorylation contribute to chromosome condensation are poorly understood. One mechanism through which this may occur is via the recruitment of condensation factors such as the condensins or Topoisomerase II. Here the interaction between the Condensin I subunit, CAPD2, and histone H1.4 is explored. CAPD2 interacts with the two most prominent linker histone subtypes, H1.4 and H1.2, through their C-terminal tails. H1.4 and CAPD2 can interact *in vitro* whilst each is phosphorylated by cyclin dependent kinase as they are during mitosis, in a manner dependent on RNA.

Overall, these results indicate that histone H1.4 is a vital component of higher order chromatin and its phosphorylation is essential for the normal progression through mitosis.

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Abbreviations

2N	Diploid
4N	Tetraploid
A	Adenine
ATP	Adenosine triphosphate
BCA	Bicinchoninic acid
BD	Becton Dickinson
bp	Base pairs
BSA	Bovine serum albumin
C	Cytosine
C-terminus	Carboxyl-terminus
CAPD2	Condensin complex subunit 1
CAPSO	3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid
CDK	Cyclin dependent kinase
cDNA	Complementary DNA
CENPA	Centromere protein A
CRG	Chromatin Research Group
CTD	C-terminal domain of CAPD2 (final 113 amino acids)
DAB+	3,3′-Diaminobenzidine
DAPI	4′,6-Diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
Dox	Doxycycline
DTT	Dithiothreitol
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
EZH2	Enhancer of zeste homolog 2
FBS	Fetal bovine serum
FFPE	Formalin fixed paraffin embedded
FRAP	Fluorescence recovery after photobleaching
g	Grams
G	Guanine
G_1 phase	Gap 1 phase
G_2 phase	Gap 2 phase
GFP	Green fluorescent protein
GST	Glutathione S-transferase
GST-CAPD2	Wild-type CAPD2 with an N-terminal GST-tag
GST-CTD	CTD of CAPD2 with an N-terminal GST-tag
GST-HP1β	HP1β with an N-terminal GST-tag

H1	Histone H1
H1.4-FLAG	Wild-type histone H1.4 with a C-terminal FLAG-tag
H1.4K26	Histone H1.4 lysine 26
H1.4K26A	H1.4-FLAG where lysine at position 26 was substituted with alanine to prevent K26 methylation
H1.4K26me3	Histone H1.4 trimethylated on lysine 26
H1.4S27	Histone H1.4 serine 27
H1.4S27A	H1.4-FLAG where serine at position 27 was substituted with alanine to prevent S27 phosphorylation
H1.4S27E	H1.4-FLAG where serine at position 27 was substituted with glutamic acid to mimic constitutive phosphorylation
H1.4S27p	H1.4 phosphorylated on serine 27
H3	Histone H3
H3K9	Histone H3 lysine 9
H3K9me3	Histone H3 trimethylated on lysine 9
H3S10	Histone H3 serine 10
H3S10A	H3 where serine at position 10 was substituted with alanine to prevent S10 phosphorylation
H3S10E	H3 where serine at position 10 was substituted with glutamic acid to mimic constitutive S10 phosphorylation
H3S10p	H3 phosphorylated on serine 10
HAT	Histone acetyltransferase
HEPES	2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
HMT	Histone methyltransferase
HP1	Heterochromatin protein 1
HRP	Horseradish peroxidase
IDT	Integrated DNA Technologies
IF	Immunofluorescence
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IP	Immunoprecipitation
IPTG	Isopropyl β-D-1-thiogalactopyranoside
K9	Lysine 9
K26	Lysine 26
Kb	Kilobase
KCM buffer	Potassium chromosome medium buffer
K_d	Dissociation constant
kDa	Kilodalton
L	Litre
LB	Lysogeny broth
M phase	Mitotic phase
Min	Minutes

MGS	Massey Genome Service
MMIC	Massey Microscopy and Imaging Centre
MNase	Micrococcal nuclease
MSK1	Mitogen- and stress-activated kinase 1
N-terminus	Amino-terminus
NEB	New England BioLabs
NFM	Non-fat milk
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PI	Propidium iodide
PP1	Protein phosphatase 1
PP2A	Protein phosphatase 2A
PRC2	Polycomb repressive complex 2
PTM	Post-translational modification
RIPA	Radioimmunoprecipitation assay buffer
RNA	Ribonucleic acid
RNase A	Ribonuclease A
rpm	Revolutions per minute
RT	Room temperature
S phase	Synthesis phase
S10	Serine 10
S27	Serine 27
SDM	Site-directed mutagenesis
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SMC	Structural maintenance of chromosomes
S.O.C	Super optimal broth with catabolite repression
Supplemented DMEM	DMEM supplemented with 10% FBS and 1% penicillin/ streptomycin
SUV39H1	Suppressor of variegation 3-9 homolog 1
SWI/SNF	Switch/Sucrose non-fermentable
T	Thymine
TAE	Tris-acetate-EDTA
TBS	Tris-buffered saline
TBST	TBS with 0.1% Tween 20
TCA	Trichloroacetic acid
TGS	Tris-glycine-SDS
U	Unit
UV	Ultraviolet
WB	Western blotting