Abstract

Histone mRNA decay (HD) is the process which ensures that histone mRNA is rapidly degraded following completion of DNA replication at the end of S-phase. Strict coordination between histone protein production and DNA replication is essential for the correct packaging of newly replicated DNA, as imbalances can lead to deleterious effects such as genomic instability.

Histone mRNA decay is controlled by the presence of a stem-loop structure at the 3' end of histone mRNA and a protein HBP/SLBP (Hairpin/stem loop binding protein). SLBP is the sole regulatory protein binding to histone mRNA regulating histone mRNA metabolism such as histone transcription, pre-mRNA processing, nucleo-cytoplasmic transport, translation and histone mRNA degradation. Moreover, the depletion of SLBP by siRNA results in diminishing histone supply during S phase, decreasing rate of DNA synthesis and consequently leading to cell-cycle arrest, confirming the importance of SLBP in ensuring S phase progression.

Importantly, HD is one functional target of an intra-S phase checkpoint activated when DNA synthesis is inhibited, ensuring that histone mRNA is rapidly destroyed when global DNA replication

is blocked. However, replication stress-induced HD does not induce SLBP destruction.

This work aimed to utilise a proteomics approach by mass spectrometry to elucidate novel aspects of the mechanism of SLBP-mediated HD during replication stress by analysis of SLBP post-translational status in addition to analysis of the SLBP interactome. I have successfully established a model system using stable Flp-In HeLa cell lines inducibly expressing Flag- and HA-tagged SLBP for the molecular analysis of SLBP function during replication stress. Using an immuno-isolation approach to purify SLBP and associated proteins for mass spectrometric analysis, Serine182 (Ser182) is identified as a novel *in vivo* phosphorylation site not previously observed in SLBP isolated from mammalian cells. Ser182 phosphorylation increases the duration of S-phase and delays histone mRNA decay after the inhibition of DNA synthesis. Bioinformatics analysis suggests WEE1 as a possible protein kinase responsible for Ser182 phosphorylation. However, experiments revealed that WEE1 does not phosphorylate SLBP *in vitro*, however, inhibition of WEE1 *in vivo* was found to induce premature SLBP degradation.

An interactome analysis by SILAC-based mass spectrometry techniques revealed that SLBP interacts with components of the initiation translation, transcription export complex (TREX), exosome complex and DNA damage response. Analysis of post-translational modification revealed interesting data that phosphorylation at Ser20 and Ser23 become significantly elevated following imposition of replication stress.

In conclusion, my finding provides novel insights into the molecular events executed by SLBP as a multi-functional protein implicated in regulation of histone mRNA degradation under DNA replication stress.

Table of Contents

List of Figures

List of Tables

List of Appendices

List of Abbreviations

ABC	Ammonium bicarbonate
ACN	Acetonitrile
APS	Ammonium persulphate
ATM	Ataxia-telangiectasia mutated
ATP	Adenosine triphosphate
ATR	ATM and Rad3-related
BSA	Bovine serum albumin
°C	Degree Celsius
CBC	Cap binding complex (CBP80/20)
cDNA	Complementary DNA
cm	Centimetre
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
ddH_2O	Double distilled water
dGTP	Deoxyguanosine triphosphate
DMEM	Dulbecco's minimum essential medium
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DNA-PK	DNA-dependent protein kinase
dNTP	Deoxynucleoside triphosphate
Dox	Doxycycline
DTT	Dithiothreitol
dTTP	Deoxythymidine triphosphate
E. Coli	*Eschericia Coli*
ECL	Enhanced chemiluminescence

EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol tetraacetic acid
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
h	Hour
HCl	Hydrochloric acid
HD	Histone mRNA decay
HeLa	Henrietta Lacks
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRP	Horseradish peroxidase
HU	Hydroxyurea
IAA	Iodoacetamide
IgG	Immunoglobulin G
IPTG	Isopropyl β-D-1-thiogalactopyranoside
KCl	Potassium chloride
kDa	Kilodalton
LB	Luria-Bertani
LC-MS	Liquid chromatography–mass spectrometry
M	Molar
mA	Milliampere
mg	Milligram
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
min	Minute
ml	Millitre
mm	Millimetre
mM	Millimolar

mRNA	Messenger RNA
mRNP	Messenger ribonucleoprotein
MS	Mass spectrometry
NaCl	Sodium chloride
Noc	Nocodazole
nm	Nanometre
NT	Non-targeting (control siRNA)
OD	Optical density
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PIKK	Phosphatidylinositol 3-kinase-related kinases
PMSF	Phenylmethanesulfonylfluoride
qPCR	Quantitative real-time PCR
RNA	Ribonucleic acid
rpm	Round per minute
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sec	Second
siRNA	Small interfering RNA
SOB	Super Optimal Broth media
SOC	Super optimal broth with catabolite repression media
ssDNA	Single-stranded DNA
ssRNA	Single-stranded RNA
TBS	Tris-buffered saline
TBST	Tris-buffered saline-Tween
TMED	NNN'N'-Tetramethylethylene-diamine

U	Enzyme unit
UTR	Untranslated region
V	Voltage
µg	Microgram
µl	Microlitre
WB	Western blotting

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"With heart and soul everything is possible"