

## **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.

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## 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 <sub>2</sub> O	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
<i>E. Coli</i>	<i>Eschericia Coli</i>
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 $\beta$ -D-1-thiogalactopyranoside
KCl	Potassium chloride
kDa	Kilodalton
LB	Luria-Bertani
LC-MS	Liquid chromatography–mass spectrometry
M	Molar
mA	Milliampere
mg	Milligram
MgCl <sub>2</sub>	Magnesium chloride
MgSO <sub>4</sub>	Magnesium sulphate
min	Minute
ml	Millilitre
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

## **Acknowledgements**

First and foremost, I owe endless thanks for my funding from the Office of the Higher Education Commission (OHEC) for the program Strategic Scholarships for Frontier Research Networks (Specific for Southern region) of Thailand, Kingdom of Thailand. It is an invaluable opportunity for my life to fulfill my dream.

I am endlessly grateful to my family. They are the wind beneath my wings for real love, care and belief in me. I would not be so lucky who I am today without their love. Especially, I am lucky to have my beloved superhero papa, Jarun Panomwan for his patience, understanding, endless love, infinite support and to let me do whatever I want. With a thankful heart to my beloved sister, Chutipapha Panomwan for taking care of our family instead of me during my study time in the UK. I also thank you to my brothers, Aek and Boy for your support and understanding me ever!

I owe deep gratitude to my supervisor, Professor Carl Smythe, who is not only a great teacher but also a generous and fun person. I was lucky to work in Smythe lab. He offered me encouragement, feedback, support and advice with many valuable suggestions. He taught me to think like a scientist and made sciences in simple explanation.

Importantly, he provides me with trust, freedom and space for development my own research, with prompt guidance when needed. I also thank him for his patience, and understanding me for my confusion and my mistakes during my study. Also for all of his immeasurable support during the past four years motivated and inspired, it made me confidence pursue the subject of science in depth with heart. This thesis would not have been completed without the big support of my supervisor, Carl Smythe.

I am deeply grateful to thank my advisors: Professor Elizabeth Smythe and Dr. Henry Roehl to offer me invaluable suggestions and provide critical inputs for my research. With my deepest thanks to Professor Elizabeth Smythe for continuous support and help towards my PhD. She is one of my role model whose passionate hard working as a teacher, scientist and mother, has been most admirable.

I am also thankful and grateful to Dr. Richard G. Beniston, Dr. Mark Collins, Dr. Edward Emmott, and Dr. Adelina E. Acosta Martin for their advice and help for mass spectrometry. Further thanks go to Mark Jones and Susan Clark for their help and advice for flow cytometry.

I am lucky to have many wonderful Smythe lab colleagues. It has been a great pleasure to work, and share my life with former, and

present friends in the lab. Special appreciation to Deborah C Sutton, Dr. Victoria Gotham, Dr. David Turton, Dr. Filipe J. Ferreira, Dr. Emma R Bowen, Marta Giralt Pujol, Dr. Zhou Zhu, Dr. Katja Vogt and Jacqueline M Price for their supports and friendships.

I am also very thankful to all of my friends in Sheffield, including Dr. Nisa Patikarnmonthon, Sujunya Boonpradit, Dr. Oratai Weeranantanapan, Nipaporn Konthapakdee, Brenda Skinner, Hataitip Tasena, Liliana Arede, Bhagyashree Kale and Chada Romcai many more, for their supports and friendships. Special thanks to my beloved Pornthip Netiparatanakul for coming into my life and giving me wonderful memorable time. With a thankful heart goes to Josephine Marsh, Vaughan Sharpe and Deborah Cobbett for their supports and friendships. They keep me warm, when it was cold outside! Further deepest thanks go to Dr. George F Turner, Deborah Cobbett and Josephine Marsh for brushing up my English writing during my study. However, I'm sorry that I could not write all my friends' names who study in Brighton, Lancaster, Liverpool, London, Manchester, Newcastle upon Tyne and Oxford for their supports and share a precious memorable experience in the UK with me. Importantly, I would like to thank all friends who remain in Thailand for always cheering me up and send love here. I never forget you. Finally, I can't thank you

enough to my special friends, Dr. Wanapat Yimsai and Dr. Phonethipsavanh Nounthong for their support, understanding and always beside me during the hardest time with love.

I am also very grateful for the support of friends, colleagues and porters at the department of Biomedical Science, University of Sheffield. I really enjoyed studying in this department. It was actually my second home where I had spent my Ph.D. time much longer than anywhere.

Finally, I would like to thank everyone for my previous experience, and former teachers who gave me lessons. That made me strong, and fulfilled my study when I was far away from home with happiness in the hardest time. In particular, I deeply thank my body and heart-determination, positive, patience, passion and enlightenment throughout my study and my life in Sheffield.

“With heart and soul everything is possible”