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ATM and p400: Characterisation of a novel interaction between a DNA repair enzyme and a chromatin remodeler

A thesis presented to Massey University in partial fulfilment of the requirement for the degree of Doctor of Philosophy in Biochemistry.

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Just keep swimming...

Dory, Finding Nemo 2003

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Abstract

The ability to maintain genomic integrity prevents unrestricted cell proliferation and the progression of cancer. DNA repair pathways such as the DNA double-strand break (DSB) response are essential in maintaining this integrity. This system requires activation of the serine/threonine kinase ataxia telangiectasia mutated (ATM) through acetylation by TIP60, a histone acetyl transferase, and subsequent ATM autophosphorylation. During DNA repair, activated ATM phosphorylates the histone variant H2AX several kilobases either side of the break site. This phosphorylation acts a signal for additional repair proteins and chromatin remodeling complexes which repairs DNA.

In a previous study, H2AX phosphorylation was induced through the over expression of TIP60 or the SWI3-ADA2-N-CoR-TFIIIB (SANT) domain of p400. It was hypothesised that over expressed TIP60 or SANT domain was able to sequester a putative negative regulator from the ATM-TIP60 complex and artificially induce activation. This study aimed to investigate if a single domain of TIP60 or if a single helix from the three helix SANT domain was responsible for the activation of the ATM-TIP60 complex. Here, the ability of the chromo domain and zinc domain of TIP60 individually and the combined zincHat domain of TIP60 to induce H2AX phosphorylation as well as three helix deletion mutants of the SANT domain of p400 was examined. While all constructs were able to be expressed in human cell lines, the induction of H2AX was variable and non-reproducible.

ATM belongs to the phosphatidylinositol 3-kinase-related kinase family (PIKK). Members of the PIKK family show domain homology, where the domain of one protein is replaced with the homologous domain of another member and the function of the protein is not altered. As p400 has been previously shown to interact with TIP60 and also Transformation/transcription domain-associated protein (TRRAP), a member of the PIKK family, it was hypothesised that p400 could interact with ATM (which also interacts with TIP60). This study confirms this novel interaction between ATM and p400 through the use of co-immunoprecipitation and protein localisation using confocal microscopy. This study provides a platform to further investigate the involvement of an ATM-p400 complex during DNA repair.

Abbreviations

-ve	Negative
A	Ampere
ADA2	Adaptor 2
Amp	Ampicillin
APS	Ammonium persulphate
AP	Apurinic or Apyrimidic
AT	Ataxia Telangiectasia
ATM	Ataxia Telangiectasia Mutated
ATP	Adenosine triphosphate
ATR	ATM and Rad3-related
BAF53	BRG1-associated factor of 53 kDa
bp	Base pair
BSA	Bovine Serum Albumin
c-Abl	Abelson murine leukemia viral oncogene homolog 1
ChIP	Chromatin ImmunoPrecipitation
CiP	Claf intestinal phosphatase
CMV	Cytomegalovirus
DAPI	4',6-diamidino-2-phenylindole
ddH ₂ O	Double distilled water
DDR	DNA damage response
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribose nucleic acid
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
dNTPs	Deoxyribonucleotide triphosphate
DSB	Double strand break
dTIP60	Drosophila TIP60 homologue
DTT	Dithiothreitol
E.coil	<i>Escherichia coli</i>
EDTA	Ethylene diamine tetra-acetic acid
EtOH	Ethanol
FAT	FRAP-ATM-TRRAP
FAT-C	FAT domain located at the C-terminus of the protein
FBS	Fetal bovine serum
FRAP	Fluorescence recovery after photobleaching
FRET	Fluorescence resonance energy transfer
g	Gram
GAS41	Glioma-amplified-sequence 41
GST	Glutathione S-Transferase
h	Hours
H2Av	Drosophila H2AZ and H2AX homologue

H2AX-P	H2AX phosphorylated at serine 139
H3K9me	Methylated H3K9
HA	Hemagglutinin
HAT	Histone acetyl transferase
HEK293T	Human embryonic kidney cell line
HD	High definition
HF	High fidelity
HDAC	Histone deacetylase
HP1	Heterochromatin binding protein 1
HRP	Horse radish peroxidase
HSA	Helicase and SANT Associated
IB	Immunoblot
IgG	Immunoglobulin G
IP	Immunoprecipitation
IPTG	Isopropyl β -D-1-thiogalactopyranoside
ISWI	Imitation SWI
K	Lysine
kb	Kilo bases
kDa	Kilo dalton
L	Liter
L	Ladder
LB	Luria Bertani bacteriological media
M2 agarose	α -FLAG antibody immobilized on agarose beads
mA	miliampere
Mb	megabase
mCi	Millicurie
mg	Milligram
min	Minute
mL	Milliliter
mM	Millimol
MRN	Mre11-Rad3-Nsb1
Mre11	Meiotic recombination 11
mTOR/FRAP	FKBP adarapamycin associated protein / mammalian target of rapamycin
MYST	MOZ, YBF2/SAS3, SAS2 and TIP60
NBS	Nijmegen breakage syndrome
NEB	New England Biolab
ng	Nano gram
NLS	Nuclear localization sequence
nm	nano meter
NP-40	Igapal CA-630
nt	Nucleotide
P1	Generation 1 of baculovirus
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction

PHYRE2	Protein Homology/analogY Recognition Engine V 2.0
PI3K	phosphatidylinositol 3-kinase
PIKK	phosphatidylinositol 3-kinase-like protein kinases
pmol	Picomol
PMSF	Phenylmethanesulfonylfluoride solution
PVDF	Polyvinylidene fluoride
Q	Glutamine
RNA	Ribose nucleic acid
S	Serine
s	second
SANT	SWI3-ADA2-N-CoR-TFIIB
SDS	Sodium dodecylsulphate
SDS-PAGE	Sodium dodecylsulphate polyacrylamide gel electrophoresis
SF9	clonal isolate of <i>Spodoptera frugiperda</i> Sf21 cells
siRNA	small interfering RNA
SV40	Simian virus 40
SWI2/SNF2	switch 2/sucrose non-fermentable 2
TAT	Transactivator of transcription
TBE	Tris Boric acid EDTA
TBS/T	Tris-buffered saline with tween 20
TEMED	N, N, N', N'- Tetramethylethylene diamine
TIP48	transactivation-domain interacting protein of 48 kDa
TIP49	transactivation-domain interacting protein of 49 kDa
TIP60	HIV-1 TAT interacting protein of 60 kDa
Tris	2-amino-2-hydroxymethyl-1,3-propanediol
TRRAP	transformation/transcription domain-associated protein
µg	microgram
µL	microliter
UV	Ultraviolet light
V	volts
WT	Wild Type
X-gal	5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside

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