Basal Transcription of Human Topoisomerase II

A thesis presented to Massey University in partial fulfillment of the requirements for the degree of Master of Science in Biochemistry

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2002

Acknowledgments

Foremost I would like to express my thanks to Dr Kathryn Stowell who has been a phenomenal supervisor, in every sense of the word. Your enthusiasm, support and understanding have been much appreciated and will never be forgotten.

Thanks to all the people in the past who have worked on topoisomerase IIα and made it every bit as interesting as it is, Agnieszka Szremska, Patricia Hintz, Sam McLenachan and Dr Richard Isaacs.

I extend my thanks to all the members of the Twilite Zone who have been an irreplaceable source of amusement and made it such fun being in the lab. Extra special thanks to Jennifer Anderson, Christin Down, Robyn Marston, Brenda Belmont, Carole Flyger, Melanie Willingham, Penny Bradbury and Kirsty Allen, all of you have inspired me in so many ways. Many warm thanks, to John Tweedie and Mark Patchett for all their encouraging words and helpful advice.

I am grateful to have such a supportive family, thank you to my Mum, Dad, Mai, Jason and Pradeep who have provided me with much love and patience. Thank you to all my family and friends that have been there for me and given me the reassurances that I needed throughout this project especially, Jennifer Goh and Roshni Nathoo.

Finally, I would like to thank my very best friend, Lawrence.

Abstract

Topoisomerase II is a ubiquitously expressed enzyme, which is required for cell survival. It has the ability to alter the topological states of DNA by introducing transient double-stranded breaks in DNA. Humans have two topoisomerase II isoforms, α and β, and both are differentially expressed and localized. Tissues with rapidly proliferating cells exhibit elevated topoisomerase IIα gene expression whereas the β isoform is ubiquitously expressed amongst tissues.

In addition to a role in cell survival, a number of anti-cancer drugs have been shown to target human topoisomerase II *in vivo*. However, the development of drug resistance is a major clinical problem; for example, approximately 60% of breast cancers treated with the topoisomerase II poison doxorubicin become resistant to this drug. Down-regulation of topoisomerase II is thought to be one of the factors involved in the development of drug resistance, where the relative levels of topoisomerase IIα and topoisomerase IIβ in cells is thought to effect drug efficacy.

The expression of topoisomerase IIα and β is regulated at the transcriptional level, through binding of transcription factors to specific elements within the promoter sequence. Therefore investigating the transcriptional regulation of both isoforms could lead to an understanding of the mechanisms involved in the development of drug resistance. The initial aim of this study was to isolate a fragment of the upstream regulatory sequence of the topoisomerase IIβ gene and carry out systematic analysis of this sequence. However, this could not be pursued, as the clones that were examined did not contain the required topoisomerase IIβ sequence.

This study progressed to examine the relevance of three elements (GC1, ICB1 and GC2) within the topoisomerase IIα minimal promoter and the importance of the cognate transcription factors NF-Y, Sp1 and Sp3 in regulating the expression of the topoisomerase IIα gene. Electrophoretic mobility shift assays and transient transfection assays were used to study protein/DNA interactions and the functional significance of these interactions, respectively. Both NF-Y and Sp1 were shown to activate the transcriptional regulation of topoisomerase IIα by binding to their respective elements; in addition functional interactions between the two proteins bound to the promoter was observed.

Abbreviations

Amp	Ampicillin
AMSA	Topoisomerase II poison
Ap-2	Activator protein 2
ATF	Activating transcription factor
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
β-gal	β-galactosidase
bp	Base pairs (DNA)
BSA	Bovine serum albumin
CAT	Chloramphenicol acetyltransferase
CDE	Cell-cycle dependant element
cDNA	Synthetic DNA, generated from RNA
cpm	counts per minute
DMSO	Dimethyl sulfoxide
Dnase	Deoxyribonuclease
dNTP	Deoxynucleoside triphosphate (dATP, dTTP, dGTP, dCTP)
DTT	Dithiothreitol
EDTA	Ethylene diamine tetra-acetic acid
EMSA	Electrophoretic mobility shift assay
FCS	Foetal calf serum
GCG	Genetics computer group
G segment	Gated segment (DNA)
GUS	β-glucuronidase
IPTG	Isopropyl thiogalactoside
HAT	Histone acetyl transferases
HeLa	Human cervical carcinoma cells
HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethane sulfonic acid]
HFM	Histone fold motif
ICB	Inverted CCAAT box
ICBP90	Inverted CCAAT box binding protein Mr 90 kDa
IgG	Immunoglobulin G
IPTG	isopropyl-β-D-thiogalactopyranoside

kb	kilobases (DNA)
KB	Human epidermoid KB cancer cells
KB/VP-2	etoposide resistant KB cells
KB/VM-4	teniposide resistant KB cells
LB	Luria Bertani bacteriological media
MCF-7	Human breast cancer cells
MCS	Multiple cloning site
MDR	Multidrug resistance
MDR1	Multidrug resistance gene
Mnase	Micrococcal nuclease
MRP	Multidrug resistance-associated protein
MEM	Eagle's minimal essential media
mt	mutated/mutant
NEB	New England Biolabs
NF-Y	Nuclear factor Y
ONPG	o-Nitrophenol β-D-Galacto-pyranoside
PAGE	Polyacrylamide gel electrophoresis
$_{P}53$	Tumour suppressor protein
PBS	Phosphate buffered saline
PBSE	Phosphate buffered saline plus EDTA
PEG	Polyethylene glycol
pGL3B	pGL3Basic vector
PIC	Pre-initiation complex
PIPES	Piperazine-n,n'-bis(2-ethane sulfonic acid)
PMSF	Phenylsulfonylmethyl fluoride
Pol II	RNA polymerase II
Q-rich	Glutamine-rich
Rb	Retinoblastoma protein
RNase	Ribonuclease
RT	Room temperature
SDS	Sodium dodecyl sulfate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
Sp1	Specificity protein 1
Sp3	Specificity protein

STET	Sucrose, Tris, EDTA and triton-X buffer
SV40	Simian virus 40
T segment	Transport segment (DNA)
T12	Human bladder cancer cells
TAE	Tris acetate EDTA buffer
TAFs	TBP associated factors
TATA	TATA box; conserved A/T rich septameter transcription sequence
TBE	Tris borate EDTA
TBP	TATA binding protein
TE	Tris-EDTA buffer
TEMED	N,N,N',N'-Tetramethylethylenediamine
TEN	Tris-EDTA buffer with sodium
TIFs	Transcription initiation factors
TFIID	Transcription initiation factor complex; TBP and TAFs
TF	Transcription factor
XK469	Topoisomerase IIβ poison (NSC 697887)
UV	Ultra-violet light
VM-26	Teniposide: topoisomerase II poison
VP-16	Etoposide
X-gal	5-bromo-4chromo-3-indolyl-β-D-galactopyranoside
wt	wild type

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