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

Natisha Magan
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Acknowledgments

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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
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<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>kb</td>
<td>kilobases (DNA)</td>
</tr>
<tr>
<td>KB</td>
<td>Human epidermoid KB cancer cells</td>
</tr>
<tr>
<td>KB/VP-2</td>
<td>etoposide resistant KB cells</td>
</tr>
<tr>
<td>KB/VM-4</td>
<td>teniposide resistant KB cells</td>
</tr>
<tr>
<td>LB</td>
<td>Luria Bertani bacteriological media</td>
</tr>
<tr>
<td>MCF-7</td>
<td>Human breast cancer cells</td>
</tr>
<tr>
<td>MCS</td>
<td>Multiple cloning site</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug resistance</td>
</tr>
<tr>
<td>MDR1</td>
<td>Multidrug resistance gene</td>
</tr>
<tr>
<td>Mnase</td>
<td>Micrococcal nuclease</td>
</tr>
<tr>
<td>MRP</td>
<td>Multidrug resistance-associated protein</td>
</tr>
<tr>
<td>MEM</td>
<td>Eagle’s minimal essential media</td>
</tr>
<tr>
<td>mt</td>
<td>mutated/mutant</td>
</tr>
<tr>
<td>NEB</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>NF-Y</td>
<td>Nuclear factor Y</td>
</tr>
<tr>
<td>ONPG</td>
<td>o-Nitrophenol β-D-Galacto-pyranoside</td>
</tr>
<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>p53</td>
<td>Tumour suppressor protein</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PBSE</td>
<td>Phosphate buffered saline plus EDTA</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>pGL3B</td>
<td>pGL3Basic vector</td>
</tr>
<tr>
<td>PIC</td>
<td>Pre-initiation complex</td>
</tr>
<tr>
<td>PIPES</td>
<td>Piperazine-n,n'-bis(2-ethane sulfonic acid)</td>
</tr>
<tr>
<td>PMSF</td>
<td>Phenylsulfonylmethyl fluoride</td>
</tr>
<tr>
<td>Pol II</td>
<td>RNA polymerase II</td>
</tr>
<tr>
<td>Q-rich</td>
<td>Glutamine-rich</td>
</tr>
<tr>
<td>Rb</td>
<td>Retinoblastoma protein</td>
</tr>
<tr>
<td>RNase</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>SDS-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>Sp1</td>
<td>Specificity protein 1</td>
</tr>
<tr>
<td>Sp3</td>
<td>Specificity protein</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>STET</td>
<td>Sucrose, Tris, EDTA and triton-X buffer</td>
</tr>
<tr>
<td>SV40</td>
<td>Simian virus 40</td>
</tr>
<tr>
<td>T segment</td>
<td>Transport segment (DNA)</td>
</tr>
<tr>
<td>T12</td>
<td>Human bladder cancer cells</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris acetate EDTA buffer</td>
</tr>
<tr>
<td>TAFs</td>
<td>TBP associated factors</td>
</tr>
<tr>
<td>TATA</td>
<td>TATA box; conserved A/T rich septamer transcription sequence</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris borate EDTA</td>
</tr>
<tr>
<td>TBP</td>
<td>TATA binding protein</td>
</tr>
<tr>
<td>TE</td>
<td>Tris-EDTA buffer</td>
</tr>
<tr>
<td>TEMED</td>
<td>N,N,N',N'-Tetramethylethylenediamine</td>
</tr>
<tr>
<td>TEN</td>
<td>Tris-EDTA buffer with sodium</td>
</tr>
<tr>
<td>TIFs</td>
<td>Transcription initiation factors</td>
</tr>
<tr>
<td>TFIID</td>
<td>Transcription initiation factor complex; TBP and TAFs</td>
</tr>
<tr>
<td>TF</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>XK469</td>
<td>Topoisomerase IIβ poison (NSC 697887)</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra-violet light</td>
</tr>
<tr>
<td>VM-26</td>
<td>Teniposide: topoisomerase II poison</td>
</tr>
<tr>
<td>VP-16</td>
<td>Etoposide</td>
</tr>
<tr>
<td>X-gal</td>
<td>5-bromo-4-chromo-3-indolyl-β-D-galactopyranoside</td>
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<tr>
<td>wt</td>
<td>wild type</td>
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