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# Regulation of Topoisomerase IIα Expression in Humans

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Biochemistry at Massey University, Palmerston North, New Zealand.

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# Abstract

In mammalian cells, the loss or down-regulation of tumour-suppressor genes and/or the mutation or overexpression of proto-oncogenes, whose products promote unregulated proliferation in cells, characterise the process of malignant transformation. This generates mitogenic signals that promote abnormal cell growth resulting in tumour progression. Topoisomerase IIα (topo IIα) is an enzyme present in elevated concentrations in highly proliferating cells due to the requirement for untwisting and unknotting of the DNA which is essential for replication. Because of this requirement, a number of anti-cancer drugs have been designed with topo IIα as their primary target. The effectiveness of these drugs however is limited by the development of resistance. One factor linked to drug resistance is the down-regulation of topo IIα at the transcription level. Expression of topo IIα appears to be regulated through various transcription factors with members of the Sp1 family having a major contribution.

Previous work has shown down regulation of topo IIα can occur at the level of transcription. Nucleotide sequencing of the topo IIα promoter in drug-resistant cell lines has not revealed any mutations thus far. Three known proteins and one uncharacterised protein are capable of interacting with the proximal topo IIα promoter region. The uncharacterised protein may act as a co-activator or a co-repressor depending on the complement of transcription factors associated with the DNA in this region. Because drug resistant cell lines showed modulated expression of these transcription factors, it is important to identify the unknown protein and characterise its role in regulating topo IIα expression.

This research aimed to identify the minimal binding site and DNA elements required for the uncharacterised protein to bind, as well as introduce mutations into this proximal region and examine their functional significance. The results of this study could provide insights into the molecular mechanisms responsible for the development of drug resistance, contributing to more efficient and effective methods for the treatment of cancer.

# Abbreviations

| | |
|---|---|
| **Amp** | Ampicillin |
| **ATP** | Adenosine triphosphate |
| **β-gal** | β-galactosidase |
| **bp** | Base pairs (DNA) |
| **BSA** | Bovine serum albumin |
| **CDE** | Cell-cycle dependent element |
| **cpm** | counts per minute |
| **CTD** | C-terminal domain |
| **DMSO** | Dimethyl sulfoxide |
| **DNA** | deoxyribose nucleic acid |
| **dNTP** | deoxynucleoside triphosphate (dATP, dTTP, dGTP, dCTP) |
| **EDTA** | Ethylene diamine tetra-acetic acid |
| **EMSA** | Electrophoretic mobility shift assay |
| **FCS** | Fetal calf serum |
| **GC1** | GGGCGGG box |
| **GC2** | GGGGCGGGG box |
| **GCG** | Genetics computer group |
| **GFP** | Green fluorescent protein |
| **G segment** | Gated segment (DNA) |
| **HAT** | Histone acetyl transferases |
| **HeLa** | Human cervical carcinoma cells (Helen Lane) |
| **HTETOP** | Human fibrosarcoma cell line HT180, tTA-expressing topo IIα |
| **ICB** | Inverted CCAAT box |
| **kb** | kilobases |
| **LB** | Luria Bertani bacteriological media |
| **MCS** | Multiple cloning site |
| **MDR1** | Multidrug resistance gene |
| **MEM** | Eagle's minimal essential media |
| **mt** | mutated/mutant |

| | |
|---|---|
| **NES** | nuclear export signal |
| **NLS** | nuclear localisation signal |
| **NF-Y** | nuclear factor Y |
| **ONPG** | o-Nitrophenyl β-D-Galacto-pyranoside |
| **PAGE** | Polyacrylimide gel electrophoresis |
| **p53** | Tumour suppressor protein |
| **PBS** | Phosphate buffered saline |
| **PBSE** | Phosphate buffered saline plus EDTA |
| **PCR** | Polymerase chain reaction |
| **pGL3B** | pGL3 Basic vector |
| **PIC** | preinitiation complex |
| **RNase** | Ribonuclease |
| **RT** | Room temperature |
| **Sp1** | Specificty protein 1 |
| **Sp3** | Specificity protein 3 |
| **STET** | Sucrose, Tris, EDTA and triton-X buffer |
| **SV40** | Simian virus 40 |
| **T segment** | Transport segment (DNA) |
| **TAE** | Tris acetate EDTA buffer |
| **TAFs** | TBP associated factors |
| **TATA** | TATA box; conserved A/T rich septameter transcriptional 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 |
| **UV** | Ultra-violet light |
| **wt** | wild type |

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