TRANSCRIPTIONAL REGULATION OF HUMAN UDP-GLUCURONOSYLTRANSFERASES

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SUMMARY

The UDP-glucuronosyltransferases (UGTs) are a superfamily of enzymes that glucuronidate small, lipophilic molecules, thereby altering their biological activity and excretion. In humans, important examples of UGT substrates include molecules of both endogenous and xenobiotic origin; thus, UGTs are considered essential contributors to homeostatic regulation and an important defence mechanism against chemical insult. In keeping with both roles, UGTs are most strongly expressed in the liver, a predominant organ involved in detoxification.

Rates of glucuronidation in humans are neither uniform among individuals, nor constant in an individual over time. Genetic determinants and non-endogenous signals are both known to influence the expression of UGTs, which in turn may affect the efficacy of certain pharmaceutical treatments or alter long-term risk of developing disease. Thus, this thesis focuses on the transcriptional regulation of *UGT* genes in humans, particularly on mechanisms that are likely to be relevant to their expression and variation in the liver. Two major approaches were used: firstly, extensive studies of several *UGT* promoters were performed to identify and characterise transcriptional elements that are important for UGT expression; and secondly, important hepatic transcription factors were investigated as potential regulators of *UGT* genes.

UGT1A3, *UGT1A4* and *UGT1A5* are a subset of highly related, but independently regulated, genes of the human *UGT1* subfamily. UGT1A3 and UGT1A4 are expressed in the liver, whereas UGT1A5 is not. The presented analysis of the *UGT1A3*, *UGT1A4* and *UGT1A5* proximal promoters demonstrates that a hepatocyte nuclear factor (HNF)1-binding site common to all three promoters is important for

UGT1A3 and *UGT1A4* promoter activity *in vitro*, but is insufficient to drive UGT1A5 expression. Two additional elements required for the maximal activity of the *UGT1A3* promoter were also identified that may distinguish this gene from *UGT1A4*. *UGT1A3* was investigated further, focusing on mechanisms that may contribute to interindividual variation in UGT1A3 expression. Polymorphisms in the *UGT1A3* proximal promoter were identified and their functional consequences tested. Known variants of HNF1α were also tested for altered activity towards the *UGT1A3* gene.

UGT1A9 is the only hepatic member of the UGT1A7-1A10 subgroup of UGT1 enzymes. Previous work had identified HNF1-binding sites in all four genes, and HNF4α as an *UGT1A9*-specific regulator. The work presented herein extends these findings to show that HNF1 factors and HNF4α synergistically regulate *UGT1A9*, and that HNF4α is not the only transcription factor responsible for the unique presence of UGT1A9 in the liver.

Liver-enriched transcription factors screened as potential *UGT* regulators were chosen from the HNF1, HNF4, HNF6, FoxA and C/EBP protein families. Functional interactions newly identified by this work were HNF4α with *UGT1A1* and *UGT1A6*, HNF6 with *UGT1A4* and *UGT2B11*, FoxA1 and FoxA3 with *UGT2B11*, *UGT2B15* and *UGT2B28* and C/EBPα with *UGT2B17*. Observations were also made regarding different patterns of interaction between each *UGT* and the transcription factors tested, particularly HNF1α.

These studies significantly advance the understanding of the transcriptional control of human *UGT* genes. In time, it is hoped that a detailed knowledge of UGTs will be useful in developing better therapeutic and prophylactic medical treatments.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Dione Gardner-Stephen

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PUBLICATIONS ARISING DIRECTLY FROM THIS THESIS

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ABBREVIATIONS

ADH	alcohol dehydrogenase
AF	activation function
AhR	aryl hydrocarbon receptor
apo	apolipoprotein
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
BAC	bacterial artificial chromosome
β-ME	β-mercaptoethanol
BSA	bovine serum albumin
bZIP	basic region leucine zipper
C/EBP	CCAAT/enhancer binding protein
CAR	constitutive androstane receptor
CBP	CREB-binding protein
CDCA	chenodeoxycholic acid
Cdx	caudal-related homeodomain protein
CIP	calf intestinal alkaline phosphatase
CMV	cytomegalovirus
CoA	coenzyme A
cpm	counts per minute
CREB	cAMP-response-element-binding protein
CYP	cytochrome P450
DBP	D-site binding protein
DCoH	dimerisation co-factor of HNF1
DD	dihydrodiol dehydrogenase
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethyl sulphoxide
dNTP	deoxynucleotide-triphosphate
DTT	dithiothreitol
E. coli	*Escherichia coli*
EDTA	ethylenediaminetetra-acetic acid
EMSA	electrophoretic mobility-shift assay
ERβ	oestrogen receptor β
fabp	fatty acid-binding protein
FoxA	forkhead box A
FXR	farnesoid X receptor
glut2	glucose transporter 2
GR	glucocorticoid receptor
GRIP	glucocorticoid receptor interacting protein
HAT	histone acetyltransferase
HCV	hepatitis C virus
HDAC	histone deacetylase
HNF	hepatocyte nuclear factor
IL	interleukin
Inr	initiator
LAP	liver-enriched transcriptional activator protein
LB	Luria broth

LETF	liver-enriched transcription factor
LIP	liver-enriched transcriptional inhibitory protein
LXR	liver X receptor
MODY	mature onset diabetes of the young
MRP	multidrug resistance protein
NCoR	nuclear receptor co-repressor
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NRRE	nuclear receptor response element
NSAID	non-steroidal anti-inflammatory drug
OAT	organic anion transporter
Oct	octamer transcription factor
P/CAF	p300/CBP-associated factor
PBREM	phenobarbital response enhancer module
PBS	phosphate buffered saline
pBSII	pBlueScript II
PCR	polymerase chain reaction
PGC	PPAR-gamma co-activator
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
POU	Pit-1, Oct-1 and Oct-2, and Unc-86
PPAR	peroxisome-proliferator-activated receptor
PXR	pregnane X receptor
QPCR	quantitative real-time PCR
rRNA	ribosomal RNA
rs	reference SNP
RXR	retinoid X receptor
SDS	sodium dodecyl sulphate
SHP	small heterodimer partner
siRNA	small interfering RNA
SMP	skim milk powder
SMRT	silencing mediator of retinoid and thyroid hormone receptor
SN-38	7-ethyl-10-hydroxycamptothecine
SNAP	*S*-nitroso-*N*-acetyl penicillamine
SNP	single nucleotide polymorphism
SRC	steroid receptor co-activator
TBE	tris-borate EDTA
TBST	tris-buffered saline/tween-20
TFII	transcription factor II
Tris	tris[hydroxymethyl]aminomethane
TSA	trichostatin A
TSS	transcription start site
UDP	uridine diphosphate
UGT	UDP-glucuronosyltransferase
XRE	xenobiotic response element