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Hepatocyte Nuclear Factor 1 and the Regulation of the Human Factor IX Gene.

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Abstract

Factor IX is a serine protease involved in the mammalian blood clotting cascade. An absence of functional factor IX protease in the bloodstream results in Haemophilia B. Mutations in the regulatory region of the factor IX gene can produce a rare form of the disease called Haemophilia B Leyden. Single nucleotide substitutions at positions -5 and -6 of the human factor IX promoter, which result in Haemophilia B Leyden, disrupt the binding of an unidentified transcription factor which interacts in the region -13 to +3. A group of transcription factors which may interact with the factor IX promoter in this region is called the hepatocyte nuclear factor 1 (HNF1) family.

The aim of this research was to investigate the potential role of the HNF1 proteins in the regulation of factor IX promoter gene expression. This study was bipartite, involving research into the ability of the HNF1 transcription factors to bind the factor IX promoter *in vitro*, and to regulate the initiation of its transcription. The HNF1 cDNAs were firstly subcloned into an expression vector suitable for use in mammalian tissue culture.

Gel mobility shift assays were employed to examine the binding of the HNF1 proteins to the wildtype factor IX promoter. The ability of these proteins to bind the factor IX promoter region carrying the -5 or -6 mutations was also investigated. Luciferase reporter gene assays using a human hepatoma cell line were used to study the regulatory effects of the HNF1 transcription factors on transcription from the normal and mutant factor IX promoters.

A variant form of the HNF1 transcription factor was shown to bind to the -14 to +6 region of the normal sequence of the factor IX promoter as well as that containing some of the -5 and -6 mutations. A protein from rat liver nuclear extracts which displayed an HNF1-like binding activity was detected using gel mobility shift assays with the factor IX promoter region. All forms of the HNF1 transcription factors could regulate the transcription of a reporter gene driven by the wildtype factor IX promoter. Two forms of the HNF1 transcription factor down-regulated the wildtype factor IX promoter-reporter gene construct by at least 50%, while the same construct was up-regulated by a third form of the transcription factor.

Unfortunately time constraints resulted in the premature conclusion of planned experimentation. The results generated during this research have been unable to confirm a role for the HNF1 family in the regulation of the factor IX promoter, but have provided a basis for further research.

CONTENTS.

	Page
Abstract.	i
Contents.	ii
List of Figures.	vi
List of Tables.	ix
Abbreviations and Definitions.	x
 CHAPTER ONE: INTRODUCTION.	 1
 1.1 Factor IX.	 1
1.1.1 Haemophilia B Leyden.	2
1.1.2 Transcription in Eukaryotes	3
<i>Promoters and Enhancers</i>	4
<i>The Action of Transcription Factors.</i>	4
1.1.3 Liver-specific expression of Factor IX.	5
1.1.4 Haemophilia B Leyden and Phenotypic Recovery.	6
1.2 HNF1 Family.	7
1.2.1 DNA binding domains.	8
1.2.2 Dimerisation domain.	10
1.2.3 HNF1 β .	10
1.2.4 Activation domains (ADs).	11
1.2.5 Isoforms of HNF1 α and HNF1 β .	12
1.2.6 DCoH.	14
1.3 The HNF1 Family and Haemophilia B.	15
1.3.1 Aims of this thesis.	16
 CHAPTER TWO: MATERIALS AND METHODS.	 18
 2.1 Materials.	 18
2.2 Methods.	20
2.2.1 Techniques for DNA Manipulation.	20
<i>General Methods.</i>	20
<i>Preparation of HNF1α and DCoH cDNAs for Subcloning.</i>	21
<i>Manual Sequencing of PCR Products.</i>	21
<i>Automatic Sequencing of PCR Products.</i>	22

2.2.2	Mammalian Tissue Culture.	22
	<i>Maintenance of Tissue Cultures.</i>	22
	<i>Transient Transfection of Tissue Culture.</i>	23
	<i>Harvesting of Transfected Cos7 Cell Cultures.</i>	23
	<i>Harvesting of Transfected Alexander Cell Cultures.</i>	23
2.2.3	Electrophoretic Mobility Shift Assays (EMSAs).	24
	<i>Labelling of DNA probes for Gel Shifts.</i>	24
	<i>The Gel Shift.</i>	24
2.2.4	Rat Liver Nuclear Extracts.	25
2.2.5	Transcriptional Assays.	25

CHAPTER THREE: RESULTS AND DISCUSSIONS. 27

Part A: Subcloning of the cDNAs for HNF1 α , HNF1 β and DCoH.

3.1	Preparation of the HNF1 α Expression Constructs.	27
3.1.1	Restriction Enzyme Digests.	27
3.1.2	PCR Amplification of the HNF1 α cDNA.	37
3.1.3	Cloning of the HNF1 α cDNA.	42
3.1.4	Sequencing of the HNF1 α cDNA.	45
3.1.5	Discussion of the Subcloning Difficulties with the HNF1 α cDNA.	50
	<i>Eco RI Restriction Endonuclease Digests</i>	50
	<i>DNA Sequencing and PCR</i>	50
3.2	pSVK3-HNF1 β Subcloning.	53
3.2.1	Restriction Enzyme Digests of the pBJ5-HNF1 β Vector	53
3.2.2	Cloning of the HNF1 β cDNA.	54
3.3	Preparation of the DCoH Expression Construct.	57
3.3.1	PCR.	57
3.3.2	Cloning of the DCoH cDNA.	58
3.3.3	Sequencing of the pSVK3-DCoH Construct	59
3.3.4	Summary of Part A.	61

Part B: Binding of the HNF1 Proteins to the Factor IX Promoter.

3.4	Introduction to Gel Shifts.	62
3.5	Gel Shifts with Rat Liver Nuclear Extracts.	64
3.5.1	Endogenous Rat Liver Proteins Bind to the Wildtype Factor IX Promoter.	64

3.5.2 HNF1 May be Interacting at the -5, -6 Region of the Factor IX Promoter.	66
3.5.3 An HNF1 Dimer May Bind at -5, -6 of the Factor IX Promoter.	68
3.6 Cos Cell Extract Gel Shifts.	68
3.6.1 Cos Cell Transfections.	68
3.6.2 Preliminary HNF1 β Gel Shifts.	69
<i>The Results for HNF1β Homodimers differ from those for Rat Liver Nuclear Extracts.</i>	73
<i>Binding of HNF1β to the Wildtype Factor IX Promoter.</i>	73
3.6.3 Gel Shifts with Cos Cell Extracts Containing HNF1 α , HNF1 β and DCoH.	76

Part C: The Effect of HNF1 Proteins on Transcription from the Factor IX Promoter.

3.7 Introduction to Luciferase Assays.	80
3.7.1 Potential Sources of Error in Luciferase Assays	82
3.8 Transcription from the Factor IX Promoters.	84
3.8.1 The Mutant Promoters are Down-regulated in Alexander Cells.	84
3.8.2 The Expression from Mutant Promoters Varies Between Patients.	86
3.9 Effects of HNF1α. on Factor IX Expression.	88
3.9.1 HNF1 α Down-regulates Transcription from the Wildtype Factor IX Promoter <i>in vitro</i> .	88
3.9.2 Is HNF1 α a Transcriptional Repressor?	90
3.9.3 The Possible Function of Repression of the Normal Factor IX Promoter by HNF1 α .	93
3.10 Effects of HNF1β on Factor IX Expression.	94
3.10.1 HNF1 β Down-regulates Transcription from the Wildtype Factor IX Reporter Gene.	94
3.10.2 HNF1 β May Possess of Bifunctional Regulatory Activity.	96
3.11 The Effects of HNF1α.HNF1β Heterodimers on Factor IX Gene Expression.	97
3.11.1 HNF1 α .HNF1 β Heterodimers up-regulate the Wildtype and -6G/C Factor IX Promoters <i>in vitro</i> .	97
3.11.2 HNF1 Heterodimers Could Potentially Activate Factor IX Gene Expression.	99
3.12 Effects of DCoH on the Activities of the HNF1 Proteins.	100
3.12.1 The <i>in vitro</i> Regulatory Ability of HNF1 α is Essentially Unchanged	

by DCoH.	101
3.12.2 The <i>in vitro</i> Regulatory Ability of HNF1 β May be Unchanged by DCoH.	102
3.12.3 DCoH has a Variable Effect on the <i>in vitro</i> Transactivating Ability of HNF1 Heterodimers in Luciferase Transfections.	104
CHAPTER FOUR: FINAL DISCUSSION AND CONCLUSIONS.	
4.1 HNF1 May Bind the Factor IX Promoter and Regulate its Transcription <i>in vitro</i>.	106
4.2 HNF1 Could Form the Basis of the Liver-specific Expression of the Factor IX Gene.	107
4.2.1 Is the Liver Protein Identified in Gel Shifts an HNF1 α .HNF1 β Heterodimer?	111
4.3 Future Work.	112
REFERENCES.	116
Appendix A: Plasmid Maps.	124
<i>pBluescript-KS-HNF1α.</i>	124
<i>pBJ5-HNF1β.</i>	125
<i>pGEX-2T-DCoH.</i>	126
<i>pSVK3.</i>	127
Appendix B: Oligonucleotides	128
<i>HNF1α PCR primers.</i>	128
<i>HNF1α Sequencing primers.</i>	128
<i>DCoH primers.</i>	128
<i>Gel Mobility Shift Oligonucleotides</i>	128
Appendix C: Lalign Results.	130

List of Figures.

Figure		Page
1.1	The blood clotting cascade.	2
1.2	The imperfect repeat found in the promoter of the gene for factor IX.	3
1.3	Haemophilia B Leyden mutations.	5
1.4	Schematic diagram of the domains of HNF1 α .	9
1.5	The consensus sequence of HP1 to which HNF1 α binds.	10
1.6	Schematic diagram of the HNF1 α and HNF1 β proteins.	12
1.7	Alignment of the HP1 site with the -5, -6 region of the Factor IX promoter.	16
3.1	Cloning strategy of the HNF1 α cDNA using restriction enzyme digests.	28
3.2	Partial digestion of pBluescript-KS-HNF1 α by <i>Eco</i> RI restriction endonuclease.	29
3.3	Digestion of the pBluescript vector with <i>Hga</i> I.	30
3.4	Identification of the HNF1 α cDNA by digestion with <i>Pst</i> I.	31
3.5	Identification of transformants carrying potential pSVK3-HNF1 α plasmids.	32
3.6	Identification of the pSVK3-HNF1 α plasmid by digestion with either <i>Pst</i> I or <i>Sma</i> I.	33
3.7	Identification of the orientation of the HNF1 α cDNA in the pSVK3-HNF1 α construct.	34
3.8	The pSVK3-HNF1 α construct with two inserts.	36
3.9	Cloning strategy for the HNF1 α cDNA using PCR.	38
3.10	Schematic diagram of the incorrect hybridisation of the 3' HNF1 α primer to the PCR template.	41
3.11	The 2.3 kb HNF1 α PCR product.	42
3.12	Identification of positive clones.	43
3.13	Identification of the correct HNF1 α expression construct.	44
3.14	The sequencing strategy for the HNF1 α cDNA.	46
3.15	The new HNF1 α cDNA sequencing strategy.	47
3.16	The internal HNF1 α PCR.	48
3.17	The potential loop of the HNF1 α cDNA.	51

3.18	Cloning strategy for the HNF1 β cDNA.	53
3.19	<i>Eco</i> RI digest of pBJ5-HNF1 β .	54
3.20	Identification of positive transformants.	55
3.21	<i>Sma</i> I diagnostic digests of the three positive plasmids.	56
3.22	Cloning of the DCoH PCR product.	57
3.23	Cloning strategy for the DCoH cDNA.	58
3.24	Sample of the manual sequencing of the DCoH cDNA.	60
3.25	Schematic diagram of a gel shift.	63
3.26	Competition gel shift 1 of rat liver nuclear extract with the wildtype factor IX promoter.	65
3.27	Competition gel shift 2 of rat liver nuclear extract with the wildtype factor IX promoter.	67
3.28	Competition gel shift 1 of HNF1 β -rich Cos cell extract with the HNF1 α site from the α 1-antitrypsin promoter.	71
3.29	Competition gel shift 2 of HNF1 β -rich Cos cell extract with the HNF1 α site from the α 1-antitrypsin promoter.	72
3.30	Alignment of the HNF1 α consensus sequence with the factor IX promoter.	74
3.31A	Gel shift to test for the presence of an HFN1 transcription factor.	77
3.31B	Gel shift to detect HNF1 α in a Cos cell extract.	78
3.32	BSA trial gel shift.	79
3.33	Schematic diagram of luciferase activities.	81
3.34	Basal transcription from the factor IX promoters.	85
3.35	Results of the co-transfection of HNF1 α with the factor IX promoters.	89
3.36	Quenching.	91
3.37	Results of the co-transfection of HNF1 β with the factor IX promoter-reporter gene constructs.	95
3.38	Results of the co-transfection of HNF1 α and HNF1 β with the factor IX promoters.	98
3.39	Results of the co-transfection of DCoH with HNF1 α and the factor IX promoters.	101
3.40	Results of the co-transfection of DCoH with HNF1 β and the factor IX promoters.	103
3.41	Results of the co-transfection of DCoH, HNF1 α and HNF1 β with the factor IX promoters.	105

4.1	The '3D puzzle' of the binding of transcription factors to various promoters.	110
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List of Tables.

Table		Page
1	HNF1 α cDNA Amplification by PCR.	39
2	HNF1 α PCR Primer Test Results.	40
3	Results of the sequencing of the pSVK3-HNF1 α clone 2.	49
4	Comparison of PCR and Sequencing Conditions.	52
5	Sample Calculation of Relative Luciferase Activities.	84
6	The blood clotting abilities of the known -5 and -6 mutant factor IX promoter carriers.	87
7	Summary of the Effecto of HNF1 α and HNF1 β on Transcription from the Factor IX Promoter.	108

Abbreviations and Definitions.

A	adenine
AR	androgen receptor
ARE	androgen response element
ATP	adenosine triphosphate
bp	base pair
BRL	Bethesda Research Laboratories
C	cytosine
cDNA	complementary DNA
Cos7	monkey fibroblast kidney cell line
cpm	counts per minute
C/EBP	CCAAT enhancer binding protein
CTF	CAAT-binding transcription factor
DBP	D-site binding protein
DCoH	Dimerisation Cofactor of HNF1 α
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DNAse I	deoxyribonuclease I
dNTPs	deoxynucleotide triphosphates
DTT	dithiothreitol
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	ethylene diamine tetra-acetate
EMSA	electrophoretic mobility shift assay
G	guanine
GST	glutathione S-transferase
HEPES	N-2-HydroxyEthylPiperazine-N'-2-Ethane Sulphonic acid
HNF1 α	hepatocyte nuclear factor 1 α
HNF1 β	hepatocyte nuclear factor 1 β
HNF4	hepatocyte nuclear factor 4
kb	kilo base
kDa	kilodalton
LF-A1	liver factor A1
mRNA	messenger ribonucleic acid

NF-1	nuclear factor 1
N terminal	amino terminal
ONPG	<i>o</i> -nitrophenyl- β -galactoside
PAGE	PolyAcrylamide Gel Electrophoresis
PBS	phosphate-buffered saline
PBSE	phosphate-buffered saline EDTA
PCR	polymerase chain reaction
<i>Pfu</i>	<i>Pyrococcus furiosus</i>
PMSF	phenyl methane sulphonyl fluoride
poly (dI-dC)	polymer of dI and dC
<i>Pwo</i>	<i>Pyrococcus woesei</i>
RNA	ribonucleic acid
RNAase	ribonuclease
RT	room temperature
<i>S. cerevisiae</i>	yeast <i>Saccharomyces cerevisiae</i>
SDS	sodium dodecyl sulphate
T	thymine
TAE	tris acetate EDTA
<i>Taq</i>	<i>Thermus aquaticus</i>
TBE	tris boric acid EDTA
TEMED	N, N, N', N' - tetra methyl ethylenediamine
TFIID	general transcription factor IID
TFIIH	general transcription factor IIH
Tris	tris- (hydroxymethyl) aminomethane
tRNA	transfer ribonucleic acid
UV	ultra violet light
³² P	radioactive isotope of phosphate