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.

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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 HNF1α
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DNAse I	deoxyribonuclease I
dNTPs	deoxynucleotide triphosphates
DTT	dithiothreitol
E. coli	*Esherichia coli*
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
HNF1α	hepatocyte nuclear factor 1 α
HNF1ß	hepatocyte nuclear factor 1 ß
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	*o*-nitrophenyl-ß-galactoside
PAGE	PolyAcrylamide Gel Electrophoresis
PBS	phosphate-buffered saline
PBSE	phosphate-buffered saline EDTA
PCR	polymerase chain reaction
Pfu	*Pyrococcus furiosus*
PMSF	phenyl methane sulphonyl fluoride
poly (dI-dC)	polymer of dI and dC
Pwo	*Pyrococcus woesei*
RNA	ribonucleic acid
RNAase	ribonuclease
RT	room temperature
S. cerevisiae	yeast *Saccaromyces cerevisiae*
SDS	sodium dodecyl sulphate
T	thymine
TAE	tris acetate EDTA
Taq	*Thermus aquaticus*
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