

Characterisation of the Multifunctional Protein, CREAP

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Declaration

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Kristy Lea Shipman

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ABBREVIATIONS

8-Br-cAMP	8-bromoadenosine cAMP
ACTH	adrenocorticotrophin releasing hormone
ATCC	American tissue culture collection
ATF	activating transcription factor
BrUTP	5-bromo-UTP
BSA	bovine serum albumin
bZIP	basic leucine zipper
cAMP	cyclic adenosine monophosphate
CBC	cap binding complex
cDNA	complementary DNA
CDXA/CDXRE	caudal type homeobox response element
CH	cysteine-histidine
CMV	cytomegalovirus
Co-IP	co-immunoprecipitation
CRE	cAMP response element
CREAP	CRE associated protein
CREB	CRE binding protein
CREM	CRE modulating protein
CRH	corticotrophin releasing hormone
CRH-BP	CRH binding protein
CROP	cisplatin resistance over-expressed protein
CsCl	cesium chloride
CTD	carboxyl terminal domain
Dex	dexamethasone
DHEAS	dehydroepiandrosterone
DMEM	Dulbecco's modified eagle's medium
DNA	dideoxy-ribonucleic acid
E.coli	escherichia coli
ECL	enhanced chemiluminescence
EcRE	ecdysone response element

EMSA	electrophoretic mobility shift assay
ERE	estrogen response element
EST	expressed sequence tag
FBS	fetal bovine serum
GFP	green fluorescent protein
GR	glucocorticoid receptor
GST	glutathione-s transferase
HBS	Hepes buffered saline
HLH	helix loop helix
hnRNPs	heteronuclear ribonuclear protein
HPA	hypothalamic pituitary adrenal axis
HRE	hybrid steroid response element
HRP	horseradish peroxidase
HTH	helix turn helix
IGC	interchromatin granule clusters
IL	interleukin
IP	immunoprecipitation
IPTG	isopropyl-beta-D-thiogalactopyranoside
IR	immunoreactivity
KAc	potassium acetate
KRE	lysine/arginine/glutamine
LB	luria broth
Luc	luciferase
lv	long-version
MAPK	mitogen activated protein kinase
Mg ₂ Cl	magnesium chloride
mRNA	messenger RNA
MTE	multiple tissue expression array
Mut	mutant
NaCl	sodium chloride
NBCS	new born calf serum
NLS	nuclear localisation signal
NTD	amino terminal domain

NuPAGE	neutral polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PG	prostaglandin
PKA	protein kinase A
PKC	protein kinase C
PMA	phorbol ester
Pol	polymerase
PR	progesterone receptor
RLU	relative luciferase units
RNA	ribonucleic acid
RRM	RNA recognition motif
rRNA	ribosomal RNA
RS domain	Arginine-serine domain
SC35	splicing component 35kDa
Scr	scrambled
SF2/ASF	splicing factor 2/alternative splicing factor
siRNA	small-interfering RNA
SNP	single nucleotide polymorphism
snRNA	small nuclear RNA
snRNP	small nuclear ribonuclear protein
SR Protein	Serine-arginine protein
sv	short-version
TBS	tris buffered saline
TBST	tris buffered saline with Tween-20
TE	tris-EDTA
TF	transcription factor
tRNA	transferase RNA
Trx	thioredoxin
UV	ultraviolet

PUBLICATIONS

“Identification of a Family of DNA-Binding Proteins with Homology to RNA Splicing Factors”. **Kristy L. Shipman**, Phillip J Robinson, Bruce R King, Roger Smith, Richard C Nicholson. Biochemistry and Cell Biology, Volume 84, Number 1, 1 February 2006, pp. 9-19(11).

CONFERENCE ABSTRACTS

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“Nuclear Localisation of the Multifunctional Protein CREAP.” 88th Annual Meeting of the Endocrine Society, 2006, Boston, USA.

“The Multifunctional Protein CREAP is a Nuclear Protein.” 48th Annual Scientific Meeting of the Endocrine Society of Australia, 2005, Perth, Australia.

“Identification of a DNA Binding Protein Family with Similarity to RNA Splicing Factors.” 47th Annual Scientific Meeting of the Endocrine Society of Australia, 2004, Sydney, Australia

“The Multifunctional Protein CREAP Inhibits CRH Promoter Activity .” 86th Annual Meeting of the Endocrine Society, 2004, New Orleans, USA

“Identification of a Novel Transcription Factor Family that Binds to the cAMP Regulatory Element.” 85th Annual Meeting of the Endocrine Society, 2003, Philadelphia, USA

“The CREAPs: A Family of CRE Binding Proteins.” 46th Annual Scientific Meeting of the Endocrine Society of Australia, 2003, Melbourne, Australia

“Analysis of Transcription Factors regulating Placental CRH through the CRE.” 45th Annual Scientific Meeting of the Endocrine Society of Australia, 2002, Adelaide, Australia.

Abstract

Pre-term birth is still the leading cause of perinatal mortality and morbidity. CRH is a hormone that is involved in the timing of labour, therefore investigation of its regulation is of importance in understanding human parturition. The CRE is a central regulatory element on the CRH promoter and in investigating proteins that bind to this element a novel protein was discovered. CREAP or cAMP Regulatory Element Associated Protein, was initially discovered by its ability to bind to the CRE. Its sequence encodes a unique set of modular domains including two zinc fingers, two leucine zippers, two coiled-coils and an RS-rich domain. These domains point to functions in both DNA binding/transcription and RNA splicing, with the leucine zippers being characteristic of bZIP transcription family and the RS domain characteristic of the SR Protein family of splicing factors, to represent a new protein family.

In this thesis, molecular reagents were produced for the study of CREAP together with a polyclonal antibody. This antibody was used in western blotting to detect a 58 kDa full-length CREAP protein and a shorter 25-30 kDa truncated splice variant. CREAP was localised to the nucleus and to intranuclear splicing speckles, with co-localisation and co-immunoprecipitation with the splicing factor SC35, strongly suggesting a role in splicing. To test the transcriptional activity of CREAP, specifically if it regulates CRH expression, luciferase reporter studies were conducted. However, CREAP showed negligible effect on CRH or CRE promoter activities suggesting that it is not involved in CRH regulation. CREAP did however react with a large number of transcription factors in an *in vitro* assay, mostly from the bZIP and zinc finger families. siRNA mediated knockout of CREAP was conducted and the effect on genome-wide expression analysed using a microarray. CREAP knockdown caused an over-representation of genes from the protein transport, metabolism, signal transduction and transcription factor processes. Overall, CREAP appears to be a multifunctional protein that is ubiquitously expressed, and is involved in both splicing and transcriptional processes.