Expression and function of *Npas4* during early development

THOMAS STEPHEN KLARIC

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The University of Adelaide

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Declaration

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Abbreviations

°C	degrees Celsius
µg	microgram
µL	microlitre
6-OHDA	6-hydroxydopamine
ACTH	adrenocorticotropic hormone
ADHD	attention deficit hyperactivity disorder
aha-1	aryl hydrocarbon receptor associated protein
Ahr	aryl hydrocarbon receptor
Akt	protein kinase B
AMP	adenosine monophosphate
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
AMPAR	AMPA receptor
ANOVA	analysis of variance
Arc	activity-regulated cytoskeleton-associated protein
Arnt	aryl hydrocarbon receptor nuclear translocator
ATP	adenosine triphosphate
βIII tubulin	Class III β-tubulin
Bax	BCL-2-associated X protein
Bcl-2	B-cell lymphoma 2
Bdnf	brain-derived neurotrophic factor
bHLH	basic helix-loop-helix
Bmal	brain and muscle ARNT-like
BMP	bone morphogenetic protein
bp	base pair
BSA	bovine serum albumin
C. elegans	*Caenorhabditis elegans*
CA	*cornu Ammonis*
Ca^{2+}	calcium
$CaCl_2$	calcium chloride
CAG	CMV/chicken β-actin hybrid
CaMKIV	Ca^{2+}/calmodulin-dependent protein kinase type IV
cAMP	cyclic AMP
$CaPO_4$	calcium phosphate
Cbp	Creb binding protein
CCG	Clock controlled gene
cDNA	complementary DNA
Chem-LTD	chemically induced LTD
Chem-LTP	chemically induced LTP
ChIP	chromatin immunoprecipitation
ChIP-Seq	ChIP followed by sequencing
Clock	circadian locomoter output kaput
cm^2	centimetres squared
CME	central midline element

CMV	cytomegalovirus
CNS	central nervous system
cNXFL	*C. elegans* NXF-like-factor
CO_2	carbon dioxide
COS7	monkey kidney cell line
CRE	cAMP response element
Creb	cAMP response element‑binding protein
CRF	corticotropin-releasing factor
CRY	cryptochrome
CSD	cortical spreading depression
CSF	cerebrospinal fluid
C-terminal	carboxy-terminal
C-terminus	carboxy-terminus
D. Melanogaster	*Drosophila melanogaster*
DAPI	4,6-Diamidino-2-phenylindole dihydrochloride
DG	dentate gyrus
DIG	digoxigenin
DIG-11-UTP	DIG-11-uridine-5'-triphosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
dNXFL	*Drosophila* NXF-like-factor
DRE	downstream regulatory element
DREAM	DRE-antagonist modulator
Drebrin	developmentally regulated brain protein
DSCR	Down's syndrome critical region
DTT	dithiothreitol
dUTP	deoxyuracil triphosphate
Dys	dysfusion
E	embryonic day
E. coli	*Escherichia coli*
EAA	Excitatory amino acid
EDTA	ethylenediaminetetraacetic acid
EF1α	elongation factor-1 alpha
eGFP	enhanced green fluorescent protein
Egr1	early growth response protein 1
EPL	early primitive ectoderm-like
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
Ern1	ER-nucleus signalling 1 protein
eRNA	enhancer RNA
ES cell	embryonic stem cell
ETS	E-twenty six
eYFP	enhanced yellow fluorescent protein
FBS	foetal bovine serum

FGF	fibroblast growth factor
Fox-3	feminizing locus on X 3
FTD	frontotemporal dementia
FTDP-17	FTD and parkinsonism linked to chromosome 17
g	the earth's gravitational acceleration
GABA	γ-aminobutyric acid
$GABA_A$-γ2	$GABA_A$-receptor γ2 subunit
GABAR	GABA receptor
GAD65	glutamic acid decarboxylase
GCL	granule cell layer
gDNA	genomic DNA
Gfap	glial fibrillary acidic protein
GFP	green fluorescent protein
GluR	glutamate receptor
Gpx2	glutathione peroxidase 2
Grp78	glucose-regulated protein 78
H^+	hydrogen
HeBS	HEPES-buffered saline
HEK	human embryonic kidney cell line
HeLa	human cervical cancer cell line
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hES cell	human embryonic stem cell
Hif	hypoxia inducible factor
HMG	high mobility group
HPA	hypothalamic-pituitary-adrenocortical
hPGK	human phosphoglycerate kinase
hr	hour
HRE	hypoxic response element
HSP	heat-shock protein
IEG	immediate-early gene
iGluR	ionotropic glutamate receptor
IP_3	inositol 1,4,5-trisphosphate
IRES	internal ribosome entry site
ISH	*in situ* hybridisation
K^+	potassium
KAR	kainate receptor
kb	kilobase
KCl	potassium chloride
KCN	potassium cyanide
KD	knock-down
kDa	kilodalton
LB	lysogeny broth
LE-PAS	limbic-enriched PAS
LIF	leukaemia inhibitory factor
LTD	long-term depression

LTP	long-term potentiation
LTR	long terminal repeat
M	molar
MAPK	mitogen-activated protein kinase
MAPT	microtubule-associated protein tau
MEK1	MAPK kinase 1
mEPSC	miniature excitatory postsynaptic current
mES cell	mouse embryonic stem cell
mGluR	metabotropic glutamate receptor
min	minutes
mIPSC	miniature inhibitory postsynaptic current
miRNA	microRNA
mL	millilitre
mm	millimetre
mM	millimolar
MOI	multiplicity of infection
MOP4	member of PAS superfamily 4
mRNA	messenger RNA
MW	molecular weight
MyoD1	myogenic differentiation 1
n	number of independent experiments
Na^+	sodium
NADE	neuronal activity-dependent enhancer
NeuroD1	neurogenic differentiation 1
NFT	neurofibrillary tangle
ng	nanograms
Ngf	nerve growth factor
NHE9	N^+/H^+ Exchanger 9
NLS	nuclear localisation sequence
nM	nanomolar
NMDA	N-methyl-D-aspartic acid
NMDAR	NMDA receptor
Npas	neuronal PAS domain protein
NPC	neural progenitor cell
NT3	neurotrophin-3
NT4	neurotrophin-4
N-terminal	amino-terminal
N-terminus	amino-terminus
$O2^-$	superoxide
Oct-4	octamer-binding transcription factor 4
$p75^{NTR}$	p75 neurotrophin receptor
Pac	puromycin N-acetyl-transferase
PAN	primitive anterior neuroectoderm
PAS	Per-ARNT-Sim
Pax6	paired box gene 6

PBS	phosphate buffered saline
PBST	PBS Tween solution
PC12	rat pheochromocytoma cell line
PCR	polymerase chain reaction
PD	Parkinson's disease
Per	period
PFA	paraformaldehyde
pg	picograms
PI3K	phosphatidylinositol 3-kinase
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
pmol	picomoles
PTZ	pentylenetetrazol
PVDF	polyvinylidene fluoride
qRT-PCR	quantitative RT-PCR
REST	RE1- silencing transcription factor
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
RNAi	RNA interference
ROS	reactive oxygen species
rpm	revolutions per minute
RRE	Rev response element
RT	reverse transcription
RTK	receptor tyrosine kinase
RT-PCR	reverse transcription PCR
s	seconds
SCN	suprachiasmatic nuclei
SERCA	sarco/endoplasmic reticulum Ca^{2+}-ATPase
SGZ	subgranular zone
shRNA	small hairpin RNA
Sim	single-minded
siRNA	small interfering RNA
SNP	single nucleotide polymorphism
SOD	superoxide dismutase
Sox	SRY-related high mobility group box
SSC	saline sodium citrate
SVZ	subventricular zone
TAD	transactivation domain
TEMED	N,N,N,N-tetramethylethylenediamine
Tgo	Tango
$TgTau^{P301L}$	Tau transgenic mice
Trh	trachealess
Trk	tropomysin-related kinase
TS	Tourette syndrome

TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labelling
U	unit
UTR	untranslated region
UV	ultra violet
V	volts
(v/v)	volume/volume
VZ	ventricular zone
(w/v)	weight/volume
XBP-1	X-box binding protein 1
XRE	xenobiotic response element

Notes

1. The literature review is current as of October 2011.
2. The gene nomenclature used throughout this thesis is as follows;

   Human: human gene symbols, transcripts and coding sequences are represented by uppercase italicised text (i.e. *NPAS4*) while proteins are represented by uppercase non-italicised text (i.e. NPAS4).

   Rodent: rodent gene symbols, transcripts and coding sequences are represented by an initial uppercase letter and are italicised (i.e. *Npas4*) while proteins are not italicised (i.e. Npas4).

   Drosophila: *Drosophila* gene symbols, transcripts and coding sequences are represented by all lowercase italicised text (i.e. *dys*) while proteins are not italicised and use an initial capital letter (i.e. Dys).

   Nematode: nematode gene symbols, transcripts and coding sequences are represented by all lowercase italicised letters (i.e. *aha-1*) while proteins are not italicised (i.e. aha-1).

Abstract

Npas4 is an activity-dependent bHLH PAS transcription factor expressed within neurons of the mammalian central nervous system where it regulates the expression of several genes that are important for neuronal survival and synaptic plasticity. In the adult brain, Npas4 plays an important role in several key aspects of neurobiology including inhibitory synapse formation, neuroprotection and memory formation. Consequently, abnormal *Npas4* expression has been implicated in a number of neurological disorders such as autism, Down's syndrome, epilepsy, cerebral ischaemia and Alzheimer's disease. While the expression and function of *Npas4* are beginning to be elucidated in the adult brain, to date little is known regarding the role of *Npas4* during neuro-development. The aim of this study was to investigate the expression and function of *Npas4* during early development.

The expression of *Npas4* during early neuro-development was investigated using several different developmental model systems. *Npas4* was found to be transiently up-regulated during neural differentiation of both mouse and human embryonic stem (ES) cells at a stage of differentiation that is marked by an increase in neural progenitor cell (NPC) proliferation. This was corroborated by analysis of *Npas4* expression in the developing mouse embryo where *Npas4* mRNA was found to be expressed between embryonic day 7.5 and 9.5. The function of *Npas4* in the context of development was investigated by using RNA interference to decrease endogenous Npas4 expression in mouse ES cells undergoing neural differentiation. *Npas4*-specific small hairpin RNA expression constructs were delivered to mouse ES cells using lentiviral transduction to create two independent Npas4 knock-down mouse ES cell lines. An Empty vector control line was also generated by transducing mouse ES cells with a construct that does not produce small hairpin RNAs. When the cell lines were assessed for their ability to undergo neural differentiation, it was found that aspects of NPC identity and neuronal maturation were affected by a reduction in Npas4 expression. The percentage of cells expressing the neuroectoderm marker Sox1 was significantly diminished in Npas4 knock-down cultures while expression of Nestin was not affected. In addition, neurite sprouting defects were also observed at a later stage of differentiation in cultures having a more severe reduction in Npas4 expression. These data suggest that *Npas4* acts upstream of *Sox1* during neural differentiation and is involved in aspects of NPC maintenance and neuritogenesis. When taken together, the data presented in this thesis provide the first evidence that *Npas4* is expressed developmentally by a population of early NPCs and that it may have a developmental role that is unrelated to its function in the adult brain.