The role of synapse-associated protein 102 in postsynaptic signalling, synaptic plasticity and learning

Peter Charles Cuthbert

Wellcome Trust Sanger Institute

Clare Hall

University of Cambridge

Dissertation submitted for the degree of Doctor of Philosophy

2005

Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as detailed in the text and below.

Blastocyst injections of targeted embryonic stem cells were performed by Jane Robinson. Assistance with PCR genotyping was given by Georgina Berry, David Fricker, Amanda Lunney, Elizabeth Sotheran and Emma Stebbings (Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, UK).

Electrophysiological experiments were performed by Ann Fink, Patricio Opazo and Tom O'Dell (Department of Physiology, University of California, Los Angeles, California, USA). Biochemical experiments on the MAP kinase pathway were performed by Marcelo Coba (Wellcome Trust Sanger Institute).

Mouse body weights were measured by Lianne Stanford, Hayley Cooke and Margaret Green (Wellcome Trust Sanger Institute). SAP102 mutant mice were tested in the water maze by Jamie Ainge (Division of Neuroscience, University of Edinburgh). All other behavioural testing was performed in collaboration with Lianne Stanford. Behavioural data analyses were performed by Lianne Stanford.

Peter Charles Cuthbert
September 2005

Acknowledgements

I owe a debt of gratitude to many people for their generosity in science and in friendship. I warmly and sincerely thank my supervisor, Seth Grant, for the opportunity to be part of an exciting research group and for his unfailing support and enthusiasm throughout my PhD. Noboru Komiyama has been a mentor and advisor like no other, giving freely his time, scientific knowledge and friendship. It has been truly a privilege to work beside him.

Other members of the lab, in Edinburgh and Cambridge, have gone out of their way to help me over the past few years. Doug Strathdee gave much valuable advice on molecular biological techniques and experimental strategies. Kris Dickson was always happy to engage in scientific discussion or help out with an experiment. Lianne Stanford was an enthusiastic collaborator and patient travelling companion. Chris Anderson taught me many aspects of immunohistochemistry and was always up for a laugh. Karen Porter's acerbic wit kept my feet on the ground and ego suitably deflated. Jane Robinson spent many hours looking after the mice. Jane Turner dealt expertly and efficiently with administrative issues. To everyone else in Team 32, thank you for your support, friendship and fond memories.

To my family, thank you for your long-suffering, unfailing support for my endeavours. Finally, to Claire, thank you for everything.

Peter Cuthbert

September 2005.

Abstract

N-methyl-D-aspartate receptors (NMDARs) are found at the postsynaptic membrane of glutamatergic synapses and play essential roles in brain development, plasticity, learning and memory. Synaptic activation of NMDARs is transduced to a large complex of intracellular postsynaptic proteins. Synapse-associated protein 102 (SAP102) is part of the MAGUK protein family whose members, including PSD-95 and PSD-93, interact directly with the NR2 subunits of NMDARs and appear to act as adaptors connecting the receptor to its intracellular signalling network. Truncating mutations in SAP102 in humans are associated with X-linked mental retardation, however the *in vivo* function of SAP102 is unknown.

This dissertation describes a gene targeting approach to elucidate the function of SAP102 in mice. A DNA cloning technique using homologous recombination in bacteria was adapted and found to provide a highly efficient and flexible tool for the production of large numbers of varied mutation types in different loci of the mouse genome. Targeting vectors were generated for the introduction of three different mutations into the SAP102 locus: a constitutive knockout; a reporter gene knock-in and a conditional mutation.

SAP102 knockout mice were generated and found to be viable and fertile with grossly normal adult brain morphology. Behavioural tests uncovered a deficit in spatial learning in the watermaze which, in contrast to PSD-95 mutant mice, could be overcome with training. SAP102 mice exhibited a specific, frequency-dependent deficit in NMDAR-mediated hippocampal synaptic plasticity, a possible physiological mechanism for learning, while basal synaptic function and NMDAR conductance were unaffected. A screen of postsynaptic protein phosphorylation states in SAP102 mutant mice showed a specific increase in phosphorylation of extracellular signal-related kinase (ERK), part of the MAP kinase signalling pathway.

Targeted mutations in SAP102 and PSD-95 were utilised to explore the functional relationship between the two proteins. PSD-95 mutants have evelated hippocampal expression of SAP102, while SAP102 knockouts have increased PSD-95 associated with NMDARs, suggesting a partial compensation in these two targeted strains arising from functional overlap between SAP102 and PSD-95. A SAP102/PSD-95 double mutation was lethal, indicating an important role for these proteins during development.

These data show that SAP102 is crucial for normal postsynaptic signalling, synaptic plasticity and learning and begins to shed light on the differential roles of NMDAR-associated MAGUKs in coordinating intracellular responses to postsynaptic activation. SAP102 null mice may prove a useful tool in discovering and testing treatments for human learning disability.

Abbreviations

A_n	Absorbance at a wavelength of *n* nanometres.
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionate
AMPAR	AMPA receptor
BAC	Bacterial artificial chromosome
bp	Base pairs
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
Dlg	Discs large protein
dNTP	Deoxynucleoside triphosphate
DAB	Diaminobenzidine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOC	Deoxycholic acid
DTA	Diptheria toxin A fragment
DTT	Dithiothreitol
ECL	Cyclic diacylhydrazide
ELISA	Enzyme-linked immunoassay
EM	Electron microscopy
EPSC	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
ES cells	Embryonic stem cells
FBS	Foetal bovine serum
FRT	Flp recognition target
hr	Hour

g	Gravity
GAP	GTPase activating protein
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GEF	Guanine nucleotide exchange factor
GMP	Guanosine monophosphate
HEK	Human embryonic kidney
HRP	Horseradish peroxidase
IRES	Internal ribosome entry site
IQ	Intelligence quotient
Kb	Kilobase pairs
loxP	Locus of recombination in P1
LTP	Long-term potentiation
LTD	Long-term depression
LB	Luria Bertani
MCS	Multiple cloning site
mGluR	Metabotropic glutamate receptor
min	Minute
mRNA	Messenger RNA
NMDA	N-methyl-D-aspartate
NMDAR	NMDA receptor
NR	NMDA receptor subunit
neo	Neomycin phosphotransferase
NS-XLMR	Non-syndromic X-linked mental retardation
pA	PolyA signal sequence
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction

rpm	Revolutions per minute
PDZ	PSD-95/dlg/zona occludens-1
PGK	Phosphoglycerate kinase
PSD	Postsynaptic density
PVDF	Polyvinyl difluoride
RNA	Ribonucleic acid
RT	Reverse transcription
SAP102	Synapse-associated protein 102
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
sharm	Short homology arm
SV40	Simian virus 40
SRF	Serum response factor
S-XLMR	Syndromic X-linked mental retardation
YENB	Yeast extract, nutrient broth
U	Units
UV	Ultraviolet
XLMR	X-linked mental retardation

List of figures

List of tables

Table of contents