



UNIVERSITY  
OF TASMANIA

# **Role of Phosphoinositides in the Biology of the Amyloid Precursor Protein**

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**Submitted in fulfillment of the requirements for the  
degree of Doctor of Philosophy**

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02/2014**

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# Table of Contents

<b>Abstract</b>	<b>VII</b>
<b>Acknowledgements</b>	<b>X</b>
<b>Publication and presentation record</b>	<b>XI</b>
<b>List of abbreviations</b>	<b>XII</b>
<b>Chapter 1 Introduction</b>	<b>1</b>
1.1.1 Clinical features of AD .....	2
1.1.2 Impact and prevalence of AD .....	4
<b>1.2 Pathological features of AD.....</b>	<b>4</b>
1.2.1 Amyloid plaques .....	5
1.2.2 Neurofibrillary tangles .....	7
1.2.2.1 Biochemical characterisation of NFTs.....	8
1.2.3 Cerebral amyloid angiopathy .....	9
<b>1.3 A<math>\beta</math> hypothesis of AD .....</b>	<b>9</b>
1.3.1 A $\beta$ production .....	10
1.3.1 A $\beta$ aggregation.....	13
1.3.2 A $\beta$ toxicity .....	14
<b>1.4 APP expression, structure and processing.....</b>	<b>17</b>
1.4.1 APP trafficking and post-translational modification.....	19
1.4.1.1 $\alpha$ -Secretase.....	21
1.4.1.2 $\beta$ -Secretase.....	22
1.4.1.3 $\gamma$ -Secretase .....	24
<b>1.5 Genetics of AD .....</b>	<b>26</b>
1.5.1 Genetics of FAD .....	26
1.5.1.1 Mutations in APP.....	27
1.5.1.2 Presenilin mutations.....	29
1.5.2 Transgenic animal models of AD .....	29
1.5.3 Genetics of LOAD .....	31
1.5.3.1 ApoE .....	33
1.5.3.2 Role of apoE in AD .....	34
1.5.3.3 Clusterin/ApoJ .....	35

1.5.3.4 PICALM .....	36
1.5.4 Summary - Genetics of AD .....	37
<b>1.6 APP function.....</b>	<b>38</b>
1.6.1 Lessons from APP family knock-out and knock-in mice .....	38
1.6.2 Trophic functions of APP.....	41
1.6.2.1 APP in stem cell proliferation and differentiation .....	42
1.6.2.2 Mechanism of the trophic effects of APP .....	45
1.6.3 Roles of APP in cell adhesion and synaptogenesis .....	46
1.6.4 Non-neuronal functions of KPI domain-containing APP isoforms (Protease nexin II) .....	47
1.6.5 AICD interactions and functions.....	49
1.6.5.1 Functional motifs and phosphorylation sites in AICD .....	49
1.6.5.2 Binding of adaptor proteins to AICD .....	51
1.6.5.3 Summary: Functional roles of the APP intracellular domain .....	57
1.6.6 Summary: Function of APP. ....	58
<b>1.7 Phosphatidylinositol phosphate lipids: Minor lipids with major roles in cellular function.....</b>	<b>59</b>
1.7.1 Structure and nomenclature of phosphatidylinositol phosphates.....	60
1.7.2 Introduction to modes of PIP signalling.....	62
1.7.3 PIP metabolism .....	62
1.7.3.1 Phosphoinositide kinases .....	64
1.7.3.2 Phosphoinositide phosphatases.....	66
1.7.4 PIP-binding domains.....	66
1.7.5 Neuronal roles of PIPs .....	73
1.7.5.1 Roles of PIPs in synaptic vesicle exocytosis .....	73
1.7.5.2 Roles of PIPs in synaptic vesicle recycling and endocytosis.....	74
1.7.5.3 Modulation of ion channel function by PIPs .....	76
1.7.5.4 PI(4,5)P <sub>2</sub> as the metabolic precursor of IP <sub>3</sub> and DAG .....	77
1.7.6 Summary .....	78
1.7.7 General links between PIP and AD.....	78
<b>1.8 Hypothesis and aims of the study. ....</b>	<b>81</b>
1.8.1 Hypothesis.....	82

1.8.2	Aims .....	83
<b>Chapter 2 Materials and methods</b>		<b>84</b>
<b>2.1</b>	<b>Materials .....</b>	<b>85</b>
2.1.1	Buffers, solutions and cell culture media .....	85
2.1.2	Antibodies and dilutions .....	85
<b>2.2</b>	<b>Methods .....</b>	<b>85</b>
2.2.1	Protein-lipid overlay assay .....	85
2.2.2	Neural stem and progenitor cell culture and conditioned medium collection .....	87
2.2.3	Primary murine hippocampal and cortical culture .....	87
2.2.4	Immunocytochemistry .....	88
2.2.5	Microscopy and image analysis .....	89
2.2.6	Western blotting .....	90
2.2.7	Computational modelling of IP <sub>3</sub> binding sites on APP .....	91
2.2.8	Lipid extraction .....	92
2.2.9	UPLC-MS lipid analysis .....	93
2.2.10	Mass spectroscopy data analysis .....	96
<b>Chapter 3 Results</b>		<b>97</b>
<b>3.1</b>	<b>Studies on the binding of APP to lipids .....</b>	<b>98</b>
3.1.1	Examination of the ability of sAPP $\alpha$ to bind to lipids .....	98
3.1.2	Identification of a PIP-binding region in the E1 domain of APP .....	101
3.1.3	Competition of APP-E1 binding to PI(4,5)P <sub>2</sub> with a water soluble PIP analogue and mucosal heparin .....	103
3.1.3.1	Computational modelling of the PIP-binding domain in the E1 domain of APP .....	107
<b>3.2</b>	<b>Studies on the binding of APP to hippocampal neurons .....</b>	<b>113</b>
3.2.1	Identification of PIPs on the extracellular surface of the cell membrane	115
3.2.1.1	Immunocytochemical detection of cell-surface PIPs .....	116
3.2.1.2	Biosensor detection of cell-surface PIP .....	119
3.2.2	Co-localisation of exogenous APP-E1 and cell-surface PI(4,5)P <sub>2</sub> .....	121
3.2.3	Competition of APP-E1 binding to cells with diC8PI(4,5)P <sub>2</sub> .....	123

3.2.4	Investigation into the role of the heparin-binding domain in the binding of APP-E1 to the cell surface .....	126
<b>3.3</b>	<b>Studies on the biological effects of APP-PIP interactions .....</b>	<b>131</b>
3.3.1	Effect of sAPP $\alpha$ on PIP levels in primary cortical cultures.....	131
3.3.2	Effect of APP-E1 on levels of cell-surface PIP .....	140
3.3.2.1	Effect of APP-E1 on cell-surface PI(4,5)P <sub>2</sub> .....	140
3.3.2.2	Effect of APP-E1 on cell-surface PI(3,4,5)P <sub>3</sub> .....	143
3.3.3	Effect of APP-E1 on Akt phosphorylation.....	149
<b>Chapter 4</b>	<b>Discussion and conclusions</b>	<b>152</b>
<b>4.1</b>	<b>Summary of results .....</b>	<b>153</b>
<b>4.2</b>	<b>Discussion.....</b>	<b>156</b>
4.2.1	PIP-binding regions in APP .....	158
4.2.2	Extracellular PIP is present on the surface of neurons and glia.....	160
4.2.3	Mechanism of APP-E1 binding to the cell surface .....	164
4.2.4	Effect of APP on levels of PIPs .....	168
4.2.5	Other possible roles for the PIP-binding domain in APP .....	170
4.2.6	Hypothetical suggestions for roles of the lipid-binding domain in APP	171
<b>4.3</b>	<b>Final conclusions .....</b>	<b>174</b>
	<b>References</b>	<b>175</b>
	<b>Appendices</b>	<b>231</b>

## Abstract

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. In countries with aging populations, such as Australia, the prevalence of AD is projected to increase substantially. AD is characterised by two distinctive pathological lesions in the brain, amyloid plaques and neurofibrillary tangles. The major component of amyloid plaques is an aggregating protein termed the beta-amyloid protein ( $A\beta$ ).  $A\beta$  is formed normally from a larger precursor protein, known as the beta-amyloid precursor protein (APP). Although APP is centrally involved in the pathogenesis of Alzheimer's disease and the production of  $A\beta$ , relatively little is known about its normal function. Deciphering the function of APP in the brain may be essential for the development of effective AD therapeutics.

APP is a type I transmembrane glycoprotein that can be proteolytically processed by  $\alpha$ ,  $\beta$ - and  $\gamma$ -secretases to produce a number of secreted ectodomain fragments termed sAPP $\beta$ , sAPP $\alpha$ ,  $A\beta$  and p3. Many studies have suggested that sAPP $\alpha$  may act in the maintenance and development of the central nervous system, by acting as a paracrine factor. In vitro, sAPP $\alpha$  has been reported to modulate the proliferation and differentiation of a variety of cell types. However, the mechanistic basis for these effects is unclear. In part, this uncertainty has arisen because the cell-surface receptor molecules that interact with sAPP $\alpha$  are not known.

Previous studies have reported that sAPP $\alpha$  may interact with a novel lipid-raft type membrane domain in the cell. Furthermore, sAPP $\alpha$  has been reported to bind to the lipid GM1-ganglioside. On the basis of these reports, the work in this thesis explored the hypothesis that an interaction of APP with cell surface lipids could facilitate binding and/or signalling by sAPP $\alpha$ .

To determine if sAPP $\alpha$  is able to interact with a sub-group of lipids. The relative ability of sAPP $\alpha$  to bind to 27 physiological lipids was examined using a protein-lipid overlay assay. This assay identified that sAPP $\alpha$  could bind selectively to phosphoinositide lipids (PIPs). Further, a recombinant fragment of APP corresponding to the E1 N-terminal domain (APP-E1) also bound selectively to PIPs, suggesting there is a PIP-binding region within the E1 domain of APP.

To investigate whether APP and PIP could interact on the cell surface, it was first necessary to demonstrate that PIPs are present on the cell surface. A live cell immunolabelling method was used to examine the location of cell surface PIPs. Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P<sub>2</sub>) immunoreactivity was found to be present on the surface of cells in primary murine hippocampal cultures in discrete puncta <1  $\mu$ m in size. This observation was also confirmed using a recombinant PI(4,5)P<sub>2</sub> biosensor protein.

To examine whether APP could interact with cell-surface PIP, studies were performed to examine the degree of colocalisation of exogenous APP-E1 and cell-surface PI(4,5)P<sub>2</sub>. APP-E1 that was added to primary hippocampal cultures bound to the surface of neurons in discrete puncta <1  $\mu$ m in size. The cell-bound APP-E1 and the cell-surface PI(4,5)P<sub>2</sub> were highly co-localised on the surface of neurons. However, cell-surface PI(4,5)P<sub>2</sub> was also present on glial cells in culture where APP-E1 did not bind. Furthermore the binding of APP-E1 to cells could not be inhibited using a water soluble analogue of PI(4,5)P<sub>2</sub>. Therefore, these data suggested that APP-E1 interacts with cell-surface PI(4,5)P<sub>2</sub>, but the interaction was not sufficient to explain why APP-E1 binds to the cell surface.

As the APP E1 domain contains a heparin-binding site, the role of this region was investigated in the binding of APP-E1 to PIP and also the binding of APP-E1 to cells. Heparin did not block the binding of APP-E1 to PIP in vitro, suggesting the heparin-binding region and the PIP-binding region in the APP E1 domain are

distinct. However, heparin did inhibit the binding of APP-E1 to cells, suggesting that the heparin-binding region of APP is required for binding to cells. Furthermore, heparitinase treatment of cells significantly reduced cell surface heparan sulfate immunoreactivity, but did not affect the binding of APP-E1 to cells. These results suggest that APP may interact with PIP on the cell surface along with another cell surface component that binds to the heparin-binding site, which is not heparan sulfate.

As PIPs are involved in many aspects of cellular physiology, it was hypothesized that APP may signal through modulation of levels of PIPs. To address this hypothesis, levels of PIPs were measured in primary cortical cultures by two methods. Firstly, a mass-spectroscopy based method was developed to measure total levels of cellular PIP. No change in total PIP levels upon sAPP $\alpha$  treatment could be detected using this method. Secondly, levels of cell-surface PIPs were determined using an array of anti-PIP biosensors and antibodies. Under resting conditions, only PI(4,5)P<sub>2</sub> was present on the surface of cells. However, in the presence of APP-E1, there was an increase in the level of cell surface PI(3,4,5)P<sub>3</sub> and an increase in the level of PI(4,5)P<sub>2</sub>, indicating that APP binding to cells may result in an increase level of cell surface PIPs.

The data presented in this thesis demonstrate that APP has a novel N-terminal PIP-binding domain. This domain may play a role in the normal function of APP, by facilitating PIP-dependent signalling.

## **Acknowledgements**

Firstly, thank you to my primary supervisor, Prof. David Small for your guidance. Thank you for giving me the freedom to explore my own ideas and also the opportunity to test them. I have learned a lot from your focus on the bigger picture but also your attention to detail. To my secondary supervisors, Dr. Rob Gasperini and Dr. Adele Vincent, thank you for your help over the last three years. Thank you Rob for sharing your expertise and support whenever it was needed. Thank you Adele for your guidance, encouragement, and feedback.

Thank you to Ivy Hu for your assistance with the neurosphere work and also to Dr. Eric Cui for your assistance with the development of the protein-lipid overlay assays. Thank you also to Assoc. Prof. Noel Davies, for your expertise and assistance in the development of the UPLC-MS procedures. I am also grateful to Dr. Adele Vincent and Dr. Dave Klaver for proofreading this thesis.

A big thank you to everyone in the Laboratory of Molecular Neurobiology, who have made the lab a fun place to be, Camilla Mitchell, Lila Landowski, Claire Hadrill, Jenny Smith, Dr. Lisa Foa, Dr. Kaylene Young, Adrian Thompson and everyone past and present. Furthermore, I am grateful to everyone at the Menzies Research Institute Tasmania for their camaraderie, especially those that have lent antibodies to me.

I also thank my family and friends for their encouragement and support. Finally, thank you to my beautiful partner Anna Niehaus for your love and care.

## Publication and presentation record

### *Peer-reviewed publications:*

Hu, Y., Hung, A. C., Cui, H., **Dawkins, E.**, Bolos, M., Foa, L., Young, K. M., and Small, D. H. (2013). Role of cystatin C in amyloid precursor protein-induced proliferation of neural stem/progenitor cells. *J Biol Chem* 288, 18853-18862.

Small, D. H., Hu, Y., Bolos, M., **Dawkins, E.**, Foa, L., and Young, K. M. (2013). Beta-amyloid precursor protein: Function in stem cell development and Alzheimer's disease brain. *Neurodegener Dis* (DOI: 10.1159/000353686)

### *Manuscripts in preparation:*

**Dawkins, E.** and Small, D.H. The N-terminal region of the amyloid precursor protein of Alzheimer's disease binds to PI(4,5)P<sub>2</sub>-rich microdomains on the surface of primary neurons.

**Dawkins, E.** and Small, D.H. Functions of the  $\beta$ -amyloid precursor protein.

### *Conference presentations:*

**Dawkins, E.**, Gasperini, R., Vincent, A.J., Foa, L. and Small, D.H. Analysis of A $\beta$  cell interactions and uptake in hippocampal culture. Oral Presentation; Australian Neuroscience Society Conference, Gold Coast, 2012

**Dawkins, E.**, Vincent, A.J., Gasperini, R., Cui, H., Foa, L., Young, K.M. and Small, D.H. A direct interaction of A $\beta$  with anionic cellular lipids mediates binding to neurons. Poster; ADPD 2013, Florence, Italy

Hu Y., Hung A.C., Cui H., **Dawkins E.**, Foa L., Young K.M. and Small D.H. APP stimulates neural stem/progenitor cell proliferation by increasing cystatin C secretion. Poster; Australian Neuroscience Society Conference, Melbourne, 2013