

Table of Contents

Chapter 1 – Introduction	7
1.1 General Introduction	9
1.2 Cell Biology of Neurons	9
1.2.1 <i>Neuronal cultures as a model for how the brain works</i>	10
1.2.2 <i>Development of neurons in culture</i>	11
1.2.3 <i>Techniques – transfection, staining, and imaging of cultured neurons</i>	12
1.3. Axon Growth, Guidance, and Targeting	13
1.3.1 <i>Growth cones</i>	14
1.3.2 <i>The cytoskeleton and axon growth</i>	14
1.3.3 <i>Directed axon growth and guidance cues</i>	15
1.4. Dendrite Development	16
1.4.1 <i>Dendritic growth and branching</i>	16
1.4.2 <i>Dendritic spines</i>	17
1.5. Formation and Function of Synapses	17
1.5.1 <i>Synaptic cell adhesion molecules</i>	17
1.5.2 <i>Synaptic scaffolding and signaling proteins</i>	18
1.5.3 <i>Synaptic vesicles and receptors</i>	19
1.6. Maintenance and Plasticity of Synapses	20
1.6.1 <i>Protein turnover</i>	20
1.6.2 <i>Modification of synapses in response to activity</i>	21
1.6.3 <i>Learning and memory</i>	21
1.7. Scope of the Thesis	22
 Chapter 2 - The Liprin-α Family of Proteins	 23
2.1. The Liprin- α Family of Proteins	25
2.2. Liprin- α and Presynaptic Development	25
2.3. Liprin- α and Postsynaptic Development	27
2.4. Liprin- α and Neurotransmitter Release	27
2.5. Neuroanatomical Expression Patterns of Liprin- α Proteins	29
2.5.1 <i>Generation and specificity of anti-liprin antibodies</i>	31
2.5.2 <i>Nervous system expression of liprin proteins</i>	31
 Chapter 3 – LAR Controls Axon Growth and Branching via Interaction with Liprin-α and Cortactin	 35
3.1. Introduction	37
3.2. Results	38
3.2.1 <i>LAR-RPTP controls axon growth and branching</i>	38
3.2.2 <i>The LAR intracellular domain is associated with liprin-α, p140Cap, and cortactin</i>	39
3.2.3 <i>LAR and cortactin co-cluster independently of liprin-α in neurons</i>	43
3.2.4 <i>Liprin-α2 and p140Cap are necessary for axon growth</i>	44
3.2.5 <i>Cortactin is responsible for promoting axon branching</i>	46

3.3. Discussion	47
3.3.1 <i>LAR controls MT and axon growth via liprin-α2, p140Cap, and EB3</i>	47
3.3.2 <i>LAR inhibits axon branching through cortactin</i>	47
Chapter 4 – Liprin-α1 Degradation by Calcium/Calmodulin-Dependent Protein Kinase II Regulates LAR Receptor Tyrosine Phosphatase Distribution and Dendrite Development	49
4.1. Introduction	51
4.2. Results	55
4.2.1 <i>Downregulation of liprin-α in hippocampal neurons by CaMKII and proteasome-mediated degradation</i>	55
4.2.2 <i>RNAi knockdown of APC increases liprin-α</i>	56
4.2.3 <i>CaMKIIα/β knockdown by RNAi increases liprin-α in hippocampal neurons</i>	58
4.2.4 <i>Active CaMKII decreases liprin-α1 protein level in COS-7 cells</i>	61
4.2.5 <i>C-terminus and PEST motif are essential for CaMKII dependent liprin-α1 degradation</i>	63
4.2.6 <i>Proteasome is not involved in CaMKIIα dependent liprin-α1 degradation in COS-7 cells</i>	68
4.2.7 <i>Interaction of CaMKII and liprin-α1 in vitro and in vivo</i>	68
4.2.8 <i>Liprin-α1ΔPESTΔC and liprin-α1ΔPEST/S-A inhibit dendrite morphogenesis and reduce synapse density</i>	71
4.2.9 <i>LAR-liprin interfering constructs and LAR shRNA inhibit dendrite morphogenesis</i>	72
4.2.10 <i>Liprin-α1 increases surface expression and clustering of LAR receptors</i>	75
4.2.11 <i>CaMKII-non-degradable liprin-α1 mutants impair dendritic targeting of LAR</i>	77
4.3. Discussion	78
4.3.1 <i>Activity-dependent regulation of liprin-α1 by two mechanisms: CaMKII and proteasome</i>	78
4.3.2 <i>Importance of CaMKII degradation of liprin-α1 for dendrite morphogenesis</i>	80
Chapter 5 – Regulation of Presynaptic Composition and Function by Distinct Liprin-α Family Proteins	83
5.1. Introduction	85
5.2. Results	86
5.2.1 <i>Liprin-α2 is abundant at synapses in the hippocampus</i>	86
5.2.2 <i>Presynaptic localization of liprin-α2 does not depend on other scaffold proteins</i>	87
5.2.3 <i>Liprin-α1 and liprin-α2 differentially regulate presynaptic function</i>	90

5.2.4 <i>Liprin-α1 and liprin-α2 selectively interact with different presynaptic proteins</i>	97
5.2.5 <i>Liprin-α2 recruits CASK to presynaptic boutons</i>	99
5.2.6 <i>Liprin-α1 reduces bassoon and piccolo localization at presynaptic boutons</i>	101
5.2.7 <i>Liprin-α1 competes with bassoon for binding to presynaptic CAST</i>	101
5.2.8 <i>CAST is critical for bassoon localization at membrane structures</i>	102
5.3. Discussion	104
5.3.1 <i>Liprin-α2 is a molecular organizer of hippocampal presynapses</i>	104
5.3.2 <i>Liprin-α1 negatively regulates SV recycling</i>	105
5.3.3 <i>Liprin-α family proteins are not functionally redundant</i>	107
Chapter 6 – Materials and Methods	109
Chapter 7 – Discussion	123
7.1 Liprin- α in Axon Growth, Guidance, and Targeting	125
7.2 Liprin- α in Dendrite Development	127
7.3 Liprin- α in Formation and Function of Synapses	128
7.4 Liprin- α in Maintenance and Plasticity of Synapses	129
7.5 Liprins in the Rest of the Brain – Future Directions	133
References	135
Summary	143
Samenvatting	144
Curriculum Vitae	145
List of Publications	146
Portfolio	147
Acknowledgements	149