DISSERTATIONES
BIOLOGICAE
UNIVERSITATIS
TARTUENSIS
318

KEIU KASK

The role of RIC8A in the development and regulation of mouse nervous system

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
318

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
318

KEIU KASK

The role of RIC8A in the development and regulation of mouse nervous system

UNIVERSITY OF TARTU
Press

Department of Developmental Biology, Institute of Molecular and Cell Biology, University of Tartu, Estonia

The dissertation is accepted for the commencement of the degree of Doctor of Philosophy (in Developmental Biology) on May 11th, 2017 by the Council of the Institute of Molecular and Cell Biology, University of Tartu.

Supervisors: Prof. Margus Pooga, PhD
Department of Developmental Biology
Institute of Molecular and Cell Biology
University of Tartu
23 Riia Street, Tartu, Estonia

Dr. Tambet Tõnissoo, PhD
Department of Developmental Biology
Institute of Molecular and Cell Biology
University of Tartu
23 Riia Street, Tartu, Estonia

Opponent: Prof. David J. Price, PhD
Professor of Developmental Neurobiology
Centre for Integrative Physiology
The University of Edinburgh
Hugh Robson Building
Edinburgh EH8 9XD, United Kingdom

Commencement: Room No 105, 23B Riia Street, Tartu, Estonia at 10:15 on June 22th, 2017

The publication of this dissertation is granted by the Institute of Molecular and Cell Biology, University of Tartu.

ISSN 1024-6479
ISBN 978-9949-77-453-1 (print)
ISBN 978-9949-77-454-8 (pdf)

University of Tartu Press
www.tyk.ee

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I. Ruisu K, **Kask K**, Meier R, Saare M, Raid R, Veraksitš A, Karis A, Tõnissoo T, Pooga M. 2013. Ablation of RIC8A function in mouse neurons leads to a severe neuromuscular phenotype and postnatal death. PLoS One. 8(8):e74031.
doi: 10.1371/journal.pone.0074031

II. **Kask K**, Ruisu K, Tikker L, Karis K, Saare M, Meier R, Karis A, Tõnissoo T, Pooga M. 2015. Deletion of RIC8A in neural precursor cells leads to altered neurogenesis and neonatal lethality of mouse. Dev. Neurobiol. 75(9):984–1002.
doi: 10.1002/dneu.22264

III. Saare M, Lulla S, Tõnissoo T, Meier R, **Kask K**, Ruisu K, Karis A, Salumets A, Pooga M. 2015. Expression pattern and localization dynamics of guanine nucleotide exchange factor RIC8 during mouse oogenesis. PLoS One. 10(6), e0129131.
doi: 10.1371/journal.pone.0129131

IV. Ruisu K, Meier R, Tõnissoo T, **Kask K**, Velling T, Pooga M. RIC8A is essential for the organisation of actin cytoskeleton and cell-matrix interaction.
Experimental Cell Research. Article accepted.

V. **Kask K**, Tikker L, Ruisu K, Lulla S, Oja E-M., Velling T, Meier R, Tõnissoo T, Pooga M. Targeted deletion of RIC8A from mouse neural precursor cells gives rise to defects resembling congenital muscular dystrophies.
Manuscript.

My contributions to the listed articles are as follows:

Ref. I – Performed and analysed a part of the experiments, participated in the manuscript drafting and finalisation

Ref. II – Participated in the design of the study, performed most of the experiments, analysed the data and wrote the manuscript draft.

Ref. III – Performed a part of the experiments and contributed to the compilation and finalisation of the manuscript.

Ref. IV – Contributed to the writing, editing, and finalisation of the manuscript.

Ref. V – Participated in the design of the study, performed most of the experiments, analysed the data and wrote the manuscript draft.

LIST OF ABBREVIATIONS

AC	Adenylyl cyclase
AP2α	Activating enhancer binding Protein 2 alpha
aPKC	Atypical protein kinase C
bIP	Basal intermediate progenitor cells
BM	Basement membrane
CMD	Congenital muscular dystrophy
CNS	Central nervous system
CSF	Cerebrospinal fluid
CXCL12	C-X-C motif chemokine ligand 12
DAG	Diacylglycerole
E	Embryonic day
ECM	Extracellular matrix
ERK	Extracellular regulated MAP kinase
ES	Embryonic stem cells
FAK	Focal adhesion kinase
FCMD	Fukuyama Congenital Muscular Dystrophy
FGF	Fibroblast growth factor
GAP	GTPase-activating protein
GDI	Guanine nucleotide dissociation inhibitor
GEF	Guanine nucleotide exhange factor
GPCR	G-protein coupled receptor
IGF	Insulin-like growth factor
ILK	Integrin linked kinase
Insc.	Inscuteable, adaptor protein
IP_3	Inositol trisphosphate
LGN	Leucine-Glycine-Asparagine
MEB	Muscle-Eye-Brain disease
MEFs	Mouse embryonic fibroblasts
NCCs	Neural crest cells
NE	Neuroepithelial cells
NuMA	Nuclear mitotic apparatus
P	Postembryonic day
Par3	Partitioning defective protein 3
Par6	Partitioning defective protein 6

PDGF	Platelet-derived growth factor
PIP	Phosphoinositides
RIC8	Resistance to Inhibitors of Cholinesterase 8
RG	Radial glial cells
RTK	Receptor tyrosine kinase
SHH	Sonic hedgehog
SVZ	Subventricular zone
VZ	Ventricular zone
WNT	Wingless/Integrated
WWS	Walker-Warburg syndrome

INTRODUCTION

Six-layered neocortex has emerged latest in the evolution of the mammalian brains and is the most expanded part of the nervous system in vertebrates. Neocortex controls nearly all aspects of behaviour, including perception, voluntary movements, cognition, language, and decision-making. Neocortex contains an immense number of neurons that can be broadly divided into two groups, excitatory neurons and inhibitory interneurons. Glutamatergic excitatory neurons comprise the majority (70–80%) of neocortical circuit neurons and are responsible for generating the output. Excitatory neurons are generated in the proliferative ventricular zone of the dorsal telencephalon and migrate radially to constitute the future neocortex. GABAergic inhibitory interneurons are produced in the proliferative zone of the ventral telencephalon and migrate tangentially to reach the neocortex, co-assemble with excitatory neurons and form functional circuits. Defects in those developmental stages lead to several malformations that severely affect mental capabilities and cytoarchitecture of the brain.

To generate neurons and guide their migration to the specific positions, cells must perceive and adequately respond to the changes in their surrounding environment. Proper interaction and communication between the cells is the key to the development and functioning of a multicellular organism. The seven-transmembrane domain G-protein coupled receptors (GPCRs) represent the most widely used system to transmit information across the cell membrane. Via coupling of such receptors to heterotrimeric G proteins and by the help of accessory proteins, numerous effectors can be activated. A chaperone and a non-canonical guanine nucleotide exchange factor RIC8A is a highly conserved protein that interacts with a subset of Gα subunits. RIC8A has been reported in different model organisms to participate in the control of mitotic cell division, cell signalling, cell migration and development. In the mammalian nervous system, RIC8A is expressed at the high level in the developing nervous system and in adult brain regions involved in the regulation of memory and emotional behaviour, which manifest as anxiety and impaired memory in the mice heterozygous for the *Ric8a* allele. However, the homozygous $Ric8a^{-/-}$ embryos die at E6.5 – E8.5 due to a gastrulation defects, hence, the function of RIC8A in the mammalian nervous system has not been sufficiently analysed.

The main goal of this thesis is to analyse the role of RIC8A in the development and function of the mammalian nervous system. Two different conditional knockout mice models were generated where *Ric8a* was specifically deleted from the differentiated neurons and from the neural precursor cells. The ablation of RIC8A function in either cell type resulted in severe neuromuscular phenotype of mice. Additionally, the deficiency of RIC8A in neural precursor cells led to a type II lissencephaly-like defect with characteristic malformations in the brain, eyes, skeletal muscle and heart. The underlying causes for these deformities are thoroughly examined in this dissertation.

REVIEW OF LITERATURE

1. Development of the mouse neocortex

During the development of the neocortex, a limited number of neural stem cells give rise to a vast array of neurons and macroglial cells. Prior to the neurogenesis the neural plate and neural tube consist of a pseudostratified neuroepithelium. All neurons of the mammalian neocortex originate from neuroepithelial cells (NE) that are apico-basally polarised multipotent neural progenitor cells (Götz and Huttner, 2005). NE cells show typical epithelial features: they are connected to each other by adherens junctions and tight junctions at the most apical end of the lateral plasma membrane; and they are attached to the pial basement membrane (BM) with integrins and α-dystroglycan, which are concentrated at the basal plasma membrane (AakuSaraste et al., 1996; Wodarz and Huttner, 2003). In concert with the mitotic cell cycle, NE cells undergo interkinetic nuclear migration where nuclei shift between the basal side (S phase) and the apical side (M phase), giving neuroepithelium a pseudostratified appearance (Götz and Huttner, 2005). Before active neurogenesis, the NE cells undergo several symmetric self-amplicative divisions in the ventricular zone (VZ) to expand their progenitor population (Miyata et al., 2010). With the onset of neurogenesis (at about E9.0 in mouse) the activation of Notch and fibroblast growth factor (FGF) pathway drive the NE cells to reveal the features typical to glial cells and lose tight junctions to become the radial glial cells (RG) (AakuSaraste et al., 1996; Hatakeyama et al., 2014; Sahara and O'Leary, 2009). RG cells are also apico-basally oriented, undergoing interkinetic nuclear migration and contribute to RG cell self-renewal but they are more fate restricted neural progenitor cells compared with NE cells (Anthony et al., 2004; Noctor et al., 2002). Most of the projection neurons form directly or indirectly through RG cell divisions (Anthony et al., 2004; Malatesta et al., 2000). Direct neurogenesis yields a neuron immediately after RG cell division producing two daughter cells with the same fate (Miyata et al., 2001; Noctor et al., 2004). Indirect neurogenesis is accomplished through asymmetric cell division where dividing RG cell gives two daughter cells with different fates: one daughter cell self-renews itself and the other loses its RG cell identity and becomes multipolar basal intermediate progenitor cell (bIP) (Miyata et al., 2001; Noctor et al., 2004). bIP cells translocate to the basal part of the VZ forming subventricular zone (SVZ) where they undergo subsequent symmetric division to produce neurons, thereby expanding the neurogenic output (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004) (Figure 1). Neuronal diversity and output are also increased using other less abundant progenitor cells that populate the mouse embryonic cortex, such as short apical intermediate progenitors (aIPs), subapical progenitors (SAPs), basal radial glial cells (bRGs), which share similarities with bIP or RG cells but differ in cell cycle kinetics and locations in the VZ and SVZ (Pilz et al., 2013; Shitamukai et al., 2011; Stancik et al., 2010; Tyler and Haydar, 2013; Wang et al., 2011b).

Upon exiting the cell cycle, newborn excitatory neurons need to migrate out of the VZ into the cortical plate, where they in response to environmental signals position themselves to appropriate layers (Hatten, 2002; Marin and Rubenstein, 2003). Processes of the RG cells provide the necessary substrate and guide to radially migrating neurons (Nadarajah et al., 2003; Nadarajah et al., 2001). There are two distinct modes for postmitotic neurons to migrate radially: somal translocation and locomotion (Nadarajah et al., 2003; Nadarajah et al., 2001). In early neocortical development, the principal mode of neuronal migration is the somal translocation, in which neurons have a long radial process attached to the pial surface and move their cell soma toward the leading edge of all (Gupta, 2002). At later stages, as the cerebral cortex grows bigger, the distance between the ventricular zone (VZ) and the marginal zone (MZ) increases, neurons predominantly start migrating using locomotion, where they use the radial processes of RG cells as a scaffold to reach their final positions (Gupta et al., 2002; Tan and Shi, 2013). The neocortical layers of II–VI are generated in an „inside-out" manner, meaning that neurons generated earlier reside in the deeper layers, whereas later-born neurons migrate past the existing neurons to occupy more superficial layers (Hatten, 1999; Nadarajah et al., 2001).

The proper arrangement of cortical plate neurons in an inside-out manner depends on the function of *Reelin* expressed by a unique group of cells in the marginal zone, the Cajal-Retzius cells (Soriano and del Rio, 2005). Cajal-Retzius cells originate from several sources outside the neocortex such as cortical hem, ventral pallium and septum (Bielle et al., 2005; Yoshida et al., 2006; Zhao et al., 2006). At the onset of neurogenesis, Cajal-Retzius cells migrate tangentially to populate developing neocortex to help future neurons to migrate to their appropriate layers (Magdaleno et al., 2002). Later in development GABAergic interneurons generated in distinct regions of the ventral telencephalon also migrate tangentially to enter the developing cortex (Jimenez et al., 2002). Interneurons invade the neocortex after their partners, excitatory neurons have reached their location, reflecting the possible requirement for signals from appropriately located excitatory neurons (Tan and Shi, 2013). As neurogenesis proceeds, the VZ shrinks and it is finally replaced by a single layer of ependymal cells that line the lateral ventricles (Kriegstein et al., 2006).

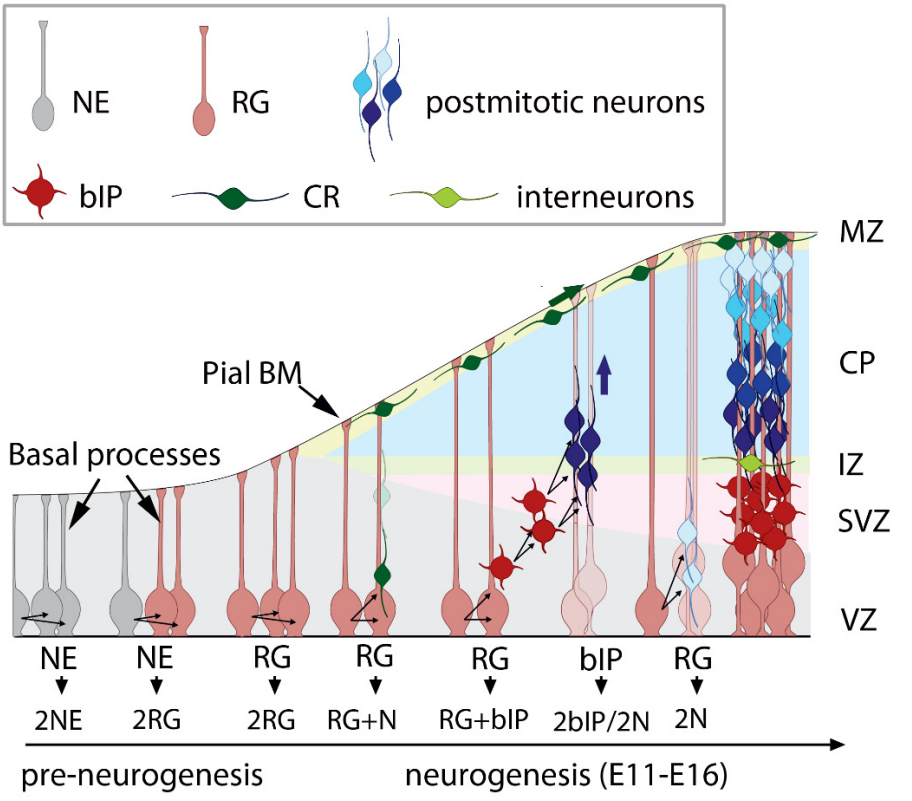

Figure 1. Neural progenitor cells and the phases of neurogenesis in mice. A limited number of neuroepithelial cells (NE) self-renew by symmetric divisions in the ventricular zone (VZ), then convert into radial glial cells (RG) to give rise to a high number of diverse neural cell types through asymmetric cell divisions. Cajal-Retzius cells (CR) migrate tangentially from ventral telencephalon to the marginal zone (MZ) to guide radial migration in neocortex. Intermediate progenitor cells (bIP) that are produced through asymmetric cell division populate the subventricular zone (SVZ). Neurons migrate along the basal processes of RG cells through the intermediate zone (IZ) to populate cortical plate (CP) from where they migrate towards their destined layer. During the radial migration, interneurons generated in the ganglionic eminences migrate tangentially in IZ and contribute to the neocortical layer formation. At later stages, RG cells undergo their final division generating symmetrically two neurons (N). Modified from (Jiang and Nardelli, 2016).

2. Cell division in mouse neurogenesis

2.1. Cell polarity in asymmetric cell division

Asymmetric cell division and the establishment of cell polarity are essential processes generating a vast variety of neuronal cell types. In order to establish the polarity and correctly locate the cell-fate determinants, the orientation of the division in animal cells requires complex coordination of external and internal cues, including signalling pathways, scaffold proteins and the mitotic spindle apparatus (Taverna et al., 2014). An axis of polarity is established in the mother cell and coordinated with the asymmetrically located fate determinants, membrane compartments and spindle orientation to create two daughter cells containing different amounts of these determinants (Götz and Huttner, 2005; Sanada and Tsai, 2005). For example, since NE and RG cells are highly polarized, their apical compartment is composed of the apical plasma membrane, the primary cilium, centrosomes and the junctional belt and it substantially differs from the basolateral compartment and the basal endfeet that are attached to the basal lamina (Kosodo et al., 2004; Paridaen et al., 2013; Peyre et al., 2011; Peyre and Morin, 2012; Tong et al., 2014).

The apical plasma membrane is in close contact with the lumen of the ventricles and mediates the signals communicated by the cerebrospinal fluid (CSF) such as IGF, SHH and WNT (Johansson, 2014; Lehtinen et al., 2011). These signals are received by primary cilium, an organelle protruding from the apical plasma membrane into the lumen of the ventricle (Arellano et al., 2012; Tong et al., 2014). When the function of primary cilium is interfered, the circulation of the cerebrospinal fluid is impaired, which, in turn, affects neurogenesis and brain homoeostasis (Boutin et al., 2014; Tong et al., 2014). The primary cilium is directly linked to the centrosome at the base of the cilium as its basal body, which forms the poles of the mitotic spindle during mitosis and after centriole duplication. Centrosomes are always asymmetrically inherited by the daughter cells because, with the self-renewing, RG cell retains the mother centriole and the differentiating cell receives the daughter centriole (Paridaen et al., 2013; Wang et al., 2009) (Figure 2).

In addition, the unequal distribution of the entire apical plasma membrane is important in generating asymmetric cell fate in daughter cells, even if the majority of divisions in the VZ occur in a planar manner (Kosodo et al., 2004). The apical membrane also embeds the cell polarity determinants such as Par3, Par6, aPKC, which are dynamically distributed in the apical membrane (Costa et al., 2008; Imai et al., 2006; Kosodo et al., 2004; Manabe et al., 2002). In RG cells the Par-complex proteins localise only in the apical endfoot and are segregated equally at the early divisions but exhibit different inheritance in later divisions (Farkas and Huttner, 2008; Manabe et al., 2002). During interphase, Par3 is localised to the lateral membrane of the ventricular endfeet, during mitosis it becomes dispersed in the apical compartment which allows Par3 protein asymmetric inheritance and distinct daughter cell fate specification by

the unequal activation of Notch signalling (Bultje et al., 2009). The daughter cell that inherits a higher amount of Par3 protein develops higher Notch signalling activity and undergoes self-renewal, whereas the daughter cell receiving less Par3 and possessing lower Notch activity acquires either neuronal or bIP fate (Bultje et al., 2009). The apical membrane contains apical junctional complexes that have crucial roles in establishing and maintaining cell polarity and cell fate. The junctions govern the association neighbouring NE and RG cells and are required for maintaining the proper tissue architecture (Aaku-Saraste et al., 1996). Adherens junctions are comprised of three membrane domains (Par-3/aPKC apically, ZO-1 and Afadin centrally, N-cadherin/β-catenin basally). During asymmetric cell division, these domains are split so that both daughter cells retain the adhesive proteins that control the cell positioning, but only one of them inherits the polarity proteins along with the apical membrane (Marthiens and Ffrench-Constant, 2009). The localisation of proteins controlling the cell polarity is regulated by the small GTPases Cdc42, RhoA, and Rac1 which are concentrated at the apical cell cortex (Cappello et al., 2006; Cappello et al., 2012). The main function of Cdc42 in mammalian neurogenesis is to activate the Par complex in order to maintain the adherens junctions coupling and progenitor cell fate. Deletion of Cdc42 caused the conversion of apical progenitors to basal SVZ progenitor cells that had also acquired the SVZ characteristic fate determinants (Cappello et al., 2006). Rac1 is required for maintaining the cell proliferation, in the absence of Rac1 cells undergo early differentiation leading to a smaller brain size (Chen et al., 2009; Leone et al., 2010). Loss of RhoA in neural progenitor cells causes the disruption of adherens junction and hyperproliferation (Katayama et al., 2011). RhoA plays an important role in maintaining the balance between actin and tubulin cyto-skeleton which regulates apical and basal anchoring and proliferation of progenitor cells (Cappello et al., 2012).

On the opposite side of the apical junctions lies the basolateral plasma membrane which forms the majority of the NE and RG cells plasma membrane. Basolateral plasma membrane surrounds the nucleus and elongates through the neuronal layers attaching to the basal lamina by the endfoot (Miyata et al., 2001; Miyata et al., 2004). The basal process is recognised as an active sub-cellular compartment involved in signalling and fate specification. During NE cells proliferation, when the neuroepithelium is relatively thin, the basal processes are bisected and inherited equally between the daughter cells (Kosodo and Huttner, 2009). During RG cell divisions and active neurogenesis, the basal process is asymmetrically inherited. During mitosis, the daughter cell inheriting the basal process often maintains its proliferative capacities (Konno et al., 2008; Miyata et al., 2001) (Figure 2). The endfoot of the basal process makes a direct contact with the basement membrane and is able to receive signals generated by the basal lamina and meninges which has an important role in the establishment of the epithelial cell polarization and the generation of differentiated cells (Halfter et al., 2002; Li et al., 2003; Zarbalis et al., 2007). A critical receptor is the GPR56 that localizes to basal endfeet and associates with extracellular

matrix (ECM) components in the basal lamina, such as collagen III, and that promotes proliferation of radial glial cells (Jeong et al., 2013; Singer et al., 2013; Zarbalis et al., 2007). Mutations of *Foxc1* which reduces retinoic acid production by cells in the dorsal meninges, delay the onset of neurogenesis and asymmetric cell division (Siegenthaler et al., 2009).

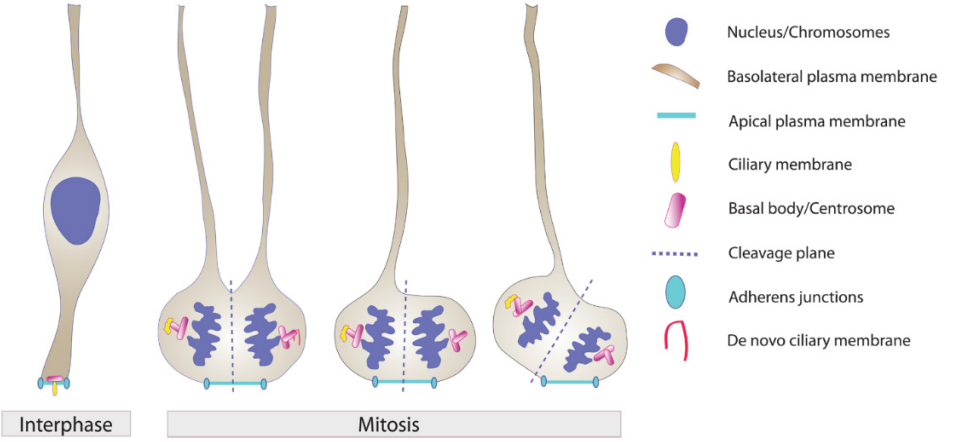

Figure 2. Asymmetric polarity in apical neural progenitor cell division. In NE and RG cells the apical polarity cues are presented by the apical plasma membrane, the adherens junctions, centrosome and ciliary membrane. The basolateral compartment contains the plasma membrane around the nucleus and the basal process. These cues can be divided symmetrically or asymmetrically which determine the cleavage plane and the fate of the daughter cells.

2.2. Spindle orientation in asymmetric cell division

The generation of multiple neurons and secondary progenitor cells from RG cells is tightly controlled by orientation of the mitotic spindle during cell division, which influences the acquisition of asymmetric cell fate determinants and apical/basolateral membrane compartment between cortical progenitors (Huttner and Kosodo, 2005; Peyre and Morin, 2012; Shitamukai and Matsuzaki, 2012). Daughter cells must be properly positioned in order to maintain the tissue structure and to contribute to tissue morphogenesis. In the mouse neurogenesis the RG cells divide mainly in a planar manner with the horizontal orientation of spindle but also exhibit oblique and vertical divisions that are suggested to be required for the bIP cell production (Konno et al., 2008; Morin et al., 2007; Peyre et al., 2011; Postiglione et al., 2011).

The mitotic spindle is formed during the prophase when the duplicated centrosomes nucleate spindle microtubules to position the chromosomes (Lancaster and Knoblich, 2012). Then, the astral microtubules elongate from the centrosomes and are fixed by capture at the plasma membrane to position the spindle (Lancaster and Knoblich, 2012). Numerous studies in different tissues in

invertebrate and vertebrate species have shown that an evolutionarily conserved complex, composed of the heterotrimeric G protein GDP-bound $G\alpha_i$ subunit, LGN (Leucine-Glycine-Asparagine) protein and nuclear mitotic apparatus (NuMA) molecules associate strongly with the spindle pole (Buchman and Tsai, 2007; Du and Macara, 2004; Du et al., 2001; Konno et al., 2008; Morin et al., 2007; Peyre et al., 2011; Schaefer and Knoblich, 2001; Schaefer et al., 2001; Zheng et al., 2013). During mitosis, $G\alpha_i$:GDP-LGN-NuMA complex localises to particular sites of basolateral membrane cortex and directs the recruitment of the minus-end-directed microtubule motor protein dynein/dynactin complex (Couwenbergs et al., 2007; Peyre et al., 2011; Zheng et al., 2013). This directed movement of dynein/dynactin complex along cortically anchored astral microtubules generates pulling forces on the spindle poles that leads to the positioning of the spindle (Siller and Doe, 2009) (Figure 3).

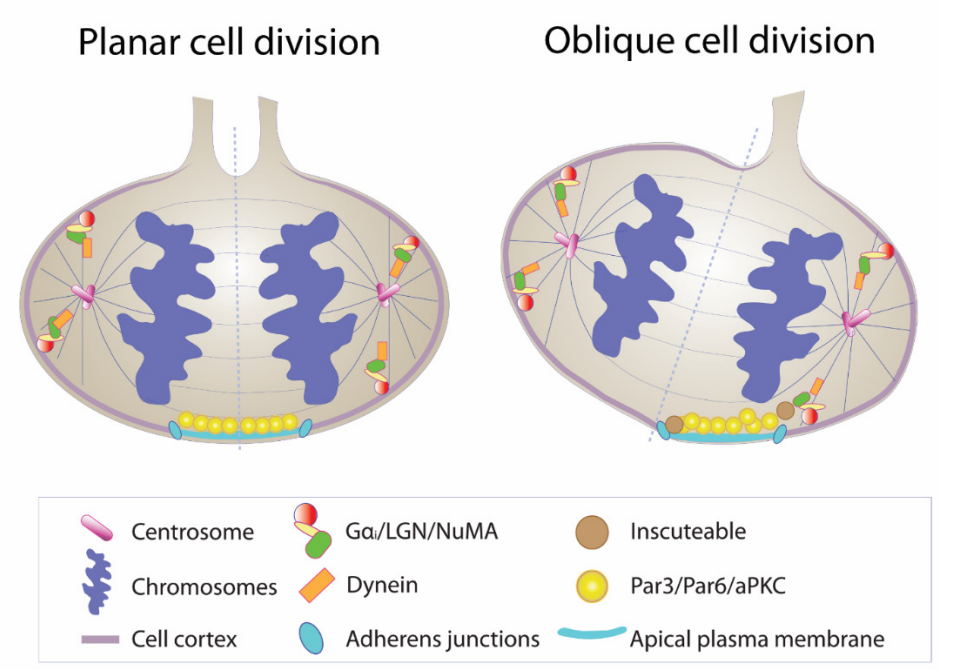

Figure 3. Spindle orientation during the planar and oblique cell division in mammalian neurogenesis. During early neurogenesis, the majority of the divisions occur in a planar manner that segregates equally apical (Par3/Par6/aPKC; apical plasma membrane) and basal compartments (basolateral membrane, basal process). Astral microtubules are nucleated to the centrosomes and are recruited to the cortex by LGN/NuMA/Gα complex. This directs the minus-end-directed microtubule motor protein dynein to move towards the centrosomes which generates pulling forces on the spindle poles. During oblique cell divisions, apical and basal compartments are segregated unequally: the self-renewing daughter cell inherits the majority of the Par-complex proteins, apical plasma membrane and the basal process, also Inscuteable promotes oblique cell division since it competes with LGN over the interaction of NuMA that is associated with spindles.

The polarity proteins Par3:Par6:aPKC promote the recruitment of $G\alpha_i$:GDP-LGN-NuMA complex via an adaptor molecule known as Inscuteable (Insc) that is shown to promote oblique and vertical divisions in the cortex (Postiglione et al., 2011; Williams et al., 2011). When mInsc is present, the communication between LGN and the spindle via NuMA is disrupted by competition with mInsc (Mapelli and Gonzalez, 2012; Zhu et al., 2011) (Figure 3). This competitive behaviour is important for the asymmetric cell division since LGN presence favours more planar spindle orientation and mInsc shifts the spindle towards the oblique orientation (Konno et al., 2008; Postiglione et al., 2011). In addition, interaction with NuMA is necessary to switch LGN to an open conformation that increases its ability to bind the $G\alpha_i$ subunits (Du and Macara, 2004). LGN is initially recruited all around the cell cortex but its localisation is restricted to two cortical crescents facing the spindle poles during metaphase and anaphase (Kiyomitsu and Cheeseman, 2012). During interphase, NuMA localises to the nucleus and after nuclear envelope breakdown, it needs to be phosphorylated by CDK1 (cyclin dependent kinase-1) (Du and Macara, 2004; Kotak et al., 2013). Then, the anaphase-specific cortical recruitment is accomplished through the interaction between phosphoinositides PIP/PIP2 and NuMA (Kotak et al., 2013, 2014). Thus, the formation of the $G\alpha_i$/LGN/NuMA cortical complex is restricted to the cortex only in mitosis (Du and Macara, 2004; Kotak et al., 2013, 2014). Increased cortical levels of NuMA in anaphase drive the recruitment of additional dynein into the cortex which is important for spindle elongation and chromosome separation (Kotak et al., 2013).

Recent studies have shown that in parallel to $G\alpha_i$/LGN/NuMA complex the intact cortex is required for the correct localisation of the spindle orientation machinery and for the stabilisation of force generators. Almost all animal cells become round or spherical as they enter mitosis which requires profound changes in cell organisation (Lancaster and Baum, 2014). Cytoskeletal remodelling begins in prophase when interphase microtubules are disassembled and a new population of shorter, more dynamic microtubules are nucleated from centrosomes (Niethammer et al., 2007). When nuclear envelope breaks down, the plus ends of centrosome-nucleated microtubules establish contacts with chromosomes at kinetochores (Lancaster and Baum, 2014). Microtubule nucleation and dynamics regulate the number of microtubules reaching the cortex, these microtubules need to establish proper contacts with the cortex and force generators (Lancaster and Baum, 2014). While cells round up and nucleate microtubules, they remain connected to the adhesive substrate through retraction fibres which are cytoplasmic extensions filled with actin filaments. These retraction fibres have been proposed to recruit polarising factors to the cell cortex, leading to spindle orientation (Fink et al., 2011; Thery et al., 2007). Moreover, the previous study has shown that amorphous clusters or membrane ruffles composed of actin filaments are formed during early prometaphase, which revolves along the cell cortex concentrating near the retraction fibres and disappear into the contractile ring upon cytokinesis (Kunda et al., 2008; Mitsushima et al., 2010). The cortical regions with attached retraction fibres

organise the adjacent cytoplasm by controlling a dynamic subcortical actin network which in turn concentrates force-generating molecules on astral microtubules (Fink et al., 2011; Kwon et al., 2015; Mitsushima et al., 2010). Microtubule binding protein Myosin-10 is required for the spindle orientation by modulating microtubule dynamics towards the polarised actin clusters and retraction fibres (Kwon et al., 2015). Myosin-10 mediated spindle positioning is acting in parallel and independently of dynein/LGN mechanism since combined depletion of myosin-10 and LGN resulted in randomised spindle orientation whereas depleting each complex individually did not impair dynein cortical localisation or Myosin-10 cortical distribution, respectively (Kwon et al., 2015).

Defects in astral microtubule stability also affect spindle orientation. Most of the studies on astral microtubules have been performed in cultured cells, but recent evidence *in vivo* has demonstrated that two different astral microtubule subpopulations regulate spindle orientation and thus proliferative or neurogenic divisions (Mora-Bermudez et al., 2014). In proliferating NE cells, there are more astral microtubules that reach the apical and basal cell cortex that collectively help to stabilise the cell shape and anchor the spindle to the cortex, which promotes symmetric divisions (Mora-Bermudez et al., 2014). In contrary, in RG cells that undergo neurogenic divisions the number of astral microtubules that reach the apical and basal cortex decreases and cells are more sensitive to other intra- and extracellular forces that can induce tilt in cleavage plane (Mora-Bermudez et al., 2014).

Intrinsic actions in cells are activated mostly by the extracellular stimuli. Integrins are transmembrane receptors that interact with extracellular matrix proteins and upon binding undergo a conformational change that induces the recruitment of integrin-interacting partners to the cortex which in turn activates a variety of processes, like cell survival, migration and proliferation. β1 integrins are implicated in regulating the mitotic spindle orientation relative to the substratum and sensing the extracellular matrix so that the cell can divide parallel to the substratum (Morris et al., 2015). The absence of β1 integrin signalling disrupts the epithelial cell polarity and correct apical localisation of the LGN complex, thus randomising the spindle orientation (Lechler and Fuchs, 2005). In addition, the direct interaction between the integrin-linked kinase (ILK) and dynactin-2 links integrins to the dynein complex independent of $G\alpha_i$/LGN/NuMA complex and controls the position of the force generators. For example, when the integrin and ILK signalling were blocked in intestinal epithelial cells, the spindle orientation was more random, which influenced the gross morphology of the bowel (Morris et al., 2015). Thus, the cell-shape-sensing mechanism contributes to the default planar orientation independently from cortical force generators (Morris et al., 2015). Also, integrin/laminin interactions are necessary for maintaining the stem cells at the apical VZ surface within their niche and preserving the architecture of the VZ (Loulier et al., 2009). After blocking the interaction between the β1 integrin and laminin α2, the apical progenitors detached from the ventricular surface. Also, divisions along the oblique and horizontal cleavage planes exhibited mostly planar divisions instead, which

suggests different outcomes and pathways acting through integrin signalling (Loulier et al., 2009).

3. The meninges in neurogenesis

Telencephalic development is accompanied by the concomitant development of meninges which comprise the layers surrounding the central nervous system: the dura mater, arachnoid mater and pia mater (last two are also considered together as leptomeninges) (Decimo et al., 2012; Radakovits et al., 2009). The meninges gives physical protection to the brain parenchyma by covering it with thick layering and by enabling circulation of cerebrospinal fluid (CSF) around the central nervous system, which cushions the brain in case of rapid movements (Nakagomi et al., 2015; Siegenthaler and Pleasure, 2011). The essentiality of pia mater lies in production and organisation of the BM covering the brain and it allows the blood vessels to traverse and nourish the cerebral cortex (Radakovits et al., 2009). The arachnoid mater is in contact with pia mater through arachnoid trabeculae which span the subarachnoid space and enable the CSF circulation (Decimo et al., 2012; Saboori and Sadegh, 2015). The dura mater is the outermost part of the meninges and is essential for the skull development (Siegenthaler and Pleasure, 2011).

Development of the meninges in mouse starts at about E9 – E10 (Siegenthaler and Pleasure, 2011). Meningeal layers need the contribution of cephalic neural crest cell (NCC) to their development since surgical removal of NCCs from posterior diencephalon, mesencephalon and rhombencephalon leads to the activation of massive cell death within the forebrain neuroepithelium (Decimo et al., 2012; Etchevers et al., 1999; Inoue et al., 2008). Cephalic NCCs altogether contribute to the development of the facial skeleton and overlying dermis and to the development of forebrain leptomeninges, the rest of the meninges in the central nervous system are entirely of mesodermal origin (Etchevers et al., 1999; Siegenthaler and Pleasure, 2011; Zarbalis et al., 2007). Thus, the presence of NCC-derived mesenchyme is necessary for the growth and survival of the telencephalic neuroepithelium and the paraxial mesodermal population near the prosencephalon is not capable of forming forebrain meninges on its own (Etchevers et al., 1999).

Little is known about the meningeal development, but few studies have revealed that the loss of presenilin-1 or transcription factor *Foxc1* disrupts the formation of forebrain meninges which accelerates the cortical BM breakdown, mislocalisation of Cajal-Retzius cells and formation of cortical dysplasias (Hartmann et al., 1999; Hecht et al., 2010; Zarbalis et al., 2007). Furthermore, recent evidence has revealed that in addition to the protective role of the meninges, they secrete several trophic factors that regulate the proliferative and migratory behaviour of neural progenitor cells (Bifari et al., 2015; Borrell and Marin, 2006; Siegenthaler et al., 2009). For example, meninges serve as a necessary substrate for the tangential spread of Cajal-Retzius cells by expressing

chemoattractive CXCL12 (Borrell and Marin, 2006; Zarbalis et al., 2007). Also, mice that fail to form complete forebrain meninges have major defects in the switch to neurogenic radial expansion due to a loss of meninges derived retinoic acid which leads to a prolonged NE cell stage and symmetric division (Siegenthaler et al., 2009).

Meningeal fibroblasts produce the key components of the extracellular matrix (ECM): laminins, collagens and nidogen that form the pial BM covering the developing neocortex (Erickson and Couchman, 2000; Siegenthaler and Pleasure, 2011). Lots of effort has been invested to the pial BM interaction with the RG cell endfeet. The pial BM and RG cell interact through transmembrane receptors, such as integrins and dystroglycan, on RG cell endfeet. Alterations in the pial BM composition and in the function of ECM-associated proteins including laminin γ1 chain, perlecan, and collagen type III, result in cortical lamination defects, accompanied by the fragility of the pial BM and detachment of the RG cell endfeet from the BM. Moreover, mutations in genes encoding cell-surface receptors for BM (β1 and α6 integrins, α-dystroglycan and GPR56), disrupt normal deposition of cortical BM and result in a disorganized type II lissencephaly-like cortex (Beggs et al., 2003; Cappello et al., 2012; Costell et al., 1999; De Arcangelis et al., 1999; Georges-Labouesse et al., 1998; Graus-Porta et al., 2001; Halfter et al., 2002; Jeong et al., 2013; Li et al., 2008; Luo et al., 2011; Moers et al., 2008; Moore et al., 2002; Niewmierzycka et al., 2005).

4. Congenital muscular dystrophies

Abnormalities in aforementioned events can cause severe neuronal defects and are associated with various diseases like lissencephaly, microcephaly, polymicrogyria, different heterotopias and epilepsy (Manzini and Walsh, 2011; Noatynska et al., 2012; Olson and Walsh, 2002).

Cobblestone lissencephaly (type II lissencephaly) is a neuronal over-migration defect where neurons and glial cells migrate through the breaches of the superficial pial BM (Olson and Walsh, 2002). It is often associated with autosomal recessive disorders like Fukuyama congenital muscle dystrophy (FCMD), Walker-Warburg syndrome (WWS) and muscle-eye-brain disease (MEB) that negatively affect skeletal muscle, central nervous system (CNS) and the development of the eyes (Barkovich et al., 2012; Devisme et al., 2012; Olson and Walsh, 2002). These syndromes are characterised by CNS symptoms such as type II lissencephaly, enlarged lateral ventricles, meningeal thickening and hydrocephalus (Bouchet et al., 2007; Brasseur-Daudruy et al., 2012; Hartmann et al., 1999; Hehr et al., 2007; Lach and Arredondo, 2013; Nabi et al., 2003; Pabuscu et al., 2003; Saito, 2006; Yoshioka and Higuchi, 2005; Yoshioka et al., 2008). In addition to brain defects, several ocular malformations and neuromuscular innervation defects characterised by lower-limb stiffness and muscle fibre atrophy have been reported (Belpaire-Dethiou et al., 1999; Nabi et al., 2003; Pabuscu et al., 2003). Also, heart and kidney defects have been reported

in some of the FCMD, WWS or MEB patients (Devisme et al., 2012). FCMD patients survive beyond infancy, ocular manifestations are rare and usually mild. Patients with WWS are severely affected from birth, and only a few live beyond infancy. In MEB, the cerebral and ocular anomalies are severe, but some patients reach adulthood. Although FCMD is frequent only in Japan, WWS has been found in many different nationalities, and MEB has been observed mainly in Finland (Silan et al., 2003).

Several studies have implicated that proteins and enzymes that are involved in glycosylation of dystroglycan cause these disorders (Grewal and Hewitt, 2003; Miyata et al., 2004; Saito et al., 2007; Satz et al., 2010; Takeda et al., 2003; van Reeuwijk et al., 2005a; van Reeuwijk et al., 2005b; Yamamoto et al., 2004). Abnormally modified α-dystroglycan is deficient in binding to extracellular matrix ligands, including laminin and agrin (Grewal and Hewitt, 2003). WWS and MEB are associated with the mutations in two genes involved in O-mannosylation, POMT1 and POMGnT1; *Fukutin* mutations are associated with FCMD (Grewal and Hewitt, 2003; Takeda et al., 2003). Despite the intensive research and genetic screening of genes involved in glycosylation of α-dystroglycan, about half of the cases remain unexplained suggesting that other genes and/or signaling pathways may be involved (Belpaire-Dethiou et al., 1999; Cormand et al., 2001; Devisme et al., 2012; Manzini et al., 2008; Vajsar and Schachter, 2006).

Integrins represent a parallel system to the dystrophin-glycoprotein complex by which the cytoskeleton is linked to the extracellular matrix. Therefore, it is possible that the signalling pathways triggered by laminin receptors (integrins and dystroglycan) are essential for BM integrity and may underlie the pathologies of these disorders. Affecting the cell signalling via integrin-mediated pathway – integrin linked kinase (Ilk), Focal Adhesion Kinase (FAK), small GTPase RhoA, G protein-coupled receptor GPR56 and G proteins $G\alpha_{12}/G\alpha_{13}$) – in the developing cerebral cortex results in type II lissencephaly (Beggs et al., 2003; Cappello et al., 2012; Jeong et al., 2013; Moers et al., 2008; Niewmierzycka et al., 2005). Few of these studies with neural precursor specific mouse models have implicated also neuromuscular disorders (Beggs et al., 2003; Niewmierzycka et al., 2005) and strong resemblance of described congenital muscular dystrophies (CMD).

5. G-Proteins

To generate neurons and guide their migration to the specific positions, cells must perceive and correctly respond to the changes in their surrounding environment. To accomplish this, cells contain receptors for chemical and physical signals and intracellular signalling molecules among which the G-proteins are one of the most prominent families. Heterotrimeric G-protein mediated signal transduction is a complex and very versatile transmembrane signalling system involving hundreds of different receptors and multiple

G-proteins and effectors. Heterotrimeric G-proteins are composed of α, β and γ subunits where β and γ are tightly associated and considered as one functional unit (Gilman, 1987; Neer, 1995). The βγ-dimer and the guanosine diphosphate (GDP) bound α-subunit are associated at the inner side of the plasma membrane, and the heterotrimer can be recognised by an appropriately activated receptor (Wettschureck and Offermanns, 2005). To dynamically couple activated receptors to effectors, the heterotrimeric G-protein undergoes activation-inactivation cycle (Bastiani and Mendel, 2006; Wettschureck and Offermanns, 2005).

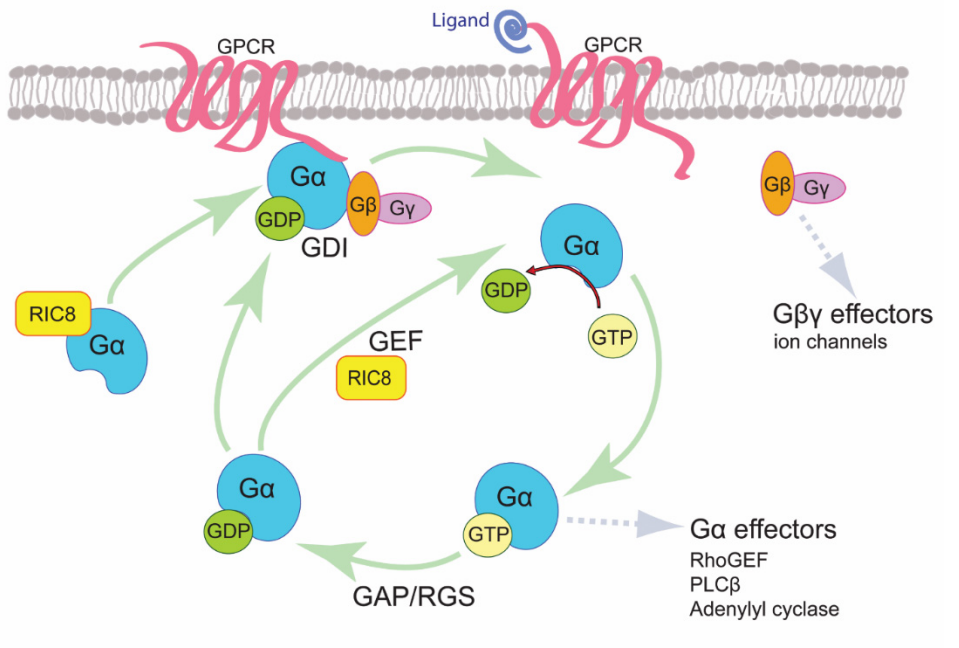

Figure 4. A classical model of the G protein signalling regulation. Heterotrimeric GDP-bound Gαβγ is associated with the transmembrane G-protein coupled receptor (GPCR). GDP-bound G-proteins are in an inactive state and the spontaneous release of GDP is inhibited by the GDI (guanine nucleotide dissociation inhibitor). The signalling of G-protein is activated by the ligand binding to the GPCR which changes the conformation of GPCR and the exchange of GDP from Gα subunit with GTP which dissociates the Gαβγ to Gα subunit and Gβγ dimer. Released functional subunits are then in an active state and can participate in downstream interactions with various cellular effectors. Gα subunit's intrinsic GTPase activity or regulator of G-protein signalling (RGS) proteins that act as GTPase-activating proteins (GAP) terminate the activity of Gα by hydrolysing the bound GTP to GDP. Inactivated Gα:GDP reassociates again with the Gβγ dimer or is activated in a receptor-independent fashion via guanine nucleotide exchange factors (GEF). RIC8 acts as a receptor-independent GEF to monomeric Gα subunits, it is also necessary for Gα plasma membrane localisation.

The classical G-protein cycle is activated by the binding of a ligand, ranging from photons to hormones and neurotransmitters, to the transmembrane G-protein coupled receptor (GPCR) (Wettschureck and Offermanns, 2005). This interaction rearranges the conformation of the GPCR so that it acts as a guanine nucleotide exchange factor (GEF) triggering the exchange of guanosine diphosphate (GDP), bound to the Gα subunit, with guanosine triphosphate (GTP) and the dissociation of Gα subunit from Gβγ dimer (Bastiani and Mendel, 2006; Siderovski and Willard, 2005). Both released functional subunits are then in an active state and can participate in further interactions with various cellular effectors (Neves et al., 2002). The signalling of the Gα subunit is terminated by the intrinsic GTPase activity of Gα, which hydrolyses the bound GTP to GDP and inactivated Gα:GDP reassociates the Gβγ dimer (Bastiani and Mendel, 2006; Neer, 1995). In addition to GPCRs, other proteins also regulate the activity of the heterotrimeric G-proteins such as GEFs, regulators of G-protein signalling (RGS), guanine nucleotide dissociation inhibitors (GDIs), GTPase-activating proteins (GAPs), and βγ-interacting proteins (Sato et al., 2006) (Figure 4). The downstream effectors activated by G-proteins interact with one another to form a network that regulates metabolic enzymes, ion channels, transporters, and other components controlling processes like transcription, motility, contractility and secretion which in turn regulate systemic functions such as embryonic development, gonad development, learning and memory, and organism homeostasis (Neves et al., 2002).

Several subtypes of α-, β- and γ-subunits have been described and G-proteins are classified into four groups by their Gα subunit sequence and functional similarities: $G\alpha_s$, $G\alpha_i$/$G\alpha_o$, $G\alpha_q$/$G\alpha_{11}$ and $G\alpha_{12}$/$G\alpha_{13}$ (Wettschureck and Offermanns, 2005). Each family consists of various members that often show very specific expression patterns. Members of one family are structurally similar and share some of their functional properties (Wettschureck and Offermanns, 2005). Currently, these families altogether comprise 18 different Gα subunits (Syrovatkina et al., 2016). In addition to Gα subunits, G-proteins also contain 5 Gβ and 12 Gγ genes in the human and mouse genomes (Syrovatkina et al., 2016). In brief, both $G\alpha_s$ and $G\alpha_i$ families regulate adenylyl cyclase (AC) where $G\alpha_s$ stimulates AC to convert ATP to cAMP which results in the activation of cAMP-regulated proteins (Wettschureck and Offermanns, 2005). $G\alpha_i$, on the other hand, can inhibit certain isotypes of AC, leading to reduced intracellular cAMP levels (Wettschureck and Offermanns, 2005). AC has a physiological influence on cardiac function and $G\alpha_s^{-/-}$ and $G\alpha_i^{-/-}$ knockout mice have shown to have a failure in cardiac contractility (Lohse et al., 2003; Rudolph et al., 1996). The $G\alpha_o$ is highly abundant in the mammalian nervous system where it constitutes up to 0.5% of membrane proteins (Offermanns, 2001). Its expression has also been shown in neuroendocrine cells as well as at low levels in the heart (Offermanns, 2001). $Ga_o^{-/-}$ mice showed no gross morphological abnormalities, but were smaller and weaker than their littermates and showed greatly reduced postnatal survival rates (Jiang et al., 1998). In addition, the $Ga_o^{-/-}$ mice had impaired motor control and they were hyperactive, running continuously in

circles (Jiang et al., 1998). The $G\alpha_q/Ga_{11}$ family of G-proteins are widely expressed in the CNS and are coupled to numerous receptors that regulate the activity of β-isoforms of phospholipase C (β1-4), which cleave the phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol trisphosphate (IP_3) and membrane-bound diacylglycerol (DAG). IP_3 opens the calcium channel IP_3-receptor on the endoplasmic reticulum membrane, and DAG activates protein kinase C (Syrovatkina et al., 2016). Mice lacking $G\alpha_q$ and $G\alpha_{11}$ genes have multiple defects including impaired motor coordination, hyperparathyroidism associated with defective cerebellar development, embryonic cardiomyocyte proliferation and craniofacial development (Dettlaff-Swiercz et al., 2005; Offermanns et al., 1997; Wettschureck et al., 2001). In addition, $G\alpha_q$ family members can induce Rho-mediated responses including the activation of RhoA in smooth muscle cells and the neurotransmitter acetylcholine release at the neuromuscular junction in *C.elegans* (Miller et al., 2000; Williams et al., 2007). The activity of RhoGEF and its related proteins is also increased by the membrane recruitment and direct interaction with $G\alpha_{13}$ from $G\alpha_{12}/G\alpha_{13}$ family (Wettschureck and Offermanns, 2005). $G\alpha_{12}$ gene deleted mice were normal, but $G\alpha_{13}^{-/-}$ mice died at E9.5 (Gu et al., 2002; Offermanns et al., 1997). $G\alpha_{13}$ is essential for blood vessel formation and is highly expressed in endothelial cells (Offermanns et al., 1997). The $G\alpha_{13}^{-/-}$ mice have a defective vascular system that shows no blood vessels (Ruppel et al., 2005). Ablation of $G\alpha_{12}$ and $G\alpha_{13}$ genes from the nervous system results in neuronal ectopia in the cerebral and cerebellar cortices suggesting they have an essential role in the proper positioning of migrating cortical plate neurons and Purkinje cells during development (Moers et al., 2008).

6. RIC8A Protein

6.1. Biochemical properties of RIC8A and cell signalling

RIC8 (*Resistant to Inhibitors of Cholinesterase 8*) is a highly conserved 63-kDa protein that was first characterised during a genetic screening of aldicarb-resistant *Caenorhabditis elegans* (*C.elegans*) mutants that were defective in synaptic transmission and suggested its interaction with $G\alpha_q$ (Miller et al., 1996; Miller et al., 2000). RIC8 mutants were able to survive the neurotoxic effects of cholinesterase inhibitors by decreasing the amount of neurotransmitter released at the synapse (Miller et al., 2000). Further purification and biochemical characterisation of the protein have revealed that RIC8 acts as a receptor-independent guanine nucleotide exchange factor (GEF) for Gα proteins (Tall et al., 2003). A single *ric8* gene has been described in *C.elegans* and in *Drosophila melanogaster* (*D.melanogaster*) and two homologues in mammals: *Ric8A* and *Ric8B* (Tall et al., 2003). RIC8A has been shown to regulate the activity of monomeric G protein α subunits such as $G\alpha_{q/}$ $G\alpha_{11}$, $G\alpha_{i/}$ $G\alpha_o$, $G\alpha_{12/}$ $G\alpha_{13}$ families and RIC8B has been mostly described in association with the $G\alpha_s$ family proteins

(Chan et al., 2011; Gabay et al., 2011; Tall et al., 2003). The structure of *Xenopus laevis* Ric8A has been suggested to contain 10 armadillo folding motifs organised in a right-twisted α-alpha super-helix (Figueroa et al., 2009). Proteins containing armadillo motif have been shown to interact with multiple partners and participate in diverse cellular functions (Figueroa et al., 2009).

Currently, RIC8A is defined as a multifunctional protein. RIC8A may act as a GEF by interacting with GDP-bound or monomeric Gα subunits forming a stable nucleotide-free Gα-RIC8A complex whereafter GTP binds to Gα-RIC8A and disrupts the complex, releasing RIC8A and activated Gα protein (Tall et al., 2003). Several studies have also implied that RIC8A may act as a molecular chaperone that regulates G protein biosynthesis and folding (Chan et al., 2013; Gabay et al., 2011) or inhibit G protein ubiquitination and degradation (Chishiki et al., 2013; Nagai et al., 2010). Moreover, recent studies have shown that RIC-8A and $G\alpha_{13}$ regulate each other: $G\alpha_{13}$ stimulates the tyrosine phosphorylation of RIC8A and subsequent translocation of RIC8A to the plasma membrane, whereas RIC8A potentiates the activation of RhoA and Cdc42 through $G\alpha_{13}$ signalling (Xing et al., 2013; Yan et al., 2015). Through $G\alpha_q$ signalling RIC8A positively regulates the $G\alpha_q$ coupled receptor-mediated ERK activation and intracellular calcium mobilisation (Nishimura et al., 2006).

6.2. The role of RIC8A in asymmetric cell division and embryogenesis

GPCR independent activation of G-proteins by RIC8A is highly conserved signaling mechanism required for the mitotic spindle orientation and asymmetric cell division in the early embryogenesis in *C.elegans* and in *D.melanogaster* and in mammalian cells (David et al., 2005; Miller et al., 2000; Miller and Rand, 2000; Wang et al., 2005; Woodard et al., 2010). These studies have shown that RIC8A triggers a conserved receptor-independent mechanism that controls the interaction between the cell membrane and microtubules, thus affecting spindle orientation and the generation of pulling forces.

Briefly, during the first division of wild-type *C.elegans* embryos, the asymmetry is dependent on the partitioning of several Par-proteins and cell fate determinants to either the anterior or the posterior cell cortex (Betschinger and Knoblich, 2004). Then, the posterior centrosome while nucleating the mitotic spindles must migrate towards the posterior pole by the end of anaphase resulting in an unequal cleavage into a larger anterior and a smaller posterior blastomere (Miller and Rand, 2000). Therefore, the $G\alpha_i$-mediated pulling activity must be greater at the posterior pole of the cell which moves the entire mitotic spindle posteriorly to help define the characteristic asymmetric cleavage plane (Afshar et al., 2004). RIC8 has been shown to localise similarly like GOA-1 ($G\alpha_i$ in mammals) in the cell cortex and on the astral microtubules of the mitotic spindle in *C.elegans* early 1-cell embryo (Afshar et al., 2004; Couwenbergs et al., 2004). RIC8 is additionally localised on the central spindles, at the nuclear envelope, around the chromatin and at the junctions

between the cells (Afshar et al., 2004; Couwenbergs et al., 2004; Hess et al., 2004).

During the cell division, RIC8 activates GOA-1 subunits to associate with GPR1/2:LIN-5 (LGN:NuMA in mammals, respectively) which binds to dynein/ dynactin complex to generate the pulling forces (Afshar et al., 2004; Afshar et al., 2005) (Analogous mechanism is shown in Figure 3). Without RIC8 the first division exhibits defects in centrosome movements and in the regulation of pulling forces. This produces equally sized blastomeres and causes embryonic lethality with phenotype identical to *goa-1* mutant embryos (Afshar et al., 2004; Afshar et al., 2005; Couwenbergs et al., 2004; Miller et al., 2000; Miller and Rand, 2000).

Also, in *D. melanogaster* neuroblasts and sensory organ precursor cells, RIC8 is localised in the cytoplasm throughout the cell cycle and accumulates to the mitotic spindle during mitosis (Hampoelz et al., 2005; Wang et al., 2005). In order to control the asymmetric cell division the adaptor protein Inscuteable (Insc.) segregates the polarity proteins (Par-proteins such as Bazooka (Par-3 in mammals), Par-6, aPKC) apically which then mediate the localization of the cell fate determinants (Numb; Brat, Miranda) to the opposite side of the membrane (Knoblich, 2008). Then, RIC8 activates the apically located $G\alpha_i$ which binds to the GoLoco domains of Pins (Partner of Inscuteable; LGN in mammals) and recruits Pins to the apical plasma membrane where it also mediates the binding of dynein/dynactin complex via Mud protein (NuMA in mammals) providing necessary pulling forces (Bowman et al., 2006; David et al., 2005; Hampoelz et al., 2005; Izumi et al., 2006; Nipper et al., 2007; Schaefer et al., 2000; Siller et al., 2006; Siller and Doe, 2009; Wang et al., 2005). $G\alpha_i$:Pins:Mud complex is linked to the apical polarity proteins by the adaptor protein Insc. which associates with Bazooka and Pins and orients the mitotic spindle (Schaefer et al., 2000; Yu et al., 2000). Thus, in *D.melanogaster* RIC8 is essential for proper spindle orientation, modulating differences in daughter cell size and in asymmetric localisation of cell-fate determinants (David et al., 2005; Hampoelz et al., 2005; Wang et al., 2005; Yu et al., 2006). In the absence of RIC8, all the G-protein subunits fail to localize to the cell cortex and subsequently the recruitment of Pins to the cortex also fails which disrupts the formation of spindle asymmetry and different daughter cell size (David et al., 2005; Hampoelz et al., 2005; Wang et al., 2005; Yu et al., 2006). In addition, *D.melanogaster ric-8* mutants exhibit embryonic lethality and have various defects during gastrulation (Hampoelz et al., 2005; Schaefer et al., 2001; Wang et al., 2005).

The role of mammalian RIC8A and $G\alpha_i$ has been studied in HeLa and MDCK cells where RIC8A localises at the cell cortex, spindle poles, centromeres, central spindle, and midbody depending on the cell cycle phase (Woodard et al., 2010). At the onset of mitosis, mammalian $G\alpha_i$-GDP:LGN:NuMA complexes form at the sites of astral microtubule regulation (Tall and Gilman, 2005). Afterwards, the GEF activity of RIC8A stimulates the release of GDP and the binding of GTP to $G\alpha_i$, which catalyses the dissociation

of the complex into $G\alpha_i$-GTP, LGN and NuMA. Finally, RGS activity (GAP) stimulates the hydrolysis of GTP on $G\alpha_i$ and the resultant $G\alpha_i$-GDP could reform the active $G\alpha_i$-GDP/LGN/NuMA complex (Tall and Gilman, 2005). Perturbation of RIC8A function reduces the localisation of LGN, NuMA and dynein at the cell cortex in metaphase, causing the failure of astral microtubule capture which leads to prolonged mitosis or mitotic arrest (Woodard et al., 2010). Without correct spindle positioning or inappropriate application of pulling forces the cell fate decisions are altered, which subsequently impede embryogenesis (Woodard et al., 2010). Compliance with the genetic studies in *C.elegans* and *D.melanogaster,* loss of *Ric8a* also results in an early embryonic lethality within E6.5 – E8.5 due to gastrulation defects in mice (Tõnissoo et al., 2006; Tõnissoo et al., 2010). The gastrulation is initiated in *Ric8a*$^{-/-}$ embryos but their growth is retarded, epiblast and mesoderm are disorganised (Tõnissoo et al., 2010). Additionally, the BM is disorganised and the folding of the amnion, the formation of allantois and cavitation are defective (Tõnissoo et al., 2010).

6.3. RIC8A in the development of the nervous system

RIC8 has a crucial role in the nervous system. First, RIC8 was characterised during a genetic screening of aldicarb-resistant *C.elegans* mutants that were defective in synaptic transmission (Miller et al., 1996; Miller et al., 2000). Neurotransmitter release in the presynapse is modulated by G-protein coupled receptors (GPCR) and by the $G\alpha_q$-$G\alpha_o$ signalling pathway. RIC8 is present throughout the *C.elegans* nervous system in the juvenile and adult worms where it functions upstream of or in conjunction with $G\alpha_q$ (Miller et al., 2000). RIC8 activates $G\alpha_q$ that activates PLCβ and leads to the production of DAG which positively regulates neurotransmitter secretion via UNC-13 interaction (Miller and Rand, 2000). $G\alpha_o$ stimulates DAG kinase to reduce the functional levels of DAG, thus negatively regulating the $G\alpha_q$ pathway (Miller and Rand, 2000). Reduction of RIC8 function in *C.elegans* results in a strong neuronal phenotype including decreased locomotion, egg laying, and body flexion (Miller et al., 2000). A recent study has shown that in the *D.melanogaster* nervous system RIC8 binds to the Ca^{2+} sensor NCS-1 to regulate the synapse number and neurotransmitter release (Romero-Pozuelo et al., 2014).

The expression of RIC8A in the murine nervous system has also been documented. During the early development of mice (E9.5 – E12.0), RIC8A is expressed in the developing nervous system in the neural tube, cranial ganglia, dorsal root ganglia and in the sympathetic chain (Tõnissoo et al., 2003). Furthermore, RIC8A is also found in the lens, vomeronasal organ, and endolymphatic sac (Tõnissoo et al., 2003). In adult mice, RIC8A is expressed in the neocortex, hippocampus, and cerebellum and has a role in the regulation of emotional behaviour and memory since haploinsufficiency of *Ric8a* in mice causes increased anxiety and impaired spatial memory (Tõnissoo et al., 2006;

Tõnissoo et al., 2003). Conditional knockout studies have additionally revealed that RIC8A is specifically required in Bergmann glia during cerebellar foliation (Ma et al., 2012). Interference of RIC8A function in neural precursor cells results in Bergmann glial disorganised scaffolding due to a decreased affinity for BM components and interaction, defective granule cell migration, and disrupted Purkinje cell positioning (Ma et al., 2012). Moreover, studies with the model organism *Xenopus tropicalis* have revealed a requirement for RIC8A also in neural crest (NCC) derived structures (Maldonado-Agurto et al., 2011). RIC8A levels are also critical for the migration of cranial NCCs and their subsequent differentiation into craniofacial cartilage (Fuentealba et al., 2013). Furthermore, cells in Ric-8A knockdown animals showed anomalous radial migration, displaying a strong reduction in cell spreading and focal adhesion formation (Fuentealba et al., 2013). Earlier, RIC8A has been linked to growth factor-induced cell migration of mouse embryonic fibroblasts (MEFs) (Wang et al., 2011a). Downregulation of RIC8A protein levels slowed down platelet-derived growth factor (PDGF)-induced dorsal ruffle turnover and inhibited PDGF-initiated cell migration (Wang et al., 2011a). Subsequent research indicated that RIC8A is critical for coupling receptor tyrosine kinase (RTK) to $G\alpha_{13}$, which is essential for actin cytoskeleton reorganisation (Wang et al., 2011a). Other studies have found that deletion of *ric8a* resulted in the reduction of the amount of total and polymerized actin which affected formation of blebs and filopodia-like structures on the ventral cellular surface of the *D.melanogaster* blastoderm cells and mouse *Ric8a*$^{-/-}$ embryonic stem cells (mES), respectively (Gabay et al., 2011; Kanesaki et al., 2013).

AIMS OF THE STUDY

RIC8A regulates the activity, stability and localisation for a subset of Gα subunits ($G\alpha_i$, $G\alpha_q$ and $G\alpha_{13}$) acting as a GEF and a chaperone. RIC8A participates in the regulation of cell division, gastrulation, cell signalling, adhesion and migration. Mammalian RIC8A is expressed in the central nervous system and it affects behavioural physiology in mice. However, *Ric8a*$^{-/-}$ embryos exhibit defects in the basement membrane integrity and die due to severe gastrulation defects.

The current dissertation is focused on the elucidation of RIC8A function in the development of the nervous system in mammals and in asymmetric cell division using knockout mouse models and primary cell cultures. The thesis covers five linked studies that are focused on four main goals:

1. To assess the role of RIC8A in the development of the nervous system and in the synaptic signal transduction. We assessed the effect of the targeted ablation of *Ric8a* in neural progenitor cells and in differentiated neurons.
2. To analyse the role of RIC8A in the cell division in mouse neurogenesis and in mouse oocytes.
3. To examine the role of RIC8A in cell migration and adhesion using RIC8A deficient mouse primary embryonic cells.
4. To characterise the similarities of the phenotypes with congenital muscular dystrophies.

RESULTS AND DISCUSSION

1. RIC8A in neuromuscular signalling (Ref. I, Ref. II and Ref. V)

1.1. Deletion of RIC8A from the developing nervous system of mouse leads to neuromuscular defects and postnatal lethality (Ref.I; Ref.II)

The expression pattern of *Ric8a* in the early stages of mouse organogenesis (E9.5 – E12.0) is highly neurospecific. In adult brains, *Ric8a* is expressed in areas that are responsible for the regulation of behaviour and memory (e.g. neocortex, cingulate cortex, caudate putamen, hippocampus, cerebellum) (Tõnissoo et al., 2003). Haploinsufficiency of *Ric8a* results in behavioural abnormalities such as increased anxiety-like behaviour and impaired spatial memory (Tõnissoo et al., 2006). The function of RIC8A in neurogenesis and in the nervous system is largely unknown since homozygous $Ric8a^{-/-}$ mice die at E6.5 – E8.5 due to multiple gastrulation defects (Gabay et al., 2011; Tõnissoo et al., 2010). In order to circumvent the embryonic lethality and examine the role of RIC8A in the mouse nervous system, we generated two conditional knockout mice models where RIC8A was specifically knocked out from neural precursor cells or from presynaptic terminals of postmitotic neurons. Transgenic mouse strains expressing Cre-recombinase under *Nestin* or *Synapsin I* promoter were introduced into the conditional *Ric8a* ($Ric8a^{lacZ/F}$) background which ablated RIC8A function in neural precursor cells (*Nes;*$Ric8a^{CKO}$ mice) and differentiated neurons (*Syn;*$Ric8a^{CKO}$ mice), respectively.

Neurospecific *Ric8a* conditional mutant mice were born at expected Mendelian ratio (22–24%), however, the genotyping data from our crossings indicated that the number of newborn pups per litter was lower than the average in *Nes;*$Ric8a^{CKO}$ mice. Nevertheless, all *Nes;*$Ric8a^{CKO}$ mice died within 12 h after birth and the majority of analysed *Syn;*$Ric8a^{CKO}$ mice died between P4 – P6 postnatally. Moreover, most *Nes;*$Ric8a^{CKO}$ pups and some *Syn;*$Ric8a^{CKO}$ were abandoned or killed by their mother during first days after their birth due to a lack of feasible viability.

The absence of RIC8A in neurons in *Syn;*$Ric8a^{CKO}$ mice and in neural precursor cells in *Nes;*$Ric8a^{CKO}$ pups give rise to a severe neuromuscular phenotype. The *Syn;*$Ric8a^{CKO}$ mice had abnormal body curvature and were not able to right themselves due to impaired motor skills and muscular spasms (Ref.I, Figure 2A–2C). The *Nes;*$Ric8a^{CKO}$ pups also showed strong neuromuscular phenotype characterised by limited capacity for movement and they always lied on one side. Moreover, they exhibited a barrel-like body shape, dropping forelimbs and stiffness of lower limbs (Ref.II, Figure 1K,1L).

The body-weight of *Nes;*$Ric8a^{CKO}$ mice varied within litters, being slightly lower or the same with the littermates, but the body weight of *Syn;*$Ric8a^{CKO}$

mice was significantly lower throughout the observed period compared to littermates (P0-P5) (Ref. I, Figure 2F). *Syn;Ric8a*^{CKO} mice were able to gain weight, but not in an exponential manner as their littermates and their weight gain stopped completely at P5. The inadequate weight-gain was probably due to a low level or absence of milk in their stomach as was also seen in *Nes;Ric8a*^{CKO} mice (Ref.I, Figure 2E; Ref.II, Figure 1O;1P). The mutant mice were probably not able to compete with their littermates for food, however, the inability to feed themselves might also be associated with neuromuscular or craniofacial defects (Turgeon and Meloche, 2009) which can be caused by the removal of RIC8A from the neural crest cells (NCC) since RIC8A has been shown to be necessary for the cranio-facial development in vertebrates (Fuentealba et al., 2013). NCCs also contribute to the palate and tongue development (Caruana and Bernstein, 2001; Liu et al., 2012). Other studies with neonatal mutant mice have revealed that nonfeeding newborns die within 12 – 24 h after birth due to the lack of nutrients or due to disturbed liquid homeostasis, which leads to dehydration (Dechiara et al., 1995; Mizushima et al., 2001; Segre et al., 1999; Turgeon and Meloche, 2009). *Nes;Ric8a*^{CKO} mice died within 12 h *postpartum* but mostly because their mother abandoned them that lead to quick dehydration and hypothermia.

However, *Syn;Ric8a*^{CKO} mice were able to feed because their stomachs contained milk in the early neonatal period, but from P3 onward, the amount of milk in the stomach decreased. The main reason for the early neonatal and postnatal death is probably malnutrition and an alteration in liquid homoeostasis. Indeed, the *Syn;Ric8a*^{CKO} mice revealed the ossification delay at P3 and it is consistent with the fact that postnatal nutrition and bone development are known to be directly linked (Land and Schoenau, 2008; Triffitt, 1987). Another indicator for malnutrition is the brain and liver weight ratio that was significantly higher in *Syn;Ric8a*^{CKO} mice confirming their malnutrition (Mitchell, 2001). Taken together, the early lethality of both conditional knockout mice was probably mostly caused by the lack of postnatal care, interfered development or defective innervation of their cranio-facial structures that lead to the inability to feed.

1.2. Deficiency of RIC8A in neurons and precursor cells leads to skeletal muscle atrophy in mice (Ref.I; Ref.V).

The analysed phenotypes revealed the evident neuromuscular defects in *Syn;Ric8a*^{CKO} mice and in *Nes;Ric8a*^{CKO} mice. The histological analyses of brains of *Syn;Ric8a*^{CKO} mice did not show any obvious malformations (Ref.I, Figure 3) indicating that the brain development has not been markedly disturbed in *Syn;Ric8a*^{CKO} mice. However, the histological examination of the skeletal muscles at P0 and P5 of *Syn;Ric8a*^{CKO} mice and P0 *Nes;Ric8a*^{CKO} mice revealed that the skeletal muscle tissue was hypoplastic compared to littermate controls. This was caused by the atrophy of myocytes, and the diameter of the muscle fibres was

substantially decreased and the fibres were also sparsely distributed and less compact resembling endomysial fibrosis (Ref.I, Figure 2G; Ref.V, Figure 4C;4D). The muscle atrophy may be the result of an insufficient neuronal stimulation at the neuromuscular junction. RIC8A has been established as a receptor-independent activator for $G\alpha_i$, $G\alpha_q$, $G\alpha_o$ and $G\alpha_{13}$ subunit families (Tall et al., 2003). This particular activity has been confirmed by separate studies that demonstrate the capacity of the RIC8A protein to potentiate the $G\alpha_q$ and $G\alpha_i$ signal (Fenech et al., 2009; Nishimura et al., 2006; Wang et al., 2007). Furthermore, RIC8A has also been suggested to function as a molecular chaperone required for Gα subunit biosynthesis (Gabay et al., 2011). The collective data on the biochemical function of RIC8A protein strongly suggests that the neuromuscular defects of *Syn;Ric8a*^{CKO} and *Nes;Ric8a*^{CKO} mice are caused by the reduced activity of G-proteins in neurons. Indeed, $G\alpha_o$ is abundantly expressed in neurons and mediates the effects of a group of receptors such as opioid, α2-adrenergic, M2 muscarinic and somatostatin receptors. $G\alpha_o^{-/-}$ mice were weaker and smaller and had impaired motor control compared to their littermates, they also displayed the neural phenotype of ataxia and impaired motor control and lived for about 7 weeks (Jiang et al., 1998; Offermanns et al., 1997).

Poor muscle innervation in *Nes;Ric8a*^{CKO} mice may also be due to defects in the peripheral nerve myelination, which develops through radial sorting that has been shown to be GPCR signalling dependent (Berti et al., 2011; Mogha et al., 2016). Mouse models where the β1 integrin, laminin α2 or α-dystroglycan functioning is deficient have myelination defects that often cause paralyses, tremor and muscular dystrophy similar to the *Nes;Ric8a*^{CKO} mice (Berti et al., 2011; Chen and Strickland, 2003; Feltri et al., 2002; Saito et al., 2007). These results suggest that RIC8A may also have a role in myelination process through Schwann cells that have neural crest origin.

1.3. Deficiency of RIC8A in neurons and neural precursor cells affects the heart development, function and morphology (Ref.I; Ref.V).

In addition to impaired neuromuscular signalling, the heart of *Syn;Ric8a*^{CKO} mice was markedly smaller than in littermate controls indicating that the heart functioning was also insufficient (Ref.I, Figure 4A;4B). Indeed, the hematoxylin-eosin stained tissue sections of mutant mice contained more blood which indicates malfunctioning of the cardiac muscle, suggesting *Syn;Ric8a*^{CKO} heart could not pump blood out after dissection (Ref.I, Figure 4C;4D). Moreover, the myocardium of these hearts was also substantially thinner than in littermates (Ref.I, Figure 4G;4H). Similarly, the majority of P0 *Nes;Ric8a*^{CKO} pups had considerably thinner myocardium and coronary artery anomalies. Furthermore, about half of the mice had severe ventricular septum defects (Ref.V, Figure 5C–5H). The closer inspection of *Ric8a* expression in E14.5 *Ric8a*$^{lacZ/+}$ mice revealed that RIC8A is expressed in the areas of developing coronary artery and

in the wall of the aorta (Ref.V, Figure 5A). The same region was also populated with cells expressing *NestinCre* transgene (Ref.V, Figure 5B). NCCs give rise to cardiac smooth muscle cells and contribute to the coronary artery and to the interseptum development (Arima et al., 2012; Dettlaff-Swiercz et al., 2005; Waldo et al., 1998). Similar defects were also described in neural crest cell-specific $G\alpha_{12}/G\alpha_{13}$-deficient mice (Dettlaff-Swiercz et al., 2005) further corroborating the essentiality of RIC8A functioning in the neural crest-derived structures.

Neural crest-derived structures were in general normally developed in *Syn;Ric8a*^{CKO} mice. However, the sinoatrial node was located more anteriorly and appeared to be substantially smaller than in littermates (Ref, I, Figure 4E;4F). The sinoatrial node is innervated by both sympathetic and parasympathetic axons, and it contains pacemaker cells, which are responsible for the generation of normal sinus rhythm (Durham and Worthley, 2002). In the case of a defective sinoatrial node, the heart rhythm becomes abnormally fast, slow or their combination (Durham and Worthley, 2002). As expected, the heart rate of *Syn;Ric8a*^{CKO} mice was significantly slower than that of littermates. The lack of RIC8A may affect the neurotransmitter release since it has been shown to participate in the regulation of neurotransmitter secretion by activating $G\alpha_q$ and PLCβ signalling in *C.elegans* (Miller et al., 2000). In mice, ubiquitously expressed $G\alpha_q$ and PLCβ proteins are mostly studied within the context of cardiac function and development (Wettschureck et al., 2001). Double knockout mice of $G\alpha_q/G\alpha_{11}$ died at E11.5 whereas mutants with a single active allele survived until birth, but then died within a couple of hours because of numerous cardiac malformations. Furthermore, they were of small size, anoxic and poorly responded to tactile stimulation (Offermanns et al., 1998). Mice lacking only $G\alpha_q$ were viable but suffered from ataxia and typical signs of motor discoordination (Offermanns et al., 1997). These results indicate that in parallel with the skeletal muscle atrophy, *Syn;Ric8a*^{CKO} mice and *Nes;Ric8a*^{CKO} mice have strong cardiac muscle hypoplasia, which affects their cardiac function. Additional defects in neural crest-derived structures in *Nes;Ric8a*^{CKO} mice strongly influence the morphology of the heart tissue which most likely further aggravates the cardiac function and thus might also be one of the causes triggering the death of *Nes;Ric8a*^{CKO} mice.

2. RIC8A in the cell-ECM interaction (Ref.II; Ref.IV; Ref.V)

2.1. Ablation of RIC8A in neural precursor cells disrupts the pial basement membrane and cortical cytoarchitecture (Ref.II).

As was mentioned earlier, *Nes;Ric8a*^{CKO} mice were readily identifiable by their appearance revealing severe neuromuscular phenotype. The analyses of the whole brain revealed an enlarged area of the neocortex in *Nes;Ric8a*^{CKO} mice and the presence of several extravasations (Ref.II, Figure 1M,1N). However, a

closer examination of histological sections exhibited much thinner cortex in the brain of newborn *Nes;Ric8a*^{CKO} than in control mice (Ref.II, Figure 2A;2B). In several cortical areas, the aberrant column-like cell clusters were discovered in the uppermost layers that had invaded to the marginal zone resembling type II lissencephaly-like cortical ectopias (Ref.II, Figure 2B, red arrowhead). In the anterior part of the *Nes;Ric8a*^{CKO} mice cortexes the cortical heterotopias were bigger and the number of ectopias was higher when compared to the posterior part of the cortexes (Ref.II, Figure 2C;2D). However, we could not detect differences in RIC8A protein expression along rostro-caudal axis (data not shown) by immunofluorescence analysis. The cortical ectopias strongly affected the overall lamination of the developing neocortex where most cortical ectopias were comprised of neurons from the upper layers but also contained occasional cells from the mid-part of the cortex (Ref.II, Figure 4A–4F compared to 4G–4L). However, with severe lamination defects, the cells from the deepest layers were detected within the over-migration in the marginal zone (Ref.II, Figure 4M–4R). These neuronal over-migrations were probably due to the defects in basement membrane (BM) since several other studies have shown analogous type II lissencephaly-like cortical ectopias accompanied with BM defects (Beggs et al., 2003; Costell et al., 1999; Georges-Labouesse et al., 1998; Graus-Porta et al., 2001; Halfter et al., 2002; Hartmann et al., 1999; Haubst et al., 2006; Hecht et al., 2010; Inoue et al., 2008; Jeong et al., 2012; Jeong et al., 2013; Li et al., 2008; Luo et al., 2011; Myshrall et al., 2012; Niewmierzycka et al., 2005; Radakovits et al., 2009; Radner et al., 2013; Singer et al., 2013; Zarbalis et al., 2007).

BMs are thin and dense sheets comprised of highly cross-linked extracellular matrix (ECM) proteins that are located at the basal side of every epithelium and endothelia and also surround muscle, fat and Schwann cells (Hohenester and Yurchenco, 2013; Yurchenco, 2011). Mutations in genes of BM constituent proteins are either embryonically lethal or lead to muscular dystrophy, vasculature ruptures, or CNS malformations (Halfter and Yip, 2014). Indeed, our research group has previously also demonstrated that the *Ric8a*$^{-/-}$ embryos died due to gastrulation defects that were accompanied with BM integrity (Tõnissoo et al., 2010). Moreover, the absence of RIC8A in cerebellum development caused impaired adhesion of the Bergmann glia to the BM, leading to the impaired migration of granular progenitor cells and to failure in the generation of cerebellar fissures (Ma et al., 2012). Therefore we analysed the morphology of BM by characterising Laminin-I localisation in murine CNS. In control mice and in *Nes;Ric8a*^{CKO} mice the BM was intact at E12.5 showing continuous Laminin-I localisation (Ref.II, Figure 5A–5D). However, at neurogenesis peak phase at E14.5 (when the first ectopias were detected) and in newborn mice, the BM was ruptured as suggested by fragmentary Laminin-I localisation pattern and numerous gaps in BM in the mutant mice (Ref.II, Figure 5E–5P). Thus, the absence of functional RIC8A in the neural precursor cells leads the loss of the pial BM integrity suggesting a putative role for RIC8A in the cell adhesion.

The assembly of the BMs depends on the meninges and radial glial endfeet that tightly associate with each other to form a barrier (*glia limitans*). The evolutionarily conserved mechanism of BM assembly is initiated through the recruitment of laminin by integrin and α-dystroglycan receptors (Beggs et al., 2003; McKee et al., 2007). Mutations in genes encoding extracellular matrix components like laminins (α5; β2 or γ1, γ3), perlecan and Collagen III disrupt normal BM maintenance and cause cortical ectopias and BM breaches (Costell et al., 1999; Haubst et al., 2006; Luo et al., 2011; Radner et al., 2013). Consistent with the ECM studies, deletion of *Integrin a6β1*, *β1*-class integrins, and conditional deletion of α-dystroglycan in mice also lead to breaks in BM integrity and allowed migration of ectopic neurons to the marginal zone (Georges-Labouesse et al., 1998; Graus-Porta et al., 2001; Myshrall et al., 2012; Radakovits et al., 2009). The meninges covering the developing brain also participate in the formation of the BM since meningeal fibroblasts secrete ECM components and organise the BM lining over the cortex (Beggs et al., 2003; Decimo et al., 2012; Radakovits et al., 2009). Defects in meninges differentiation lead to the breakdown of the pial BM in the development of mouse brain cortex and cause severe cortical dysplasia associated with the marginal zone heterotopias and dyslamination (Hecht et al., 2010; Inoue et al., 2008; Zarbalis et al., 2012). It seems that in *Nes;Ric8a*^{CKO} mice Laminin-I production was not affected as the BM was organised correctly at E12.5. However, considering the fragmented and scattered localisation of Laminin-I in between and around the cells at E14.5 and P0, the ability to maintain an intact BM is lost after E12.5. We hypothesise that intactness of BM is lost due to the impaired polarisation of the RIC8A deficient pial cells. In fact, the polarised distribution of RhoA and microtubule dynamics has been shown to play a role in the disruption of the BM maintenance during gastrulation (Nakaya, 2008). Cells surrounded by Laminin-I, in an analogous manner to the RIC8A deficient pial cells, were also detected in the primitive streak region of the *Ric8a*$^{-/-}$ embryo at E7.5 (Tõnissoo et al., 2010). Moreover, our *in vitro* studies showed that RIC8A plays an important role in the organisation and remodelling of actin cytoskeleton since *Ric8a*$^{-/-}$ mouse embryonic stem cells (mES) were not able to form stress fibres or spread properly (Ref.IV, Figure, 3A;3A';3B;3B'). Also, recent studies have proposed the participation of RIC8A in the activation of RhoA and Cdc42 which play a crucial role in cell polarisation (Gabay et al., 2011; Yan et al., 2015). Hence, abnormal localisation of laminin accompanying the RIC8A deficiency also implies the malfunctioning of the RhoA pathway leading to a defective epithelial tissue polarity (Cappello et al., 2012; Daley et al., 2012). Therefore we suggest that insufficient activation of RhoA pathway could be the reason for the breakdown of the pial BM seen in *Nes;Ric8a*^{CKO} mutants.

2.2. RIC8A is needed for the attachment of radial glial endfeet to BM and Cajal-Retzius cell positioning (Ref.V).

Intact pial BM is necessary for the anchorage of radial glial endfeet using integrins or α-dystroglycan. Cortical abnormalities in laminar organization were also found in studies in mice with deletion of nidogen-binding site of laminin γ1, integrin-linked-kinase (Ilk), focal adhesion kinase (FAK), adhesion G protein-coupled receptor GPR56, $G\alpha_{12}/G\alpha_{13}$ (Beggs et al., 2003; Halfter et al., 2002; Jeong et al., 2012; Jeong et al., 2013; Li et al., 2008; Moers et al., 2008; Niewmierzycka et al., 2005). Consistent with the results found in *Nes;Ric8a*^{CKO} mice where the anterior cortical heterotopias were bigger, the GPR56 expression pattern mimics the anterior-to-posterior gradient of defects associated with loss of GPR56 in mice (Jeong et al., 2012; Li et al., 2008; Singer et al., 2013). These results strongly suggest that RIC8A might associate with GPR56 and $G\alpha_{13}$ since GPR56 has been shown to function using interaction with $G\alpha_{13}$ (Luo et al., 2011). All of the aforementioned studies also reported that the radial glial endfeet were detached from the BM and the Cajal-Retzius cells were mislocalized around the ectopias. In *Nes;Ric8a*^{CKO} mice the first ectopias were detected at (data not shown) and the first BM breach already at E13.5 and these were not present in control mice (Ref.V, Figure 2G;2H;2G′;2H′). In ectopias the Cajal-Retzius cells were randomly distributed, the radial glial processes were disorganised and the Laminin-I lining was fragmentary (Ref.V, Figure 2A′–2F′, respectively). Since Cajal-Retzius cells did not express RIC8A, the mislocalization was probably a concurrent feature and dependent on the detachment of radial glia and BM defects as reported earlier (Kwon et al., 2011). Taken together, the absence of RIC8A in the neural precursor cells affects the attachment of radial glia to the BM and the localisation of CR cells which in turn may affect the signal molecules secreted by them.

2.3. RIC8A deficiency causes impaired cell migration (Ref.II, Ref. IV)

Along with the defects in the BM integrity, different signals from the surrounding environment (e.g signal molecules secreted by Cajal-Retzius cells) can also lead to mislocalization of the migrating neurons. Removal of RIC8A function from neural progenitors in *Nes;Ric8a*^{CKO} mice did not influence the generation of neural progenitor cell types at early embryonic ages or the onset of neurogenesis. Furthermore, newborn neurons were able to start the migration towards the pial surface (Ref.II, Figure 3), and to form cortical layers in an „inside-out“ manner (Ref.II, Figure 4). However, the cells were not correctly positioned in the layers in *Nes;Ric8a*^{CKO} mice. The binding partners of RIC8A – $G\alpha_{12}/G\alpha_{13}$ – have been shown to provide the positioning cues for the cortical neurons during brain development and ablation of the genes encoding these α-subunits in neural precursor cells resulted in a cobblestone-like cortical mal-

formation (Moers et al., 2008). One of the possibilities for these cortical over-migrations is that the cells have lost the ability to respond to the repulsive mediators that signal via GPCRs. Indeed, embryonic cortical neurons lacking $G\alpha_{12}/G\alpha_{13}$ did not retract, the neurites in response to repulsive mediators, indicating that they had lost the ability to transmit the stop signals from $G\alpha_{12}/G\alpha_{13}$ coupled receptors and therefore cortical plate neurons were not correctly positioned during development (Moers et al., 2008). $G\alpha_{12}/G\alpha_{13}$ stimulate the small GTPase RhoA-dependent actomyosin-based contractility and it is likely that the loss of this regulatory pathway interferes with the normal regulation of cell migration (Buhl et al., 1995). Consistently, the genetic deletion of RhoA in the developing neocortex lead to two migrational disorders: the cobblestone lissencephaly and subcortical band heterotopia (SBH) which were perhaps caused by partial or incomplete migration of neurons to their cortical locations (Cappello et al., 2012). *In vivo* and *in vitro* studies have shown that $RhoA^{-/-}$ neurons were able to initiate migration, however, they showed decreased the formation of F-actin and reached the cortical plate faster (Cappello et al., 2012). Ablation of RhoA in the RG cells caused profound destabilisation of the actin and tubulin cytoskeleton in RG cells and loss of apical anchoring as well as defects in formation or maintenance of basal process (Cappello et al., 2012). In the RIC8A-deficient neural precursor cells, the levels of $G\alpha_{13}$ were decreased (Ref.II, Figure S2), which incorporates RIC8A into the RhoA-mediated signalling pathway as also shown before (Gabay et al., 2011; Yan et al., 2015). Therefore, the cortical ectopias forming in $Nes;Ric8a^{CKO}$ mutants may be caused by defects in $G\alpha_{12}/G\alpha_{13}$ and RhoA signalling pathways.

Next, we studied the migratory capacity of RIC8A deficient cells in more detail. However, instead of neural cells, for simplicity, we used mouse embryonic stem (mES) cells and mouse primary fibroblasts (MEFs) where first four exons were flanked by loxP sites and the ablation of functional RIC8A was achieved by transfection of cells with Cre-recombinase-expressing vector. We discovered the deficiency of RIC8A indeed affects the cell migration but this is highly dependent on the substrate. Migration of $Ric8a^{-/-}$ cells was impaired on laminin 521 (Ref.IV, Figure 5C) when no chemotactic stimulus was introduced. However, upon chemotactic stimulation with foetal bovine serum (FBS) the migration of RIC8A deficient mES cells was increased on type IV collagen and on laminin 521 compared to control cells (Ref. IV, Figure 5E). MEFs displayed similar tendencies with decreased migration on type I collagen and increased migration when the chemotactic stimulus was added. Cells mostly bind to laminin and collagens using β1 integrin subfamily integrins (Humphries et al., 2006). These results indicated that RIC8A is involved in the regulation of cell migration, which is dependent on the ECM substrate, probably through β1-integrin signalling. To verify this hypothesis, we analysed whether RIC8A regulates the activity of β1-integrins by quantifying the amount of β1-integrins active in conformation on the cell surface using flow cytometry. We discovered that the activation of β1-integrins upon cell adhesion to type I collagen was decreased in RIC8A-deficient cells as was the activating phosphorylation of Akt

downstream of integrins when compared to control cells (Ref.IV, Figure 6C;6D). These results suggest that the lack of RIC8A does not impair the cell migration as such, rather RIC8A-deficient cells are unable to properly interact with specific ECM components and this interaction is most likely integrin β1 dependent.

Integrins link the ECM to F-actin in focal adhesion complexes, hence we analysed the formation of focal adhesion complexes. RIC8A deficient cells did not assemble ordinary focal adhesion complexes since β1-integrin was distributed rather randomly in the plasma membrane (Ref.IV, Figure 4B;4B') whereas it had accumulated into sprouting clusters in RIC8A expressing cells (Ref.IV, Figure 4A;4A'). Vinculin, which is a major component of focal adhesions, also showed similar localisation pattern in RIC8A deficient cells (Ref.IV, Figure 4D; 4D'). The reduction of focal adhesion complexes in RIC8A deficient conditions was also detected in *X.laevis* NCCs (Fuentealba et al., 2013). The downregulation of RIC8A in *X.laevis* resulted in reduced adhesion of neural crest cells to fibronectin (Fuentealba et al., 2013) and upon its deletion from mouse neural progenitor cells reduced adhesion to laminin (Ma et al., 2012).

The lack of focal adhesion complexes was probably due to an inability to properly organise actin cytoskeleton. No stress-fibre-like structures were found in the RIC8A-deficient cells in contrary to control mES colonies (Ref.IV, Figure 3). This is probably due to the downregulation of $G\alpha_{13}$ in RIC8A deficient cells (Ref.IV, Figure 1F) and reduction of RhoA activity since the activation of RhoA is known to be required for the formation of actin stress fibres (Ridley and Hall, 1992) and RIC8A has previously been shown to affect RhoA activity (Gabay et al., 2011). Another key component of this pathway is the focal adhesion kinase (FAK) which has a similar phenotype with *Ric8a*$^{-/-}$ cells since *Fak* $^{-/-}$ cells have an increased number of immature focal adhesions, resulting in cell rounding and reduced cell migration as well as the altered regulation of the actin cytoskeleton (Beggs et al., 2003). FAK is a nonreceptor tyrosine kinase that is activated following integrin binding to various components of ECM (Parsons, 2003). Interference with FAK function in neural precursor cells or in meningeal cells leads to a severe cortical dysplasia resembling typeII lissencephaly (Beggs et al., 2003). These observed defects resemble the phenotype of *Nes;Ric8a*^{CKO} mice and *Ric8a*$^{-/-}$ cells. Therefore, based on our results and studies by others, we can conclude that RIC8A is an essential regulator of cell-matrix interactions and can modulate the cell migration in early cerebral cortex development.

4. RIC8A in asymmetric cell division (Ref. II; Ref. III)

Dorsal meninges and radial glial endfeet lie in close proximity to each other and their interactions may be crucial for cell specification through extrinsic signals (Siegenthaler and Pleasure, 2011). As reported earlier, the disruption of the pial BM in mice could lead to miscommunication between the meninges and the RG

endfeet and may trigger neurogenic fate (Siegenthaler et al., 2009). The reduction of the cortical thickness of *Nes;Ric8a*^{CKO} mice at P0 is indicative of the premature neurogenesis where postmitotic cells are generated too early at the expense of progenitor cells. Therefore, we analysed the division of mitotic cells by measuring their cleavage plane angles in relation to the VZ surface and grouped the angles using 10° intervals. The results were somewhat surprising, since usually planar divisions are analysed using the angle intervals of 30° (0–30°; 30–60°; 60–90°) (Haubst et al., 2006; Kosodo et al., 2004; Noctor et al., 2002), but our results showed significant increase of the cleavage planes between 70–90° range and obvious decrease in the range of 0–70° in *Nes;Ric8a*^{CKO} mice (Ref.II, Figure 6E). These results indicate that the loss of RIC8A in neural progenitor cells shifts the balance between the planar and oblique cell divisions towards planar divisions. In addition, studying the cell lineage of daughter cells after mitosis revealed an imbalance between the direct or indirect neurogenesis since the amount of radial glial cells and neurons (could be produced through planar divisions) increased while the number of intermediate progenitors (produced through oblique divisions) decreased in *Nes;Ric8a*^{CKO} cortices (Ref.II, Figure 6G–6L).

Cleavage plane is oriented through the correct attachment of astral microtubules to the cell cortex which requires the formation of $G\alpha_i$-LGN-NuMA complex (Morin and Bellaiche, 2011; Nipper et al., 2007; Schaefer et al., 2001). RIC8A is required for the asymmetric cell division and it catalyses the release of $G\alpha_i$-GTP and NuMA from $G\alpha_i$-GDP:LGN:NuMA complex (Tall and Gilman, 2005). $G\alpha_i$, that serves as an attachment point for astral microtubules at the plasma membrane, locates mostly in the cytoplasm and only occasionally at the cell cortex in the neural precursor cells of *Nes;Ric8a*^{CKO} mutants at E14.5 (Ref.II, Figure S2B-S2E). Such localisation of $G\alpha_i$ is in good concordance with the studies where RIC8A was shown to function as a chaperone that governs the membrane-association of nascent G-protein α-subunit (Chan et al., 2013; Gabay et al., 2011; Tall et al., 2013). The $G\alpha_i$-LGN-NuMA complex on the ends of astral microtubules can also associate with Inscuteable (Postiglione et al., 2011). The loss and gain of function analysis of the mouse Inscuteable (mInsc) gene indicated that mInsc interferes with the horizontal orientation of mitotic spindle during RG cell division and increases the number of forming neurons. The oblique spindle orientation is required for the production of intermediate progenitor cells and thereby causes the increase in the final brain size (Postiglione et al., 2011). In our studies, the number of cells in mitosis did not differ in brains of control and *Nes;Ric8a*^{CKO} mice. However, the number of cells in anaphase differed remarkably, especially the number of planarly dividing cells was significantly higher in the brain of mutant mice than in control cortices (Ref.II, Figure 6F). These results are consistent with the results from an earlier study where RIC8A removal in HeLa cells interfered with the localisation of $G\alpha_i$ subunit to the cell cortex in metaphase and disrupted correct mitotic spindle alignment, which in turn caused the occasional mitotic arrest and prolonged mitosis (Woodard et al., 2010).

When RIC8A regulated asymmetric cell division is excluded, the resulting LGN-complex-independent actin spindle orientation could lead to unregulated and rather symmetric divisions as a default state (Kwon et al., 2015). Moreover, aforementioned proteins that mediate cell adhesion also participate in orientation of the mitotic spindle. For example, the main function for Cdc42 in mammalian neurogenesis is to activate the Par complex in order to maintain the adherens junctions coupling and progenitor cell fate. In line with this, the deletion of Cdc42 caused the conversion of apical progenitors to bIP cells that had also acquired the SVZ characteristic fate determinants (Cappello et al., 2006). Furthermore, during cell division, Ilk localises to the centrosome and plays an essential role in mitotic spindle assembly and colocalizes with tubulin-interacting proteins (Fielding et al., 2008). Overall, Ilk has been implicated in regulating migration, cell survival, proliferation, and IP_3 dependent signal transduction. Similarly to Ilk, RIC8A has also been shown to localise in centrosomes, in the mitotic spindles and in the midbody in HeLa cells (Miller and Rand, 2000; Woodard et al., 2010). Additionally, in *C.elegans* RIC8 has been shown to participate in IP_3 signalling, thus, RIC8 may also be involved in mitotic spindle assembly and other centrosome-mediated processes (Miller and Rand, 2000). Accordingly, mice deficient in centrosomal protein Pericentrin have similar spindle orientation defects in neural progenitor cells and also possess malformations in the heart septum (Chen et al., 2014). Pericentrin is necessary for spindle orientation and functions by regulating the astral microtubule length and density. Based on extensive similarities of the phenotype of $Pcnt^{-/-}$ and $Nes;Ric8a^{CKO}$ mice, it seems feasible that RIC8A may act in the regulatory network of astral microtubule length and density. Moreover, a recent study revealed the importance of astral microtubule density and dynamics on the stability of mitotic spindle orientation (Mora-Bermudez et al., 2014). The authors suggested that in neuroepithelial cells (NE) the astral microtubule density is high which keeps the cells parallel to the substratum, but when NE cells transform into RG cells, which is at about the same time when RIC8A is upregulated, the density of astral microtubules decreases and they become more dynamic (Mora-Bermudez et al., 2014). The orientation of spindle that becomes less tightly anchored is, therefore, more easily readjusted by intra- and extracellular forces that can induce tilts (Mora-Bermudez et al., 2014). Thus, the regulation of astral microtubule assembly, density, and dynamics may be influenced by the $G\alpha_i$-RIC8A interplay during cell division, but this is a completely unexplored area in mammals and definitely needs further investigations before solid conclusions can be drawn.

In a separate line of investigation, we studied the RIC8A role in mammalian oocyte divisions, since oocyte undergoes highly asymmetric cell divisions resulting in the formation of small polar bodies and one large oocyte. The size difference between the daughter cells is achieved by the asymmetric spindle positioning before the cytokinesis. Gene expression analyses have shown that *Ric8a* is upregulated at the beginning of meiosis (Olesen et al., 2007). Moreover, xRic-8 is maternally expressed in amphibian oocytes where it

participates in the maintenance of meiotic arrest (Maldonado-Agurto et al., 2011; Romo et al., 2008). In meiosis, RIC8A localises to the cytoplasm at the early and in germinal vesicle at later stages. Upon meiotic spindle formation, RIC8A shifted to the spindle in metaphase and retained there during the anaphase and telophase of meiosis I and II (Ref. III, Figure 2). RIC8 also co-localizes with its known interaction partners NuMA, LGN and $G\alpha_{i1/2}$ in the meiotic spindle (Ref.III, Figure 4; Figure 5). LGN has been shown to participate in chromosome alignment by regulating the spindle elongation and cortical localisation (Guo and Gao, 2009). NuMA is associated with the centrosome core structure in meiotic cells and functions during meiotic maturation. Accurate translocation to the meiotic poles is important for maturation that leads to functional spindle poles (Schatten and Sun, 2011). RIC8A may regulate these processes since the downregulation of RIC8A synthesis by RNA interference during oocyte maturation interfered with the correct localisation of $G\alpha_{i1/2}$ and reduced its level in the cell cortex (Ref.III, Figure 8G). Although the downregulation of *Ric8* had no statistically significant effect on morphology, we observed a tendency for some unfertilized oocytes to divide abnormally (forming two or three almost equal cells). Furthermore, meiosis I lasted longer in *Ric8* siRNA treated cells and also some oocytes could not maintain the correct positioning in metaphase.

Meiotic spindle positioning in mouse oocytes relies mostly on actin-dependent mechanisms but not microtubules (Lancaster and Baum, 2014). In mammalian oocytes, there are no true centrosomes and astral microtubules, therefore different pools of F-actin meshworks in the cortex and in the cytoplasm play the key role in the positioning of spindles (Almonacid et al., 2014; Chaigne et al., 2012). To compensate the lack of centrosomes and astral microtubules, oocytes use several alternative strategies depending on the species (Almonacid et al., 2014). Although, we have shown that RIC8A is important in F-actin assembly, in oocyte meiotic divisions there are possibly other regulators that could compensate for RIC8A downregulation after siRNA treatment.

5. RIC8A and neural crest-derived structures (Ref.II, Ref.V)

Previous results have suggested that functionality and behaviour of the neural crest cells may be affected in *Nes;Ric8a*^{CKO} mice. The cranial neural crest cells migrate first rostrally between E9 and E10 in mouse embryo to contribute to the initial layer of meningeal cells that becomes a part of the leptomeninges (Etchevers et al., 1999; Siegenthaler and Pleasure, 2011). Without this migration, the development of the telencephalon and cranio-facial development are interrupted (Etchevers et al., 1999). The genotyping data of *Nes;Ric8a*^{CKO} mice indicated that the number of newborn pups per litter was smaller than the average, therefore the embryos at different development stages were evaluated. The phenotypic evaluation revealed that at E10.5, almost 40% of *Nes;Ric8a*^{CKO} embryos had severe developmental abnormalities and they died around that

period or shortly after. These mutant mice displayed neural tube closure defects in the trunk and head regions, morphologically defective brain vesicles, twisted body shape and cranio-facial defects that were not detected in the littermate controls (Ref.II, Figure 1A–1D). A larger group of *Nes;Ric8a*^{CKO} embryos had rather mild phenotypic defects or were even indistinguishable from littermates. Therefore, in some cases, the absence of RIC8A in the neural progenitors affects more severely the migrating cranial NCCs and results in the defective telencephalon and cranio-facial development. However, at the peak phase of mouse neurogenesis at E14.5, the majority of *Nes;Ric8a*^{CKO} embryos (~80%) were indistinguishable from the control embryos. Nevertheless, at the age of E18.5 the *Nes;Ric8a*^{CKO} mutant animals were again easily recognisable by their neuromuscular phenotype (described earlier in Results and Discussion Chapter 1.1.). Some of the observed defects in *Nes;Ric8a*^{CKO} mice again point to developmental aberrations in the neural crest-derived structures, such as the short snout and steep forehead. The RIC8A expression (E9.5) and *NestinCre* upregulation (E9.5) in the neuroepithelium (Ref.V, Figure S1) probably overlap with the time of cranial neural crest migration and the deficiency of RIC8A sum to randomly affect the neural crest cells that migrate to the rostral region or influences the rear part of cranial NCCs.

NCCs also provide all parasympathetic innervation of the heart which influences the normal myocardial function (Kirby et al., 1983). Therefore, we analysed RIC8A and *NestinCre* transgene expression at E14.5 in the area of coronary artery and in the wall of the aorta (Ref. V, Figure 5A,5B). The NCCs contribute to the heart development in the same area giving rise to cardiac smooth muscle cells which contribute to the development of coronary artery and interseptum (Arima et al., 2012; Dettlaff-Swiercz et al., 2005; Waldo et al., 1998). Moreover, in the heart development, it has been demonstrated that the early migration of preotic NCCs, rather than postotic NCCs, gives rise to smooth muscle cells and contributes to coronary artery development (Arima et al., 2012). According to the above-mentioned results in some cases, RIC8A deletion affects the very early migrating cranial NCCs, however, in the majority of cases RIC8A deletion affects cranial NCCs in later stages resulting in malformations of heart and brain.

Moreover, poor muscle innervation described in *Nes;Ric8a*^{CKO} mice may also be due to defects in NCCs derived structures that in turn affect myelination of peripheral nerve and their development through radial sorting. Myelination in the peripheral nervous system is accomplished by Schwann cells which originate from NCCs (Witt and Brady, 2000). Schwann cells deposit polarised BM around themselves and the myelinated axon during the radial sorting. This process is dependent on the interaction between laminin and β1-integrins or α-dystroglycan (McKee et al., 2012). Interference with this interaction in Schwann cells by ablation of laminins (α2; γ2), integrin β1, Ilk, FAK, RhoGTPases, GPR56 or α-dystroglycan function causes dysmyelination and subsequent paralysis, tremors and muscular dystrophies (Berti et al., 2011; Berti et al., 2006; Feltri et al., 2008; Gheyara et al., 2007; Giera et al., 2015; Grove et al.,

2007; Pereira et al., 2009; Postel et al., 2008). These results strongly support the idea that RIC8A functionality is essential for nerve myelination by regulating the BM organisation through RhoA pathway. However, further studies are needed to unravel the role of RIC8A in nerve myelination.

In order to migrate along their cranial routes, cranial NCCs need to integrate positional and guidance cues to dynamically interact with each other and with their surrounding extracellular matrix (Deakin and Turner, 2008). Clusters of migrating cells firmly associate with each other while only transiently adhere to a substrate (Friedl et al., 2004). A myriad of molecular signals trigger the cascade of coordinated events like induction, specification, polarisation and migration (Theveneau et al., 2010). Signal transduction of heterotrimeric G-proteins has been described to regulate each of these events by promoting actin cytoskeleton reorganisation via activation of small GTPases such as Cdc42, Rac and Rho (Cotton and Claing, 2009; Kjoller and Hall, 1999; Nobes and Hall, 1995). It is well documented that Cdc42 has an essential role in NCC development. Cdc42 is activated by integrins and focal adhesion kinase (FAK) and loss of integrin β1 or FAK in NCCs result in craniofacial and cardiovascular developmental defects (Pietri et al., 2004; Vallejo-Illarramendi et al., 2009). The total deletion of Cdc42 caused embryonic lethality and aberrant actin cytoskeleton organisation (Chen et al., 2000; Liu et al., 2013). *Cdc42* conditional knockout studies have indicated that Cdc42 plays a crucial role in the renewal of neural progenitor cell and cerebral hemisphere separation (Cappello et al., 2006; Chen et al., 2000; Peng et al., 2013). Moreover, deletion of Cdc42 in NCCs induced embryonic lethality with craniofacial morphogenetic defects showing similar defects in craniofacial and cardiovascular development as in *Nes;Ric8a*^{CKO} embryos (Liu et al., 2013). A recent study by Yan and colleagues also affirmed that RIC8A participates in Cdc42 activation through $G\alpha_{13}$ (Yan et al., 2015). Furthermore, the evidence that heterotrimeric G-protein signalling controls the collective and directional migration of NCCs was also provided recently (Theveneau et al., 2010; Theveneau and Mayor, 2011). However, the craniofacial defects mostly associate with defective signalling via $G\alpha_q/G\alpha_{11}$ rather than $G\alpha_{12}/G\alpha_{13}$ which insufficiency has been shown to contribute to the cardiac malformations such as coronary artery dilation and various VSD defects that were also manifested in newborn *Nes;Ric8a*^{CKO} pups (Dettlaff-Swiercz et al., 2005; Ref.V, Figure 5C-5H). Furthermore, similarly to *Nes;Ric8a*^{CKO} mice and *Ric8a*$^{-/-}$ mES cells, Ric8A loss-of-function completely abolishes the ability of cranial NCCs to spread and migrate in *Xenopus laevis*, suggesting impaired cell adhesion, which in turn leads to craniofacial defects (Fuentealba et al., 2013; Maldonado-Agurto et al., 2011). These results strongly suggest the requirement for RIC8A functionality in migration and subsequent differentiation of NCCs that contribute to craniofacial development.

Intrigued by this data, I decided to identify possible neural crest migration defects in *Nes;Ric8a*^{CKO} embryos for this dissertation by labelling migrating neural crest cells using transcription factor AP2α (Activating enhancer binding Protein 2 alpha) as a marker. The results indicated that the neural crest cells

were able to migrate to their destination areas also in *Nes;Ric8a*^{CKO} embryos. AP2α-positive cells were found in leptomeninges of the telencephalic vesicles (Figure 5A;5A'), but they were not present in the meninges of mesencephalic vesicle (Figure 5B;5B'). AP2α-positive cells were also detected in the pharyngeal arches from where they migrate to the heart (Figure 5C;5C'), also in dorsal root ganglion and in the epidermis where NCCs contribute to the development of melanocytes (Figure 5D;5D'). Moreover, the deletion of RIC8A did not alter the initiation of cell migration in the cerebral cortex development (Ref. II, Figure 3G,3H), but showed aberrant positioning afterwards. Thus, incorrect positioning of cranial NCCs may be one of the reasons for cranio-facial defects in E10.5 in *Nes;Ric8a*^{CKO} mice.

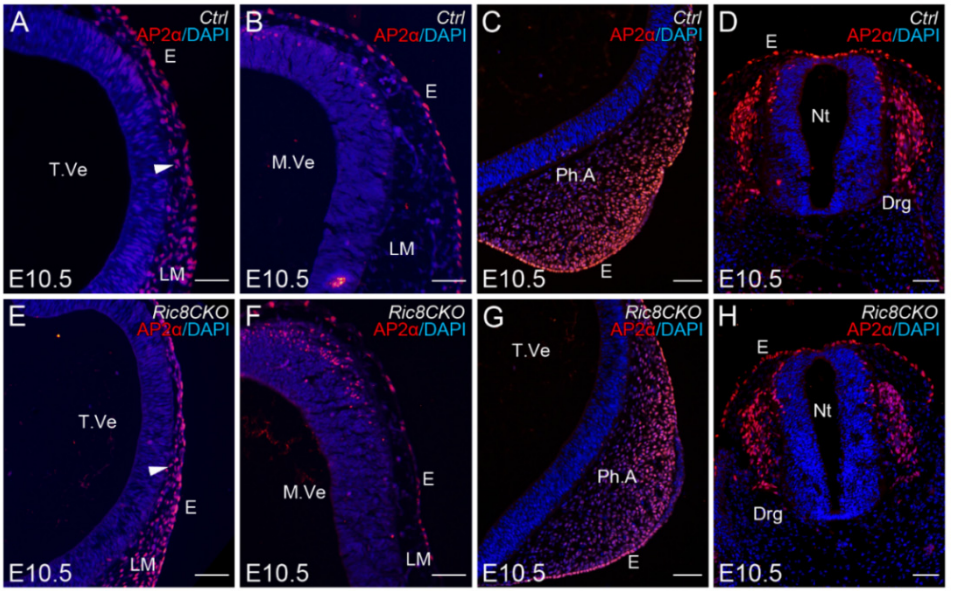

Figure 5. AP2α positive migrating neural crest cells in control (*Ctrl*) and *Nes;Ric8a*^{CKO} embryos (*Ric8CKO*) at E10.5. (A;A') AP2α positive neural crest cells (white arrowhead) in the telencephalic meninges. (B,B') The lack of neural crest cells in the mesencephalic meninges (C,C') Neural crest cell population in the pharyngeal arch and (D) in the dorsal root ganglion. Abbreviations: T.Ve – telencephalic vesicle; M.Ve – mesencephalic vesicle; LM – leptomeninges; Ph.A – pharyngeal arch; Nt – neural tube; Drg – dorsal root ganglion; E – epidermis. Scale bars: 100 µm.

6. The role of RIC8A in the development of congenital muscular dystrophies (Ref. II and Ref. V)

Nes;Ric8a^{CKO} mice exhibit a severe neuromuscular phenotype which is mainly due to multiple developmental defects. The enlarged ventricles, multiple cerebral cortical ectopias and breaches in the BM (Ref.II, Figure 1I–1N; Figure 2; Figure 4 and Figure 5, respectively) strongly resemble the symptoms found in the congenital muscular dystrophies, especially Fukuyama Congenital Muscular Dystrophy (FCMD), Walker-Warburg syndrome (WWS) and Muscle-Eye-Brain (MEB) disease (Bouchet et al., 2007; Pabuscu et al., 2003; Saito, 2006; Yoshioka and Higuchi, 2005). These syndromes are characterised by ocular defects, muscle dystrophy, dysmyelination and occasionally heart, kidney or thymus function failure.

In the current thesis, the brain, muscle and heart defects have been characterised earlier. In addition to these, *Nes;Ric8a*^{CKO} mice also display defective lens development revealing abnormal vacuoles between the secondary fibres (Ref.V, Figure 3). These vacuoles and aberrant Y-suture formation in lens secondary fibre elongation are linked to defective adhesion and migration, associated with aberrant RhoA signaling (Cammas et al., 2012; Maddala et al., 2011; Maddala et al., 2004; Maddala et al., 2003; Maddala et al., 2008). All of the deficiencies that are found in *Nes;Ric8a*^{CKO} mice add up to a phenotype that highly resembles congenital muscular dystrophies.

Since the WWS is the most severe syndrome among these disorders (Barkovich et al., 2012; Cormand et al., 2001; Devisme et al., 2012), we presume that the lack of RIC8A in neural progenitor cells mostly generates the phenotype resembling WWS. Persons with lissencephaly and only mild eye abnormalities develop muscle dysplasia but can live beyond infancy like FCMD and MEB patients (Jang et al., 2013; Yoda et al., 2011). However, WWS patients mostly live only for a few months, and never reach over 3 years. FCMD patients form a distinct group from other two having the mildest phenotype and mostly lacking ocular defects (Cormand et al., 2001; Vajsar and Schachter, 2006). MEB patients are usually floppy, mentally retarded with suspected visual problems, however, they are able to learn of few words and learn to walk (Cormand et al., 2001). The defects described in WWS individuals are the most severe and can be diagnosed already prenatally because of aggravated hydrocephalus (Cormand et al., 2001). However, a lot of patients possess clinical features somewhere in between these typical groups described (Cormand et al., 2001). Despite intensive research and genetic screening of genes involved in glycosylation of α-dystroglycan, a lot of the cases remain unexplained suggesting that other genes and/or signalling pathways may be involved in these pathologies (Belpaire-Dethiou et al., 1999; Devisme et al., 2012; Vajsar and Schachter, 2006).

Our hypothesis is that in addition to described features, the NCC with impaired functionality, due to the absence of RIC8A, may also cause additional defects in the heart development and nerve myelination. Our hypothesis is also

supported by case studies where cleft palate defects were described in WWS patients (Pratap et al., 2007; Vajsar et al., 2008). Our research also showed that RIC8A is expressed in neural crest cell derived structures (heart, meninges) and it is involved in regulation of cell shape, division adhesion, and migration. However, further studies are needed to clarify how the absence of RIC8A impairs the NCC migration and differentiation contributes to multiple developmental processes defects that could cause CMD.

To sum up, our findings demonstrate that the functional RIC8A is required for the development and normal functioning of the central nervous system in mammals as well as for the development of the peripheral nervous system. The removal of RIC8A from the neural precursor cells impairs differentiation and migration of progenitor cells in the neuroepithelium and also neural crest cells. On the cellular level RIC8A regulates reorganisation of BM and cell's morphogenetic changes which both require proper actin cytoskeleton organisation, and probably RIC8A modulates the activity of RhoA pathway. The phenotype of created mutant mice has features that are characteristic to CMD symptoms i.e the development of brain, eyes, muscles and heart is affected. Among CMD disorders, *Nes;Ric8a*^{CKO} mouse model could be considered the most similar to Walker-Warburg syndrome, but being more severe and causing early lethality. In principle malfunctioning of RIC8A may be one of the factors generating WWS due to the failure in cell-ECM interaction and insufficient signalling via G-protein mediated RhoA pathway. We also suggest that defective neural crest cells might contribute to the emergence of the CMDs if the integrin-mediated interactions with ECM are defective.

CONCLUSIONS

RIC8A is a highly conserved protein that regulates the activity of a subset of G protein α subunits and is irreplaceable in the normal development and functioning of the brain regions that influence the emotional behaviour and memory. The total loss of RIC8A function is embryonically lethal and causes severe gastrulation defects in mice. To characterise the role of RIC8A function in the nervous system and its development we generated two knockout mouse models where RIC8A was knocked out from the postmitotic neurons and neural precursor cells, respectively. Additionally, the mechanism of RIC8A action in regulating the adhesion of cells to ECM, and in migration was analysed *in vitro* in primary cells.

The main results of this dissertation can be concluded as follows

1. Deletion of RIC8A from the nervous system during its development leads to the severe defects in neuromuscular signalling that affect the functioning of skeletal muscles and heart. The muscles of these animals suffer from progressive atrophy and fibrosis, the heart function is severely impaired due to several morphological defects.
2. RIC8A has an essential role in neurogenesis by maintaining the integrity of pial basement membrane. The breaches in the basement membrane cause further defects in the attachment of arachnoid trabeculae and radial glial endfeet to it, which in turn leads to wrong positioning of Cajal-Retzius cells.
3. RIC8A functions as one of the organisers of actin filaments assembly. RIC8A deficient cells fail to activate β1-integrin and form proper focal adhesion complexes or stress fibres, which subsequently impair cell migration.
4. Deletion of RIC8A from neural precursor cells shift the ratio of the planar and oblique cell division toward planar divisions and thereby the proportion of direct or indirect neurogenesis.
5. RIC8A also regulates the development of neural crest-derived structures such as meninges, cranio-facial development, the formation of coronary artery and interseptum in heart and innervation of nerves in muscles.
6. The phenotype and histological studies of the RIC8A deficient knockout mice reveal its high resemblance with the symptoms of congenital muscular dystrophies such as Walker-Warburg disease, Muscle-Eye-Brain disease and Fukuyama congenital muscular dystrophy.

SUMMARY IN ESTONIAN

RIC8A roll hiire närvisüsteemis ja selle arengus

Kesknärvisüsteemi ja perifeerse närvisüsteemi häireteta toimimine on olulise tähtsusega täisväärtusliku elu tagamiseks. Närvisüsteemi funktsioneerimiseks on vajalik neuraalsete eellasrakkude, gliiarakkude ja neuronite õigeaegne moodustumine ning paigutumine. Sellele paneb aluse korrektselt läbitud neurogenees, mis hõlmab endas neuraalsete eellasrakkude jagunemist, neuronite diferentseerumist ja migreerumist ning rakkudevaheliste võrgustike loomist (Bjornsson et al., 2015). Üheks üliolulisteks ja väga kompleksseks struktuuriks imetajate neurogeneesis on evolutsiooniliselt noor 6-kihiline neokorteks, mis vastutab tunnetuslike ja õppimis- ning tajufunktsioonide eest (Buchman and Tsai, 2007). Neokorteksi suurus oleneb aga embrüonaalse neurogeneesi käigus tekkinud neuronite arvust ning õigest paiknemisest kihtides (Fernandez et al., 2016).

Rakkude jagunemist, adhesiooni ja liikumist õigetesse piirkondadesse koordineerivad erinevad signaalide võrgustikud, mis vahendavad signaali ülekannet rakuvälisest keskkonnast rakku (Bastiani and Mendel, 2006). Loomariigis konserveerunud G-valkude vahendatud signaali ülekanne enim kasutatud mehhanismiks rakuvälise signaali viimiseks rakusisesesse keskkonda (Bastiani and Mendel, 2006). G-valkude aktiviseerimisel osalevad transmembraansed retseptorid ning rakusisesed G-valgu aktiivsuse regulaatorid. Üheks neist on RIC8A, mis mõjutab G-valgu $G\alpha_q/G\alpha_{11}$; $G\alpha_i/G\alpha_o$ and $G\alpha_{12}/G\alpha_{13}$ subühikute toimimist. Seni teadaolevalt omab RIC8A kahte funktsiooni, millest esmalt kirjeldati nukleotiidivahetuse võime (GEF) G valgu α subühiku aktiveerimisel ning hiljem G-valgu stabiliseerimine biosünteesil ja membraani suunamine (Chan et al., 2011; Gabay et al., 2011; Tall et al., 2013).

Imetaja närvisüsteemis on RIC8A täpselt kaardistatud koduhiires (*Mus musculus*). RIC8A ekspressioon on aktiivsel organogeneesi staadiumil (E9.5–E12.5) peamiselt neurospetsiifiline. Täiskasvanud hiire kesknärvisüsteemis on RIC8A avaldunud piirkondades, mis on seotud tunnetuslike ja õppimis- ning tajufunktsioonidega (Tõnissoo et al., 2003; Tõnissoo et al., 2006). Käitumiskatsed haplodefitsiitsete *Ric8a*$^{+/-}$ hiirtega näitasid, et neil esinevad ärevushäired, neil on vähenenud ruumiline taju ning õppimisvõime (Tõnissoo et al., 2006). Homosügootsed *Ric8a*$^{-/-}$ embrüod surevad gastrulatsiooni staadiumis (E6.5 – E8.5) tekkinud arenguanomaaliate tõttu (Tõnissoo et al., 2010).

Käesoleva töö eesmärgiks oli uurida RIC8A rolli närvisüsteemi arengus. Selleks loodi hiireliin, kus *Ric8a* geen oli välja lülitatud neuraalsetest eellasrakkudest. Lisaks loodi hiireliin, kus *Ric8a* oli inaktiveeritud diferentseerunud neuronites. RIC8A valgu puudumine hiire kesknärvisüsteemi ja perifeerse närvisüsteemi rakkudest põhjustab tugevat neuromuskulaarset fenotüüpi, mida iseloomustab liikumisvõime puudumine, värisemine ja tõmblused ning mis põhjustab hiirte sünnijärgse suremuse. Mõlemal närvisüsteemi-põhisel mutandil esinesid skeleti- ja südamelihaste atroofia. Hiirtel, kellel RIC8A puudus närvi-

süsteemi arengu ajal esinesid lisaks veel morfoloogilised defektid südame ja näo-kolju arengus.

Neuraalsetes eellasrakkudes RIC8A puudumine põhjustas rakkude migratsiooni häired neurogeneesis. Mutantsete hiirte ajukoor oli õhem ning ebakorrektse morfoloogiaga. Nende ajukoor sisaldas anomaalseid ektoopilisi väljakasve, mis olid eelkõige tekkinud pehmekesta basaalmembraani (BM) purunemise tagajärel. BM katked põhjustasid omakorda sellest sõltuvate rakkude jätkete kinnitumise (radiaalgliia rakud, ämblikvõrk-kelme trabeekulid) ning paiknemise häireid (Cajal-Retzius rakud). Lähemalt uuriti edasi RIC8A funktsiooni raku ja rakuvälise maatriksi interaktsioonil *in vitro*. Tulemused näitasid, et RIC8A defitsiitsed rakud ei ole võimelised moodustama rakk-maatriks interaktsioonil olulisi fokaalse adhesiooni komplekse ega ka stressifiibreid, mis põhjustasid ka rakkude vähenenud migreerumist. Need häired on peamiselt põhjustatud β1-integriini vahendatud signaaliraja defektsusega.

RIC8A puudus neuraalsetest eellasrakkudest mõjutas ka neurogeneesis toimuvaid rakujagunemisi, kus vähenes viltuste jagunemiste osakaal ning kasvas planaarselt jagunevate rakkude osakaal. Selline tasakaalu muutus mõjutab oluliselt tekkivate rakkude arvu ajukoore arengus, kus viltuste jagunemistega suurendatakse rakkude mitmekesisust erinevate eellasrakkude abil (kaudne neurogenees) ning samas säilitatakse eellasrakkude õige arvukus. Rohkete planaarsete jagunemiste käigus tekib pigem kaks sama saatusega rakku (otsene neurogenees) ning neurogenees peatub enneaegselt, põhjustades õhema ajukoore tekke.

RIC8A geeni inaktiveerimine närvisüsteemi arenemise ajal mõjutas ka neuraalharja rakkudest moodustatavate struktuuride arengut, põhjustades näo-kolju arenguhäireid ning südames pärgarterite ja vatsakeste vaheseina defekte. Samuti võib RIC8A puudus ajukelmetes põhjustada kõrvalekaldeid BM struktuuris ja koostises ning mõjutada närvide müeliniseerumist ja seeläbi ka lihaste innervatsiooni. Nii ajukelmete tekkesse kui ka müeliniseerumisse panustavad oluliselt omavad neuraalharja rakud.

Kirjeldatud defektid RIC8A puudulikel hiirtel sarnanevad kaasasündinud lihasdüstroofiate tunnustega, mis näiteks Fukuyama lihasdüstroofia, Walker-Warburgi sündroomi ja lihase-silma-aju haiguse korral patsientidel esinevad. Need haigused on eelkõige seotud häirunud rakk-maatriks vahendatud signalisatsiooni tõttu läbi düstroglükaanide ja integriinide. Seega, häired RIC8A funktsioonis koostöös G-valkude ja β1-integriinide vahendatud signalisatsiooniga võib olla seotud nende haiguste kujunemisel.

REFERENCES

AakuSaraste, E., Hellwig, A., Huttner, W.B., 1996. Loss of occludin and functional tight junctions, but not ZO-1, during neural tube closure – Remodeling of the neuroepithelium prior to neurogenesis. Developmental Biology 180, 664–679.

Afshar, K., Willard, F.S., Colombo, K., Johnston, C.A., McCudden, C.R., Siderovski, D.P., Gönczy, P., 2004. RIC-8 is required for GPR-1/2-dependent Galpha function during asymmetric division of C. elegans embryos. Cell 119, 219–230.

Afshar, K., Willard, F.S., Colombo, K., Siderovski, D.P., Gönczy, P., 2005. Cortical localization of the Galpha protein GPA-16 requires RIC-8 function during C. elegans asymmetric cell division, Development, England, pp. 4449–4459.

Almonacid, M., Terret, M.E., Verlhac, M.H., 2014. Actin-based spindle positioning: new insights from female gametes. Journal of Cell Science 127, 477–483.

Anthony, T.E., Klein, C., Fishell, G., Heintz, N., 2004. Radial glia serve as neuronal progenitors in all regions of the central nervous system. Neuron 41, 881–890.

Arellano, J.I., Guadiana, S.M., Breunig, J.J., Rakic, P., Sarkisian, M.R., 2012. Development and distribution of neuronal cilia in mouse neocortex. Journal of Comparative Neurology 520, 848–873.

Arima, Y., Miyagawa-Tomita, S., Maeda, K., Asai, R., Seya, D., Minoux, M., Rijli, F.M., Nishiyama, K., Kim, K.S., Uchijima, Y., Ogawa, H., Kurihara, Y., Kurihara, H., 2012. Preotic neural crest cells contribute to coronary artery smooth muscle involving endothelin signalling. Nature Communications 3.

Barkovich, A.J., Guerrini, R., Kuzniecky, R.I., Jackson, G.D., Dobyns, W.B., 2012. A developmental and genetic classification for malformations of cortical development: update 2012. Brain 135, 1348–1369.

Bastiani, C., Mendel, J., 2006. Heterotrimeric G proteins in C. elegans. WormBook : the online review of C. elegans biology, 1–25.

Beggs, H.E., Schahin-Reed, D., Zang, K.L., Goebbels, S., Nave, K.A., Gorski, J., Jones, K.R., Sretavan, D., Reichardt, L.F., 2003. FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. Neuron 40, 501–514.

Belpaire-Dethiou, M.C., Saito, K., Fukuyama, Y., Kondo-Iida, E., Toda, T., Duprez, T., Verellen-Dumoulin, C., Van den Bergh, P.Y.K., 1999. Congenital muscular dystrophy with central and peripheral nervous system involvement in a Belgian patient. Neuromuscular Disorders 9, 251–256.

Berti, C., Bartesaghi, L., Ghidinelli, M., Zambroni, D., Figlia, G., Chen, Z.L., Quattrini, A., Wrabetz, L., Feltri, M.L., 2011. Non-redundant function of dystroglycan and beta 1 integrins in radial sorting of axons. Development 138, 4025–4037.

Berti, C., Nodari, A., Wrabetz, L., Feltri, M.L., 2006. Role of integrins in peripheral nerves and hereditary neuropathies. Neuromolecular Medicine 8, 191–204.

Betschinger, J., Knoblich, J.A., 2004. Dare to be different: Asymmetric cell division in Drosophila, C-elegans and vertebrates. Current Biology 14, R674–R685.

Bielle, F., Griveau, A., Narboux-Neme, N., Vigneau, S., Sigrist, M., Arber, S., Wassef, M., Pierani, A., 2005. Multiple origins of Cajal-Retzius cells at the borders of the developing pallium. Nature Neuroscience 8, 1002–1012.

Bifari, F., Berton, V., Pino, A., Kusalo, M., Malpeli, G., Di Chio, M., Bersan, E., Amato, E., Scarpa, A., Krampera, M., Fumagalli, G., Decimo, I., 2015. Meninges harbor cells expressing neural precursor markers during development and adulthood. Frontiers in Cellular Neuroscience 9.

Bjornsson, C.S., Apostolopoulou, M., Tian, Y.Z., Temple, S., 2015. It Takes a Village: Constructing the Neurogenic Niche. Developmental Cell 32, 435–446.

Borrell, V., Marin, O., 2006. Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. Nature Neuroscience 9, 1284–1293.

Bouchet, C., Vuillaumier-Barrot, S., Gonzales, M., Boukari, S., Le Bizec, C., Fallet, C., Delezoide, A.L., Moirot, H., Laquerriere, A., Encha-Razavi, F., Durand, G., Seta, N., 2007. Detection of an Alu insertion in the POMT1 gene from three French Walker Warburg syndrome families. Molecular Genetics and Metabolism 90, 93–96.

Boutin, C., Labedan, P., Dimidschstein, J., Richard, F., Cremer, H., Andre, P., Yang, Y.Z., Montcouquiol, M., Goffinet, A.M., Tissir, F., 2014. A dual role for planar cell polarity genes in ciliated cells. Proceedings of the National Academy of Sciences of the United States of America 111, E3129–E3138.

Bowman, S.K., Neumuller, R.A., Novatchkova, M., Du, Q.S., Knoblich, J.A., 2006. The Drosophila NuMA homolog mud regulates spindle orientation in asymmetric cell division. Developmental Cell 10, 731–742.

Brasseur-Daudruy, M., Vivier, P.H., Ickowicz, V., Eurin, D., Verspyck, E., 2012. Walker-Warburg syndrome diagnosed by findings of typical ocular abnormalities on prenatal ultrasound. Pediatric Radiology 42, 488–490.

Buchman, J.J., Tsai, L.-H., 2007. Spindle regulation in neural precursors of flies and mammals. Nature Reviews Neuroscience 8, 89–100.

Buhl, A.M., Johnson, N.L., Dhanasekaran, N., Johnson, G.L., 1995. G-alpha(12) and G-alpha(13) stimulate Rho-dependent stress fiber formation and focal adhesion assembly. Journal of Biological Chemistry 270, 24631–24634.

Bultje, R.S., Castaneda-Castellanos, D.R., Jan, L.Y., Jan, Y.-N., Kriegstein, A.R., Shi, S.-H., 2009. Mammalian Par3 Regulates Progenitor Cell Asymmetric Division via Notch Signaling in the Developing Neocortex. Neuron 63, 189–202.

Cammas, L., Wolfe, J., Choi, S.Y., Dedhar, S., Beggs, H.E., 2012. Integrin-Linked Kinase Deletion in the Developing Lens Leads to Capsule Rupture, Impaired Fiber Migration and Non-Apoptotic Epithelial Cell Death. Investigative Ophthalmology & Visual Science 53, 3067–3081.

Cappello, S., Attardo, A., Wu, X.W., Iwasato, T., Itohara, S., Wilsch-Brauninger, M., Eilken, H.M., Rieger, M.A., Schroeder, T.T., Huttner, W.B., Brakebusch, C., Götz, M., 2006. The Rho-GTPase cdc42 regulates neural progenitor fate at the apical surface. Nature Neuroscience 9, 1099–1107.

Cappello, S., Boehringer, C.R.J., Bergami, M., Conzelmann, K.-K., Ghanem, A., Tomassy, G.S., Arlotta, P., Mainardi, M., Allegra, M., Caleo, M., van Hengel, J., Brakebusch, C., Götz, M., 2012. A Radial Glia-Specific Role of RhoA in Double Cortex Formation. Neuron 73, 911–924.

Caruana, G., Bernstein, A., 2001. Craniofacial dysmorphogenesis including cleft palate in mice with an insertional mutation in the discs large gene. Molecular and Cellular Biology 21, 1475–1483.

Chaigne, A., Verlhac, M.H., Terret, M.E., 2012. Spindle positioning in mammalian oocytes. Experimental Cell Research 318, 1442–1447.

Chan, P., Gabay, M., Wright, F.A., Tall, G.G., 2011. Ric-8B is a GTP-dependent G protein alphas guanine nucleotide exchange factor. J Biol Chem 286, 19932–19942.

Chan, P., Thomas, C.J., Sprang, S.R., Tall, G.G., 2013. Molecular chaperoning function of Ric-8 is to fold nascent heterotrimeric G protein alpha subunits. Proceedings of the National Academy of Sciences of the United States of America 110, 3794–3799.

Chen, C.T., Hehnly, H., Yu, Q., Farkas, D., Zheng, G.Q., Redick, S.D., Hung, H.F., Samtani, R., Jurczyk, A., Akbarian, S., Wise, C., Jackson, A., Bober, M., Guo, Y., Lo, C., Doxsey, S., 2014. A Unique Set of Centrosome Proteins Requires Pericentrin for Spindle-Pole Localization and Spindle Orientation. Current Biology 24, 2327–2334.

Chen, F., Ma, L., Parrini, M.C., Mao, X., Lopez, M., Wu, C., Marks, P.W., Davidson, L., Kwiatkowski, D.J., Kirchhausen, T., Orkin, S.H., Rosen, F.S., Mayer, B.J., Kirschner, M.W., Alt, F.W., 2000. Cdc42 is required for PIP2-induced actin polymerization and early development but not for cell viability. Current Biology 10, 758–765.

Chen, L., Melendez, J., Campbell, K., Kuan, C.Y., Zheng, Y., 2009. Rac1 deficiency in the forebrain results in neural progenitor reduction and microcephaly. Developmental Biology 325, 162–170.

Chen, Z.L., Strickland, S., 2003. Laminin gamma 1 is critical for Schwann cell differentiation, axon myelination, and regeneration in the peripheral nerve. Journal of Cell Biology 163, 889–899.

Chishiki, K., Kamakura, S., Yuzawa, S., Hayase, J., Sumimoto, H., 2013. Ubiquitination of the heterotrimeric G protein alpha subunits G alpha i2 and G alpha q is prevented by the guanine nucleotide exchange factor Ric-8A. Biochemical and Biophysical Research Communications 435, 414–419.

Cormand, B., Pihko, H., Bayes, M., Valanne, L., Santavuori, P., Talim, B., Gershoni-Baruch, R., Ahmad, A., van Bokhoven, H., Brunner, H.G., Voit, T., Topaloglu, H., Dobyns, W.B., Lehesjoki, A.E., 2001. Clinical and genetic distinction between Walker-Warburg syndrome and muscle-eye-brain disease. Neurology 56, 1059–1069.

Costa, M.R., Wen, G., Lepier, A., Schroeder, T., Götz, M., 2008. Par-complex proteins promote proliferative progenitor divisions in the developing mouse cerebral cortex. Development 135, 11–22.

Costell, M., Gustafsson, E., Aszodi, A., Morgelin, M., Bloch, W., Hunziker, E., Addicks, K., Timpl, R., Fassler, R., 1999. Perlecan maintains the integrity of cartilage and some basement membranes. Journal of Cell Biology 147, 1109–1122.

Cotton, M., Claing, A., 2009. G protein-coupled receptors stimulation and the control of cell migration. Cellular Signalling 21, 1045–1053.

Couwenbergs, C., Labbe, J.C., Goulding, M., Marty, T., Bowerman, B., Gotta, M., 2007. Heterotrimeric G protein signaling functions with dynein to promote spindle positioning in C. elegans. Journal of Cell Biology 179, 15–22.

Couwenbergs, C., Spilker, A.C., Gotta, M., 2004. Control of embryonic spindle positoning and G alpha activity by C-elegans RIC-8. Current Biology 14, 1871–1876.

Daley, W.P., Gervais, E.M., Centanni, S.W., Gulfo, K.M., Nelson, D.A., Larsen, M., 2012. ROCK1-directed basement membrane positioning coordinates epithelial tissue polarity. Development 139, 411–422.

David, N.B., Martin, C.A., Segalen, M., Rosenfeld, F., Schweisguth, F., Bellaiche, Y., 2005. Drosophila Ric-8 regulates G alpha i cortical localization to promote G alpha i-dependent planar orientation of the mitotic spindle during asymmetric cell division. Nature Cell Biology 7, 1083–1090.

De Arcangelis, A., Mark, M., Kreidberg, J., Sorokin, L., Georges-Labouesse, E., 1999. Synergistic activities of alpha 3 and alpha 6 integrins are required during apical ectodermal ridge formation and organogenesis in the mouse. Development 126, 3957–3968.

Deakin, N.O., Turner, C.E., 2008. Paxillin comes of age. Journal of Cell Science 121, 2435–2444.

Dechiara, T.M., Vejsada, R., Poueymirou, W.T., Acheson, A., Suri, C., Conover, J.C., Friedman, B., McClain, J., Pan, L., Stahl, N., Ip, N.Y., Kato, A., Yancopoulos, G.D., 1995. Mice lacking the CNTF receptor, unlike mice lacking CNTF, exhibit profound motor-neuron deficits at birth. Cell 83, 313–322.

Decimo, I., Fumagalli, G., Berton, V., Krampera, M., Bifari, F., 2012. Meninges: from protective membrane to stem cell niche. American journal of stem cells 1, 92–105.

Dettlaff-Swiercz, D.A., Wettschureck, N., Moers, A., Huber, K., Offermanns, S., 2005. Characteristic defects in neural crest cell-specific G alpha(q)/G alpha(11)- and G alpha(12)/G alpha(13)-deficient mice. Developmental Biology 282, 174–182.

Devisme, L., Bouchet, C., Gonzales, M., Alanio, E., Bazin, A., Bessieres, B., Bigi, N., Blanchet, P., Bonneau, D., Bonnieres, M., Bucourt, M., Carles, D., Clarisse, B., Delahaye, S., Fallet-Bianco, C., Figarella-Branger, D., Gaillard, D., Gasser, B., Delezoide, A.-L., Guimiot, F., Joubert, M., Laurent, N., Laquerriere, A., Liprandi, A., Loget, P., Marcorelles, P., Martinovic, J., Menez, F., Patrier, S., Pelluard, F., Perez, M.-J., Rouleau, C., Triau, S., Attie-Bitach, T., Vuillaumier-Barrot, S., Seta, N., Encha-Razavi, F., 2012. Cobblestone lissencephaly: neuropathological subtypes and correlations with genes of dystroglycanopathies. Brain 135, 469–482.

Du, Q.S., Macara, I.G., 2004. Mammalian pins is a conformational switch that links NuMA to heterotrimeric G proteins. Cell 119, 503–516.

Du, Q.S., Stukenberg, P.T., Macara, I.G., 2001. A mammalian Partner of inscuteable binds NuMA and regulates mitotic spindle organization. Nature Cell Biology 3, 1069–1075.

Durham, D., Worthley, L.I., 2002. Cardiac arrhythmias: diagnosis and management. The bradycardias. Crit Care Resusc 4, 54–60.

Erickson, A.C., Couchman, J.R., 2000. Still more complexity in mammalian basement membranes. Journal of Histochemistry & Cytochemistry 48, 1291–1306.

Etchevers, H.C., Couly, G., Vincent, C., Le Douarin, N.M., 1999. Anterior cephalic neural crest is required for forebrain viability. Development 126, 3533–3543.

Farkas, L.M., Huttner, W.B., 2008. The cell biology of neural stem and progenitor cells and its significance for their proliferation versus differentiation during mammalian brain development. Current Opinion in Cell Biology 20, 707–715.

Feltri, M.L., Porta, D.G., Previtali, S.C., Nodari, A., Migliavacca, B., Cassetti, A., Littlewood-Evans, A., Reichardt, L.F., Messing, A., Quattrini, A., Mueller, U., Wrabetz, L., 2002. Conditional disruption of beta 1 integrin in Schwann cells impedes interactions with axons. Journal of Cell Biology 156, 199–209.

Feltri, M.L., Suter, U., Relvas, J.B., 2008. The Function of RhoGTPases in Axon Ensheathment and Myelination. Glia 56, 1508–1517.

Fenech, C., Patrikainen, L., Kerr, D.S., Grall, S., Liu, Z., Laugerette, F., Malnic, B., Montmayeur, J.P., 2009. Ric-8A, a Galpha protein guanine nucleotide exchange factor potentiates taste receptor signaling. Front Cell Neurosci 3, 11.

Fernandez, V., Llinares-Benadero, C., Borrell, V., 2016. Cerebral cortex expansion and folding: what have we learned? Embo Journal 35, 1021–1044.

Fielding, A.B., Dobreva, I., McDonald, P.C., Foster, L.J., Dedhar, S., 2008. Integrin-linked kinase localizes to the centrosome and regulates mitotic spindle organization. Journal of Cell Biology 180, 681–689.

Figueroa, M., Hinrichs, M.V., Bunster, M., Babbitt, P., Martinez-Oyanedel, J., Olate, J., 2009. Biophysical studies support a predicted superhelical structure with armadillo repeats for Ric-8. Protein Sci 18, 1139–1145.

Fink, J., Carpi, N., Betz, T., Betard, A., Chebah, M., Azioune, A., Bornens, M., Sykes, C., Fetler, L., Cuvelier, D., Piel, M., 2011. External forces control mitotic spindle positioning. Nature Cell Biology 13, 771–U401.

Friedl, P., Hegerfeldt, Y., Tusch, M., 2004. Collective cell migration in morphogenesis and cancer. International Journal of Developmental Biology 48, 441–449.

Fuentealba, J., Toro-Tapia, G., Arriagada, C., Riquelme, L., Beyer, A., Henriquez, J.P., Caprile, T., Mayor, R., Marcellini, S., Hinrichs, M.V., Olate, J., Torrejon, M., 2013. Ric-8A, a guanine nucleotide exchange factor for heterotrimeric G proteins, is critical for cranial neural crest cell migration. Developmental Biology 378, 74–82.

Gabay, M., Pinter, M.E., Wright, F.A., Chan, P., Murphy, A.J., Valenzuela, D.M., Yancopoulos, G.D., Tall, G.G., 2011. Ric-8 proteins are molecular chaperones that direct nascent G protein alpha subunit membrane association. Sci Signal 4, ra79.

Georges-Labouesse, E., Mark, M., Messaddeq, N., Gansmuller, A., 1998. Essential role of alpha 6 integrins in cortical and retinal lamination. Current Biology 8, 983–986.

Gheyara, A.L., Vallejo-Illarramendi, A., Zang, K., Mei, L., St-Arnaud, R., Dedhar, S., Reichardt, L.F., 2007. Deletion of integrin-linked kinase from skeletal muscles of mice resembles muscular dystrophy due to alpha 7 beta 1-integrin deficiency. American Journal of Pathology 171, 1966–1977.

Giera, S., Deng, Y.Y., Luo, R., Ackerman, S.D., Mogha, A., Monk, K.R., Ying, Y.Q., Jeong, S.J., Makinodan, M., Bialas, A.R., Chang, B.S., Stevens, B., Corfas, G., Piao, X.H., 2015. The adhesion G protein-coupled receptor GPR56 is a cell-autonomous regulator of oligodendrocyte development. Nature Communications 6.

Gilman, A.G., 1987. G-Proteins – Transducers of receptor-generated signals. Annual Review of Biochemistry 56, 615–649.

Götz, M., Huttner, W.B., 2005. The cell biology of neurogenesis. Nature Reviews Molecular Cell Biology 6, 777–788.

Graus-Porta, D., Blaess, S., Senften, M., Littlewood-Evans, A., Damsky, C., Huang, Z., Orban, P., Klein, R., Schittny, J.C., Muller, U., 2001. beta 1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. Neuron 31, 367–379.

Grewal, P.K., Hewitt, J.E., 2003. Glycosylation defects: a new mechanism for muscular dystrophy? Human Molecular Genetics 12, R259–R264.

Grove, M., Komiyama, N.H., Nave, K.A., Grant, S.G., Sherman, D.L., Brophy, P.J., 2007. FAK is required for axonal sorting by Schwann cells. Journal of Cell Biology 176, 277–282.

Gu, J.L., Muller, S., Mancino, V., Offermanns, S., Simon, M.I., 2002. Interaction of G alpha(12) with G alpha(13) and G alpha(q) signaling pathways. Proceedings of the National Academy of Sciences of the United States of America 99, 9352–9357.

Guo, X.Z., Gao, S.R., 2009. Pins homolog LGN regulates meiotic spindle organization in mouse oocytes. Cell Research 19, 838–848.

Gupta, A., Tsai, L.H., Wynshaw-Boris, A., 2002. Life is a journey: A genetic look at neocortical development. Nature Reviews Genetics 3, 342–355.

Halfter, W., Dong, S.C., Yip, Y.P., Willem, M., Mayer, U., 2002. A critical function of the pial basement membrane in cortical histogenesis. Journal of Neuroscience 22, 6029–6040.

Halfter, W., Yip, J., 2014. An organizing function of basement membranes in the developing nervous system. Mechanisms of Development 133, 1–10.

Hampoelz, B., Hoeller, O., Bowman, S.K., Dunican, D., Knoblich, J.A., 2005. Drosophila Ric-8 is essential for plasma-membrane localization of heterotrimeric G proteins. Nat Cell Biol 7, 1099–1105.

Hartmann, D., De Strooper, B., Saftig, P., 1999. Presenilin-1 deficiency leads to loss of Cajal-Retzius neurons and cortical dysplasia similar to human type 2 lissencephaly. Current Biology 9, 719–727.

Hatakeyama, J., Wakamatsu, Y., Nagafuchi, A., Kageyama, R., Shigemoto, R., Shimamura, K., 2014. Cadherin-based adhesions in the apical endfoot are required for active Notch signaling to control neurogenesis in vertebrates. Development 141, 1671–1682.

Hatten, M.E., 1999. Central nervous system neuronal migration. Annual Review of Neuroscience 22, 511–539.

Hatten, M.E., 2002. Neuroscience – New directions in neuronal migration. Science 297, 1660–1663.

Haubensak, W., Attardo, A., Denk, W., Huttner, W.B., 2004. Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: A major site of neurogenesis. Proceedings of the National Academy of Sciences of the United States of America 101, 3196–3201.

Haubst, N., Georges-Labouesse, E., De Arcangelis, A., Mayer, U., Goetz, M., 2006. Basement membrane attachment is dispensable for radial glial cell fate and for proliferation, but affects positioning of neuronal subtypes. Development 133, 3245–3254.

Hecht, J.H., Siegenthaler, J.A., Patterson, K.P., Pleasure, S.J., 2010. Primary Cellular Meningeal Defects Cause Neocortical Dysplasia and Dyslamination. Annals of Neurology 68, 454–464.

Hehr, U., Uyanik, G., Gross, C., Walter, M.C., Bohring, A., Cohen, M., Oehl-Jaschkowitz, B., Bird, L.M., Shamdeen, G.M., Bogdahn, U., Schuierer, G., Topaloglu, H., Aigner, L., Lochmuller, H., Winkler, J., 2007. Novel POMGnT1 mutations define broader phenotypic spectrum of muscle-eye-brain disease. Neurogenetics 8, 279–288.

Hess, H.A., Roper, J.C., Grill, S.W., Koelle, M.R., 2004. RGS-7 completes a receptor-independent heterotrimeric G protein cycle to asymmetrically regulate mitotic spindle positioning in C. elegans, Cell, United States, pp. 209–218.

Hohenester, E., Yurchenco, P.D., 2013. Laminins in basement membrane assembly. Cell Adhesion & Migration 7, 56–63.

Humphries, J.D., Byron, A., Humphries, M.J., 2006. Integrin ligands at a glance. Journal of Cell Science 119, 3901–3903.

Huttner, W.B., Kosodo, Y., 2005. Symmetric versus asymmetric cell division during neurogenesis in the developing vertebrate central nervous system. Current Opinion in Cell Biology 17, 648–657.

Imai, F., Hirai, S., Akimoto, K., Koyama, H., Miyata, T., Ogawa, M., Noguchi, S., Sasaoka, T., Noda, T., Ohno, S., 2006. Inactivation of aPKC lambda results in the loss of adherens junctions in neuroepithelial cells without affecting neurogenesis in mouse neocortex. Development 133, 1735–+.

Inoue, T., Ogawa, M., Mikoshiba, K., Aruga, J., 2008. Zic deficiency in the cortical marginal zone and meninges results in cortical lamination defects resembling those in type II lissencephaly. Journal of Neuroscience 28, 4712–4725.

Izumi, Y., Ohta, N., Hisata, K., Raabe, T., Matsuzaki, F., 2006. Drosophila Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. Nature Cell Biology 8, 586–593.

Jang, D.H., Sung, I.Y., Ko, T.S., 2013. Peripheral Nerve Involvement in Fukuyama Congenital Muscular Dystrophy: A Case Report. Journal of Child Neurology 28, 132–137.

Jeong, S.-J., Luo, R., Li, S., Strokes, N., Piao, X., 2012. Characterization of G protein-coupled receptor 56 protein expression in the mouse developing neocortex. Journal of Comparative Neurology 520, 2930–2940.

Jeong, S.-J., Luo, R., Singer, K., Giera, S., Kreidberg, J., Kiyozumi, D., Shimono, C., Sekiguchi, K., Piao, X., 2013. GPR56 Functions Together with alpha 3 beta 1 Integrin in Regulating Cerebral Cortical Development. Plos One 8.

Jiang, M., Gold, M.S., Boulay, G., Spicher, K., Peyton, M., Brabet, P., Srinivasan, Y., Rudolph, U., Ellison, G., Birnbaumer, L., 1998. Multiple neurological abnormalities in mice deficient in the G protein Go. Proc Natl Acad Sci U S A 95, 3269–3274.

Jiang, X.N., Nardelli, J., 2016. Cellular and molecular introduction to brain development. Neurobiology of Disease 92, 3–17.

Jimenez, D., Lopez-Mascaraque, L.M., Valverde, F., De Carlos, J.A., 2002. Tangential migration in neocortical development. Developmental Biology 244, 155–169.

Johansson, P.A., 2014. The choroid plexuses and their impact on developmental neurogenesis. Frontiers in Neuroscience 8.

Kanesaki, T., Hirose, S., Grosshans, J., Fuse, N., 2013. Heterotrimeric G protein signaling governs the cortical stability during apical constriction in Drosophila gastrulation. Mechanisms of Development 130, 132–142.

Katayama, K.I., Melendez, J., Baumann, J.M., Leslie, J.R., Chauhan, B.K., Nemkul, N., Lang, R.A., Kuan, C.Y., Zheng, Y., Yoshida, Y., 2011. Loss of RhoA in neural progenitor cells causes the disruption of adherens junctions and hyperproliferation. Proceedings of the National Academy of Sciences of the United States of America 108, 7607–7612.

Kirby, M.L., Gale, T.F., Stewart, D.E., 1983. Neural crest cells contribute to normal aorticopulmonary septation. Science 220, 1059–1061.

Kiyomitsu, T., Cheeseman, I.M., 2012. Chromosome- and spindle-pole-derived signals generate an intrinsic code for spindle position and orientation. Nature Cell Biology 14, 311–+.

Kjoller, L., Hall, A., 1999. Signaling to Rho GTPases. Experimental Cell Research 253, 166–179.

Knoblich, J.A., 2008. Mechanisms of asymmetric stem cell division. Cell 132, 583–597.

Konno, D., Shioi, G., Shitamukai, A., Mori, A., Kiyonari, H., Miyata, T., Matsuzaki, F., 2008. Neuroepithelial progenitors undergo LGN-dependent planar divisions to maintain self-renewability during mammalian neurogenesis. Nature Cell Biology 10, 93–U78.

Kosodo, Y., Huttner, W.B., 2009. Basal process and cell divisions of neural progenitors in the developing brain. Development Growth & Differentiation 51, 251–261.

Kosodo, Y., Roper, K., Haubensak, W., Marzesco, A.M., Corbeil, D., Huttner, W.B., 2004. Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. Embo Journal 23, 2314–2324.

Kotak, S., Busso, C., Gönczy, P., 2013. NuMA phosphorylation by CDK1 couples mitotic progression with cortical dynein function. Embo Journal 32, 2517–2529.
Kotak, S., Busso, C., Gönczy, P., 2014. NuMA interacts with phosphoinositides and links the mitotic spindle with the plasma membrane. Embo Journal 33, 1815–1830.
Kriegstein, A., Noctor, S., Martinez-Cerdeno, V., 2006. Patterns of neural stem and progenitor cell division may underlie evolutionary cortical expansion. Nature Reviews Neuroscience 7, 883–890.
Kunda, P., Pelling, A.E., Liu, T., Baum, B., 2008. Moesin controls cortical rigidity, cell rounding, and spindle morphogenesis during mitosis. Current Biology 18, 91–101.
Kwon, H.J., Ma, S., Huang, Z., 2011. Radial glia regulate Cajal-Retzius cell positioning in the early embryonic cerebral cortex. Developmental Biology 351, 25–34.
Kwon, M.J., Bagonis, M., Danuser, G., Pellman, D., 2015. Direct Microtubule-Binding by Myosin-10 Orients Centrosomes toward Retraction Fibers and Subcortical Actin Clouds. Developmental Cell 34, 323–337.
Lach, B., Arredondo, J., 2013. Cobblestone Lissencephaly in Schinzel-Giedion Syndrome. Journal of Child Neurology 28, 259–263.
Lancaster, M.A., Knoblich, J.A., 2012. Spindle orientation in mammalian cerebral cortical development. Current Opinion in Neurobiology 22, 737–746.
Lancaster, O.M., Baum, B., 2014. Shaping up to divide: Coordinating actin and microtubule cytoskeletal remodelling during mitosis. Seminars in Cell & Developmental Biology 34, 109–115.
Land, C., Schoenau, E., 2008. Fetal and postnatal bone development: reviewing the role of mechanical stimuli and nutrition. Best Pract Res Clin Endocrinol Metab 22, 107–118.
Lechler, T., Fuchs, E., 2005. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature 437, 275–280.
Lehtinen, M.K., Zappaterra, M.W., Chen, X., Yang, Y.J., Hill, A.D., Lun, M., Maynard, T., Gonzalez, D., Kim, S., Ye, P., D'Ercole, A.J., Wong, E.T., LaMantia, A.S., Walsh, C.A., 2011. The Cerebrospinal Fluid Provides a Proliferative Niche for Neural Progenitor Cells. Neuron 69, 893–905.
Leone, D.P., Srinivasan, K., Brakebusch, C., McConnell, S.K., 2010. The Rho GTPase Rac1 is Required for Proliferation and Survival of Progenitors in the Developing Forebrain. Developmental Neurobiology 70, 659–678.
Li, S., Jin, Z., Koirala, S., Bu, L., Xu, L., Hynes, R.O., Walsh, C.A., Corfas, G., Piao, X., 2008. GPR56 regulates pial basement membrane integrity and cortical lamination. Journal of Neuroscience 28, 5817–5826.
Li, S.H., Edgar, D., Fassler, R., Wadsworth, W., Yurchenco, P.D., 2003. The role of laminin in embryonic cell polarization and tissue organization. Developmental Cell 4, 613–624.
Liu, H.-X., Komatsu, Y., Mishina, Y., Mistretta, C.M., 2012. Neural crest contribution to lingual mesenchyme, epithelium and developing taste papillae and taste buds. Developmental Biology 368, 294–303.
Liu, Y., Jin, Y.X., Li, J.L., Seto, E., Kuo, E., Yu, W., Schwartz, R.J., Blazo, M., Zhang, S.Y.L., Peng, X., 2013. Inactivation of Cdc42 in neural crest cells causes craniofacial and cardiovascular morphogenesis defects. Developmental Biology 383, 239–252.
Lohse, M.J., Engelhardt, S., Eschenhagen, T., 2003. What is the role of beta-adrenergic signaling in heart failure? Circulation Research 93, 896–906.

Loulier, K., Lathia, J.D., Marthiens, V., Relucio, J., Mughal, M.R., Tang, S.-C., Coksaygan, T., Hall, P.E., Chigurupati, S., Patton, B., Colognato, H., Rao, M.S., Mattson, M.P., Haydar, T.F., Ffrench-Constant, C., 2009. beta 1 Integrin Maintains Integrity of the Embryonic Neocortical Stem Cell Niche. Plos Biology 7.

Luo, R., Jeong, S.-J., Jin, Z., Strokes, N., Li, S., Piao, X., 2011. G protein-coupled receptor 56 and collagen III, a receptor-ligand pair, regulates cortical development and lamination. Proceedings of the National Academy of Sciences of the United States of America 108, 12925–12930.

Ma, S., Kwon, H.J., Huang, Z., 2012. Ric-8a, a Guanine Nucleotide Exchange Factor for Heterotrimeric G Proteins, Regulates Bergmann Glia-Basement Membrane Adhesion during Cerebellar Foliation. Journal of Neuroscience 32, 14979–14993.

Maddala, R., Chauhan, B.K., Walker, C., Zheng, Y., Robinson, M.L., Lang, R.A., Rao, P.V., 2011. Rac1 GTPase-deficient mouse lens exhibits defects in shape, suture formation, fiber cell migration and survival. Developmental Biology 360, 30–43.

Maddala, R., Deng, P.F., Costello, J.M., Wawrousek, E.F., Zigler, J.S., Rao, V.P., 2004. Impaired cytoskeletal organization and membrane integrity in lens fibers of a Rho GTPase functional knockout transgenic mouse. Laboratory Investigation 84, 679–692.

Maddala, R., Reddy, V.N., Epstein, D.L., Rao, V., 2003. Growth factor induced activation of Rho and Rac GTPases and actin cytoskeletal reorganization in human lens epithelial cells. Molecular Vision 9, 329–336.

Maddala, R., Reneker, L.W., Pendurthi, B., Rao, P.V., 2008. Rho GDP dissociation inhibitor-mediated disruption of Rho GTPase activity impairs lens fiber cell migration, elongation and survival. Developmental Biology 315, 217–231.

Magdaleno, S., Keshvara, L., Curran, T., 2002. Rescue of ataxia and preplate splitting by ectopic expression of reelin in reeler mice. Neuron 33, 573–586.

Malatesta, P., Hartfuss, E., Götz, M., 2000. Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. Development 127, 5253–5263.

Maldonado-Agurto, R., Toro, G., Fuentealba, J., Arriagada, C., Campos, T., Albistur, M., Henriquez, J.P., Olate, J., Hinrichs, M.V., Torrejon, M., 2011. Cloning and spatiotemporal expression of RIC-8 in Xenopus embryogenesis. Gene Expression Patterns 11, 401–408.

Manabe, N., Hirai, S.I., Imai, F., Nakanishi, H., Takai, Y., Ohno, S., 2002. Association of ASIP/mPAR-3 with adherens junctions of mouse neuroepithelial cells. Developmental Dynamics 225, 61–69.

Manzini, M.C., Gleason, D., Chang, B.S., Hill, R.S., Barry, B.J., Partlow, J.N., Poduri, A., Currier, S., Galvin-Parton, P., Shapiro, L.R., Schmidt, K., Davis, J.G., Basel-Vanagaite, L., Seidahmed, M.Z., Salih, M.A.M., Dobyns, W.B., Walsh, C.A., 2008. Ethnically Diverse Causes of Walker-Warburg Syndrome (WWS): FCMD Mutations Are a More Common Cause of WWS Outside of the Middle East. Human Mutation 29, E231–E241.

Manzini, M.C., Walsh, C.A., 2011. What disorders of cortical development tell us about the cortex: one plus one does not always make two. Current Opinion in Genetics & Development 21, 333–339.

Mapelli, M., Gonzalez, C., 2012. On the inscrutable role of Inscuteable: structural basis and functional implications for the competitive binding of NuMA and Inscuteable to LGN. Open Biology 2.

Marin, O., Rubenstein, J.L.R., 2003. Cell migration in the forebrain. Annual Review of Neuroscience 26, 441–483.

Marthiens, V., Ffrench-Constant, C., 2009. Adherens junction domains are split by asymmetric division of embryonic neural stem cells. Embo Reports 10, 515–520.
McKee, K.K., Harrison, D., Capizzi, S., Yurchenco, P.D., 2007. Role of laminin terminal globular domains in basement membrane assembly. Journal of Biological Chemistry 282, 21437–21447.
McKee, K.K., Yang, D.-H., Patel, R., Chen, Z.-L., Strickland, S., Takagi, J., Sekiguchi, K., Yurchenco, P.D., 2012. Schwann cell myelination requires integration of laminin activities. Journal of Cell Science 125, 4609–4619.
Miller, K.G., Alfonso, A., Nguyen, M., Crowell, J.A., Johnson, C.D., Rand, J.B., 1996. A genetic selection for Caenorhabditis elegans synaptic transmission mutants. Proceedings of the National Academy of Sciences of the United States of America 93, 12593–12598.
Miller, K.G., Emerson, M.D., McManus, J.R., Rand, J.B., 2000. RIC-8 (synembryn): A novel conserved protein that is required for G(q)alpha signaling in the C-elegans nervous system. Neuron 27, 289–299.
Miller, K.G., Rand, J.B., 2000. A role for RIC-8 (Synembryn) and GOA-1 (G(o)alpha) in regulating a subset of centrosome movements during early embryogenesis in Caenorhabditis elegans. Genetics 156, 1649–1660.
Mitchell, M.L., 2001. Fetal brain to liver weight ratio as a measure of intrauterine growth retardation: analysis of 182 stillborn autopsies. Mod Pathol 14, 14–19.
Mitsushima, M., Aoki, K., Ebisuya, M., Matsumura, S., Yamamoto, T., Matsuda, M., Toyoshima, F., Nishida, E., 2010. Revolving movement of a dynamic cluster of actin filaments during mitosis. Journal of Cell Biology 191, 453–462.
Miyata, T., Kawaguchi, A., Okano, H., Ogawa, M., 2001. Asymmetric inheritance of radial glial fibers by cortical neurons. Neuron 31, 727–741.
Miyata, T., Kawaguchi, A., Saito, K., Kawano, M., Muto, T., Ogawa, M., 2004. Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. Development 131, 3133–3145.
Miyata, T., Kawaguchi, D., Kawaguchi, A., Gotoh, Y., 2010. Mechanisms that regulate the number of neurons during mouse neocortical development. Current Opinion in Neurobiology 20, 22–28.
Mizushima, N., Yamamoto, A., Hatano, M., Kobayashi, Y., Kabeya, Y., Suzuki, K., Tokuhisa, T., Ohsumi, Y., Yoshimori, T., 2001. Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. Journal of Cell Biology 152, 657–667.
Moers, A., Nuernberg, A., Goebbels, S., Wettschureck, N., Offermanns, S., 2008. G alpha(12)/G alpha(13) deficiency causes localized overmigration of neurons in the developing cerebral and cerebellar cortices. Molecular and Cellular Biology 28, 1480–1488.
Mogha, A., D'Rozario, M., Monk, K.R., 2016. G Protein-Coupled Receptors in Myelinating Glia. Trends in Pharmacological Sciences 37, 977–987.
Moore, S.A., Saito, F., Chen, J.G., Michele, D.E., Henry, M.D., Messing, A., Cohn, R.D., Ross-Barta, S.E., Westra, S., Williamson, R.A., Hoshi, T., Campbell, K.P., 2002. Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. Nature 418, 422–425.
Mora-Bermudez, F., Matsuzaki, F., Huttner, W.B., 2014. Specific polar subpopulations of astral microtubules control spindle orientation and symmetric neural stem cell division. Elife 3.

Morin, X., Bellaiche, Y., 2011. Mitotic Spindle Orientation in Asymmetric and Symmetric Cell Divisions during Animal Development. Developmental Cell 21, 102–119.

Morin, X., Jaouen, F., Durbec, P., 2007. Control of planar divisions by the G-protein regulator LGN maintains progenitors in the chick neuroepithelium. Nature Neuroscience 10, 1440–1448.

Morris, E.J., Assi, K., Salh, B., Dedhar, S., 2015. Integrin-Linked Kinase links Dynactin-1/Dynactin-2 with cortical Integrin receptors to orient the mitotic spindle relative to the substratum. Scientific Reports 5.

Myshrall, T.D., Moore, S.A., Ostendorf, A.P., Satz, J.S., Kowalczyk, T., Huy, N., Daza, R.A.M., Lau, C., Campbell, K.P., Hevner, R.F., 2012. Dystroglycan on Radial Glia End Feet Is Required for Pial Basement Membrane Integrity and Columnar Organization of the Developing Cerebral Cortex. Journal of Neuropathology and Experimental Neurology 71, 1047–1063.

Nabi, N.U., Mezer, E., Blaser, S.I., Levin, A.A., Buncic, J.R., 2003. Ocular findings in lissencephaly. Journal of Aapos 7, 178–184.

Nadarajah, B., Alifragis, P., Wong, R.O.L., Parnavelas, J.G., 2003. Neuronal migration in the developing cerebral cortex: Observations based on real-time imaging. Cerebral Cortex 13, 607–611.

Nadarajah, B., Brunstrom, J.E., Grutzendler, J., Wong, R.O.L., Pearlman, A.L., 2001. Two modes of radial migration in early development of the cerebral cortex. Nature Neuroscience 4, 143–150.

Nagai, Y., Nishimura, A., Tago, K., Mizuno, N., Itoh, H., 2010. Ric-8B Stabilizes the alpha Subunit of Stimulatory G Protein by Inhibiting Its Ubiquitination. Journal of Biological Chemistry 285, 11114–11120.

Nakagomi, T., Nakano-Doi, A., Matsuyama, T., 2015. Leptomeninges: a novel stem cell niche harboring ischemia-induced neural progenitors. Histology and Histopathology 30, 391–399.

Nakaya, 2008. RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation (vol 10, pg 765, 2008). Nature Cell Biology 10, 1012–1012.

Neer, E.J., 1995. Heterotrimeric G-Proteins – organizers of transmembrane signals. Cell 80, 249–257.

Neves, S.R., Ram, P.T., Iyengar, R., 2002. G protein pathways. Science 296, 1636–1639.

Niethammer, P., Kronja, I., Kandels-Lewis, S., Rybina, S., Bastiaens, P., Karsenti, E., 2007. Discrete states of a protein interaction network govern interphase and mitotic microtubule dynamics. Plos Biology 5, 190–202.

Niewmierzycka, A., Mills, J., St-Arnaud, R., Dedhar, S., Reichardt, L.F., 2005. Integrin-linked kinase deletion from mouse cortex results in cortical lamination defects resembling cobblestone lissencephaly. Journal of Neuroscience 25, 7022–7031.

Nipper, R.W., Siller, K.H., Smith, N.R., Doe, C.Q., Prehoda, K.E., 2007. G alpha i generates multiple Pins activation states to link cortical polarity and spindle orientation in Drosophila neuroblasts. Proceedings of the National Academy of Sciences of the United States of America 104, 14306–14311.

Nishimura, A., Okamoto, M., Sugawara, Y., Mizuno, N., Yamauchi, J., Itoh, H., 2006. Ric-8A potentiates Gq-mediated signal transduction by acting downstream of G protein-coupled receptor in intact cells. Genes Cells 11, 487–498.

Noatynska, A., Gotta, M., Meraldi, P., 2012. Mitotic spindle (DIS)orientation and DISease: Cause or consequence? Journal of Cell Biology 199, 1025–1035.
Nobes, C.D., Hall, A., 1995. Rho, Rac and Cdc42 GTPases – Regulators of actin structures, cell-adhesion and motility. Biochemical Society Transactions 23, 456–459.
Noctor, S.C., Flint, A.C., Weissman, T.A., Wong, W.S., Clinton, B.K., Kriegstein, A.R., 2002. Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. Journal of Neuroscience 22, 3161–3173.
Noctor, S.C., Martinez-Cerdeno, V., Ivic, L., Kriegstein, A.R., 2004. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nature Neuroscience 7, 136–144.
Offermanns, S., 2001. In vivo functions of heterotrimeric G-proteins: studies in G alpha-deficient mice. Oncogene 20, 1635–1642.
Offermanns, S., Hashimoto, K., Watanabe, M., Sun, W., Kurihara, H., Thompson, R.F., Inoue, Y., Kano, M., Simon, M.I., 1997. Impaired motor coordination and persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking Galphaq. Proc Natl Acad Sci U S A 94, 14089–14094.
Offermanns, S., Zhao, L.P., Gohla, A., Sarosi, I., Simon, M.I., Wilkie, T.M., 1998. Embryonic cardiomyocyte hypoplasia and craniofacial defects in G alpha(q)/G alpha(11)-mutant mice. Embo Journal 17, 4304–4312.
Olesen, C., Nyeng, P., Kalisz, M., Jensen, T.H., Moller, M., Tommerup, N., Byskov, A.G., 2007. Global gene expression analysis in fetal mouse ovaries with and without meiosis and comparison of selected genes with meiosis in the testis. Cell Tissue Res 328, 207–221.
Olson, E.C., Walsh, C.A., 2002. Smooth, rough and upside-down neocortical development. Current Opinion in Genetics & Development 12, 320–327.
Pabuscu, Y., Bulakbasi, N., Kocaoglu, M., Ucoz, T., 2003. Walker-Warburg syndrome variant (vol 26, pg 453, 2002). Computerized Medical Imaging and Graphics 27, 537–537.
Paridaen, J., Wilsch-Brauninger, M., Huttner, W.B., 2013. Asymmetric Inheritance of Centrosome-Associated Primary Cilium Membrane Directs Ciliogenesis after Cell Division. Cell 155, 333–344.
Parsons, J.T., 2003. Focal adhesion kinase: the first ten years. Journal of Cell Science 116, 1409–1416.
Peng, X., Lin, Q., Liu, Y., Jin, Y.X., Druso, J.E., Antonyak, M.A., Guan, J.L., Cerione, R.A., 2013. Inactivation of Cdc42 in embryonic brain results in hydrocephalus with ependymal cell defects in mice. Protein & Cell 4, 231–242.
Pereira, J.A., Benninger, Y., Baumann, R., Goncalves, A.F., Ozcelik, M., Thurnherr, T., Tricaud, N., Meijer, D., Fassler, R., Suter, U., Relvas, J.B., 2009. Integrin-linked kinase is required for radial sorting of axons and Schwann cell remyelination in the peripheral nervous system. Journal of Cell Biology 185, 147–161.
Peyre, E., Jaouen, F., Saadaoui, M., Haren, L., Merdes, A., Durbec, P., Morin, X., 2011. A lateral belt of cortical LGN and NuMA guides mitotic spindle movements and planar division in neuroepithelial cells. Journal of Cell Biology 193, 141–154.
Peyre, E., Morin, X., 2012. An oblique view on the role of spindle orientation in vertebrate neurogenesis. Development Growth & Differentiation 54, 287–305.
Pietri, T., Eder, O., Breau, M.A., Topilko, P., Blanche, M., Brakebusch, C., Fassler, R., Thiery, J.P., Dufour, S., 2004. Conditional beta 1-integrin gene deletion in neural

crest cells causes severe developmental alterations of the peripheral nervous system. Development 131, 3871–3883.
Pilz, G.A., Shitamukai, A., Reillo, I., Pacary, E., Schwausch, J., Stahl, R., Ninkovic, J., Snippert, H.J., Clevers, H., Godinho, L., Guillemot, F., Borrell, V., Matsuzaki, F., Götz, M., 2013. Amplification of progenitors in the mammalian telencephalon includes a new radial glial cell type. Nature Communications 4.
Postel, R., Vakeel, P., Topczewski, J., Knoll, R., Bakkers, J., 2008. Zebrafish integrin-linked kinase is required in skeletal muscles for strengthening the integrin-ECM adhesion complex. Developmental Biology 318, 92–101.
Postiglione, M.P., Jueschke, C., Xie, Y., Haas, G.A., Charalambous, C., Knoblich, J.A., 2011. Mouse Inscuteable Induces Apical-Basal Spindle Orientation to Facilitate Intermediate Progenitor Generation in the Developing Neocortex. Neuron 72, 269–284.
Pratap, A., Agrawal, A., Tiwari, A., Lakshmi, R., Rajbanshi, S., 2007. The Walker-Warburg syndrome with cleft lip and palate. Singapore Med J, 48(2): e66–e67.
Radakovits, R., Barros, C.S., Belvindrah, R., Patton, B., Mueller, U., 2009. Regulation of Radial Glial Survival by Signals from the Meninges. Journal of Neuroscience 29, 7694–7705.
Radner, S., Banos, C., Bachay, G., Li, Y.N., Hunter, D.D., Brunken, W.J., Yee, K.T., 2013. 2 and 3 laminins are critical cortical basement membrane components: Ablation of Lamb2 and Lamc3 genes disrupts cortical lamination and produces dysplasia. Developmental Neurobiology 73, 209–229.
Ridley, A.J., Hall, A., 1992. The small GTP-binding protein Rho regulates the assembly of focal adhesions and actin stress fibers in response to growth-factors. Cell 70, 389–399.
Romero-Pozuelo, J., Dason, J.S., Mansilla, A., Banos-Mateos, S., Sardina, J.L., Chaves-Sanjuan, A., Jurado-Gomez, J., Santana, E., Atwood, H.L., Hernandez-Hernandez, A., Sanchez-Barrena, M.J., Ferrus, A., 2014. The guanine-exchange factor Ric8a binds to the Ca2+ sensor NCS-1 to regulate synapse number and neurotransmitter release. Journal of Cell Science 127, 4246–4259.
Romo, X., Pasten, P., Martinez, S., Soto, X., Lara, P., De Arellano, A.R., Torrejon, M., Montecino, M., Hinrichs, M.V., Olate, J., 2008. xRic-8 is a GEF for Gs alpha and participates in maintaining meiotic arrest in Xenopus laevis oocytes. Journal of Cellular Physiology 214, 673–680.
Rudolph, U., Spicher, K., Birnbaumer, L., 1996. Adenylyl cyclase inhibition and altered G protein subunit expression and ADP-ribosylation patterns in tissues and cells G(i2)alpha–/– mice. Proceedings of the National Academy of Sciences of the United States of America 93, 3209–3214.
Ruppel, K.M., Willison, D., Kataoka, H., Wang, A., Zheng, Y.W., Cornelissen, L., Yin, L.Y., Xu, S.M., Coughlin, S.R., 2005. Essential role for G alpha(13) in endothelial cells during embryonic development. Proceedings of the National Academy of Sciences of the United States of America 102, 8281–8286.
Saboori, P., Sadegh, A., 2015. Histology and Morphology of the Brain Subarachnoid Trabeculae. Anatomy research international 2015, 279814–279814.
Sahara, S., O'Leary, D.D.M., 2009. Fgf10 Regulates Transition Period of Cortical Stem Cell Differentiation to Radial Glia Controlling Generation of Neurons and Basal Progenitors. Neuron 63, 48–62.
Saito, F., Masaki, T., Saito, Y., Nakamura, A., Takeda, S., Shimizu, T., Toda, T., Matsumura, K., 2007. Defective peripheral nerve myelination and neuromuscular

junction formation in fukutin-deficient chimeric mice. Journal of Neurochemistry 101, 1712–1722.
Saito, K., 2006. Prenatal diagnosis of Fukuyama congenital muscular dystrophy. Prenatal Diagnosis 26, 415–417.
Sanada, K., Tsai, L.H., 2005. G protein beta gamma subunits and AGS3 control spindle orientation and asymmetric cell fate of cerebral cortical progenitors. Cell 122, 119–131.
Sato, M., Blumer, J.B., Simon, V., Lanier, S.M., 2006. Accessory proteins for G proteins: Partners in signaling. Annual Review of Pharmacology and Toxicology 46, 151–187.
Satz, J.S., Ostendorf, A.P., Hou, S., Turner, A., Kusano, H., Lee, J.C., Turk, R., Nguyen, H., Ross-Barta, S.E., Westra, S., Hoshi, T., Moore, S.A., Campbell, K.P., 2010. Distinct Functions of Glial and Neuronal Dystroglycan in the Developing and Adult Mouse Brain. Journal of Neuroscience 30, 14560–14572.
Schaefer, M., Knoblich, J.A., 2001. Protein localization during asymmetric cell division. Experimental Cell Research 271, 66–74.
Schaefer, M., Petronczki, M., Dorner, D., Forte, M., Knoblich, J.A., 2001. Heterotrimeric G proteins direct two modes of asymmetric cell division in the Drosophila nervous system. Cell 107, 183–194.
Schaefer, M., Shevchenko, A., Knoblich, J.A., 2000. A protein complex containing inscuteable and the G alpha-binding protein Pins orients asymmetric cell divisions in Drosophila. Current Biology 10, 353–362.
Schatten, H., Sun, Q.Y., 2011. Centrosome Dynamics During Mammalian Oocyte Maturation With a Focus on Meiotic Spindle Formation. Molecular Reproduction and Development 78, 757–768.
Segre, J.A., Bauer, C., Fuchs, E., 1999. Klf4 is a transcription factor required for establishing the barrier function of the skin. Nature Genetics 22, 356–360.
Shitamukai, A., Konno, D., Matsuzaki, F., 2011. Oblique Radial Glial Divisions in the Developing Mouse Neocortex Induce Self-Renewing Progenitors outside the Germinal Zone That Resemble Primate Outer Subventricular Zone Progenitors. Journal of Neuroscience 31, 3683–3695.
Shitamukai, A., Matsuzaki, F., 2012. Control of asymmetric cell division of mammalian neural progenitors. Development Growth & Differentiation 54, 277–286.
Siderovski, D.P., Willard, F.S., 2005. The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. International Journal of Biological Sciences 1, 51–66.
Siegenthaler, J.A., Ashique, A.M., Zarbalis, K., Patterson, K.P., Hecht, J.H., Kane, M.A., Folias, A.E., Choe, Y., May, S.R., Kume, T., Napoli, J.L., Peterson, A.S., Pleasure, S.J., 2009. Retinoic Acid from the Meninges Regulates Cortical Neuron Generation. Cell 139, 597–609.
Siegenthaler, J.A., Pleasure, S.J., 2011. We have got you 'covered': how the meninges control brain development. Current Opinion in Genetics & Development 21, 249–255.
Silan, F., Yoshioka, M., Kobayashi, K., Simsek, E., Tunc, M., Alper, M., Cam, M., Guven, A., Fukuda, Y., Kinoshita, M., Kocabay, K., Toda, T., 2003. A new mutation of the fukutin gene in a non-Japanese patient. Annals of Neurology 53, 392–396.
Siller, K.H., Cabernard, C., Doe, C.Q., 2006. The NuMA-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts. Nature Cell Biology 8, 594–600.

Siller, K.H., Doe, C.Q., 2009. Spindle orientation during asymmetric cell division. Nature Cell Biology 11, 365–374.
Singer, K., Luo, R., Jeong, S.-J., Piao, X., 2013. GPR56 and the Developing Cerebral Cortex: Cells, Matrix, and Neuronal Migration. Molecular Neurobiology 47, 186–196.
Soriano, E., del Rio, J.A., 2005. The cells of Cajal-Retzius: Still a mystery one century after. Neuron 46, 389–394.
Stancik, E.K., Navarro-Quiroga, I., Sellke, R., Haydar, T.F., 2010. Heterogeneity in Ventricular Zone Neural Precursors Contributes to Neuronal Fate Diversity in the Postnatal Neocortex. Journal of Neuroscience 30, 7028–7036.
Syrovatkina, V., Alegre, K.O., Dey, R., Huang, X.Y., 2016. Regulation, Signaling, and Physiological Functions of G-Proteins. Journal of Molecular Biology 428, 3850–3868.
Takeda, S., Kondo, M., Sasaki, J., Kurahashi, H., Kano, H., Arai, K., Misaki, K., Fukui, T., Kobayashi, K., Tachikawa, M., Imamura, M., Nakamura, Y., Shimizu, T., Murakami, T., Sunada, Y., Fujikado, T., Matsumura, K., Terashima, T., Toda, T., 2003. Fukutin is required for maintenance of muscle integrity, cortical histiogenesis and normal eye development. Human Molecular Genetics 12, 1449–1459.
Tall, G.G., Gilman, A.G., 2005. Resistance to inhibitors of cholinesterase 8A catalyzes release of G alpha i-GTP and nuclear mitotic apparatus protein (NuMA) from NuMA/LGN/G alpha i-GDP complexes. Proceedings of the National Academy of Sciences of the United States of America 102, 16584–16589.
Tall, G.G., Krumins, A.M., Gilman, A.G., 2003. Mammalian Ric-8A (synembryn) is a heterotrimeric Galpha protein guanine nucleotide exchange factor. J Biol Chem 278, 8356–8362.
Tall, G.G., Patel, B.R., Chan, P., 2013. Ric-8 folding of G proteins better explains Ric-8 apparent amplification of G protein-coupled receptor signaling. Proceedings of the National Academy of Sciences of the United States of America 110, E3148–E3148.
Tan, X., Shi, S.-H., 2013. Neocortical neurogenesis and neuronal migration. Wiley Interdisciplinary Reviews-Developmental Biology 2, 443–459.
Taverna, E., Götz, M., Huttner, A.B., 2014. The Cell Biology of Neurogenesis: Toward an Understanding of the Development and Evolution of the Neocortex. Annual Review of Cell and Developmental Biology, Vol 30 30, 465–502.
Thery, M., Jimenez-Dalmaroni, A., Racine, V., Bornens, M., Juelicher, F., 2007. Experimental and theoretical study of mitotic spindle orientation. Nature 447, 493–U496.
Theveneau, E., Marchant, L., Kuriyama, S., Gull, M., Moepps, B., Parsons, M., Mayor, R., 2010. Collective Chemotaxis Requires Contact-Dependent Cell Polarity. Developmental Cell 19, 39–53.
Theveneau, E., Mayor, R., 2011. Collective Cell Migration of the Cephalic Neural Crest: The Art of Integrating Information. Genesis 49, 164–176.
Tong, C.K., Han, Y.G., Shah, J.K., Obernier, K., Guinto, C.D., Alvarez-Buylla, A., 2014. Primary cilia are required in a unique subpopulation of neural progenitors. Proceedings of the National Academy of Sciences of the United States of America 111, 12438–12443.
Tõnissoo, T., Kõks, S., Meier, R., Raud, S., Plaas, M., Vasar, E., Karis, A., 2006. Heterozygous mice with Ric-8 mutation exhibit impaired spatial memory and decreased anxiety. Behav Brain Res 167, 42–48.

Tõnissoo, T., Lulla, S., Meier, R., Saare, M., Ruisu, K., Pooga, M., Karis, A., 2010. Nucleotide exchange factor RIC-8 is indispensable in mammalian early development. Dev Dyn 239, 3404–3415.

Tõnissoo, T., Meier, R., Talts, K., Plaas, M., Karis, A., 2003. Expression of ric-8 (synembryn) gene in the nervous system of developing and adult mouse. Gene Expr Patterns 3, 591–594.

Triffitt, J.T., 1987. Initiation and enhancement of bone formation. A review. Acta Orthop Scand 58, 673–684.

Turgeon, B., Meloche, S., 2009. Interpreting Neonatal Lethal Phenotypes in Mouse Mutants: Insights Into Gene Function and Human Diseases. Physiological Reviews 89, 1–26.

Tyler, W.A., Haydar, T.F., 2013. Multiplex Genetic Fate Mapping Reveals a Novel Route of Neocortical Neurogenesis, Which Is Altered in the Ts65Dn Mouse Model of Down Syndrome. Journal of Neuroscience 33, 5106–5119.

Vajsar, J., Baskin, B., Swoboda, K., Biggar, D.W., Schachter, H., Ray, P.N., 2008. Walker-Warburg Syndrome with POMT1 mutations can be associated with cleft lip and cleft palate. Neuromuscular Disorders 18, 675–677.

Vajsar, J., Schachter, H., 2006. Walker-Warburg syndrome. Orphanet Journal of Rare Diseases 1.

Vallejo-Illarramendi, A., Zang, K.L., Reichardt, L.F., 2009. Focal adhesion kinase is required for neural crest cell morphogenesis during mouse cardiovascular development. Journal of Clinical Investigation 119, 2218–2230.

van Reeuwijk, J., Brunner, H.G., van Bokhoven, H., 2005a. Glyc-O-genetics of Walker-Warburg syndrome. Clinical Genetics 67, 281–289.

van Reeuwijk, J., Janssen, M., van den Elzen, C., de Bernabe, D.B.V., Sabatelli, P., Merlini, L., Boon, M., Scheffer, H., Brockington, M., Muntoni, F., Huynen, M.A., Verrips, A., Walsh, C.A., Barth, P.G., Brunner, H.G., van Bokhoven, H., 2005b. POMT2 mutations cause alpha-dystroglycan hypoglycosylation and Walker-Warburg syndrome. Journal of Medical Genetics 42, 907–912.

Waldo, K., Miyagawa-Tomita, S., Kumiski, D., Kirby, M.L., 1998. Cardiac neural crest cells provide new insight into septation of the cardiac outflow tract: Aortic sac to ventricular septal closure. Developmental Biology 196, 129–144.

Wang, H., Ng, K.H., Qian, H., Siderovski, D.P., Chia, W., Yu, F., 2005. Ric-8 controls Drosophila neural progenitor asymmetric division by regulating heterotrimeric G proteins. Nat Cell Biol 7, 1091–1098.

Wang, X., Tsai, J.-W., Imai, J.H., Lian, W.-N., Vallee, R.B., Shi, S.-H., 2009. Asymmetric centrosome inheritance maintains neural progenitors in the neocortex. Nature 461, 947–U206.

Wang, L., Guo, D., Xing, B., Zhang, J.J., Shu, H.B., Guo, L., Huang, X.Y., 2011a. Resistance to inhibitors of cholinesterase-8A (Ric-8A) is critical for growth factor receptor-induced actin cytoskeletal reorganization. J Biol Chem 286, 31055–31061.

Wang, S.C., Lai, H.L., Chiu, Y.T., Ou, R., Huang, C.L., Chern, Y., 2007. Regulation of type V adenylate cyclase by Ric8a, a guanine nucleotide exchange factor. Biochem J 406, 383–388.

Wang, X., Tsai, J.-W., LaMonica, B., Kriegstein, A.R., 2011b. A new subtype of progenitor cell in the mouse embryonic neocortex. Nature Neuroscience 14, 555–U534.

Wettschureck, N., Offermanns, S., 2005. Mammalian G proteins and their cell type specific functions. Physiological Reviews 85, 1159–1204.

Wettschureck, N., Rutten, H., Zywietz, A., Gehring, D., Wilkie, T.M., Chen, J., Chien, K.R., Offermanns, S., 2001. Absence of pressure overload induced myocardial hypertrophy after conditional inactivation of G alpha(q)/G alpha(11) in cardiomyocytes. Nature Medicine 7, 1236–1240.

Williams, S.E., Beronja, S., Pasolli, H.A., Fuchs, E., 2011. Asymmetric cell divisions promote Notch-dependent epidermal differentiation. Nature 470, 353–358.

Williams, S.L., Lutz, S., Charlie, N.K., Vettel, C., Ailion, M., Coco, C., Tesmer, J.J.G., Jorgensen, E.M., Wieland, T., Miller, K.G., 2007. Trio's Rho-specific GEF domain is the missing G alpha(q) effector in C-elegans. Genes & Development 21, 2731–2746.

Witt, A., Brady, S.T., 2000. Unwrapping new layers of complexity in axon/glial relationships. Glia 29, 112–117.

Wodarz, A., Huttner, W.B., 2003. Asymmetric cell division during neurogenesis in Drosophila and vertebrates. Mechanisms of Development 120, 1297–1309.

Woodard, G.E., Huang, N.N., Cho, H., Miki, T., Tall, G.G., Kehrl, J.H., 2010. Ric-8A and Gi alpha recruit LGN, NuMA, and dynein to the cell cortex to help orient the mitotic spindle. Mol Cell Biol 30, 3519–3530.

Xing, B., Wang, L., Guo, D., Huang, J., Espenel, C., Kreitzer, G., Zhang, J.J., Guo, L., Huang, X.Y., 2013. Atypical Protein Kinase Clambda Is Critical for Growth Factor Receptor-induced Dorsal Ruffle Turnover and Cell Migration. J Biol Chem 288, 32827–32836.

Yamamoto, T., Kato, Y., Kawaguchi, M., Shibata, N., Kobayashi, M., 2004. Expression and localization of fukutin, POMGnT1, and POMT1 in the central nervous system: consideration for functions of fukutin. Medical electron microscopy : official journal of the Clinical Electron Microscopy Society of Japan 37, 200–207.

Yan, M., Ha, J.H., Dhanasekaran, D.N., 2015. Galpha13 Stimulates the Tyrosine Phosphorylation of Ric-8A. Journal of molecular signaling 10, 3–3.

Yoda, M., Tanabe, H., Nishino, I., Suma, H., 2011. Left ventriculoplasty for dilated cardiomyopathy in Fukuyama-type muscular dystrophy. European Journal of Cardio-Thoracic Surgery 40, 514–516.

Yoshida, M., Assimacopoulos, S., Jones, K.R., Grove, E.A., 2006. Massive loss of Cajal-Retzius cells does not disrupt neocortical layer order. Development 133, 537–545.

Yoshioka, M., Higuchi, Y., 2005. Long-term prognosis of epilepsies and related seizure disorders in Fukuyama-type congenital muscular dystrophy. Journal of Child Neurology 20, 385–391.

Yoshioka, M., Higuchi, Y., Fujii, T., Aiba, H., Toda, T., 2008. Seizure-genotype relationship in Fukuyama-type congenital muscular dystrophy. Brain & Development 30, 59–67.

Yu, F.W., Kuo, C.T., Jan, Y.N., 2006. Drosophila neuroblast asymmetric cell division: Recent advances and implications for stem cell biology. Neuron 51, 13–20.

Yu, F.W., Morin, X., Cai, Y., Yang, X.H., Chia, W., 2000. Analysis of partner of inscuteable, a novel player of Drosophila asymmetric divisions, reveals two distinct steps in inscuteable apical localization. Cell 100, 399–409.

Yurchenco, P.D., 2011. Basement Membranes: Cell Scaffoldings and Signaling Platforms. Cold Spring Harbor Perspectives in Biology 3.

Zarbalis, K., Choe, Y., Siegenthaler, J.A., Orosco, L.A., Pleasure, S.J., 2012. Meningeal defects alter the tangential migration of cortical interneurons in Foxc1hith/hith mice. Neural Development 7.

Zarbalis, K., Siegenthaler, J.A., Choe, Y., May, S.R., Peterson, A.S., Pleasure, S.J., 2007. Cortical dysplasia and skull defects in mice with a Foxc1 allele reveal the role of meningeal differentiation in regulating cortical development. Proceedings of the National Academy of Sciences of the United States of America 104, 14002–14007.

Zhao, C.J., Guan, W., Pleasure, S.J., 2006. A transgenic marker mouse line labels Cajal-Retzius cells from the cortical hem and thalamocortical axons. Brain Research 1077, 48–53.

Zheng, Z., Wan, Q., Liu, J., Zhu, H., Chu, X., Du, Q., 2013. Evidence for dynein and astral microtubule-mediated cortical release and transport of G alpha(i)/LGN/NuMA complex in mitotic cells. Molecular Biology of the Cell 24, 901–913.

Zhu, J.W., Wen, W.Y., Zheng, Z., Shang, Y., Wei, Z.Y., Xiao, Z.N., Pan, Z., Du, Q.S., Wang, W.N., Zhang, M.J., 2011. LGN/mInsc and LGN/NuMA Complex Structures Suggest Distinct Functions in Asymmetric Cell Division for the Par3/mInsc/LGN and G alpha i/LGN/NuMA Pathways. Molecular Cell 43, 418–431.

ACKNOWLEDGEMENTS

I am very grateful to my supervisors, Prof. Margus Pooga and Dr. Tambet Tõnissoo, for their support and guidance throughout all these years. I thank Margus for encouraging me to pursue the bigger goals and for always finding the solution whenever it was needed. I thank Tambet for the motivation and advice in every step in my PhD studies. You have also been the soul of the Department of Developmental Biology providing the environment where students and supervisors can become lifelong friends. Thank you for that!

I am also very thankful to Prof. Arnold Kristjuhan, who reviewed the current thesis and suggested helpful notes and corrections.

Furthermore, I am very grateful to my non-official supervisor Riho Meier who has the best quality to support the seeds of the fragile new ideas which has given me the courage to go on with them. Also, I appreciate the useful conversations about RIC8A and the development of the nervous system and about whatever topic one could think of. I would like to thank Mall Kure for teaching me all the tricks of histological sample handling, they have served me well. I thank Sulev Kuuse and the staff of IMCB vivarium for their technical assistance and for being always cheerful and supportive. I also thank Viljar Jaks and his research group for the positive attitude and support.

I would like to thank all the co-authors of the publications and of course the wonderful family and friends of the lab of Developmental Biology. I especially thank my academic sister Katrin Ruisu, who has been very helpful and superb companion throughout all these years. We have had tons of enjoyable conversations about work and life inside and outside the lab. You have even introduced me to your friends who have now become my friends.

I am forever grateful to my wonderful Year´2003 biologists who have given me the numerous adventures and exciting student-life stories to tell to my grandchildren one day. I especially thank Triin, Kärt, Kätlin, Sten, Teet, Putku, Veiko, Kaido for giving me the annual Christmas dinners and the best years of the university. I also thank my childhood friends: Reelika, Rellu, Triin, Merili & Co and my favourite girl-power-gang in Tartu: Marit, Piret, Elen, Triin, Reilika, Ingrid, who have always been there for me and have always cheering „Go, Keiu!“ next to my endless educational journey.

My most loving gratitude goes to my family. Especially to my mother, who has given me the privileged opportunity to follow my dreams. I have to write a book to express all the appreciation for the support, encouragement and love you have given me. And last, but not least, my warmest hugs and kisses go to my favourite person in the world, my wonderful daughter Hanna Eliisa, who has given me the strength and motivation throughout all her entire existence. Thank you!

PUBLICATIONS

CURRICULUM VITAE

Name: Keiu Kask
Date of birth: 07.05.1984
Nationality: Estonian
Contact: The Institute of Molecular and Cell Biology, University of Tartu, Riia 23, 51010, Tartu, Estonia
E-mail: keiu.kask@ut.ee

Education:

1991–2003	Tallinn Järveotsa Gymnasium
2003–2006	BSc, Biology, University of Tartu, Faculty of Science and Technology
2006–2010	MSc, Biology, University of Tartu, Faculty of Science and Technology
2010–…	PhD, Molecular and Cell Biology, specialised in Developmental Biology, University of Tartu, Institute of Molecular and Cell Biology

Working experience:

2015–2016	Institute of Molecular and Cell Biology, technology specialist

Laboratory experience:

2005–2006	Member of the *C.elegans* research group in the Department of the Developmental Biology, University of Tartu
2009–…	Member of the RIC8A research group in the Department of Developmental Biology, University of Tartu

Main fields of research:
The function of RIC8A, a regulator for G protein activity, in the development of the nervous system, using the mouse (*Mus musculus*) as a model.

Professional self-improvement:

2006	Practical course "Introduction to *C.elegans*: Laboratory Course", Tallinn, Estonia
2010	Practical course "3D reconstruction by serial sectioning of histological samples", Tartu, Estonia
2012	EMBO Workshop "Cell Biology of Early Mouse Development", Cambridge, UK
2014	Laboratory Animal Science I/II, Tartu, Estonia

Publications:

Ruisu K, Kask K, Meier R, Saare M, Raid R, Veraksitš A, Karis A, Tõnissoo T, Pooga M. 2013. Ablation of RIC8A function in mouse neurons leads to a severe neuromuscular phenotype and postnatal death. PLoS One. 2013 Aug 16; 8(8):e74031.

Kask K, Ruisu K, Tikker L, Karis K, Saare M, Meier R, Karis A, Tõnissoo T, Pooga M. 2015. Deletion of RIC8A in neural precursor cells leads to altered neurogenesis and neonatal lethality of mouse. Dev. Neurobiol. 2015 Jan 16; 75(9):984–1002.

Saare M, Lulla S, Tõnissoo T, Meier R, Kask K, Ruisu K, Karis A, Salumets A, Pooga M. 2015. Expression pattern and localization dynamics of guanine nucleotide exchange factor RIC8 during mouse oogenesis. PLoS One. 10(6), e0129131.

Ruisu K, Meier R, Tõnissoo T, Kask K, Velling T, Pooga M. 2017. RIC8A is essential for the organisation of actin cytoskeleton and cell-matrix interaction. Experimental Cell Research. (2017), http://dx.doi.org/10.1016/j.yexcr.2017.05.012

Other scientific- and organisational activities

2012–2015	IMBC Council member
2005–…	Tartu Students' Nature Conservation Circle, member
2004–…	University Cultural Club, member

Supervised dissertations:

2012–2014	Laura Tikker, MSc, Department of Developmental Biology, Institute of Molecular and Cell Biology, University of Tartu
2011–2012	Epp Kaleviste, BSc, Department of Developmental Biology, Institute of Molecular and Cell Biology, University of Tartu
2013–2014	Kirstin Karis, BSc, Department of Developmental Biology, Institute of Molecular and Cell Biology, University of Tartu
2015–2016	Eva-Maria Oja, BSc, Department of Developmental Biology, Institute of Molecular and Cell Biology, University of Tartu

Dissertations under supervision:

2016–…	Anet Tammemäe, BSc studies, Department of Developmental Biology, Institute of Molecular and Cell Biology, University of Tartu
2016–…	Hanna Antson (co-supervisor), BSc studies, Department of Developmental Biology, Institute of Molecular and Cell Biology, University of Tartu
2016–…	Gundra Raissar, research study in gymnasium program, supervisor, Hugo Treffner Gymnasium, Tartu

ELULOOKIRJELDUS

Nimi: Keiu Kask
Sünniaeg: 07.05.1984
Kodakondsus: Eesti
Kontaktandmed: Molekulaar- ja rakubioloogia instituut, Tartu Ülikool, Riia23, 51010, Tartu, Eesti
E-mail: keiu.kask@ut.ee

Hariduskäik:

1991–2003	Tallinna Järveotsa Gümnaasium
2003–2006	Tartu Ülikool, Loodusteaduste bakalaureus (BSc) bioloogia erialal
2006–2010	Tartu Ülikool, Loodusteaduste magister (MSc) bioloogia erialal
2010–…	Tartu Ülikool, Doktoriõpingud arengubioloogia erialal, molekulaar- ja rakubioloogia õppekaval

Töökogemus:

2014–2015	Tartu Ülikool, Molekulaar- ja Rakubioloogia Instituut, tehnoloogia spetsialist

Laboripraktika:

2005–2006	Arengubioloogia õppetooli laboratooriumi mudelorganismi *C.elegans* uurimisgrupi liige, Tartu Ülikool
2009–…	Arengubioloogia õppetooli laboratooriumi RIC8A uurimisgrupi liige, Tartu Ülikool

Peamised uurimisvaldkonnad:
G valkude regulaatori RIC8A funktsiooni uurimine närvisüsteemis ja selle arengus kasutades mudelsüsteemina koduhiirt (*Mus musculus*).

Erialane enesetäiendus:

2006	Praktiline kursus „Introduction to *C.elegans*: Laboratory Course, Tallinn, Eesti
2010	Praktiline kursus „Histoloogiliste proovide seerialõikude 3D rekonstruktsioon“, Tartu Estonia
2012	EMBO kursus „Cell Biology of Early Mouse Development“, Cambridge, Suurbritannia
2014	Katseloomateadus I/II, Tartu, Eesti

Publikatsioonid:

Ruisu K, Kask K, Meier R, Saare M, Raid R, Veraksitš A, Karis A, Tõnissoo T, Pooga M. 2013. Ablation of RIC8A function in mouse neurons leads to a severe neuromuscular phenotype and postnatal death. PLoS One. 2013 Aug 16; 8(8):e74031.

Kask K, Ruisu K, Tikker L, Karis K, Saare M, Meier R, Karis A, Tõnissoo T, Pooga M. 2015. Deletion of RIC8A in neural precursor cells leads to altered neurogenesis and neonatal lethality of mouse. Dev. Neurobiol. 2015 Jan 16; 75(9):984–1002.

Saare M, Lulla S, Tõnissoo T, Meier R, Kask K, Ruisu K, Karis A, Salumets A, Pooga M. 2015. Expression pattern and localization dynamics of guanine nucleotide exchange factor RIC8 during mouse oogenesis. PLoS One. 10(6), e0129131.

Ruisu K, Meier R, Tõnissoo T, Kask K, Velling T, Pooga M. 2017. RIC8A is essential for the organisation of actin cytoskeleton and cell-matrix interaction. Experimental Cell Research. (2017), http://dx.doi.org/10.1016/j.yexcr.2017.05.012

Muu teaduslik- ja organisatsiooniline tegevus:

2012–2015	TÜMRI Teadusnõukogu liige
2005–…	TÜ Looduskaitseringi liige
2004–…	Ülikooli Kultuuriklubi liige

Juhendatud väitekirjad:

2012–2014	Laura Tikker, MSc väitekiri, arengubioloogia õppetool, TÜ Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool
2011–2012	Epp Kaleviste, BSc väitekiri, arengubioloogia õppetool, TÜ Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool
2013–2014	Kirstin Karis, BSc väitekiri, arengubioloogia õppetool, TÜ Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool
2015–2016	Eva-Maria Oja, BSc väitekiri, arengubioloogia õppetool, TÜ Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool

Juhendamisel väitekirjad:

2016–…	Anet Tammemäe, BSc õpingud, arengubioloogia õppetool, TÜ Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool.
2016–…	Hanna Antson (kaasjuhendaja), BSc õpingud, arengubioloogia õppetool, TÜ Molekulaar- ja Rakubioloogia Instituut, Tartu Ülikool
2016–…	Gundra Raissar, Gümnaasiumi uurimustöö juhendaja, Hugo Treffneri Gümnaasium, Tartu.

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida.* Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.
19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.

20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro*. Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous grassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.
40. **Arnold Kristjuhan**. Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.
41. **Sulev Ingerpuu**. Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.

42. **Veljo Kisand**. Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.
43. **Kadri Põldmaa**. Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa**. Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik**. Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo**. Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo**. Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots**. Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero**. Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees**. Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.
51. **Sulev Kõks**. Cholecystokinin (CCK) – induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and serotonin. Tartu, 1999, 123 p.
52. **Ebe Sild**. Impact of increasing concentrations of O_3 and CO_2 on wheat, clover and pasture. Tartu, 1999, 123 p.
53. **Ljudmilla Timofejeva**. Electron microscopical analysis of the synaptonemal complex formation in cereals. Tartu, 1999, 99 p.
54. **Andres Valkna**. Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.
55. **Taavi Virro**. Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.
56. **Ana Rebane**. Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.
57. **Tiina Tamm**. Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.
58. **Reet Kurg**. Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.
59. **Toomas Kivisild**. The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.
60. **Niilo Kaldalu**. Studies of the TOL plasmid transcription factor XylS. Tartu, 2000, 88 p.
61. **Dina Lepik**. Modulation of viral DNA replication by tumor suppressor protein p53. Tartu, 2000, 106 p.

62. **Kai Vellak**. Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu, 2000, 122 p.
63. **Jonne Kotta**. Impact of eutrophication and biological invasionas on the structure and functions of benthic macrofauna. Tartu, 2000, 160 p.
64. **Georg Martin**. Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000, 139 p.
65. **Silvia Sepp**. Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.
66. **Jaan Liira**. On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000, 96 p.
67. **Priit Zingel**. The role of planktonic ciliates in lake ecosystems. Tartu, 2001, 111 p.
68. **Tiit Teder**. Direct and indirect effects in Host-parasitoid interactions: ecological and evolutionary consequences. Tartu, 2001, 122 p.
69. **Hannes Kollist**. Leaf apoplastic ascorbate as ozone scavenger and its transport across the plasma membrane. Tartu, 2001, 80 p.
70. **Reet Marits**. Role of two-component regulator system PehR-PehS and extracellular protease PrtW in virulence of *Erwinia Carotovora* subsp. *Carotovora*. Tartu, 2001, 112 p.
71. **Vallo Tilgar**. Effect of calcium supplementation on reproductive performance of the pied flycatcher *Ficedula hypoleuca* and the great tit *Parus major,* breeding in Nothern temperate forests. Tartu, 2002, 126 p.
72. **Rita Hõrak**. Regulation of transposition of transposon Tn*4652* in *Pseudomonas putida*. Tartu, 2002, 108 p.
73. **Liina Eek-Piirsoo**. The effect of fertilization, mowing and additional illumination on the structure of a species-rich grassland community. Tartu, 2002, 74 p.
74. **Krõõt Aasamaa**. Shoot hydraulic conductance and stomatal conductance of six temperate deciduous tree species. Tartu, 2002, 110 p.
75. **Nele Ingerpuu**. Bryophyte diversity and vascular plants. Tartu, 2002, 112 p.
76. **Neeme Tõnisson**. Mutation detection by primer extension on oligonucleotide microarrays. Tartu, 2002, 124 p.
77. **Margus Pensa**. Variation in needle retention of Scots pine in relation to leaf morphology, nitrogen conservation and tree age. Tartu, 2003, 110 p.
78. **Asko Lõhmus**. Habitat preferences and quality for birds of prey: from principles to applications. Tartu, 2003, 168 p.
79. **Viljar Jaks**. p53 – a switch in cellular circuit. Tartu, 2003, 160 p.
80. **Jaana Männik**. Characterization and genetic studies of four ATP-binding cassette (ABC) transporters. Tartu, 2003, 140 p.
81. **Marek Sammul**. Competition and coexistence of clonal plants in relation to productivity. Tartu, 2003, 159 p
82. **Ivar Ilves**. Virus-cell interactions in the replication cycle of bovine papillomavirus type 1. Tartu, 2003, 89 p.

83. **Andres Männik**. Design and characterization of a novel vector system based on the stable replicator of bovine papillomavirus type 1. Tartu, 2003, 109 p.
84. **Ivika Ostonen**. Fine root structure, dynamics and proportion in net primary production of Norway spruce forest ecosystem in relation to site conditions. Tartu, 2003, 158 p.
85. **Gudrun Veldre**. Somatic status of 12–15-year-old Tartu schoolchildren. Tartu, 2003, 199 p.
86. **Ülo Väli**. The greater spotted eagle *Aquila clanga* and the lesser spotted eagle *A. pomarina*: taxonomy, phylogeography and ecology. Tartu, 2004, 159 p.
87. **Aare Abroi**. The determinants for the native activities of the bovine papillomavirus type 1 E2 protein are separable. Tartu, 2004, 135 p.
88. **Tiina Kahre**. Cystic fibrosis in Estonia. Tartu, 2004, 116 p.
89. **Helen Orav-Kotta**. Habitat choice and feeding activity of benthic suspension feeders and mesograzers in the northern Baltic Sea. Tartu, 2004, 117 p.
90. **Maarja Öpik**. Diversity of arbuscular mycorrhizal fungi in the roots of perennial plants and their effect on plant performance. Tartu, 2004, 175 p.
91. **Kadri Tali**. Species structure of *Neotinea ustulata*. Tartu, 2004, 109 p.
92. **Kristiina Tambets**. Towards the understanding of post-glacial spread of human mitochondrial DNA haplogroups in Europe and beyond: a phylogeographic approach. Tartu, 2004, 163 p.
93. **Arvi Jõers**. Regulation of p53-dependent transcription. Tartu, 2004, 103 p.
94. **Lilian Kadaja**. Studies on modulation of the activity of tumor suppressor protein p53. Tartu, 2004, 103 p.
95. **Jaak Truu**. Oil shale industry wastewater: impact on river microbial community and possibilities for bioremediation. Tartu, 2004, 128 p.
96. **Maire Peters**. Natural horizontal transfer of the *pheBA* operon. Tartu, 2004, 105 p.
97. **Ülo Maiväli**. Studies on the structure-function relationship of the bacterial ribosome. Tartu, 2004, 130 p.
98. **Merit Otsus**. Plant community regeneration and species diversity in dry calcareous grasslands. Tartu, 2004, 103 p.
99. **Mikk Heidemaa**. Systematic studies on sawflies of the genera *Dolerus, Empria,* and *Caliroa* (Hymenoptera: Tenthredinidae). Tartu, 2004, 167 p.
100. **Ilmar Tõnno**. The impact of nitrogen and phosphorus concentration and N/P ratio on cyanobacterial dominance and N_2 fixation in some Estonian lakes. Tartu, 2004, 111 p.
101. **Lauri Saks**. Immune function, parasites, and carotenoid-based ornaments in greenfinches. Tartu, 2004, 144 p.
102. **Siiri Rootsi**. Human Y-chromosomal variation in European populations. Tartu, 2004, 142 p.
103. **Eve Vedler**. Structure of the 2,4-dichloro-phenoxyacetic acid-degradative plasmid pEST4011. Tartu, 2005. 106 p.

104. **Andres Tover**. Regulation of transcription of the phenol degradation *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 126 p.
105. **Helen Udras**. Hexose kinases and glucose transport in the yeast *Hansenula polymorpha*. Tartu, 2005, 100 p.
106. **Ave Suija**. Lichens and lichenicolous fungi in Estonia: diversity, distribution patterns, taxonomy. Tartu, 2005, 162 p.
107. **Piret Lõhmus**. Forest lichens and their substrata in Estonia. Tartu, 2005, 162 p.
108. **Inga Lips**. Abiotic factors controlling the cyanobacterial bloom occurrence in the Gulf of Finland. Tartu, 2005, 156 p.
109. **Kaasik, Krista**. Circadian clock genes in mammalian clockwork, metabolism and behaviour. Tartu, 2005, 121 p.
110. **Juhan Javoiš**. The effects of experience on host acceptance in ovipositing moths. Tartu, 2005, 112 p.
111. **Tiina Sedman**. Characterization of the yeast *Saccharomyces cerevisiae* mitochondrial DNA helicase Hmi1. Tartu, 2005, 103 p.
112. **Ruth Aguraiuja**. Hawaiian endemic fern lineage *Diellia* (Aspleniaceae): distribution, population structure and ecology. Tartu, 2005, 112 p.
113. **Riho Teras**. Regulation of transcription from the fusion promoters generated by transposition of Tn*4652* into the upstream region of *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 106 p.
114. **Mait Metspalu**. Through the course of prehistory in india: tracing the mtDNA trail. Tartu, 2005, 138 p.
115. **Elin Lõhmussaar**. The comparative patterns of linkage disequilibrium in European populations and its implication for genetic association studies. Tartu, 2006, 124 p.
116. **Priit Kupper**. Hydraulic and environmental limitations to leaf water relations in trees with respect to canopy position. Tartu, 2006, 126 p.
117. **Heili Ilves**. Stress-induced transposition of Tn*4652* in *Pseudomonas Putida.* Tartu, 2006, 120 p.
118. **Silja Kuusk**. Biochemical properties of Hmi1p, a DNA helicase from *Saccharomyces cerevisiae* mitochondria. Tartu, 2006, 126 p.
119. **Kersti Püssa**. Forest edges on medium resolution landsat thematic mapper satellite images. Tartu, 2006, 90 p.
120. **Lea Tummeleht**. Physiological condition and immune function in great tits (*Parus major* l.): Sources of variation and trade-offs in relation to growth. Tartu, 2006, 94 p.
121. **Toomas Esperk**. Larval instar as a key element of insect growth schedules. Tartu, 2006, 186 p.
122. **Harri Valdmann**. Lynx (*Lynx lynx*) and wolf (*Canis lupus*) in the Baltic region: Diets, helminth parasites and genetic variation. Tartu, 2006. 102 p.
123. **Priit Jõers**. Studies of the mitochondrial helicase Hmi1p in *Candida albicans* and *Saccharomyces cerevisia*. Tartu, 2006. 113 p.
124. **Kersti Lilleväli**. Gata3 and Gata2 in inner ear development. Tartu, 2007, 123 p.

125. **Kai Rünk**. Comparative ecology of three fern species: *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *D. expansa* (C. Presl) Fraser-Jenkins & Jermy and *D. dilatata* (Hoffm.) A. Gray (Dryopteridaceae). Tartu, 2007, 143 p.
126. **Aveliina Helm**. Formation and persistence of dry grassland diversity: role of human history and landscape structure. Tartu, 2007, 89 p.
127. **Leho Tedersoo**. Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. Tartu, 2007, 233 p.
128. **Marko Mägi**. The habitat-related variation of reproductive performance of great tits in a deciduous-coniferous forest mosaic: looking for causes and consequences. Tartu, 2007, 135 p.
129. **Valeria Lulla**. Replication strategies and applications of Semliki Forest virus. Tartu, 2007, 109 p.
130. **Ülle Reier**. Estonian threatened vascular plant species: causes of rarity and conservation. Tartu, 2007, 79 p.
131. **Inga Jüriado**. Diversity of lichen species in Estonia: influence of regional and local factors. Tartu, 2007, 171 p.
132. **Tatjana Krama**. Mobbing behaviour in birds: costs and reciprocity based cooperation. Tartu, 2007, 112 p.
133. **Signe Saumaa**. The role of DNA mismatch repair and oxidative DNA damage defense systems in avoidance of stationary phase mutations in *Pseudomonas putida.* Tartu, 2007, 172 p.
134. **Reedik Mägi**. The linkage disequilibrium and the selection of genetic markers for association studies in european populations. Tartu, 2007, 96 p.
135. **Priit Kilgas**. Blood parameters as indicators of physiological condition and skeletal development in great tits (*Parus major*): natural variation and application in the reproductive ecology of birds. Tartu, 2007, 129 p.
136. **Anu Albert**. The role of water salinity in structuring eastern Baltic coastal fish communities. Tartu, 2007, 95 p.
137. **Kärt Padari**. Protein transduction mechanisms of transportans. Tartu, 2008, 128 p.
138. **Siiri-Lii Sandre**. Selective forces on larval colouration in a moth. Tartu, 2008, 125 p.
139. **Ülle Jõgar**. Conservation and restoration of semi-natural floodplain meadows and their rare plant species. Tartu, 2008, 99 p.
140. **Lauri Laanisto**. Macroecological approach in vegetation science: generality of ecological relationships at the global scale. Tartu, 2008, 133 p.
141. **Reidar Andreson**. Methods and software for predicting PCR failure rate in large genomes. Tartu, 2008, 105 p.
142. **Birgot Paavel**. Bio-optical properties of turbid lakes. Tartu, 2008, 175 p.
143. **Kaire Torn**. Distribution and ecology of charophytes in the Baltic Sea. Tartu, 2008, 98 p.
144. **Vladimir Vimberg**. Peptide mediated macrolide resistance. Tartu, 2008, 190 p.
145. **Daima Örd**. Studies on the stress-inducible pseudokinase TRB3, a novel inhibitor of transcription factor ATF4. Tartu, 2008, 108 p.

146. **Lauri Saag**. Taxonomic and ecologic problems in the genus *Lepraria* (*Stereocaulaceae*, lichenised *Ascomycota*). Tartu, 2008, 175 p.
147. **Ulvi Karu**. Antioxidant protection, carotenoids and coccidians in greenfinches – assessment of the costs of immune activation and mechanisms of parasite resistance in a passerine with carotenoid-based ornaments. Tartu, 2008, 124 p.
148. **Jaanus Remm**. Tree-cavities in forests: density, characteristics and occupancy by animals. Tartu, 2008, 128 p.
149. **Epp Moks**. Tapeworm parasites *Echinococcus multilocularis* and *E. granulosus* in Estonia: phylogenetic relationships and occurrence in wild carnivores and ungulates. Tartu, 2008, 82 p.
150. **Eve Eensalu**. Acclimation of stomatal structure and function in tree canopy: effect of light and CO_2 concentration. Tartu, 2008, 108 p.
151. **Janne Pullat**. Design, functionlization and application of an *in situ* synthesized oligonucleotide microarray. Tartu, 2008, 108 p.
152. **Marta Putrinš**. Responses of *Pseudomonas putida* to phenol-induced metabolic and stress signals. Tartu, 2008, 142 p.
153. **Marina Semtšenko**. Plant root behaviour: responses to neighbours and physical obstructions. Tartu, 2008, 106 p.
154. **Marge Starast**. Influence of cultivation techniques on productivity and fruit quality of some *Vaccinium* and *Rubus* taxa. Tartu, 2008, 154 p.
155. **Age Tats**. Sequence motifs influencing the efficiency of translation. Tartu, 2009, 104 p.
156. **Radi Tegova**. The role of specialized DNA polymerases in mutagenesis in *Pseudomonas putida.* Tartu, 2009, 124 p.
157. **Tsipe Aavik**. Plant species richness, composition and functional trait pattern in agricultural landscapes – the role of land use intensity and landscape structure. Tartu, 2009, 112 p.
158. **Kaja Kiiver**. Semliki forest virus based vectors and cell lines for studying the replication and interactions of alphaviruses and hepaciviruses. Tartu, 2009, 104 p.
159. **Meelis Kadaja**. Papillomavirus Replication Machinery Induces Genomic Instability in its Host Cell. Tartu, 2009, 126 p.
160. **Pille Hallast**. Human and chimpanzee Luteinizing hormone/Chorionic Gonadotropin beta (*LHB/CGB*) gene clusters: diversity and divergence of young duplicated genes. Tartu, 2009, 168 p.
161. **Ain Vellak**. Spatial and temporal aspects of plant species conservation. Tartu, 2009, 86 p.
162. **Triinu Remmel**. Body size evolution in insects with different colouration strategies: the role of predation risk. Tartu, 2009, 168 p.
163. **Jaana Salujõe**. Zooplankton as the indicator of ecological quality and fish predation in lake ecosystems. Tartu, 2009, 129 p.
164. **Ele Vahtmäe**. Mapping benthic habitat with remote sensing in optically complex coastal environments. Tartu, 2009, 109 p.

165. **Liisa Metsamaa**. Model-based assessment to improve the use of remote sensing in recognition and quantitative mapping of cyanobacteria. Tartu, 2009, 114 p.
166. **Pille Säälik**. The role of endocytosis in the protein transduction by cell-penetrating peptides. Tartu, 2009, 155 p.
167. **Lauri Peil**. Ribosome assembly factors in *Escherichia coli.* Tartu, 2009, 147 p.
168. **Lea Hallik**. Generality and specificity in light harvesting, carbon gain capacity and shade tolerance among plant functional groups. Tartu, 2009, 99 p.
169. **Mariliis Tark**. Mutagenic potential of DNA damage repair and tolerance mechanisms under starvation stress. Tartu, 2009, 191 p.
170. **Riinu Rannap**. Impacts of habitat loss and restoration on amphibian populations. Tartu, 2009, 117 p.
171. **Maarja Adojaan**. Molecular variation of HIV-1 and the use of this knowledge in vaccine development. Tartu, 2009, 95 p.
172. **Signe Altmäe**. Genomics and transcriptomics of human induced ovarian folliculogenesis. Tartu, 2010, 179 p.
173. **Triin Suvi**. Mycorrhizal fungi of native and introduced trees in the Seychelles Islands. Tartu, 2010, 107 p.
174. **Velda Lauringson**. Role of suspension feeding in a brackish-water coastal sea. Tartu, 2010, 123 p.
175. **Eero Talts**. Photosynthetic cyclic electron transport – measurement and variably proton-coupled mechanism. Tartu, 2010, 121 p.
176. **Mari Nelis**. Genetic structure of the Estonian population and genetic distance from other populations of European descent. Tartu, 2010, 97 p.
177. **Kaarel Krjutškov**. Arrayed Primer Extension-2 as a multiplex PCR-based method for nucleic acid variation analysis: method and applications. Tartu, 2010, 129 p.
178. **Egle Köster**. Morphological and genetical variation within species complexes: *Anthyllis vulneraria* s. l. and *Alchemilla vulgaris* (coll.). Tartu, 2010, 101 p.
179. **Erki Õunap**. Systematic studies on the subfamily Sterrhinae (Lepidoptera: Geometridae). Tartu, 2010, 111 p.
180. **Merike Jõesaar**. Diversity of key catabolic genes at degradation of phenol and *p*-cresol in pseudomonads. Tartu, 2010, 125 p.
181. **Kristjan Herkül**. Effects of physical disturbance and habitat-modifying species on sediment properties and benthic communities in the northern Baltic Sea. Tartu, 2010, 123 p.
182. **Arto Pulk**. Studies on bacterial ribosomes by chemical modification approaches. Tartu, 2010, 161 p.
183. **Maria Põllupüü**. Ecological relations of cladocerans in a brackish-water ecosystem. Tartu, 2010, 126 p.
184. **Toomas Silla**. Study of the segregation mechanism of the Bovine Papillomavirus Type 1. Tartu, 2010, 188 p.

185. **Gyaneshwer Chaubey**. The demographic history of India: A perspective based on genetic evidence. Tartu, 2010, 184 p.
186. **Katrin Kepp**. Genes involved in cardiovascular traits: detection of genetic variation in Estonian and Czech populations. Tartu, 2010, 164 p.
187. **Virve Sõber**. The role of biotic interactions in plant reproductive performance. Tartu, 2010, 92 p.
188. **Kersti Kangro**. The response of phytoplankton community to the changes in nutrient loading. Tartu, 2010, 144 p.
189. **Joachim M. Gerhold**. Replication and Recombination of mitochondrial DNA in Yeast. Tartu, 2010, 120 p.
190. **Helen Tammert**. Ecological role of physiological and phylogenetic diversity in aquatic bacterial communities. Tartu, 2010, 140 p.
191. **Elle Rajandu**. Factors determining plant and lichen species diversity and composition in Estonian *Calamagrostis* and *Hepatica* site type forests. Tartu, 2010, 123 p.
192. **Paula Ann Kivistik**. ColR-ColS signalling system and transposition of Tn*4652* in the adaptation of *Pseudomonas putida.* Tartu, 2010, 118 p.
193. **Siim Sõber**. Blood pressure genetics: from candidate genes to genome-wide association studies. Tartu, 2011, 120 p.
194. **Kalle Kipper**. Studies on the role of helix 69 of 23S rRNA in the factor-dependent stages of translation initiation, elongation, and termination. Tartu, 2011, 178 p.
195. **Triinu Siibak**. Effect of antibiotics on ribosome assembly is indirect. Tartu, 2011, 134 p.
196. **Tambet Tõnissoo**. Identification and molecular analysis of the role of guanine nucleotide exchange factor RIC-8 in mouse development and neural function. Tartu, 2011, 110 p.
197. **Helin Räägel**. Multiple faces of cell-penetrating peptides – their intracellular trafficking, stability and endosomal escape during protein transduction. Tartu, 2011, 161 p.
198. **Andres Jaanus**. Phytoplankton in Estonian coastal waters – variability, trends and response to environmental pressures. Tartu, 2011, 157 p.
199. **Tiit Nikopensius**. Genetic predisposition to nonsyndromic orofacial clefts. Tartu, 2011, 152 p.
200. **Signe Värv**. Studies on the mechanisms of RNA polymerase II-dependent transcription elongation. Tartu, 2011, 108 p.
201. **Kristjan Välk**. Gene expression profiling and genome-wide association studies of non-small cell lung cancer. Tartu, 2011, 98 p.
202. **Arno Põllumäe**. Spatio-temporal patterns of native and invasive zooplankton species under changing climate and eutrophication conditions. Tartu, 2011, 153 p.
203. **Egle Tammeleht**. Brown bear (*Ursus arctos*) population structure, demographic processes and variations in diet in northern Eurasia. Tartu, 2011, 143 p.

205. **Teele Jairus**. Species composition and host preference among ectomycorrhizal fungi in Australian and African ecosystems. Tartu, 2011, 106 p.
206. **Kessy Abarenkov**. PlutoF – cloud database and computing services supporting biological research. Tartu, 2011, 125 p.
207. **Marina Grigorova**. Fine-scale genetic variation of follicle-stimulating hormone beta-subunit coding gene (*FSHB*) and its association with reproductive health. Tartu, 2011, 184 p.
208. **Anu Tiitsaar**. The effects of predation risk and habitat history on butterfly communities. Tartu, 2011, 97 p.
209. **Elin Sild**. Oxidative defences in immunoecological context: validation and application of assays for nitric oxide production and oxidative burst in a wild passerine. Tartu, 2011, 105 p.
210. **Irja Saar**. The taxonomy and phylogeny of the genera *Cystoderma* and *Cystodermella* (Agaricales, Fungi). Tartu, 2012, 167 p.
211. **Pauli Saag**. Natural variation in plumage bacterial assemblages in two wild breeding passerines. Tartu, 2012, 113 p.
212. **Aleksei Lulla**. Alphaviral nonstructural protease and its polyprotein substrate: arrangements for the perfect marriage. Tartu, 2012, 143 p.
213. **Mari Järve**. Different genetic perspectives on human history in Europe and the Caucasus: the stories told by uniparental and autosomal markers. Tartu, 2012, 119 p.
214. **Ott Scheler**. The application of tmRNA as a marker molecule in bacterial diagnostics using microarray and biosensor technology. Tartu, 2012, 93 p.
215. **Anna Balikova**. Studies on the functions of tumor-associated mucin-like leukosialin (CD43) in human cancer cells. Tartu, 2012, 129 p.
216. **Triinu Kõressaar**. Improvement of PCR primer design for detection of prokaryotic species. Tartu, 2012, 83 p.
217. **Tuul Sepp**. Hematological health state indices of greenfinches: sources of individual variation and responses to immune system manipulation. Tartu, 2012, 117 p.
218. **Rya Ero**. Modifier view of the bacterial ribosome. Tartu, 2012, 146 p.
219. **Mohammad Bahram**. Biogeography of ectomycorrhizal fungi across different spatial scales. Tartu, 2012, 165 p.
220. **Annely Lorents**. Overcoming the plasma membrane barrier: uptake of amphipathic cell-penetrating peptides induces influx of calcium ions and downstream responses. Tartu, 2012, 113 p.
221. **Katrin Männik**. Exploring the genomics of cognitive impairment: whole-genome SNP genotyping experience in Estonian patients and general population. Tartu, 2012, 171 p.
222. **Marko Prous**. Taxonomy and phylogeny of the sawfly genus *Empria* (Hymenoptera, Tenthredinidae). Tartu, 2012, 192 p.
223. **Triinu Visnapuu**. Levansucrases encoded in the genome of *Pseudomonas syringae* pv. tomato DC3000: heterologous expression, biochemical characterization, mutational analysis and spectrum of polymerization products. Tartu, 2012, 160 p.

224. **Nele Tamberg**. Studies on Semliki Forest virus replication and pathogenesis. Tartu, 2012, 109 p.
225. **Tõnu Esko**. Novel applications of SNP array data in the analysis of the genetic structure of Europeans and in genetic association studies. Tartu, 2012, 149 p.
226. **Timo Arula**. Ecology of early life-history stages of herring *Clupea harengus membras* in the northeastern Baltic Sea. Tartu, 2012, 143 p.
227. **Inga Hiiesalu**. Belowground plant diversity and coexistence patterns in grassland ecosystems. Tartu, 2012, 130 p.
228. **Kadri Koorem**. The influence of abiotic and biotic factors on small-scale plant community patterns and regeneration in boreonemoral forest. Tartu, 2012, 114 p.
229. **Liis Andresen**. Regulation of virulence in plant-pathogenic pectobacteria. Tartu, 2012, 122 p.
230. **Kaupo Kohv**. The direct and indirect effects of management on boreal forest structure and field layer vegetation. Tartu, 2012, 124 p.
231. **Mart Jüssi**. Living on an edge: landlocked seals in changing climate. Tartu, 2012, 114 p.
232. **Riina Klais**. Phytoplankton trends in the Baltic Sea. Tartu, 2012, 136 p.
233. **Rauno Veeroja**. Effects of winter weather, population density and timing of reproduction on life-history traits and population dynamics of moose (*Alces alces*) in Estonia. Tartu, 2012, 92 p.
234. **Marju Keis**. Brown bear (*Ursus arctos*) phylogeography in northern Eurasia. Tartu, 2013, 142 p.
235. **Sergei Põlme**. Biogeography and ecology of *alnus*- associated ectomycorrhizal fungi – from regional to global scale. Tartu, 2013, 90 p.
236. **Liis Uusküla**. Placental gene expression in normal and complicated pregnancy. Tartu, 2013, 173 p.
237. **Marko Lõoke**. Studies on DNA replication initiation in *Saccharomyces cerevisiae.* Tartu, 2013, 112 p.
238. **Anne Aan**. Light- and nitrogen-use and biomass allocation along productivity gradients in multilayer plant communities. Tartu, 2013, 127 p.
239. **Heidi Tamm**. Comprehending phylogenetic diversity – case studies in three groups of ascomycetes. Tartu, 2013, 136 p.
240. **Liina Kangur**. High-Pressure Spectroscopy Study of Chromophore-Binding Hydrogen Bonds in Light-Harvesting Complexes of Photosynthetic Bacteria. Tartu, 2013, 150 p.
241. **Margus Leppik**. Substrate specificity of the multisite specific pseudouridine synthase RluD. Tartu, 2013, 111 p.
242. **Lauris Kaplinski**. The application of oligonucleotide hybridization model for PCR and microarray optimization. Tartu, 2013, 103 p.
243. **Merli Pärnoja**. Patterns of macrophyte distribution and productivity in coastal ecosystems: effect of abiotic and biotic forcing. Tartu, 2013, 155 p.
244. **Tõnu Margus**. Distribution and phylogeny of the bacterial translational GTPases and the Mqsr/YgiT regulatory system. Tartu, 2013, 126 p.

245. **Pille Mänd**. Light use capacity and carbon and nitrogen budget of plants: remote assessment and physiological determinants. Tartu, 2013, 128 p.
246. **Mario Plaas**. Animal model of Wolfram Syndrome in mice: behavioural, biochemical and psychopharmacological characterization. Tartu, 2013, 144 p.
247. **Georgi Hudjašov**. Maps of mitochondrial DNA, Y-chromosome and tyrosinase variation in Eurasian and Oceanian populations. Tartu, 2013, 115 p.
248. **Mari Lepik**. Plasticity to light in herbaceous plants and its importance for community structure and diversity. Tartu, 2013, 102 p.
249. **Ede Leppik**. Diversity of lichens in semi-natural habitats of Estonia. Tartu, 2013, 151 p.
250. **Ülle Saks**. Arbuscular mycorrhizal fungal diversity patterns in boreo-nemoral forest ecosystems. Tartu, 2013, 151 p.
251. **Eneli Oitmaa**. Development of arrayed primer extension microarray assays for molecular diagnostic applications. Tartu, 2013, 147 p.
252. **Jekaterina Jutkina**. The horizontal gene pool for aromatics degradation: bacterial catabolic plasmids of the Baltic Sea aquatic system. Tartu, 2013, 121 p.
253. **Helen Vellau**. Reaction norms for size and age at maturity in insects: rules and exceptions. Tartu, 2014, 132 p.
254. **Randel Kreitsberg**. Using biomarkers in assessment of environmental contamination in fish – new perspectives. Tartu, 2014, 107 p.
255. **Krista Takkis**. Changes in plant species richness and population performance in response to habitat loss and fragmentation.Tartu, 2014, 141 p.
256. **Liina Nagirnaja**. Global and fine-scale genetic determinants of recurrent pregnancy loss. Tartu, 2014, 211 p.
257. **Triin Triisberg**. Factors influencing the re-vegetation of abandoned extracted peatlands in Estonia. Tartu, 2014, 133 p.
258. **Villu Soon**. A phylogenetic revision of the *Chrysis ignita* species group (Hymenoptera: Chrysididae) with emphasis on the northern European fauna. Tartu, 2014, 211 p.
259. **Andrei Nikonov**. RNA-Dependent RNA Polymerase Activity as a Basis for the Detection of Positive-Strand RNA Viruses by Vertebrate Host Cells. Tartu, 2014, 207 p.
260. **Eele Õunapuu-Pikas**. Spatio-temporal variability of leaf hydraulic conductance in woody plants: ecophysiological consequences. Tartu, 2014, 135 p.
261. **Marju Männiste**. Physiological ecology of greenfinches: information content of feathers in relation to immune function and behavior. Tartu, 2014, 121 p.
262. **Katre Kets**. Effects of elevated concentrations of CO_2 and O_3 on leaf photosynthetic parameters in *Populus tremuloides*: diurnal, seasonal and interannual patterns. Tartu, 2014, 115 p.

263. **Külli Lokko**. Seasonal and spatial variability of zoopsammon communities in relation to environmental parameters. Tartu, 2014, 129 p.
264. **Olga Žilina**. Chromosomal microarray analysis as diagnostic tool: Estonian experience. Tartu, 2014, 152 p.
265. **Kertu Lõhmus**. Colonisation ecology of forest-dwelling vascular plants and the conservation value of rural manor parks. Tartu, 2014, 111 p.
266. **Anu Aun**. Mitochondria as integral modulators of cellular signaling. Tartu, 2014, 167 p.
267. **Chandana Basu Mallick**. Genetics of adaptive traits and gender-specific demographic processes in South Asian populations. Tartu, 2014, 160 p.
268. **Riin Tamme**. The relationship between small-scale environmental heterogeneity and plant species diversity. Tartu, 2014, 130 p.
269. **Liina Remm**. Impacts of forest drainage on biodiversity and habitat quality: implications for sustainable management and conservation. Tartu, 2015, 126 p.
270. **Tiina Talve**. Genetic diversity and taxonomy within the genus *Rhinanthus.* Tartu, 2015, 106 p.
271. **Mehis Rohtla**. Otolith sclerochronological studies on migrations, spawning habitat preferences and age of freshwater fishes inhabiting the Baltic Sea. Tartu, 2015, 137 p.
272. **Alexey Reshchikov**. The world fauna of the genus *Lathrolestes* (Hymenoptera, Ichneumonidae). Tartu, 2015, 247 p.
273. **Martin Pook**. Studies on artificial and extracellular matrix protein-rich surfaces as regulators of cell growth and differentiation. Tartu, 2015, 142 p.
274. **Mai Kukumägi**. Factors affecting soil respiration and its components in silver birch and Norway spruce stands. Tartu, 2015, 155 p.
275. **Helen Karu**. Development of ecosystems under human activity in the North-East Estonian industrial region: forests on post-mining sites and bogs. Tartu, 2015, 152 p.
276. **Hedi Peterson**. Exploiting high-throughput data for establishing relationships between genes. Tartu, 2015, 186 p.
277. **Priit Adler**. Analysis and visualisation of large scale microarray data, Tartu, 2015, 126 p.
278. **Aigar Niglas**. Effects of environmental factors on gas exchange in deciduous trees: focus on photosynthetic water-use efficiency. Tartu, 2015, 152 p.
279. **Silja Laht**. Classification and identification of conopeptides using profile hidden Markov models and position-specific scoring matrices. Tartu, 2015, 100 p.
280. **Martin Kesler**. Biological characteristics and restoration of Atlantic salmon *Salmo salar* populations in the Rivers of Northern Estonia. Tartu, 2015, 97 p.
281. **Pratyush Kumar Das**. Biochemical perspective on alphaviral nonstructural protein 2: a tale from multiple domains to enzymatic profiling. Tartu, 2015, 205 p

282. **Priit Palta**. Computational methods for DNA copy number detection. Tartu, 2015, 130 p.
283. **Julia Sidorenko**. Combating DNA damage and maintenance of genome integrity in pseudomonads. Tartu, 2015, 174 p.
284. **Anastasiia Kovtun-Kante**. Charophytes of Estonian inland and coastal waters: distribution and environmental preferences. Tartu, 2015, 97 p.
285. **Ly Lindman**. The ecology of protected butterfly species in Estonia. Tartu, 2015, 171 p.
286. **Jaanis Lodjak**. Association of Insulin-like Growth Factor I and Corticosterone with Nestling Growth and Fledging Success in Wild Passerines. Tartu, 2016, 113 p.
287. **Ann Kraut**. Conservation of Wood-Inhabiting Biodiversity – Semi-Natural Forests as an Opportunity. Tartu, 2016, 141 p.
288. **Tiit Örd.** Functions and regulation of the mammalian pseudokinase TRIB3. Tartu, 2016, 182. p.
289. **Kairi Käiro.** Biological Quality According to Macroinvertebrates in Streams of Estonia (Baltic Ecoregion of Europe): Effects of Human-induced Hydromorphological Changes. Tartu, 2016, 126 p.
290. **Leidi Laurimaa**. *Echinococcus multilocularis* and other zoonotic parasites in Estonian canids. Tartu, 2016, 144 p.
291. **Helerin Margus.** Characterization of cell-penetrating peptide/nucleic acid nanocomplexes and their cell-entry mechanisms. Tartu, 2016, 173 p.
292. **Kadri Runnel**. Fungal targets and tools for forest conservation. Tartu, 2016, 157 p.
293. **Urmo Võsa**. MicroRNAs in disease and health: aberrant regulation in lung cancer and association with genomic variation. Tartu, 2016, 163 p.
294. **Kristina Mäemets-Allas**. Studies on cell growth promoting AKT signaling pathway – a promising anti-cancer drug target. Tartu, 2016, 146 p.
295. **Janeli Viil.** Studies on cellular and molecular mechanisms that drive normal and regenerative processes in the liver and pathological processes in Dupuytren's contracture. Tartu, 2016, 175 p.
296. **Ene Kook**. Genetic diversity and evolution of *Pulmonaria angustifolia* L. and *Myosotis laxa sensu lato* (Boraginaceae). Tartu, 2016, 106 p.
297. **Kadri Peil.** RNA polymerase II-dependent transcription elongation in *Saccharomyces cerevisiae*. Tartu, 2016, 113 p.
298. **Katrin Ruisu.** The role of RIC8A in mouse development and its function in cell-matrix adhesion and actin cytoskeletal organisation. Tartu, 2016, 129 p.
299. **Janely Pae**. Translocation of cell-penetrating peptides across biological membranes and interactions with plasma membrane constituents. Tartu, 2016, 126 p.
300. **Argo Ronk.** Plant diversity patterns across Europe: observed and dark diversity. Tartu, 2016, 153 p.

301. **Kristiina Mark**. Diversification and species delimitation of lichenized fungi in selected groups of the family Parmeliaceae (Ascomycota). Tartu, 2016, 181 p.
302. **Jaak-Albert Metsoja**. Vegetation dynamics in floodplain meadows: influence of mowing and sediment application. Tartu, 2016, 140 p.
303. **Hedvig Tamman.** The GraTA toxin-antitoxin system of *Pseudomonas putida*: regulation and role in stress tolerance. Tartu, 2016, 154 p.
304. **Kadri Pärtel**. Application of ultrastructural and molecular data in the taxonomy of helotialean fungi. Tartu, 2016, 183 p.
305. **Maris Hindrikson**. Grey wolf (*Canis lupus*) populations in Estonia and Europe: genetic diversity, population structure and -processes, and hybridization between wolves and dogs. Tartu, 2016, 121 p.
306. **Polina Degtjarenko**. Impacts of alkaline dust pollution on biodiversity of plants and lichens: from communities to genetic diversity. Tartu, 2016, 126 p.
307. **Liina Pajusalu**. The effect of CO_2 enrichment on net photosynthesis of macrophytes in a brackish water environment. Tartu, 2016, 126 p.
308. **Stoyan Tankov**. Random walks in the stringent response. Tartu, 2016, 94 p.
309. **Liis Leitsalu**. Communicating genomic research results to population-based biobank participants. Tartu, 2016, 158 p.
310. **Richard Meitern**. Redox physiology of wild birds: validation and application of techniques for detecting oxidative stress. Tartu, 2016, 134 p.
311. **Kaie Lokk**. Comparative genome-wide DNA methylation studies of healthy human tissues and non-small cell lung cancer tissue. Tartu, 2016, 127 p.
312. **Mihhail Kurašin.** Processivity of cellulases and chitinases. Tartu, 2017, 132 p.
313. **Carmen Tali.** Scavenger receptors as a target for nucleic acid delivery with peptide vectors. Tartu, 2017, 155 p.
314. **Katarina Oganjan**. Distribution, feeding and habitat of benthic suspension feeders in a shallow coastal sea. Tartu, 2017, 132 p.
315. **Taavi Paal.** Immigration limitation of forest plants into wooded landscape corridors. Tartu, 2017, 145 p.
316. **Kadri Õunap**. The Williams-Beuren syndrome chromosome region protein WBSCR22 is a ribosome biogenesis factor. Tartu, 2017, 135 p.
317. **Riin Tamm**. In-depth analysis of factors affecting variability in thiopurine methyltransferase activity. Tartu, 2017, 170 p.