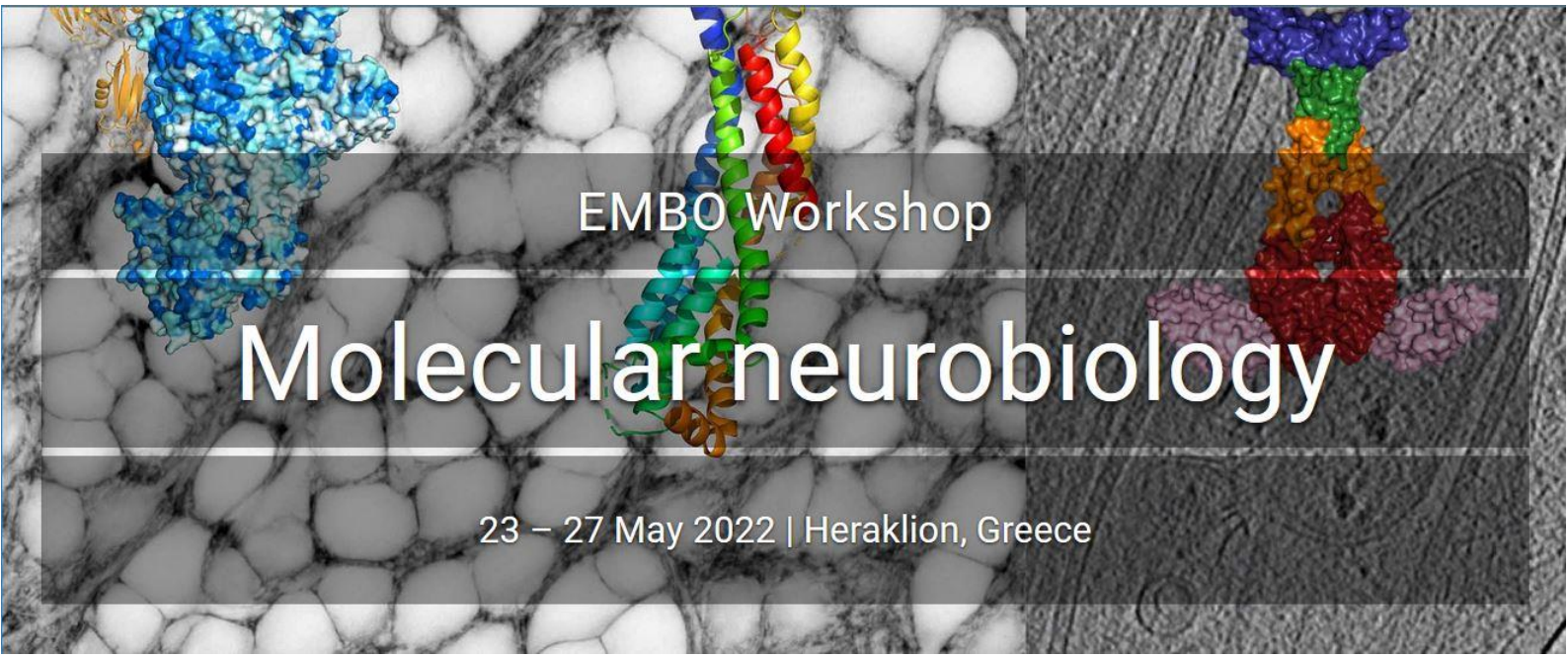

Organizers

Amparo ACKER-PALMER [Goethe Universität Frankfurt, Germany]
Alex KOLODKIN [Johns Hopking University, USA]
Naoko MIZUNO [NIH, USA]
Valentin NÄGERL [Université de Bordeaux, France]
Elena SEIRADAKE [University of Oxford, UK]
Marc TESSIER-LAVIGNE [Stanford University, USA]

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Programme of the Workshop

Monday, 23 May 2022

10.00 - Opening of the Molecular Neurobiology Workshop *– Welcome to the event*

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10:35-11:05	**Yvonne JONES** [University of Oxford, UK] *Semaphorin and Plexin Receptor Signalling Mechanisms*
11:05-11:20	**Alexander JAWORSKI** [Brown University, Germany] *A Novel Axon Guidance Cue that Signals through Divergent Dcc Family Receptors*
11:20-11:50	**Beatriz RICO** [King's College, UK] *Synapse-Specific Regulation of Protein Synthesis for Cortical Wiring*
11:50-12:20	**Rob MEIJERS** [Institute of Protein Innovation, USA] *Developing Antibodies to Study Neural Receptors*
12:20-14:00	***Lunch Break***
14:00-14:30	**Christian SIEBOLD** [University of Oxford, UK] *Molecular Mechanisms for Processing and Signal Reception of the Hedgehog Morphogen and Axon Guidance Molecule*
14:30-15:00	**Ruediger KLEIN** [MPI of Neurobiology, Germany] *Regulation of Cortical Folding by Controlling Progenitor Expansion and Neuronal Migration via Cell-Cell Communication*
15:00-15:15	**Emily ROWLAND** [University of Oxford, UK] *Structural and Binding Studies of Semaphorin-3G and its Receptor Neuropilin-2*
15:15-15:30	**Dietmar SCHMUCKER** [LIMES, University of Bonn, Germany] *Molecular Mechanisms of Neurite Branching and Central Synapse Formation*
15:30-16:30	**Poster Presenters:** *Poster Flash Talks*
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19:30	***Dinner***

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09:30-10:00	**Elena SEIRADAKE** [University of Oxford, UK] *GPC3-Unc5 Complex Structure and Role in Cell Migration*
10:00-10:30	**Alex KOLODKIN** [Johns Hopking University, USA] *Molecular Mechanisms Underlying Neural Circuit Assembly in the Mammalian Visual System*
10:30-10:45	**Yehuda SALZBERG** [Weizmann Institute of Science, Israel] *Post-Developmental Roles of the Netrin Receptor UNC-40/DCC Mediated by a Novel Phosphodegron Motif*
10:45-11:00	**Francesca HOUGHTON** [The Francis Crick Institute, UK] *A GDNF-GFRa1 Synaptic Trans-Adhesion Complex is Disassembled by RET Receptor Interaction*
11:00-11:30	***Coffee Break***
11:30-12:00	**Guillermina LOPEZ-BENDITO** [Instituto de Neurosciencias, Spain] *Programming and Reprogramming Sensory Circuits*
12:00-12:30	**Bert JANSSEN** [Utrecht University, Netherlands] *Protein Structure, Flexibility and Interactions in Control of Myelin-Axon Contacts*
12:30-12:45	**Dimphna MEIJER** [Delft University of Technology, Netherlands] *Structural Insights into the Teneurin-4 Dimer Reveal a Compact Conformation for Trans-Interactions*
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15:15-15:45	**Yarden OPATOWSKY** [Bar-Ilan University Ramat-Gan, Israel] *SARM1 Ring to Rule Them All*
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19:30	***Dinner***

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SYNAPSE BIOLOGY & WIRING

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09:30-10:00	**Daniel CHOQUET** [University of Bordeaux, France] *New Tools to Study Nanoscale Synapse Organization, Function and Plasticity*
10:00-10:30	**Ryan HIBBS** [UT Southwestern, USA] *Structural Pharmacology of the Muscle-Type Nicotinic Acetylcholine Receptor*
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11:30-12:00	**Ingo GREGER** [MRC-LMB, UK] *Mechanisms Underlying AMPA Receptor Activation and Modulation*
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12:30-13:00	**Cordelia IMIG** [University of Copenhagen, Denmark] *Caught in the Act: Ultrastructural Imaging of Synaptic Vesicle Pools*
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NEUROVASCULAR BIOLOGY AND DISEASE

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10:00-10:30	**Nektarios TAVERNARAKIS** [University of Crete & IMBB-FORTH, Greece] *Autophagy in Neuronal Systems and Aging*
10:30-11:00	**Carmen RUIZ DE ALMONDOVAR** [University of Bonn, Germany] *Premature Vascularization of Motor Neuron Columns Alters Motor Neuron Development: A Role for Semaphorin3C-Plexind1 Signaling*
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11:30-11:45	**Dario BONANOMI** [San Raffaele Scientific Institute, Italy] *Push-Pull Signals From Motor Axons Direct Endothelial Remodeling to Ensure Assembly of Neuromuscular Connectivity*
11:45-12:00	**Xin DUAN** [University of California, San Francisco, USA] *Retinal Neurons Instruct Vascular Scaffold Patterning via Piezo2-mediated Direct Endothelial Contacts*
12:00-12:15	**Monica SOUSA** [University of Porto, Portugal] *Sensory Neurons Have an Axon Initial Segment that Initiates Spontaneous Activity in Neuropathic Pain*
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14:30-15:30	***Career Discussion for Students and Postdocs***
15:30-17:30	**POSTER SESSION III** ***with Cretan fruit and savoury hors d' oeuvre, drinks***
19:00	***Departure for the Conference Dinner – includes Poster Prizes***

Friday, 27 May 2022

Optional Excursion to Sights of Interest

Opportunities for relaxed interaction and networking

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TALKS TO POSTERS

POSTER PRESENTATIONS – FLASH TALKS

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Frabrice ANGO [Institute for Neurociences of Montpellier (INM), France]

Poster Nr **07** - ***Cardiolipin in Mitochondrial Dysfunction and Neurodegenerative Disease***
Charlie COLLINGHAM [University of Reading, UK]

Poster Nr **10** - ***Structural Basis of the Cytokine-Mediated Activation of ALK Family Receptors***
Steven DE MUNCK [CNRS-IINS, France]

Poster Nr **11** - ***Guardian of the Nociception: A New Role of the Microtubule Regulator Protein Kif2a in the Remodeling of Post-Developmental Nociceptive Neurons***
Swagata DEY [Weizmann Institute of Science, Israel]

Poster Nr **15** - ***The Ancestry of Neurosecretory Vesicles***
Ronja GÖHDE [Sars International Centre for Marine Molecular Biology, Norway]

Poster Nr **16** - ***Mice Lacking a Novel Phosphosite on MET Display ASD-Associated Behavior Pattern***
Liana HAYRAPETYAN [Bern University Hospital, Switzerland]

Poster Nr **23** - ***Posttranslational Polyglutamylation of Microtubules in Neuronal Health and Degeneration***
Maria MAGIERA [CNRS / Institut Curie, France]

Poster Nr **26** - ***Structural Snapshots of the Alpha 7 Nicotinic Acetylcholine Receptor Gating Cycle***
Colleen NOVIELLO [University of Texas Southwestern Medical Center, USA]

Poster Nr **30** - ***The Role of the Proton Inhibited DEG/ENaC Channel DEL-4 on Neuronal Ionstasis and Survival under Stress***
Dionysia PETRATOU [University of Crete & IMBB-FORTH, Greece]

Poster Nr **35** - ***Profiling of the Neuron-Specific Dystroglycan Interactome Reveals New Factors Contributing to Cobblestone Lissenchephaly and Neurodegeneration***
Halyna SHCHERBATA [Hannover Medical School, Germany]

Poster Nr **36** - ***The Developmental Changes in Intrinsic and Synaptic Properties of Prefrontal Neurons Enhance Local Network Activity from the Second to the Third Postnatal Weeks in Mice***
Kyriaki SIDIROPOULOU [University of Crete & IMBB-FORTH, Greece]

Poster Nr **37** - ***Dissecting the Molecular Mechanisms of Neurodegeneration in a Drosophila SWS/NTE Neuropathy Model***
Mariana SLIUSARENKO [Medizinische Hochschule Hannover (MHH), Germany]

Poster Nr **40** - ***Physiological Phosphorylation of tau at Disease-Relevant Sites Is Required for Dynamic Microtubule Interaction***
Nataliya TRUSHINA [Osnabrück University, Germany]

The Molecular Biology of Axon Guidance and Pruning

Mark Tessier-Lavigne

Stanford University, Stanford, USA

In developing brain, neuronal axons are guided by molecular cues over long distances to appropriate targets, and often branch to connect to multiple synaptic partners. Many branches are later pruned to sculpt a final pattern of connections, often in an activity-dependent fashion. This talk will describe progress in deciphering mechanisms of axon guidance, with a focus on how axons switch their responses to guidance cues at defined choice points. It will also discuss novel insights into mechanisms regulating a Caspase-dependent pathway of axonal pruning triggered by loss of trophic support, with implications for non-apoptotic roles of Caspases in neuronal physiology.

BACK

Semaphorin and Plexin Receptor Signalling Mechanisms

Gergely Nagy[1], Jing Yu[1], Emily Rowland[1], Richard Karlsson[2], Rebecca Miller[2], Henrik Clausen[2], **E. Yvonne Jones**[1]

[1] *Division of Structural Biology, Wellcome Centre for Human Genetics University of Oxford.*
[2] *Copenhagen Centre for Glycomics, Department of Cellular and Molecular Medicine, Faculty of Heath Sciences, University of Copenhagen.*

In my laboratory we combine x-ray crystallographic analyses with biophysical, electron and light microscopy based approaches to study the assembly and modulation of cell surface signalling complexes involved in neurobiology. We aim to generate mechanistic insights, at atomic resolution, which can be tested by functional studies in vitro and in vivo. I will discuss some of the recent results (Mehta et al 2020 and unpublished) we have generated by applying this approach to the signalling mechanism of the semaphorin-plexin cell guidance system. In particular, I will discuss insights provided, and questions raised, concerning the role of plexin conformation and clustering in signalling outcome.

V. Mehta, K.L. Pang, D. Rozbesky, K. Nather, A. Keen, D. Lachowski, Y. Kong, D. Karia, M. Ameismeier, J. Huang, Y. Fang, A. del Rio Hernandez, J.S. Reader*, E.Y. Jones* and E. Tzima*. (2020) 'The Guidance Receptor Plexin D1 is a mechanosensor in endothelial cells.' *Nature* **578**, 290-295.

BACK

A Novel Axon Guidance Cue that Signals through Divergent Dcc Family Receptors

Kelsey Nickerson [1, 2], Jhair Torres [1, 2], Lino Gonzalez [3], **Alexander Jaworski** [1, 2]

[1] *Department of Neuroscience, Brown University, Providence, RI 02912*
[2] *Robert J. and Nancy D. Carney Institute for Brain Science, Providence, RI 02912*
[3] *Department of Protein Chemistry, Genentech, South San Francisco, CA 94080*

Guidance of axons to their targets is a key step in nervous system wiring, and it is mediated by attractive and repulsive cues that activate receptors on the axonal growth cone. The Netrin-DCC ligand-receptor pair mediates axon attraction and instructs neuronal wiring in various organisms. The vertebrate DCC family contains four additional receptors; for three of these – Punc, Nope, and Prtg – no functional ligand had been discovered, and roles in axon guidance had remained elusive. Here, we deorphanize these receptors by identifying a secreted multi-domain protein that binds Punc, Nope, and Prtg, but not the other DCC family members. We show that this ligand is expressed in mesodermal structures surrounding the developing spinal cord, while its receptors are expressed in embryonic DRG sensory neurons and spinal motor neurons. Further, using in vitro axon guidance assays, we demonstrate that the Punc/Nope/Prtg ligand repels sensory axons and attracts motor axons. Lastly, analysis of knockout mice reveals that these molecules prevent straying of peripheral sensory axons from their normal trajectories in vivo. Taken together, our work identifies a novel, bifunctional axon guidance cue that acts through three previously orphan receptors of the DCC family to help wire sensory and motor neurons.

BACK

Synapse-Specific Regulation of Protein Synthesis for Cortical Wiring

Beatriz Rico

King's College, UK

Tuberous sclerosis complex (TSC) is a genetic condition caused by inactivating mutations in TSC1 or TSC2 genes. The proteins encoded by TSC1 and TSC2 cooperate to inhibit mTORC1, a protein complex critical for protein synthesis.

Consistently, genes encoding proteins involved in regulating translation at the synapse are frequently mutated in autism spectrum disorder (ASD), which has led to the suggestion that altered protein synthesis is a core pathophysiological mechanism of intellectual disability and autism. The identification of molecules regulated by local translation remains a major challenge in the field.

We found that synaptic Tsc2 is specifically regulated in a cell-type and synaptic-type specific manner in parvalbumin-expressing (PV+) cortical interneurons during the period of synaptogenesis in the mouse. We demonstrate that Tsc2 and local translation is important for the formation of excitatory synapses onto cortical PV+ interneurons. Then, we identify mRNA associated to Ribosomes that are coding for a set of synaptic proteins regulated by the tyrosine kinase receptor ErbB4. Interesting, some of these proteins depending on ErbB4-Tsc2 translation are associated with ASD. The synapse-specific molecular program unveiled in this study reinforces the idea that this connection is a sensitive hub for maladaptive network responses in neurodevelopmental disorders.

BACK

Developing Antibodies to Study Neural Receptors

Rob Meijers

Institute of Protein Innovation, USA

Kkkkkkkk

BACK

Molecular Mechanisms for Processing and Signal Reception of the Hedgehog Morphogen and Axon Guidance Molecule

Christian Siebold

Division of Structural Biology, Nuffield Department of Medicine, University of Oxford, U
Email: christian@strubi.ox.ac.uk
Website: www.strubi.ox.ac.uk/research/professor-christian-siebold

Hedgehog (HH) ligands function as classical, extracellular morphogens during the development of all animals. They pattern tissues ranging from the Drosophila wing to the developing spinal cord in dependence of their concentration. However, the HH morphogen can also take on a different role – as an axon guidance molecule being temporally "recycled" and serving as both attractive or repulsive axon guidance cues.
A key step in signalling is transfer of a palmitate group to the HH N terminus, catalysed by the multi-pass transmembrane enzyme Hedgehog acyltransferase (HHAT). I will present high-resolution cryo-EM structures of HHAT bound to substrates, Sonic HH (SHH)-mimetic megabodies as well as a potent small-molecule inhibitor. Our multidisciplinary analysis provides a detailed view of the mechanism by which HHAT adapts the membrane environment to transfer an acyl chain across the endoplasmic reticulum membrane. It also provides a blueprint for other protein-substrate acyltransferases (e.g Wnts and Ghrelin) and a template for future drug discovery. I will then discuss the molecular mechanisms of how the HH ligand uses its lipid modifications to interact and potentially signal via its receptor Patched and its co-receptors to mediate morphogen function and axon guidance.

BACK

Regulation of Cortical Folding by Controlling Progenitor Expansion and Neuronal Migration via Cell-Cell Communication

Ruediger Klein

MPI of Neurobiology, Germany

Cerebral cortex folding into sulci and gyri represents an important evolutionary mechanism that is incompletely understood. Present evidence suggests that cortex folding is favored by two expansion of progenitor cells and divergent radial migration of neurons. We have previously generated a loss-of-function mouse model (Flrt1/3 cDKO; del Toro et al., 2017) with sulci-like folding induced by divergent radial neuronal migration without amplification of progenitor cells. We have used this mouse model to ask if cortex folding induced by divergent radial migration could be enhanced by expansion of progenitor cells and if expansion of different types of progenitors would lead to qualitatively different cortical folds.

I will report that increasing the length of the early cortical stem cell expansion phase by deletion of fibroblast growth factor 10 (FGF10) in the Flrt1/3 cDKO model (FGF10;Flrt1/3 cTKO) leads to cortical folding with much increased penetrance and, importantly, with gyrus-like protrusions. Conversely, overproduction of intermediate progenitors by deletion of centrosomal protein 83 (Cep83) in the Flrt1/3 cDKO model (Cep83;Flrt1/3 cTKO) leads to cortical folding with much increased sulci-like appearance. Similar results were obtained by amplification of intermediate progenitors by activation of the Shh signaling pathway. These results indicate that expansion of progenitor cells and divergent radial migration of neurons synergize in vivo to induce folding of the smooth mouse neocortex. They further suggest that expanding different types of progenitors leads to qualitatively different folding, suggesting that the formation of gyri and sulci requires the timely expansion of distinct progenitors.

BACK

Growing Tip-localized Microtubule Organizing Center Determines Microtubule Orientation in Dendrites

Emily Rowland, Gergely N. Nagy, Karl Harlos, Weixian Lu, E. Yvonne Jones

Division of Structural Biology, University of Oxford

Semaphorins are a large family of cell guidance molecules commonly studied for their near ubiquitous roles in neural development. The secreted Class 3 Semaphorins are one of many sub-classes, and largely require cell surface receptors Plexins and Neuropilins to facilitate their signaling. Of these, Sema3G forms a ternary complex with hippocampal PlexinA4 and Neuropilin-2 to promote synaptic plasticity and dendritic spine formation, and ultimately support memory recall. Processing by Furin proteases modulates Sema3G signaling by controlling its specificity for Neuropilin-2 and Neuropilin-1. Previous structural studies of a Sema3G homologue, Sema3A, depict a 2:2:2 complex with Neuropilin-1 and PlexinA2/A4, in which Sema3A dimers are anchored to their Plexin counterparts by cross-bracing Neuropilin molecules. However, the molecular basis of Sema3G ternary complex formation and Neuropilin specificity remains uncharted. We present two crystal structures of furin-processed Sema3G, which together reveal a dimeric architecture similar to Sema3A. This concentration-dependent dimerization of Sema3G was confirmed by AUC and MALS. Further, we detected a Ca2+-dependent association of Sema3G to Neuropilin-2 using an in vitro Biolayer Interferometry binding assay. Our work aims to pursue a better understanding not only of Sema3G-dependent hippocampal development, but also of general mechanisms of Neuropilin specificities and complex formation of Sema3s.

BACK

Molecular Mechanisms of Neurite Branching and Central Synapse Formation

Dietmar Schmucker

Life and Medical Sciences Institute (LIMES), University Bonn, Germany

The functional complexity, capacity, as well as plasticity, of neuronal circuits depend to a high degree on the morphological diversity of neurons. In this context branching and compartmentalization of neurons play a central role. Despite huge amounts of transcriptome data on neuron type classifications, the developmental mechanisms that account for the acquisition of diversity of neuronal morphologies remain largely uncharacterized. We developed, therefore, an experimental system for systematic genetic analysis of cell-intrinsic mechanisms contributing to neurite branching and synaptogenesis in Drosophila CNS. Among a large collection of novel factors we identified signaling networks directly linked to axonal branching and/or central synapse formation. I present results that are based on our genetic, molecular, and biochemical approaches. I aim to discuss:

a) new mechanisms that specify number and spatial compartmentalization of pre-synapses.
b) new signaling molecules that are essential for selection and stabilization of axonal branches in development as well as maintenance in mature neural circuits.
c) how dysregulation of developmental signaling networks may contribute to neuro-degeneration in mature neural circuits.
d) evidence that initiation as well as termination of axonal branching requires a “growth cone to nucleus” signaling.
e) whether and how these novel mechanisms might be conserved in vertebrates.

BACK

X-ray Nanoimaging to Probe the 3D Structure and Elemental Composition of Neuronal Tissues

Alexandra Pacureanu

ESRF, France

High-energy X-rays can be a valuable illumination source for neuronal tissues as their combined high penetration power and sub-angstrom wavelength make it possible to explore thick samples with high precision. Holographic X-ray microscopy offers exceptional contrast in soft tissues and is well suited for multi-scale 3D imaging with spatial resolutions in the range of tens to hundreds of nanometres. X-ray fluorescence imaging can generate quantitative maps of native trace elements with sub-ppm precision and sub-100 nm spatial resolution. Both technologies can be applied in cryogenic conditions on frozen-hydrated specimens. This talk will aim to give insight into the capabilities of hard X-ray 3D microscopy for exploration of neuronal tissues and cells. Applications include in-depth incursions into neuronal structures, neurovascular and neuromuscular interactions, axonal alterations underlying neuropathies and elemental changes inside cells triggered by neurodegenerative conditions. Integration with downstream or upstream complementary measurements on the same specimens will be discussed as well, together with future perspectives.

BACK

GPC3-Unc5 Complex Structure and Role in Cell Migration

Akkermans O[1]*, Delloye-Bourgeois C[2]*, Peregrina C [3]*, Carrasquero-Ordaz M[1], Kokolaki M[1], Berbeira-Santana M[1], Chavent M[4], Reynaud F[2], Ritu Raj[5], Agirre J[6], Aksu M[1], White E[1], Lowe E[1], Ben Amar D[2], Zaballa S[3], Huo J[7,8], McCubbin P.T.N.[1], Comoletti D[9,10], Owens R[7,8], Robinson C.V.[5], Castellani V[2]#, del Toro D[3]#, **Seiradake E**[1]#

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[3] *Department of Biological Sciences, Institute of Neurosciences, IDIBAPS, CIBERNED, University of Barcelona, Barcelona, Spain*
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[6] *York Structural Biology Laboratory, Department of Chemistry, University of York, YO10 5DD, UK.*
[7] *Structural Biology, The Rosalind Franklin Institute, Harwell Science Campus, Didcot, UK.*
[8] *Division of Structural Biology, The Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK*
[9] *Child Health Institute of New Jersey New Brunswick, NJ 08901, USA*
[10] *School of Biological Sciences, Victoria University of Wellington, Wellington 6012, New Zealand*

Neural migration is a critical step during brain development that requires the interactions of cell-surface guidance receptors. Cancer cells often hijack these mechanisms to disseminate. Here we reveal crystal structures of Uncoordinated-5 receptor D (Unc5D) in complex with morphogen receptor glypican-3 (GPC3), forming an octameric glycoprotein complex. In the complex, four Unc5D molecules pack into an antiparallel bundle, flanked by four GPC3 molecules. Central glycan-glycan interactions are formed by N-linked glycans emanating from GPC3 and C-mannosylated tryptophans of the Unc5D thrombospondin-like domains. MD simulations, mass-spectrometry and structure-based mutants validate the crystallographic data. Anti-GPC3 nanobodies enhance or weaken Unc5-GPC3 binding. Using these tools in vivo, we show that Unc5/GPC3 guide migrating pyramidal neurons in the mouse cortex, and cancer cells in an embryonic xenograft neuroblastoma model. The results demonstrate a conserved structural mechanism of cell-guidance, with the potential for wide-ranging biomedical implications.

BACK

Molecular Mechanisms Underlying Neural Circuit Assembly in the Mammalian Visual System

Alex Kolodkin

Kavli Neuroscience Discovery Institute, The Johns Hopkins School of Medicine

The assembly of neural circuits critical for visual system function includes the differentiation of select subtypes of amacrine cells (ACs) and retinal ganglion cells (RGCs), the elaboration of precise connections within the retina among ACs and RGCs, and targeting of RGC axons to their appropriate retino-recipient regions within the CNS. These events will be considered in the context of the mammalian accessory optic system (AOS), which is tuned to detect slow directional motion in order to stabilize images on the retina. Using high-complexity transcriptomic profiling of AOS RGCs tuned to detect motion in different directions allows for the identification of closely related, rare, direction-selective ganglion cells (DSGCs). Mutations in several genes selectively expressed in AOS DSGC subtypes disrupt the image stabilizing optokinetic reflex (OKR), yielding information critical for understanding the differentiation of DSGC subtypes and the regulation of their morphology. In addition to providing insight into directional tuning of RGCs, this work implicates mutations in certain human genes that encode orthologues of proteins critical for assembling murine AOS circuits in phylogenetically conserved aspects of visual system function.

BACK

Post-Developmental Roles of the Netrin Receptor UNC-40/DCC Mediated by a Novel Phosphodegron Motif

Yehuda Salzberg, Sapir Sela, Meital Oren-Suissa

Department of Brain Sciences, Weizmann Institute of Science, Israel

Netrin and its receptor DCC are mostly studied for their developmental roles in neuronal navigation. Much less is known about their roles in the mature nervous system, despite the known genetic association of DCC variants with many neurological conditions in adults. We report two post-developmental processes in C. elegans that are regulated by UNC-40 (homolog of DCC).
First, we found that UNC-40 acts to maintain specific synapses in adult male worms. In hermaphrodites, however, UNC-40 is degraded by the conserved E3 ubiquitin ligase SEL-10/FBW7, thus leading to the sex-specific removal of these synapses. We defined a conserved phosphodegron sequence within UNC-40 that mediates the binding of SEL-10 and show that mutating this sequence prevents UNC-40 degradation and stabilizes the synapses.
Second, we found that UNC-40 promotes the loss of dopaminergic (DA) neurons in a C. elegans model for neurodegeneration (induced by 6-OHDA). In UNC-40-null animals, DA neuron degeneration was attenuated under 6-OHDA treatment. Remarkably, in animals with a mutated phosphodegron, DA neuron degradation was significantly enhanced even without 6-OHDA treatment, in a mechanism involving the parthanatos pathway. Together, our results reveal new roles for UNC-40/DCC in the mature nervous system and identify a conserved motif that mediates these functions.

BACK

A GDNF-GFRa1 Synaptic Trans-Adhesion Complex is Disassembled by RET Receptor Interaction

Francesca M Houghton [1], Sarah E Adams [1], Andrew G Purkiss [3], David C Briggs [1], Mia Zol-Hanlon [1], Andrea Nans [3], Svend Kjaer [3], Fernanda Ledda [4], Neil Q McDonald [1, 2]

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Glial-cell line derived neurotrophic factor is a promising treatment for Parkinson's disease through its ability to stimulate the RET receptor tyrosine kinase and support dopaminergic neuronal survival. However, several RET-independent functions for GDNF have been described including ligand-dependent cell adhesion at synapses, the basis of which remains unclear. Here we show that GDNF-GFRa1 form multimers in vitro and provide crystallographic evidence for a barrel-shaped assembly in which two GFRa1 pentamers are crosslinked by five GDNF covalent dimers. The GFRa1:GFRa1 pentameric interface is validated in vitro and its role in a trans-synaptic GDNF-GFRa1 complex is demonstrated by cell-aggregation (HEKs) and dendritic spine assay (dissociated hippocampal neurons). The GFRa1 pentamer interface overlaps with the known high affinity RET-GFRa1 site suggesting mutually exclusive complexes. By reconstituting GDNF-dependent GFRa1 trans-adhesion using a liposome assay, we show that these complexes are disrupted by interaction with RET extracellular module (ECM), through competing with GFRa1 homotypic interactions. Our results reveal an unexpected GDNF-dependent trans-synaptic complex that is negatively regulated by cell surface RET, implicating GFRa1 as a versatile co-receptor that mediates distinct GDNF functions.

BACK

Programming and Reprogramming Sensory Circuits

Guillermina Lopez-Bendito

Instituto de Neurosciencias, Spain

Our research team runs several related projects studying the cellular and molecular mechanisms involved in the development of axonal connections in the brain. In particular, our aim is to uncover the principles underlying thalamocortical axonal wiring, maintenance and ultimately the reprogramming of connections, through an integrated and innovative experimental programme. The development of the thalamocortical wiring requires a precise topographical sorting of its connections. Each thalamic nucleus receives specific sensory information from the environment and projects topographically to its corresponding cortical. A second level of organization is achieved within each area, where thalamocortical connections display an intra-areal topographical organization, allowing the generation of accurate spatial representations within each cortical area. Therefore, the level of organization and specificity of the thalamocortical projections is much more complex than other projection systems in the CNS. The central hypothesis of our laboratory is that thalamocortical wiring influences and maintains the functional architecture of the brain. We also believe that rewiring and plasticity events can be triggered by activity-dependent mechanisms in the thalamus. Here in this talk, I will present our recent data on the thalamic activity-dependent mechanisms involved in sensory cortical maps development. I will also present data on the role of this activity in the thalamus in promoting neuroplastic cortical changes following sensory deprivation. Finally, I will provide our new data in reprogramming thalamic astrocytes into specific thalamic sensory neurons. Within these projects we are using several experimental programmes, these include: optical imaging, manipulation of gene expression in vivo, cell and molecular biology, biochemistry, cell culture, sensory deprivation paradigms and electrophysiology.

BACK

Protein Structure, Flexibility and Interactions in Control of Myelin-Axon Contacts

Bert Janssen

Bijvoet Centre for Biomolecular Research, Utrecht University

Myelin and axons interact intimately to facilitate saltatory conduction. A selective set of intercellular adhesion complexes establish and maintain specialized axon-myelin junctions; contactin1-neurofascin155 at the paranode, contactin2-contactin2 at the juxtaparanode, and MAG-gangliosides at the internode. Their dysfunction, from mutations or autoantibodies, leads to mental and movement disorders. We have determined a series of detailed structures that inform on the interaction mechanisms, extracellular specificity, and higher-order organization of these complexes. Contactin1-neurofascin155 and contactin2-contactin2 interaction modes, established through domains Ig1-2, are similar, implying a conserved mechanism for neuronal adhesion. Neurofascin155 homodimerization competes with contactin1 heterodimerization, whereas contactin1 forms low-affinity clusters through Ig3-6 interfaces. MAG dimerizes in cis at the membrane proximal site and interacts with gangliosides at its N-terminus. The structures explain how the heterophilic Ig1-Ig4 horseshoe combination in the contactin1-neurofascin155 complex defines the 7.4 paranodal spacing and how the MAG-ganglioside dimer complex determines a 10 nm internodal cell-cell distance. Conformational flexibility in the remaining six domains of contactin1, contactin2 and neurofascin155 may allow the bridging of wider intercellular distances, such as the synapse. Our work identifies a combination of protein structure, interactions, and flexibility that controls adhesion in the nervous system.

BACK

Structural Insights into the Teneurin-4 Dimer Reveal a Compact Conformation for Trans-Interactions

Dimphna Meijer[1, 2], Christos Gogou[1], Cátia Frias[1], Wouter Beugelink[2], Bert Janssen[2]

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Synaptic partner matching is a crucial step during neural circuitry formation. The neuronal transmembrane protein family of Teneurins is required for partner finding in the visual and hippocampal systems in vertebrates. It remains unclear how individual Teneurin molecules form macromolecular cis- and trans-synaptic protein complexes. Here, we present a 2.7 Å cryo-EM structure of the dimeric ectodomain of human Teneurin4. The structure reveals a compact conformation of the dimer that is stabilized by C-rich, YD-shell and ABD-mediated interactions. A 1.5 Å crystal structure of the C-rich domain shows three conserved calcium binding sites and we demonstrate using thermal unfolding and SAXS-based rigid-body modelling that the compactness and stability of Teneurin4 dimers is calcium-dependent. Atomic force microscopy in combination with cellular assays reveal that the compact cis dimer is compatible with homomeric trans interactions. Together, these findings support a role for Teneurins as a scaffold for macromolecular complex assembly and the establishment of cis- and trans-synaptic interactions to construct functional neuronal circuits.

BACK

In situ Cryo-Electron Tomography Reveals Local Orchestration of Cellular Machineries for Axon Branch Development

Naoko Mizuno

NIH, USA

Neurons are highly polarized cells with an intricate network of protrusions that form dendrites and axons. The protrusions are shaped by the dynamic reorganization of cytoskeleton components and cellular organelles. Axon branches allow the formation of new axonal paths and increase circuit complexity. However, our understanding of their formation and function is sparse due to the lack of molecular snapshots of the cellular reorganization at axon branches. Using in situ cellular cryo-electron tomography on primary mouse neurons, we directly visualized the remodeling of organelles and the cytoskeleton at premature and mature axon branches. In contrast to axon shafts that have few non-cytoskeletal organelles, branched areas function as hotspots by concentrating organelles to support dynamic cellular activities. In premature branches, filopodia formed protrusions that were accompanied by fragments of unaligned actin filaments at the base of filopodia. Upon branch maturation, filopodia diminished while short actin filaments remained, and microtubules and ER co-migrated into the preformed branch to support its outgrowth. In premature and mature axon branches, compact mitochondria of ~500 nm at the roots of branches were adjacent to locally clustered ribosomes, which are not found in axon shafts. Interestingly, clustered ribosomes formed polysomes at the axon branches. We present the direct evidence of local protein synthesis selectively taking place at axon branches, allowing them to serve as unique control hubs for axon development and downstream neural network formation.

BACK

Development and Evolution of Visual Projections

Alain Chédotal

Institute de la Vision, France

In most animal species including humans, commissural axons connect neurons on the left and right side of the nervous system. This communication between the two sides of the brain and spinal cord is necessary for a series of complex function, including binocular vision, coordinated locomotor movements, and sound direction localization. In humans, abnormal axon midline crossing during development causes a whole range of neurological disorders ranging from congenital mirror movements, horizontal gaze palsy, scoliosis or binocular vision deficits. The mechanisms which guide axons across the CNS midline were thought to be evolutionary conserved but our recent results suggesting that they differ across vertebrates. I will discuss the evolution of visual projection laterality. In most vertebrates, camera-style eyes contain retinal ganglion cell (RGC) neurons projecting to visual centers on both sides of the brain. However, in fish, RGCs are thought to only innervate the contralateral side. This suggested that bilateral visual projections appeared in tetrapods as an adaptation to aerial vision. Using 3D imaging and tissue clearing we found that bilateral visual projections exist in non-teleost fishes. We also found that the developmental program specifying visual system laterality differs between fishes and mammals.

BACK

SARM1 Ring to Rule Them All

Yarden Opatowsky

The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

SARM1 is an executor of Wallerian axonal degeneration. Remarkably, neurons from SARM1 knock-out mice (which appear to be normal in many respects) show prolonged resistance for neuronal degeneration after mechanical damage, oxidative stress, and chemotherapy treatments. Mechanistically, SARM1 contains NADase activity, which, in response to nerve injury, depletes the key cellular metabolite, NAD+. To gain structural knowledge of SARM1 we use X-ray crystallography of isolated SARM1 domains and single particle EM 3D reconstruction of the intact protein. We discovered that SARM1, like other apoptotic complexes, assembles into an oligomeric ring. Structure analysis and additional experiments in cultured cells points at a surprising molecular mechanism by which SARM1 is kept inactive during homeostasis and how it becomes activated in response to metabolic and oxidative stress conditions.

BACK

4D Neuronal Imaging at the Nanoscale

Ilaria Testa

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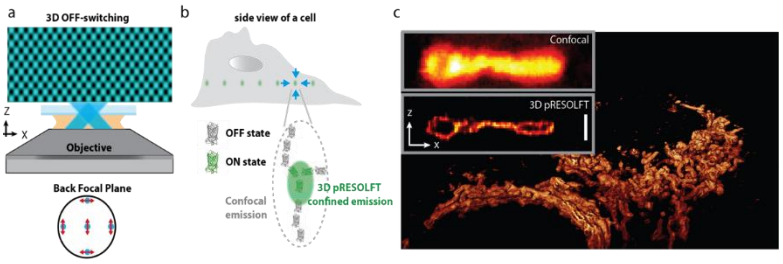
The development of parallelized STED/RESOLFT super resolution microscopy techniques have shown impressive results combining diffraction unlimited lateral spatial resolution (< 70 nm) with increasingly fast acquisition speeds (~ 1-30Hz) and optical sectioning. However, the diffraction-unlimited spatial resolution in these systems are often limited to the lateral dimension. Here we present a novel design of illumination patterns that allows for 3-dimensional isotropic diffraction unlimited resolution while maintaining the rapid recording speed of a parallelized imaging system. Much like in the original RESOLFT-MoNaLISA set-up[1] the system is based on controlling the spatial distribution of ON-OFF states in a sample labelled with reversibly switchable fluorescent proteins (rsFPs)[2]. Using rsFPs as labels allows for high resolution imaging at minimal light doses and is thus suitable for live cell imaging. Designing illumination patterns that are modulated in all three spatial dimensions and combining these with the complete OFF-switching of rsFP allow to confine the emission spots in all the three spatial dimensions to sub-diffraction sized. The extended frequency content in the emission pattern manifests itself as isotropic diffraction unlimited resolution in the final images [3].

The spatial modulation in all three dimensions is made possible by the incoherent superposition of several independent illumination patterns, each pattern highly modulated in one spatial direction. Proper co-alignment of these patterns results in sharply confined zero intensity volumes that, together with saturation of the fluorophore OFF-state, can create sub-diffraction sized emission volumes. Quantification and reassignment of the emitted photons from these volumes allows for reconstruction of the final isotropic super resolution images.



We recorded 3D stacks across time of moving organelles and cytoskeleton in living human cells with spatial details < 80 nm in all the three dimensions.

Fig. 1 a. Interference pattern for 3D OFF-switching; b. Confinement of the emission point spread function in 3D with reversibly switching rsFPs; c. Live cell recording of mitochondria with < 80 nm isotropic spatial resolution.

The new 3D ability enables imaging of packed structures inside living cells such as the vimentin and actin cytoskeleton even in the nuclear region with 3D super-resolution. The entire mitochondria network in a living cell, free from fixation and 3D distortion induced by immersion media, can be recorded in a few tens of seconds. The 3D resolution allows visualization of distinct compartments in small organelles such as exosomal outer shell, mitochondrial derived vesicles and spheroids for the first time using light microscopy. The 3D recording can also be done over time enabling to study volumetric structural alterations and synaptic protein plasticity in hippocampal neurons.

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BACK

Critical Role of Bioenergetics in Neuronal Remodeling

Sumita Chackraborty, Oren Schuldiner

Department of Molecular Neuroscience, Weizmann Institute of Science, Israel

Neuronal remodelling is an evolutionary conserved and essential process for development and maturation of both vertebrate and invertebrate nervous systems. Remodelling of neuronal connections includes elimination of exuberant synapses and neurites, often followed by regeneration or stabilization of mature axons and dendrites. A unique system to explore the cellular and molecular mechanism of remodelling is the stereotypic remodelling of Drosophila mushroom body (MB) ɣ neurons during metamorphosis. Despite the crucial role of remodelling in neurodevelopment, and its emerging contribution to neuronal plasticity and neuropsychiatric disorders, the molecular mechanisms that drive remodelling are largely unknown. We have uncovered a critical role of endoplasmic reticulum (ER)-mitochondrial calcium transfer in ɣ neuron pruning. Perturbation of ER Ca2+ release and mitochondrial Ca2+ uptake result in pruning defects of MB ɣ neurons. Furthermore, disruption of mitochondrial ATP synthesis as well as extracellular uptake of monocarboxylic acids result in severe pruning defects. Finally, knockdown of AMPK□, a key cellular metabolic sensor, also results in pruning defects. Combined, these data suggest an essential role of ATP in the pruning of MB ɣ neurons. We are presently continuing our investigation of the role of ER-mitochondria dynamics in ATP homeostasis during MB ɣ neuron pruning.

BACK

Structural Neuropharmacology of NMDA Receptors

Hiro Furukawa

Cold Spring Harbor Laboratory, USA

Neurotransmission and cellular signaling mediated by N-methyl-D-aspartate receptors (NMDARs) are fundamental to brain development and functions including learning and memory. Dysfunctional NMDARs are implicated in various neurological diseases and disorders including depression, Alzheimer's disease, stroke, and schizophrenia making them relevant targets for therapy. NMDARs are ligand-gated ion channels, which open their transmembrane ion channels upon application of glycine, glutamate, and voltage. Various combinations of NMDAR subunits including GluN1, GluN2(A-D), and GluN3(A-B) give rise to heterotetrameric ion channels with distinct ion channel properties and spatio-temporal expression patterns. In this talk, I will first go over mechanistic insights into NMDARs functions gained by a combination of structural biology, electrophysiology, and MD simulations. I will also present our recent effort in understanding the mechanism of binding and unbinding of therapeutically important channel blockers, and exploring a possibility of targeting and controlling the functions of specific NMDAR subtypes by antibodies.

BACK

New Tools to Study Nanoscale Synapse Organization, Function and Plasticity

Daniel Choquet

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The spatio-temporal organization of neurotransmitter receptors in the postsynaptic membrane is a fundamental determinant of synaptic transmission and thus information processing by the brain. Ionotropic AMPA glutamate receptors (AMPAR) mediate fast excitatory synaptic transmission in the central nervous system. Using a combination of high resolution single molecule superresolution imaging and tracking techniques, we have established that AMPARs are not all stable in the synapse as thought initially, but in large part undergo continuous entry and exit to and from the post-synaptic density through lateral diffusion. The other fraction of AMPAR are highly concentrated inside synapses into a few clusters of around seventy nanometers. These results have opened the new possibility that glutamatergic synaptic transmission is controlled by the regulation at the nanometer scale of the position and composition of these highly concentrated nanodomains. The dynamic exchange of AMPAR within the PSD and between synaptic and extrasynaptic sites is highly regulated by neuronal activity. We have demonstrated that AMPAR conformation strongly impacts their mobility, desensitized receptors being more mobile than naïve ones. This property likely explains how post-synaptic AMPAR receptor mobility can regulate short term synaptic plasticity, a feature previously ascribed to pre-synaptic mechanisms. Recently, using new methods to exogenously control AMPAR surface diffusion, we have demonstrated that AMPAR surface diffusion directly controls the establishment of long term synaptic plasticity. We will now present a series of new tools to study nanoscale synapse organization, function and plasticity 1) to label hard to access AMPA receptor auxiliary subunits, 2) to measure and manipulate endogenous AMPAR trafficking, 3) to establish the role of AMPAR surface diffusion in short and long term plasticity.

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BACK

Structural Pharmacology of the Muscle-Type Nicotinic Acetylcholine Receptor

Ryan Hibbs

Md. Mahfuzur Rahman[1], Jinfeng Teng[1], Myeongseon Lee[2], Brady T. Worrell[2], Michael H.B. Stowell[2] and **Ryan E. Hibbs**[1]

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Our lab focuses on using structural and functional approaches to understand signaling by ligand-gated ion channels in the nervous system. A major emphasis is on understanding nicotinic receptor pharmacology from a structural perspective. Here I will present some of our recent work using the muscle-type nicotinic receptor from the Torpedo ray as a structurally tractable reference for the human receptor at the neuromuscular junction. I will focus on how toxins from animals and plants act on the receptor, and how we can use this information to understand state transitions that underlie ion channel gating. These studies have revealed a new class of allosteric site at the junction of the extracellular and transmembrane domains that small molecules can leverage to modulate channel desensitization. Our findings suggest that stabilizing a desensitized-like non-conducting state, through this allosteric site, may be an important alternative mechanism for antagonizing the receptor.

BACK

The Neuronal Membrane Proteasome (NMP): A Modulator of Nervous System Signaling

Seth Margolis

The Johns Hopkins University School of Medicine

We discovered a protein degradation pathway in the mammalian nervous system that modulates neuronal signaling. The is composed of uncapped 20S proteasome-complexes associated with the neuronal plasma membrane and exposed to the neurons surface in a manner allowing for degradation of intracellular substrates into peptides released directly into the extracellular space. The products of this neuronal membrane proteasome (NMP) are a diverse class of novel peptides that when purified and added to naïve neurons are sufficient to rapidly induce changes in neuronal calcium signaling. This is impart dependent on NMDA receptors. To determine the endogenous relevance of the NMP we developed membrane-impermeant proteasome inhibitors that selectively inhibits NMPs and not cytosolic proteasomes. Addition of this inhibitor to neurons blocks extracellular peptide production and attenuates activity-dependent neuronal signaling within seconds. We now hypothesize that NMPs mediate a program of extracellular peptide-receptor signaling critical for modulating neuronal communication. Despite our progress, critical questions remain: What are the molecular mechanisms required for NMP assembly and function? What are the peptide sequences that specify distinct peptide-receptor interactions and mediate changes in neuronal signaling? Our efforts will provide critical insight into a new modality of neuronal communication with emerging roles in brain health and disease.

BACK

The Role of AMPA Receptor Surface Mobility in Short-Term Synaptic Plasticity

Agata Nowacka [1, 2], Angela Getz [1, 2], Christelle Breillat [1, 2], Sophie Daburon [1, 2], Diogo Bessa-Neto [1, 2], Cecile Lemoigne [1, 2], Matthieu Sainlos [1, 2], Daniel Choquet [1, 2]

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Short-term synaptic plasticity (STP) is important for information processing in the brain, serving particularly for temporal integration. However, its precise regulation, function and impact on information processing remain to be fully understood. Here, we study the functional role of AMPA receptor (AMPAR) surface diffusion in STP. We use the AP-GluA2KI mouse model where GluA2 subunits of AMPARs can be specifically biotinylated when co-expressed with a biotin ligase BirA, and immobilized with a biotin-binding protein. We show that immobilization of endogenous AMPARs modulates STP by decreasing synaptic facilitation in the SC-CA1 synapse of organotypic hippocampal slices. This is achieved by preventing the replacement of desensitized AMPARs in the synapse. In ex vivo brain slices, we show that the effect of mobility on STP varies between synapse types and is strongest in the L2/L3 pyramidal neurons of the somatosensory cortex. We examine why AMPAR mobility doesn't affect STP in all synapse types equally, focusing on the role of AMPAR auxiliary protein GSG1L. Moreover, we aim to identify natural processes which act upon AMPAR kinetics and mobility to regulate STP in the brain. Altogether, this research will contribute to a better understanding of mechanisms underlying STP expression and its functions in the brain.

BACK

Mechanisms Underlying AMPA Receptor Activation and Modulation

Ingo Greger

MRC-LMB, Cambridge, UK

AMPA glutamate receptors (AMPARs) mediate fast excitatory neurotransmission throughout the brain, and are central players in various forms of synaptic plasticity that underlies learning. In addition to four core subunits (GluA1-4), an array of auxiliary subunits render AMPAR response properties uniquely versatile, tuned to the needs of a given circuitry. These assemble with the receptor core in various stoichiometries, and in a brain-region specific fashion.

Recent cryo-EM structural data, combined with functional studies, have started to shed light on the arrangement of both, core and auxiliary subunits. Hence, despite tremendous versatility, unique to AMPA-type glutamate receptors, organizing principles are beginning to emerge. This information will ultimately allow us to decipher the activation mechanisms underlying synaptic AMPAR responses. In this talk I will discuss our current understanding of AMPAR organization, and latest insights into its modulation by prominent auxiliary subunits.

BACK

Lactate Supply Overtakes Glucose when Neural Computational and Cognitive Loads Scale Up

Yulia Dembitskaya [1], Charlotte Piette [1], Sylvie Perez [1], Hugues Berry [2, 3], Pierre Magistretti [4], Laurent Venance [1]

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The neural computational power is determined by neuroenergetics, but how and which energy substrates are allocated to various forms of memory engram is unclear. To solve this question, we asked whether neuronal fueling by glucose or lactate scales differently upon increasing neural computation and cognitive loads. Here, using electrophysiology, two-photon imaging, cognitive tasks and mathematical modeling, we show that both glucose and lactate are involved in engram formation, with lactate supporting long-term synaptic plasticity evoked by high stimulation load activity patterns and high attention load in cognitive tasks, and glucose being sufficient for less demanding neural computation and learning tasks. Overall, these results demonstrate that glucose and lactate metabolisms are differentially engaged in neuronal fueling depending on the complexity of the activity-dependent plasticity and behavior.

BACK

Caught in the Act: Ultrastructural Imaging of Synaptic Vesicle Pools

Cordelia Imig

University of Copenhagen, Denmark

Synapses in the brain can exhibit strikingly different functional properties including the probability of neurotransmitter release and short-term plasticity characteristics. Electron microscopy (EM) is a powerful technique to resolve the organisation of synaptic vesicle pools at individual active zone neurotransmitter release sites with nanometer precision. However, to link ultrastructural observations with defined synaptic activity states that operate on the millisecond to second timescale, fast sample cryo-fixation approaches in temporal register with controlled synaptic stimulation are required. The capture of activity-dependent exo- and endocytic events in distinct and functionally specialized synapses embedded in a tissue-context has remained particularly challenging. In this talk, I will discuss our methodological approach to apply optogenetic stimulation-coupled cryofixation ("flash-and-freeze) for EM to enable the study of synaptic ultrastructure of genetically identified neurons in cultured mouse brain tissue. I will highlight the power, but also the challenges of this technology, for the study of structurally and functionally highly complex synapse types, such as hippocampal mossy fiber synapses.

BACK

The novel TBC family Rab-GAP Blobby operates as an active zone assembly chaperone

Stephan Sigrist, Janine Lützkendorf

Freie Universität Berlin

Every presynapse builds a core 'active zone' scaffold, which clusters ion channels and forms release sites for synaptic vesicles. While the active zone scaffold composition appears rather well characterized, the regulations controlling their initial assembly and plastic remodeling potentially relevant for behavior remain insufficiently understood. We identified a Rab-GAP domain protein ("Blobby") as a new active zone scaffold resident protein of Drosophila synapses. Blobby executes Rab3 GTPase activity through an evolutionary conserved TBC domain. Both, elimination of Blobby and equally loss of its GAP-activity via point mutations resulted in reduced and structurally aberrant active zone scaffolds displaying reduced release due to decreased number and recruitment of release ready synaptic vesicles. Acute active zone plasticity also was eliminated in blobby mutants. In brains, Blobby expressed highly within several behavior-relevant regions, and blobby mutants showed severely increased base-line sleep but defective sleep homeostasis.
Recently, liquid-liquid phase separation was shown to drive active zone assembly. Our data suggest that Blobby evolved as an assembly chaperone promoting liquid character of the active zone scaffold in antagonism to Rab3 activity promoting transition into a final stable active zone structure.

BACK

Clusters or Condensate? How Arc Regulate AMPA Receptor Level

Martina Damenti [1], Giovanna Coceano [1], Jonatan Alvelid [1], Mariline Mendes Silva [1], Lea Rems [2], Yvonne Johansson [3], Erdinc Sezdig [3], Lucie Delemotte [2], Gilad Gilad Silderberg [3], Ilaria Testa [1]

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The Activity Regulated-Cytoskeleton associated-protein Arc is pivotal to mediate plastic responses in neuronal cells. In vitro studies suggest its ability to form large and small order oligomers which are potentially involved in interneuronal trafficking. Despite its important function, no direct observation of Arc oligomers in cells has been presented due to the small size, and lack of appropriate labelling strategies.

Here, we take advantage of STED microscopy to study Arc nanoscale organization in cellular environment especially at the synapses. Arc oligomers role in the regulation of AMPA receptor surface levels, together with their close association to the plasma membrane, were addressed via chemical mutagenesis and molecular dynamic simulation studies. Furthermore, for the first time, Arc-Arc molecular interaction and its liquid-liquid phase separation properties were uncovered in cellular system.

Together, our observations support the model by which Arc oligomerization at the post-synaptic endocytic zone, favors AMPA receptors endocytosis inducing plasma membrane curvature.

BACK

Role of Molecular Chaperones in Aggregation Prevention and Removal

Ulrich Hartl

Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Martinsried, Germany

The past two decades have witnessed a paradigm shift in our understanding of cellular protein folding. While the three-dimensional structures of functional proteins are determined by their amino acid sequences, newly-synthesized polypeptides in the crowded cellular environment depend on molecular chaperones to reach their folded states efficiently and at a biologically relevant time scale. Chaperone machineries play an important role in preventing the accumulation of potentially harmful protein aggregates, both by interfering with de novo aggregate formation and by dissociating preexistent aggregates. These functions are of central relevance in delaying the manifestation of neurodegenerative syndromes including Alzheimer's disease and tauopathies. I will discuss recent findings describing a novel pathway of amyloid disaggregation, whereby the AAA+ chaperone VCP engages ubiquitylated Tau fibrils, resulting in their efficient dissociation and clearance in cooperation with the proteasome system. A potential trade-off of this activity is the production of seeding-competent Tau aggregates that can be taken up by cells in culture and induce the aggregation of endogenous Tau protein.

BACK

Promoting Axon Regeneration by Elevating Intrinsic Growth Ability of Mature Neurons

Zhigang He

Boston Children's Hospital, Harvard Medical School

In adult CNS, an axonal damage often triggers retrograde neuronal death and regenerative failure, which contributes to permanent functional deficits after CNS injury and neurodegenerative diseases. In adult mice, optic nerve crush (ONC) injury, which severs all axons of retinal ganglion cells (RGCs), results in massive death of axotomized RGCs and regenerative failure of survivors. With this model, our previous studies demonstrated that elevating neuronal intrinsic growth ability, by manipulating molecules in PTEN/mTOR and/or SOCS3/STAT pathways, is able to promote neuronal survival and axon regeneration. However, major questions remain. For example, it is unclear why these available interventions have only partial effects. Also, these interventions suffer from obvious issues such as tumor growth and inflammation, limiting their translational potential. To address these questions, we utilized single cell RNA-seq analysis to profile transcriptomics of injured RGCs with or without several interventions. We first define the contribution of different cell types to the observed effects of partial effects. Together with other analyses, we identified molecular programs that account for injury-induced neuronal degeneration and axon regeneration elicited by different interventions. Strikingly, both computational prediction and functional verification support a pro-regenerative role of neuropeptides/hormone-related genes, pointing to a potential translatable neural repair strategy.

BACK

Neuro-Vascular Interactions in the CNS

Chengua Gu

Harvard Medical School
Howard Hughes Medical Institute

The relationship between the brain and its vasculature is different than that of other organs. The brain vasculature has two distinct features that cater to the brain's unique functions. First, neurons in the brain are extremely sensitive to their extracellular chemical environment and each neuron is at most 15 microns away from a capillary. Brain endothelial cells forming the blood vessel walls constitute a blood-brain barrier (BBB) to provide a safe and homeostatic environment for the brain. Second, despite representing only 2% of the body weight, our brain consumes 20% of body's energy at rest and has very limited ability to store energy. So, to meet moment-to-moment changes in regional brain energy demand, neural activity rapidly increases local blood flow, a process called neurovascular coupling. I will present our recent progress on the molecular mechanistic understanding of how the brain vasculature executes these functions, and how unique vascular demands of the brain have led to molecular, cellular, and trans-cellular specializations unlike those found in other tissues.

BACK

Wiring Specificity of Neural Circuits

Liquin Luo

Stanford University, USA

Developing brains use a limited number of molecules to specify connection specificity of a much larger number of neurons and synapses. How is this feat achieved? I will first describe our work using the Drosophila olfactory circuit as a model to address this question. I will then discuss functions of homologs of wiring molecules we identified in the fly olfactory circuits in determining wiring specificity of complex circuits in the mouse hippocampal network.

BACK

Blood-Brain Barrier Regulation of Brain Function and Behavior

Richard Daneman

University of California San Diego, USA

Vascular endothelial cells in the central nervous system (CNS) form a barrier that restricts the movement of molecules and ions between the blood and the brain. This blood-brain barrier (BBB) is crucial to ensure proper neuronal function and protect the CNS from injury and disease. Although the properties of the BBB are manifested in the endothelial cells, transplantation studies have demonstrated that the BBB is not intrinsic to the endothelial cells, but is induced by interactions with the neural cells. We are interested in identifying how the BBB interacts with the neural circuitry to regulate brain function and behavior, addressing the following questions: How does neural activity dynamically regulate the BBB? How do changes to the BBB regulate brain function and behavior? Are there regional specializations of the BBB that are important for local circuit function? Here we use a genomic, genetic and molecular approach to elucidate these questions. We have found that neural activity robustly alters the gene expression of CNS endothelial cells, regulating key BBB properties including efflux transport. We have further identified roles for vascular metabolism in regulating behavior, and have identified significant regional heterogeneity of the BBB.

BACK

Neurovascular Cross-Talk in Brain Development

Amparo Acker-Palmer

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Endothelial cells build interfaces with themselves and with other cells in many different organs and, receptor crosstalk at such interfaces is necessary for proper cell signaling that ultimately regulates cell-cell communication in vivo to achieve organ development and function. We use a multiscale approach ranging from molecules to cells/tissues to complex organ function in vivo in mice and Zebrafish to unravel the emergence of a structural-functional connection at the neuronal-vascular interface and elucidate the key molecular players important for the development of functional cellular networks in the brain. The molecules important for crosstalk between endothelial cells and neuronal cells are called angioneurins. Our work in recent years has contributed to the discovery of new angioneurins such as Reelin and the Fibronectin leucine-rich transmembrane proteins (FLRTs). We have recently shown that the vasculature orchestrates neuroglial developmental processes through the novel function of the Reelin signaling pathway in the vascular compartment. Thus, the vasculature is critical for cerebral cortex development by regulating neuronal migration and glial organization as well as neurovascular unit development to maintain the integrity of the blood-brain barrier. Bi-directional neurovascular communication seems to be essential for CNS development. Thus, our work also uncovered that FLRTs expressed in the neuroretina negatively influence postnatal retina vascularization through an Uncoordinated-5 receptors B (Unc5B)-mediated repulsive responses in developing blood vessels. Interestingly, our most recent data indicates that FLRTs are also expressed in the vessels and modulate angiogenesis and the cross-talk to the neuronal compartment.

BACK

Autophagy in Neuronal Systems and Aging

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Ageing is driven by the inexorable and stochastic accumulation of damage in biomolecules vital for proper cellular function. Although this process is fundamentally haphazard and uncontrollable, senescent decline and ageing is broadly influenced by genetic and extrinsic factors. Numerous gene mutations and treatments have been shown to extend the lifespan of diverse organisms ranging from the unicellular Saccharomyces cerevisiae to primates. It is becoming increasingly apparent that most such interventions ultimately interface with cellular stress response mechanisms, suggesting that longevity is intimately related to the ability of the organism to effectively cope with both intrinsic and extrinsic stress. Key determinants of this capacity are the molecular mechanisms that link ageing to main stress response pathways, and mediate age-related changes in the effectiveness of the response to stress. How each pathway contributes to modulate the ageing process is not fully elucidated. A better understanding of the dynamics and reciprocal interplay between stress responses and ageing is critical for the development of novel therapeutic strategies that exploit endogenous stress combat pathways against age-associated pathologies. Mitochondria, the indispensable and highly dynamic, energy-generating organelles in all eukaryotic cells, play essential roles in fundamental cellular processes. Neuronal cells depend, perhaps more than any other cell type, on proper mitochondrial function. Mitochondrial impairment is a major hallmark of several age-related neurodegenerative pathologies, including Alzheimer's disease. Interestingly, accumulation of damaged mitochondria has been observed in post-mortem brain of Alzheimer's disease patients. Although disease-associated tau and amyloid β are known to deregulate mitochondrial function, it remains elusive whether they also directly influence the efficiency of mitophagy. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradation pathway, by which cells regulate mitochondrial number in response to their metabolic state. However, little is known about the role of mitophagy in the pathogenesis of Alzheimer's disease. To address this question, we developed an in vivo imaging system to monitor mitophagy in neurons. We demonstrated that neuronal mitophagy is impaired in C. elegans models of Alzheimer's disease. Urolithin A- and nicotinamide mononucleotide-induced mitophagy ameliorates several pathological features of Alzheimer's disease, including cognitive defects. Mitophagy stimulation restores memory impairment through PINK-1-, PDR-1 or DCT-1-dependent pathways. Our findings suggest that impaired removal of damaged mitochondria is a pivotal event in Alzheimer's disease pathogenesis highlighting mitophagy as a potential therapeutic intervention.

BACK

Premature Vascularization of Motor Neuron Columns Alters Motor Neuron Development: A Role for Semaphorin3C-Plexind1 Signaling

José Ricardo Vieira[1,2], Isidora Paredes[1,2], Patricia Himmels[2], Géza Schermann[1,3], Heike Adler[1], Lea Gärtner[1], Dario Bonanomi[5], Christiana Ruhrberg[6], **Carmen Ruiz de Almodóvar**[1,3,7]

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Development of the vascular and neural compartments within the central nervous system occurs concomitantly and in a highly stereotypical fashion. However, how these two different systems cooperate to achieve such complex and highly specialized structures is unclear. Here, we reveal a novel developmental crosstalk between motor neurons (MN) and endothelial cells (EC) necessary for the coordinated development of MNs. Through a comprehensive analysis of cell-to-cell communication profiles from single-cell RNA sequencing of the mouse developing spinal cord we identified Semaphorin 3C (sema3C) and PlexinD1 as a communication axis between MNs and ECs. Using cell-specific knockout mice and in vitro assays, we demonstrate that removal of SEma3C in MNs, or its receptor PlexinD1 in ECs, results in premature and aberrant vascularization of MN columns. Those vascular defects lead to impaired MN axon exiting to the periphery at early developmental stages, and to MN maturation defects at later stages. Overall, this study highlights the importance of Sema3C-PlexinD1 signaling for MNs-ECs communication and reveals that a timely and spatially controlled vascularization process is essential for proper spinal cord development.

BACK

Push-Pull Signals from Motor Axons Direct Endothelial Remodeling to Ensure Assembly of Neuromuscular Connectivity

Luis Martins [1], Ilaria Brambilla [1], Alessia Motta [1], Stefano De Pretis [1], Ganesh Bhat [1], Yutaka Yoshida [3], Samuel Pfaff [2], **Dario Bonanomi** [1]

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Neurons and blood vessels form interconnected networks that support the high energy demand of the brain and neural control of circulatory function. Neurovascular interactions are typically viewed through the principle of congruency aimed at metabolic coupling. However, the close proximity between growing axons and vessels during embryo development carries the risk that uncontrolled neurovascular contacts may interfere with neural circuit wiring. We show that such conflicts arise during assembly of neuromuscular connectivity and are prevented through push-pull interactions orchestrated by neuronal signals. Through a mouse mutagenesis screen, we found that Plexin-D1 receptor is required in endothelial cells for axon targeting of a conserved motor neuron subtype. Motor neurons attract vessels with VEGF while releasing Sema3C to elicit short-range repulsion via Plexin-D1, thus displacing endothelial cells that obstruct axon growth. When this signaling pathway is disrupted, motor neurons are blocked from reaching their muscle targets and concomitantly vascular patterning in the spinal cord is altered. Thus, an integrative system of opposing cues ensures detrimental axon-endothelial encounters are avoided while enabling vascularization within the nervous system and along peripheral nerves. Our findings indicate that neurons safeguard pathfinding by repurposing guidance signals to remodel the cellular environment along their projection routes.

BACK

Neuronal Piezo2 Patterns Three-Dimensional Retinal and Cerebellar Vascular Scaffolds

Xin Duan

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The retinal vasculatures are assembled into parallel planes to support accurate visual functions. Mechanisms controlling parallel planar vascular organization are largely unknown. Retroorbital delivery of AAV2-Br1-GFP revealed a specific subset of Fam19a4/Nts-positive retinal ganglion cells (RGC) that physically contact endothelial cells with unique perisomatic endfeet. Genetic ablation of Nts-RGCs led to disorganized vascular sprouts close to the RGC layer, disrupting the planar organization of the vascular bed. We identified Piezo2 as an Nts-RGCs-enriched protein during retinal vascular development. Pan-neuronal and Nts-RGC specific deletion of Piezo2 led to the loss of Nts-RGC-vascular contacts and phenocopied the vascular defects upon the Nts-RGC ablation. These data indicate that Nts-RGC controls vascular organization via direct physical contacts, established through a Piezo2-dependent mechanism. Additionally, we identified a cerebellar granule cell subset regulating cerebellar vascular scaffold patterning using a similar Piezo2-dependent mechanism, generalizing a unique neuronal role in guiding the three-dimensional vascular patterning during neural circuit assembly.

BACK

Sensory Neurons Have an Axon Initial Segment that Initiates Spontaneous Activity in Neuropathic Pain

Ana Isabel Nascimento, Boris Safronov, **Monica Sousa**

IBMC/i3S, University of Porto, Portugal

The axon initial segment (AIS) is a specialized compartment of the proximal axon of CNS neurons where action potentials are initiated. However, it remains unknown whether this domain is assembled in sensory dorsal root ganglion neurons, in which spikes are initiated in peripheral terminals. We investigated whether sensory neurons have an AIS and if it contributes to spontaneous activity in neuropathic pain. We demonstrate that myelinated dorsal root ganglion neurons assemble an AIS in the proximal region of their stem axon, enriched in the voltage-gated sodium channels Nav1.1 and Nav1.7. Using correlative immunofluorescence and calcium imaging, we demonstrate that the Nav1.7 channels at the AIS are associated with spontaneous activity. Computer simulations further indicate that the AIS plays a key role in the initiation of spontaneous discharges by lowering their voltage threshold. Finally, using a Cre-based mouse model for time-controlled AIS disassembly, we demonstrate that this compartment is a major source of spontaneous discharges causing mechanical allodynia in neuropathic pain. Thus, an AIS domain is present in dorsal root ganglion neurons and facilitates their spontaneous activity. This study provides a new insight in the cellular mechanisms that cause pathological pain and identifies a new potential target for chronic pain management.

BACK

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Complex Regulation of Gephyrin Splicing is a Determinant of Inhibitory Postsynaptic Diversity

Frabrice Ango

INM, Univ Montpellier, INSERM, CNRS, Montpellier, France

Gephyrin (GPHN) regulates the clustering of postsynaptic components at inhibitory synapses and is involved in pathophysiology of neuropsychiatric disorders. Here, we uncover an extensive diversity of GPHN transcripts that are tightly controlled by splicing during mouse and human brain development. Proteomics analysis reveals at least a hundred isoforms of GPHN incorporated at inhibitory Glycine and GABA-A receptors containing synapses. They exhibit different localization and postsynaptic clustering properties, and altering the expression level of one isoform is sufficient to affect the number, size, and density of inhibitory synapses in cerebellar Purkinje cells. Furthermore, we discovered that splicing defects reported in neuropsychiatric disorders are carried by multiple alternative GPHN transcripts, demonstrating the need for a thorough analysis of the GPHN transcriptome in patients. Overall, we show that alternative splicing of GPHN is an important genetic variation to consider in neurological diseases and a determinant of the diversity of postsynaptic inhibitory synapses.

BACK

Identification of New Genes Involved in the Development of Commissural Neurons

Manuela Argentini [1], Sergi Roig-Puiggros [1], François Friocourt [1], Quentin Rappenau [1], Yvrick Zagar [1], Luisa Riancho [1], Annie Munier [2], Chédotal Alain [1]

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During neuronal development, axons of commissural neurons cross the midline to reach contralateral targets. To gain insight into the molecular diversity of commissural circuits we performed a transcriptional analysis of Robo3+ neurons. Robo3 is specifically expressed in commissural neurons and its deletion prevents commissural axons to cross the midline. We used the Robo3Cre mouse line to drive the expression of the ribosomal HA-tagged Rpl22a gene into the embryonic commissural neurons. After spinal cord and hindbrain dissections, HA-purified ribosomal-bound RNA was submitted to NGS. Among different candidates, Dmbx1 appeared as a highly enriched gene in commissural neurons. We found that Dmbx1 was specifically expressed in commissural neuron subpopulations of the spinal cord, the caudal hindbrain and the cerebellar primordium. Interestingly, at E12, Robo3 was still expressed in cerebellar Dmbx1+ neurons while it was no longer found in the other Dmbx1+ cells. These results suggest that the axons of Dmbx1+ commissural subpopulations could cross the midline at different developmental timepoints. We then looked at Dmbx1 expression in published hindbrain single cell data sets. At E12, in the hindbrain, two transcriptionally distinct Dmbx1+ subpopulations were present, only one of which co-expressing Robo3. These findings further highlight the diversity of commissural neurons developmental programs.

BACK

Unc5D and GPC3 form an Octameric Protein Complex That Directs Neuronal Migration

Onno Akkermans [1], Celine Delloye-Bourgeois [2], Claudia Peregrina [3], Maria Carrasquero [1], **Maria Kokolaki** [1], **Miguel Berbeira-Santana** [1], Mathieu Chavent [4], Florie Reynaud [2], Ritu Raj [5], Carol Robinson [5], Valerie Castellani [2], Daniel Del Toro [3], Elena Seiradake [1]

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Cell surface receptors play an important role in cell migration, an event crucial for brain development. This process can be also used by cancerous cells in order to disperse and metastasize. Our study shows that the morphogen receptor Glypican-3 (GPC3) and the Uncoordinated-5D receptor (Unc5D) are able to form an octameric hetero-complex, containing four copies of each of the proteins. Molecular dynamics simulations, mass-spectrometry data and structure-based mutants validated the crystal structure. In addition, we showed that the complex is able to form in trans as well as in cis using cell aggregation assays. Interestingly, there is an abolition of the trans interaction when GPC3 and Unc5 are expressed in the same cell. We also characterised nanobodies against GPC3, which either enhance or weaken the Unc5-GPC3 interaction and they were used in different in vivo and in vitro experiments. In vitro assays, such as stripe assays, display a repulsive effect of the Unc5-GPC3 interaction on cells. These results reveal a conserved cell guidance mechanism for the Unc5/GPC3 interaction.

Note: this abstract is the same as the one submitted by Miguel Berbeira-Santana as we will present it together

BACK

Building Axonal Branches: Molecular Insights from Cryo-Electron Tomography

Satish Bodakuntla, Nirakar Basnet, Hana Nedozralova, Kenichiro Taira, christian Biertuempfel, Naoko Mizuno

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Axonal branching is a key event during neuronal development that is built by an extraordinary orchestration of many cellular processes including the dynamic reorganization of cytoskeleton and relocation of cellular organelles. Our current understanding of how the intracellular elements interact and coordinate is sparse due to the technical limitations. Using in situ cellular cryo-electron tomography on primary mouse neurons, we observed how different cellular processes including organelle dynamics and transport, local protein synthesis and cytoskeletal rearrangements take place at different stages of building the axon branch. Currently, we are exploring how endoplasmic reticulum interacting proteins influence the ER morphology and thereby affect the axonal branching process. This bottom-up approach will help in dissecting the intricate relationship between different intracellular entities during neuronal development and further provide insights into the molecular mechanisms of mental health disorders.

BACK

Neuroblastoma Dissemination is Instructed by Embryonic Olfactomedin1/NOGOR and UNC5/GPC3 Signaling Pathways

Dounia Ben Amar [1], Florie Reynaud [1], Maria Carrasquero [2], Daniel Del Toro Ruiz [3], Elena Seiradake [2], Celine Delloye-Bourgeois [1], **Castellani Valerie** [1]

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Neuroblastoma (NB) is a childhood malignancy of the peripheral nervous system with prenatal occurrence, arising from the embryonic trunk neural crest (NC). While lineage relationships between malignant cells and their physiological cells of origin are extensively investigated, whether malignant cells take advantage of environmental cues expressed during embryogenesis is poorly known. In previous work, we modeled NB in the chicken embryo by transplanting human NB cells back to their neural crest site of origin. We found that NB cells adopt a directional ventral migration, first properly selecting and colonizing the sympathoadrenal territories, second establishing primary tumors and third disseminating to form distal metastatic foci as in NB patients. We reported contribution of Sema3C/Neuropilin/PlexinA signaling in this migratory sequence (Delloye-Bourgeois et al, Cancer cell, 2017). Here, using combinations of functional assays and omics approaches, we identified two additional developmental signaling that enable NB cells to migrate, target the sympathetic chain and disseminate: UNC5/GPC3, and Olfactomedin1/NOGOR.

BACK

Impact of Reduced Tubulin Polyglutamylation on Neuronal Functions and Degeneration

Shreyangi Chakraborty [1, 2], Maria Rodrigo Riestra [1, 2], Silvia Poggini [3], Naomi Ciano Albanese [3], Igor Branchi [3], Carsten Janke [1, 2], Maria M. Magiera [1, 2]

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Microtubules (MTs) exert a multitude of essential functions in neurons, which are expected to be orchestrated by a variety of posttranslational modifications (PTMs) taking place directly on the MTs. We have previously demonstrated that excessive accumulation of the PTM polyglutamylation perturbs neuronal transport and leads to neurodegeneration in mice and humans.
To determine the role of polyglutamylation as a physiological modulator of MT functions, we generated novel mouse models with reduced polyglutamylation. Analyses of these mice show signs of late-age onset degeneration in the olfactory bulb and pre-frontal cortex, suggesting a key role of polyglutamylation in neuronal homeostasis. To determine the precise sequence of events leading to neurodegeneration, we use immuno-histochemistry on brain sections and cleared whole brains of different ages. To further investigate whether cellular anomalies in the brains with hypoglutamylation translate into brain malfunction, we asses learning, memory and other cognitive parameters of our mouse models using an automated 'Intellicage' system.
By determining the role of tubulin polyglutamylation in the regulation of physiological functions, we will gain insight into its potential role in the pathogenesis of human neurodegenerative disorders. This might open a promising new prospect for the development of novel therapies for these so-far untreatable disorders.

BACK

Cardiolipin in Mitochondrial Dysfunction and Neurodegenerative Disease

Charlie Collingham

University of Reading

Background: Mitochondrial dysfunction and disruption of mitochondrial dynamics act as underlying features in most diseases, and therefore stand as a potential targets for different fields of therapy. Cardiolipin (CL), a mitochondrial lipid, refolds misfolded α-synuclein, preventing the build-up of aggregated deposits. In patients suffering with Parkinson's disease (PD), this system is overwhelmed, CL levels are depleted and the mitochondria are destroyed, leading to death of the cell.

Methods & findings: Treatment of Drug X restores CL levels and mitochondrial function in PD patient fibroblast cells and rotenone treated C. elegans. The aim of this project is to determine the process responsible for the restoration of CL levels. X-ray crystallisation studies produced a 3D structure of Drug X interacting with upstream proteins. We are using knockdown studies to observe upstream and downstream effects of Drug X within the worm on CL levels, viability and mitochondrial function.

Future: It would be interesting to explore advanced imaging techniques and label upstream proteins and lipids involved in this process in order to further study their interaction in vivo. This workshop will provide me with insight of complementary techniques and networking opportunities to help further my research into the mechanism behind this interaction.

BACK

Role of Intracellular Trafficking of AMPAR during LTP

Françoise Coussen [1, 2], Caroline Bonnet [1], Valeria Pecoraro [1], Justine Charpentier [1], Daniel Choquet [1, 2]

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Abundance of AMPA receptors (AMPAR) at synapse is essential for the establishment and maintenance of synaptic function. Their synaptic localization is dependent on a highly dynamic exocytosis, endocytosis and plasma membrane mobility events.

Using our new biochemical tool combined with photonic live imaging, we controlled and followed the intracellular transport of tagged GluA1 containing receptors in cultured rat hippocampal neurons. Analyzes are performed for GluA1 WT and mutants of GluA1 C-terminus domain in basal condition and during LTP. In organotypic hippocampal slices we combine imaging and electrophysiology experiments to analyze the impact of intracellular transport of AMPAR on LTP.
Localization of AMPAR is regulated by their intracellular trafficking thru interaction of their C-terminus domains with different intracellular partners. These interactions play a major rule in the exocytosis and localization of the receptor at the plasma membrane both in basal condition of during cLTP. In hippocampal slice intracellular transport of AMPAR plays a major role during LTP.

BACK

Motor Neurons Instruct Spinal Cord Vascularization via Sema3C-PlexinD1 Signaling

José Ricardo Vieira [1, 2], Patricia Himmels [2, 3], Isidora Paredes [1], Heike Adler [1], Dario Bonanomi [4], Christiana Ruhrberg [5], Carmen Ruiz de Almodóvar [1, 2]
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Formation of the vascular and neural compartments in the developing spinal cord (SC) occurs concomitantly and in a highly stereotypical fashion. However, whether and how these two different systems signal to orchestrate each other's development in order to achieve such complex structures is not well understood.
Here, we reveal a novel crosstalk between motor neurons (MN) and endothelial cells (EC) necessary to keep MN columns avascular. From inferring and analyzing cell-to-cell communication from previously published single-cell RNA sequencing of the mouse developing SC and, by using cell-specific knockout mice, we demonstrate that removal of semaphorin 3C in MNs, or its receptor PlexinD1 in ECs, results in premature vascularization of MN columns. Furthermore, in vitro assays similarly show that removal of one of the factors in the respective cell results in increased contact between MNs and ECs. This premature vascular misspatterning leads to MN developmental defects such as impaired MN axon exiting the SC at early stages and MN maturation defects at later developmental stages.
This study shows the importance of Sema3C-PlxinD1 signaling as a communication path between MNs and ECs. It also demonstrates that for proper SC development a timely and spatially controlled vascularization is required.

BACK

Structural Basis of the Cytokine-Mediated Activation of ALK Family Receptors

Steven De Munck [1, 2], Mathias Provost [1, 2], Michiko Kurikawa [3], Ikuko Omori [3], Junko Mukohyama [3], Jan Felix [1, 2], Yehudi Bloch [1, 2], Omar Abdel-Wahab [4], J.Fernando Bazan [5], Akihide Yoshimi [3], Savvas N. Savvides [1, 2]

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Anaplastic lymphoma kinase (ALK) and the related leukocyte tyrosine kinase (LTK) are recently deorphanized receptor tyrosine kinases (RTK) involved in neural development, cancer, and autoimmune diseases. Furthermore, ALK has emerged as a surprising key regulator of energy expenditure and weight gain through signaling in the hypothalamus. Despite such pleiotropy in physiology and disease, structural insights into ALK and LTK and their activation mediated by the cytokines ALKAL1 and ALKAL2 has remained elusive.

Using X-ray crystallography, we show for the first time that human ALK and LTK feature cytokine-binding segments of a novel fold with long polyglycine stretches arranged in a hexagonal lattice. Structures of the ternary complexes reveal a single ALKAL molecule inducing the homodimerization of ALK and LTK in twofold symmetric assemblies. Additional validation of these structures via biophysical and cellular assays uncovers that ALK family receptors display cytokine specificity via contributions of their membrane-adjacent EGF-like domains.

These structure-function findings, enable us to propose a blueprint for extracellular complexes of ALK family receptors, thereby extending the structural repertoire of cytokine-driven dimerization mechanisms adopted by human RTK's. Additionally, our structures provide opportunities for the design of therapeutics blocking and modulating ALK family signaling in cancer, autoimmune and metabolic diseases.

BACK

Flrt2 Functions during Zebrafish Retinal Development

Lukas Euler [1], Marta Parrilla [1], Bettina Carmen Kirchmaier [1], Amparo Acker-Palmer [1, 2, 3]

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The angioneurin Flrt2 belongs to the fibronectin leucine-rich repeat transmembrane (Flrt) protein family. Flrt2 has been shown to guide the development of nervous and vascular systems through adhesive and/or repulsive signaling in mouse and Xenopus. Here we investigated the function of Flrt2 during zebrafish retinal development. We found flrt2 expression within most of the zebrafish's retinal cell types, including a pronounced expression in a proliferative stem cell niche called ciliary marginal zone (CMZ). In fish and amphibians, the CMZ is maintained in adult stages, allowing the constant addition of new cells to the retina. Using CRISPR/Cas9 we generated two flrt2 loss of function mutant alleles with a 401bp and a 341bp deletion. Both deletions targeted conserved homo- and heterophilic binding regions within the leucine-rich repeat domain. At 4dpf our flrt2 knockout zebrafish lines displayed deficits in neuronal cell adhesion within the stem and the progenitor cell populations of the CMZ. These deficits resulted in an increased CMZ size, decreased cell density and changes in cell orientation. Furthermore, both retinal ganglion cell and amacrine cell processes showed aberrant organization and laminar targeting to the inner plexiform layer. Our data sheds light on a novel role for Flrt2 during zebrafish retinal development.

BACK

Generation of a Gal4/UAS Driven Reporter Line to Unravel Spatial and Temporal Expression Dynamics of the Angioneurin Flrt2 during Early Zebrafish Development

Bettina Carmen Kirchmaier [1], Lukas Euler [1], Philippe Eschmann [1], Marta Parrilla [1], Andreas van Impel [4], Stefan Schulte-Merker [4], Amparo Acker-Palmer [1, 2, 3]

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As a molecular guidance cue of the angioneurin class, Flrt2, a member of the fibronectin leucine-rich repeat transmembrane (Flrt) protein family, exerts critical functions during neuronal and vascular development through adhesive and/or repulsive signaling. Information about the spatio-temporal expression dynamics of flrt2 is crucial for developing experimental approaches to assess the function of Flrt2 in tissues. Here we show how to supplement the traditionally used in-situ hybridization-based mRNA detection by the implementation of a Gal4/UAS reporter system in zebrafish that is driven by the regulatory genomic elements of the flrt2 gene. Using those two approaches, we demonstrate flrt2 expression in the developing zebrafish nervous and vascular systems of the brain, eye, and trunk. For dorsal trunk areas this includes the mechanosensory Rohon-Beard neurons mediating early touch responsiveness. Within the brain vascular system flrt2 is expressed, among others, by the primordial hindbrain channels (PHBCs), which are among the first vessels to invade the brain during its vascularization. Interestingly, in the absence of flrt2, in vivo imaging of sprouting angiogenesis from the PHBCs in the zebrafish revealed decreased sprout and filopodia lengths. By combining the reporter line with different UAS responders, additional experimental approaches can be pursued to investigate Flrt2 function.

BACK

An Electric Fields-Mediated Compass in Neurons Mediates Axonal Adaptive Responses in Misguidance Contexts

Sarah Dinvaut [1], Sophie Calvet [1], Jean-Christophe Comte [2], Olivier Pascual [3], Rosaria Rosaria [4], Jérôme Honnorat [3], Frédéric Moret [1], **Julien Falk** [1], Valérie Castellani [1]

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In children with early developmental diseases affecting physiological axon navigation, plasticity is manifested by the presence of axonal tracts that succeeded to reach their target by traveling through alternate routes. We studied whether the neurons could locate their target at a distance, thereby assisting misguided axons. We designed a model of misguidance for spinal cord neurons, using the chicken embryo. We found that the neurons have an internal compass pointing to the target, that utilizes embryonic electric fields. A core component of the compass is the ATP1A3 gene coding for Na+/K+ ATPase subunit. This reveals a mechanism mediating neuronal adaptive responses when stepwise axon guidance is compromised, that could ultimately ensure circuit wiring.

BACK

Guardian of the Nociception: A New Role of the Microtubule Regulator Protein Kif2a in the Remodeling of Post-Developmental Nociceptive Neurons

Swagata Dey [1, 4], Irena Gokhman [1, 4], Sapir Suissa [1, 4], Rotem Ben Tov Perry [2, 4], Noa Wigoda [3], Ester Feldmesser [3], Shifra Ben-Dor [3], Andrew Kovalenko [1, 4], Avraham Yaron [1, 4]

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Sensory axons undergo morphogenesis during development and remodeling in response to injury. However, how the innervation is modulated in homeostatic conditions throughout life is poorly understood.
Kif2a, a microtubule destabilizer protein, have an indispensable role in developmental axon pruning in sensory neurons. We investigated the role of Kif2a in sensory neurons in the post-developmental period by generating conditional knockout of Kif2a mouse and analyzed it by anatomical, behavioral and genomic approaches.
We found that ablation of Kif2a in sensory neurons causes specific hypersensitivity to acute pain, but not to touch or proprioceptive stimulus, which is associated with skin hyperinnervation. Interestingly, this pain hypersensitivity largely disappeared in six-month- old animals.
Transcriptome profiling of WT and Kif2a cKO animals at 3 months revealed an induction of stress genes and sterile inflammatory program in knockout DRGs. In 6 m/o mice, the overexpression of inflammatory genes was significantly decreased. Instead, a highly specific downregulation of the expression of multiple genes responsible for pain sensation was observed.
Overall, our work suggests that Kif2a operates as a guardian of target innervation and noxious response. Its loss leads to aberrant innervation, pain sensitization and sterile inflammation, which is resolved by homeostatic transcriptional response of nociceptive neurons.

BACK

The Ancestry of Neurosecretory Vesicles

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Many key neurosecretory vesicle proteins are present in organisms even outside of the animal kingdom and hence seem to predate neurons. So far, characterizations of neurosecretory vesicle proteins are mainly limited to vertebrates making it difficult to draw conclusions about their evolutionary origin. By comparing 28 core neurosecretory vesicle proteins in 13 different species, we demonstrate that most of the assessed proteins are present in unicellular relatives of animals. Choanoflagellates, the closest unicellular relatives of animals, possess a surprisingly diverse repertoire of neuronal protein homologues even though they lack neurons. Using 3D electron microscopy reconstruction analysis, we revealed the diverse and polarized vesicular landscapes of the choanoflagellate species Salpingoeca rosetta and Monosiga brevicollis. In combination with immunostainings of the vesicle-localized SNARE protein synaptobrevin at the apical and basal pole of Salpingoeca rosetta, our results support the presence of putative secretory vesicles at both poles of a choanoflagellate cell. Strikingly, this organization is shared by synapses which secrete the content of neurosecretory vesicles into the synaptic cleft. Our study sheds light on the ancestral molecular machinery of neurosecretory vesicles and provides a framework to understand the origin and evolution of secretory cells, synapses, and neurons.

BACK

Mice Lacking a Novel Phosphosite On MET Display ASD-Associated Behavior Pattern

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Background. Receptor tyrosine kinase MET is an oncogene highly expressed in patients with autism spectrum disorders (ASD). MET is a key player in synapse formation, maturation, and process outgrowth. Within a phosphoproteomics study carried out by our group, previously unreported phosphorylation on S1014 of MET was identified. To study this site, we generated a knock-in mouse model lacking the phosphorylation on S1014 (METS1014A/S1014A). As the mice showed stereotypical, circling movements, we aimed to assess the impact of MET S1014A on the behavior and neuronal features of these animals.

Methods. A behavioral battery targeting symptoms of ASD such as social interaction, repetitive behavior, and cognition has been employed. Neuronal density in the cortex, hippocampus, amygdala, and striatum was quantified by Nissl and immunostaining.

Results. METS1014A/S1014A homozygous mice show higher level of dominance over WT mates in social behavioral tests (n=12, METS1014A/S1014A = 71% vs WT = 29%, $p \leq 0.05$). Additionally, we have detected a significant increase in the number of parvalbumin-expressing interneurons in the striatum. We did not observe any significant differences in other behavioral tests or neuron counts. To conclude, the lack of MET S1014 phosphorylation impacts social behavior and alters the expression of inhibitory neurons.

BACK

Structural Basis of Sema3E-PlexinD1 Recognition

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The structure and function of semaphorins and their receptor plexins have been extensively studied since this signaling mediates multiple important processes including vasculature and neuronal development, also cancer. Previous studies demonstrated that a dimeric semaphorin ligand binding to two plexin receptors to form a 2:2 complex is key for plexin activation. In vertebrates, Plexins can be categorised into four classes (PlexinA-D). In the past decades, the structures of class A, B and C plexins have been characterized either by X-ray crystallography or cryo-electron microscopy (cryo-EM). PlexinD1 is the only member in class D and hasn't been understood yet.
Here we present a complex structure of PlexinD1 ectodomain with its canonical ligand Sema3E at 3.0 Å resolution by single particle cryo-EM. The overall structure contains a dimeric Sema3E and two PlexinD1. PlexinD1 ectodomain adopts a closed-ring conformation and the tenth domains of the two PlexinD1s form an interface. The tail of PlexinD1 ectodomain is relatively flexible, suggesting it can adopt different conformations on binding of co-receptors, such as neuropilins. We will conduct biophysical and functional assays based on the structure guided mutagenesis in vitro and in vivo to explore the interactions between intermolecular and intramolecular interfaces.

BACK

Effects of Autophagy Impairment during Adolescence on Dendritic Spine Density in Prefrontal Cortical Neurons and Cognitive Functions

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Autophagy has been associated with pruning of excitatory synapses that normally occurs during development. The prefrontal cortex (PFC) is a brain area involved in higher-order cognitive function and exhibits slower maturation compared to other cortical areas. Here, we aimed to understand whether inhibiting autophagy during the PFC synaptic pruning period (postnatal day (P)35-P45) affects dendritic spine density in the PFC and its function. For this purpose, thy1-CreERT2;atg5f/f (KO) mice were generated and tamoxifen was injected to mice on P31-35 or P61-65, to induce Cre activity and lead to autophagy impairment. After P80, all mice were tested in the novel object (NOR) and temporal order object recognition (TOR) tasks. KO mice that had received tamoxifen at P31-35 exhibited reduced discrimination index in the NOR and TOR tasks, compared to het P30 mice as well as control groups. Golgi-cox staining was used to investigate dendritic spine density. Increased density of mature dendritic spines is observed in KO and het animals in both P30 and P60 treatments, compared to control animals, only in PFC and not hippocampus. Therefore, our results suggest that deficient autophagy after P30 results in increased dendritic spine density in the PFC and impaired performance in NOR and TOR tests.

BACK

Probing Structural Determinants for Partial Agonism and a Novel Allosteric Binding Site at the Full-Length Human α4β2 Nicotinic Acetylcholine Receptor

Guipeun Kang
UT Southwestern medical center

The α4β2 nicotinic receptor subtype is expressed at high levels in the brain and is a major pharmaceutical target for nicotine addiction and neurodegenerative disorders. The α4β2 nicotinic receptor binds neurotransmitter at orthosteric binding sites in its extracellular domain. Agonist binding shifts the conformational equilibrium from a closed-channel resting state to an open-channel activated state and then in the sustained presence of agonist the channel adopts a closed, desensitized state. Partial agonists bind at the orthosteric binding site with full occupancy,, but produce a submaximal response. Here, we utilized varenicline, a drug for nicotine addiction and partial agonist for the α4β2 receptor, to investigate the structural basis of subtype-selective partial agonism. We further used the negative allosteric modulator KAB-18 to probe a novel allosteric binding site in the presence of nicotine. New cryo-EM structures reveal two distinctive binding sites for the modulator in the receptor's transmembrane domain. Together, we combine functional analysis of purified, reconstituted receptor with cryo-EM based structural analysis of the full-length receptor to investigate principles underlying partial agonism and agonist and modulator recognition. Our results offer a structural basis for better understanding subtype-selective partial agonism and how a negative modulator acts through stabilizing a desensitized receptor conformation.

BACK

CKII Mediated Axonal Plasticity via Mitochondria NCLX Ca2+ Handling

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Mitochondrial Ca2+ -[Ca2+]m signaling has several key roles in cell function. Ca2+ enters the mitochondria through the mitochondrial Ca2+ uniporter and is extruded through Na+/Ca2+ exchanger, NCLX. Mass spectral analysis of NCLX revealed the presence of a Casein Kinase 2 (CKII) phosphorylation site, on the regulatory loop on Ser271 residue. Previous studies showed that the axon initial segment (AIS) plasticity is dependent on CKII activity. The AIS can exhibit plasticity over time, a vital proses for a dynamic neuronal network. One method of triggering AIS plasticity is via direct blocking of axonal M-type K+ channels, which induces a distal relocation of Na+ and M-channels. We hypothesized that this AIS plasticity is directly linked to [Ca2+]m handling by NCLX. First, we tested whether CKII inhibitor TBCA affect NCLX activity, and found that its application downregulated NCLX dependent [Ca2+]m efflux. Furthermore, we showed that phosphomimetic mutant S271A and S271D, a constitutively active or inhibited NCLX mutants, respectively and independently of TBCA, had a similar effect on NCLX activity. Finally, we found that CKII dependent AIS distal plasticity, is blocked in NCLX KO hippocampal neurons. Thus, our results indicate that CKII is a crucial regulator of NCLX and thereby controls AIS plasticity.

BACK

Unc5D and GPC3 Form an Octameric Protein Complex that Directs Neuronal Migration

Onno Akkermans [1], Celine Delloye-Bourgeois [2], Claudia Peregrina [3], Maria Carrasquero [1], **Maria Kokolaki** [1], Miguel Berbeira-Santana [1], Mathieu Chavent [4], Florie Reynaud [2], Ritu Raj [5], Carol Robinson [5], Valerie Castellani [2], Daniel Del Toro [3], Elena Seiradake [1]

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Cell surface receptors play an important role in cell migration, an event crucial for brain development. This process can be also used by cancerous cells in order to disperse and metastasize. Our study shows that the morphogen receptor Glypican-3 (GPC3) and the Uncoordinated-5D receptor (Unc5D) are able to form an octameric hetero-complex, containing four copies of each of the proteins. Molecular dynamics simulations, mass-spectrometry data and structure-based mutants validated the crystal structure. In addition, we showed that the complex is able to form in trans as well as in cis using cell aggregation assays. Interestingly, there is an abolition of the trans interaction when GPC3 and Unc5 are expressed in the same cell. We also characterised nanobodies against GPC3, which either enhance or weaken the Unc5-GPC3 interaction and they were used in different in vivo and in vitro experiments. In vitro assays, such as stripe assays, display a repulsive effect of the Unc5-GPC3 interaction on cells. These results reveal a conserved cell guidance mechanism for the Unc5/GPC3 interaction.

Note: this abstract is the same as the one submitted by Miguel Berbeira-Santana as we will present it together

BACK

Molecular Architecture of the Glycogen-Committed PP1/PTG Holoenzyme

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Lafora disease (LD) is the most severe form of teenage-onset progressive epilepsy. Seizures increase in frequency accompanied by cognitive decline. Affected individuals die usually within 10 years of onset, in a vegetative state and constant myoclonus. No therapy is available.

LD is characterized by the accumulation in neurons of Lafora bodies (LB), which are composed of insoluble starch-like polyglucosans. The delicate alternation between glycogen synthesis and degradation is governed by the interplay between key regulatory enzymes altering the activity of glycogen synthase and phosphorylase. Among these, the PP1 phosphatase promotes glycogenesis while inhibiting glycogenolysis. PP1 is, however, a master regulator of a variety of cellular processes, being conveniently directed to each of them by scaffolding subunits. PTG, Protein Targeting to Glycogen, addresses PP1 action to glycogen granules. In LD, genetic alterations leading to PTG accumulation cause the deposition of LB in neurons.

The crystallographic structure of the ternary complex PP1/PTG/carbohydrate here reported is the first determined for a glycogen-directed PP1 holoenzyme. We refined the mechanism of the PTG-mediated PP1 recruitment to glycogen by identifying i) an unusual combination of recruitment sites, ii) their contributions to the overall binding affinity, and iii) the dynamic nature of this complex by SAXS analyses.

BACK

Posttranslational Polyglutamylation of Microtubules in Neuronal Health and Degeneration

Maria M. Magiera [1, 2], Davide Boido [3], Shreyangi Chakraborty [1, 2], Luisa Cuibanu [3], Carsten Janke [1, 2]

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The microtubule cytoskeleton plays crucial roles in many aspects of neuronal cyto-architecture, function and longevity. How these multiple roles are orchestrated remains poorly understood. One possibility is that microtubules would be adapted to their functions by the tubulin code – the use of different tubulin gene products and the addition of posttranslational modifications. In support of this hypothesis, we demonstrated that excessive polyglutamylation, one of the microtubule modifications enriched in neurons, leads to deregulated axonal transport, and neurodegeneration in mice and humans. Our recent results further suggest that equilibrated levels of glutamylation might be essential for neuronal homeostasis.

To assess the physiological role of polyglutamylation in neurons and the whole nervous system, we generated novel mouse models with reduced glutamylation. Magnetic resonance imaging (MRI) analyses revealed late-onset neurodegeneration, and functional MRI uncovered altered neuronal activity. We now investigate whether these changes lead to behavioral abnormalities, by assessing learning and memory of our mouse models. In parallel, we test the molecular impact of perturbed glutamylation by analyzing its effects on the cellular level.

Our results indicate that polyglutamylation is an important controller of microtubule functions and neuronal homeostasis. It could therefore be relevant in the so-far untreatable human late-onset neurodegenerative disorders.

BACK

In silico Analysis of the Conformational Features of Botulinic Toxins A1 and E1

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Botulinum neurotoxins (BoNTs) are among the most powerful toxic compounds found in nature. They bind to pre-synaptic gangliosides and protein receptor complexes, which get internalized into recycling vesicles. Then, the inner acidification of vesicles induces a translocation of the catalytic domain into the cytosol, which next cleaves SNARE proteins to impair the action potential-mediated release of neurotransmitter into the neuromuscular cleft. The succession of these events is far from being elucidated at the atomic level, although numerous structures of the interaction partners have been determined. This is the reason for which we undertook a study of BoNTs by molecular dynamics simulations.
Systems were prepared for the subtypes BoNT/A1 and BoNT/E1, with protonation levels defined by neutral and acidic pH values. The toxins flexibility is mainly described by the relative displacements of domains, variations of flexibility being observed in the ganglioside binding site in the domain HCC, the HN switch and the belt alpha helix. The belt alpha helix fluctuations are connected with the larger accessibility of residues in HN, allowing to propose a model for the translocation, in which the mobility of belt is transmitted to HN, inducing an easier interaction of HN residues with the non-polar membrane environment.

BACK

Structural and Specificity Analysis of Sema5A Glycosaminoglycan Interactions

Gergely N Nagy [1], Rebecca L Miller [2], Richard Karlsson [2], Ramona Duman [3], Karl Harlos [1], Kamel El Omari [3], Armin Wagner [3], Henrik Clausen [2], E Yvonne Jones [1]
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Precise wiring of neurons relies on guidance cue proteins that steer them to target tissues during neuronal development. The transmembrane protein semaphorin-5A (Sema5A) is a bifunctional guidance cue exerting both attractive and inhibitory effects based on interactions of its thrombospondin repeat (TSR) domains with heparan sulfate proteoglycans (HSPGs) and chondroitin sulfate proteoglycans, (CSPGs), respectively. Selective and specific Sema5A-glycosaminoglycan (GAG) interactions are crucial for the formation of proper neuronal circuitry, but the underlying molecular mechanisms are uncharacterized. Our heparin affinity tests of Sema5A ectodomain constructs indicate that the TSR3-4 region has a decisive contribution to GAG binding. Using X-ray crystallography, we determined high-resolution structures of this segment to reveal its disulfide-linked dimer form and a unique structural fold variation within TSR4. This architecture enables the formation of two symmetrically positioned, positively charged cavities verified as the GAG binding sites of Sema5A based on co-crystallization experiments, in silico docking and structure-guided mutagenesis . Analysis of Sema5A-GAG interactions from cell-based glycan array and in vitro biophysical assays (BLI and mass photometry) shed light on the specific GAG modifications required for Sema5A binding and suggest that Sema5A-GAG higher-order organization may provide a molecular mechanism for modulation of signalling response.

BACK

Structural Snapshots of the Alpha 7 Nicotinic Acetylcholine Receptor Gating Cycle

Colleen Noviello [1], Anant Gharpure [1], Yuxuan Zhuang [2], Nuriya Mukhtasimova [3], Rico Cabuco [1], Leah Baxter [1], Dominika Borek [1], Rebecca Howard [3], Erik Lindahl [3], Ryan Hibbs [1]

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The α7 nicotinic acetylcholine receptor is a member of the Cys-loop receptor superfamily of pentameric ligand gated ion channels. It is unusual in that it assembles as a homopentamer, is highly permeable to Ca2+, and desensitizes faster than any other Cys-loop receptor. These unique biochemical and biophysical properties influence its physiological function in the central nervous system and in non-neuronal cells, especially in the cholinergic inflammatory pathway. We present the first cryo-EM structures of the human α7 nicotinic receptor. We have determined cryo-EM structures of this receptor in a lipidic environment in the principal steps in the gating cycle: resting, activated, and desensitized. The structures revealed a novel substructural element we call the C-terminal latch that is permissive for channel opening. Surprisingly, the activated state structure contained an anionic ring in the extracellular vestibule that influences both the conductance and calcium permeability of this receptor. Transition to the activated state also involve a compression of the local membrane, as revealed by molecular dynamics simulations. Comparisons among the α7 structures provide a foundation for mapping the gating cycle and reveal divergence in gating mechanisms in the Cys-loop receptor superfamily.

BACK

Vascular Wnt5a Orchestrates Cerebellar Neuronal Architecture and Circuitry

Marta Parrilla [1], Robert Hülse [1], Anna D'Errico [1], Nensi Alivodej [1, 2], Jing Jin [1], Maria R. Aburto [1], Marta Segarra [1, 3], Amparo Acker-Palmer [1, 2, 3]

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Vessels emerge as potent instructive players in central nervous system development. Here, we identified Wnt5a as a vascular signal that controls postnatal development of the neuronal compartment in the cerebellum. We show that Reelin-mediated activation of the adaptor protein Dab1 in endothelial cells (ECs) leads to an upregulation of Wnt5a expression. Pial vessels as well as the vascular plexus underneath the Purkinje cell (PC) layer are sources of Wnt5a. In mice with vascular Dab1 deletion (Dab1iΔEC) from postnatal day 1 to 3, the reduced Wnt5a secretion by ECs results in increased granule cell progenitor (GCP) proliferation and reduced PC arborization analyzed at P7. Importantly, treatment of Dab1iΔEC organotypic cerebellar cultures with Wnt5a rescued the GCP phenotype. Perinatal deletion of Dab1 in vessels resulted in the adult in a misalignment of parallel fibers (PF), the GC axons projecting to the molecular layer, and a persistent aberrant dendritic arborization of PCs. Functionally, these defects were associated with reduced PF-PC synapses as well as reduced synaptic contacts between climbing fibers (CF) and PCs. Long term potentiation at the PF-PC synapse was also impaired. All in all, we demonstrate that the vasculature is an important regulator of neuronal development in the cerebellum.

BACK

Role of Developmental Regulators of Axonal Local Translation in Adult Axons

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Local mRNA translation (LT) is vital for axon development. Impaired LT is linked to the emergence of neurodevelopmental disorders. Also, LT is related to the intrinsic regenerative capacity of mature neurons. Axonal regeneration displays similarities with axon growth during development, exhibiting various cellular processes that require LT. Although mature PNS axons maintain the ability for LT, adult CNS axons exhibit a gradually decreasing LT potential, highly correlated with limited regenerative capacity. The regulatory mechanisms behind LT and its role in post-injury regeneration remain elusive. We have previously identified a ribonucleoprotein complex (Mena-RNP) that regulates LT in the developing brain. It consists of the cytoskeleton-associated protein Mena that interacts with known translation regulators (i.e. HnrnpK, PCBP1), controlling the translation of the RNP-bound mRNAs. We investigate the conservation of Mena-RNP in the adult nervous system, in an attempt to elucidate the role of Mena in axon regeneration. Employing both in vivo and ex vivo injury assays we observe that the Mena-RNP components differ between the developing and adult nervous system and in CNS vs PNS axons. We are currently exploring other molecules that function locally in axons and could potentially explain the differential capacity of adult CNS and PNS axons for regeneration.

BACK

Unc5-GPC3 Interaction Controls Cortical Migration by Repulsion

Claudia Peregrina [1], Sofía Zaballa [1], María Carrasquero [2], Marilee Kokolaki [2], Elena Seiradake [2], Daniel del Toro [1]

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During development, pyramidal neurons are born in the proliferative zone and radially migrate to settle in one of six cortical layers. We and others have shown that Uncoordinated-5 receptor D (Unc5D) regulates radial migration of cortical neurons independent of Netrin-1, suggesting the presence of other ligands. Our collaborators found that Unc5 receptors interact with the morphogen receptor glypican-3 (GPC3). Glypicans are expressed during central nervous system development but little is known about their function. By taking advantage of structured-based engineered mutants and anti-GPC3 nanobodies developed by our collaborators, we elucidate their role during cortical migration.

Here we show that Unc5D is enriched in migrating cells while GPC3 is expressed in in radial glial cells during cortical development. Unc5D present in migrating neurons binds GPC3 in trans on opposing radial glial fibers. In stripe assays we show that GPC3 induces repulsion that is partially mediated by Unc5 receptors. Disrupting Unc5D-GPC3 interaction in vivo produces a strong delay in neuronal migration. Similar effects were seen after GPC3 knockdown in the developing cortex. Together out results show that Unc5D-GPC3 interaction controls the migration of cortical neurons in vivo.

BACK

The Role of the Proton Inhibited DEG/ENaC Channel DEL-4 on Neuronal Ionstasis and Survival under Stress

Dionysia Petratou [1, 2], Martha Gjikolaj [1, 2], Eva Kaulich [3], William Schafer [3], Nektarios Tavernarakis [1, 2]

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Neuronal ionstasis is of utmost importance for the function of nerve cells. Several diseases of the nervous system have been linked with an imbalance of neuronal sodium homeostasis. However, little is known about how stress affects neurons' ionstasis. In the present study, we identified in C. elegans an ENaC/DEG family member, the DEL-4 sodium channel, to be regulated by distinct types of stress and trigger appropriate organismal stress responses and motor adaptation. We show that DEL-4 may form a homomeric proton-gated channel and that it localizes on the cell membrane of dopaminergic, serotonergic, sensory and motor neurons. Heat stress and starvation reduce DEL-4 expression levels. In turn, the DEL-4 channel, through regulating the resting membrane potential, affects neurotransmission of dopaminergic and motor neurons and alters the locomotory rate of the animal. At the same time, DEL-4 depletion activates HSF-1, SKN-1 and ERUPR and impedes activation of DAF-16. Utilizing humanized models of Parkinson's and Alzheimer's disease in C. elegans, we demonstrate that DEL-4 promotes neuronal survival in the context of these proteinopathies. Our findings provide insight into the molecular mechanisms via which sodium channels uphold neuronal function and promote adaptation upon stress.

BACK

Is the NMDA Receptor Targeted to Synapses by Liquid-Liquid Phase Separation?

Emily C. Pheasant [1, 2, 3], Macarena Gomez de Salazar [1, 2, 3], Xavier L. Warnet [1, 2, 3], Sadegh Nabavi [1, 2, 3], Magnus Kjærgaard [1, 2, 3]

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The postsynaptic density (PSD) recruits and organises the synaptic signalling machinery, including the NMDA type glutamate receptors. The NMDA receptors' intrinsically disordered C-terminal tails control receptor trafficking and synaptic targeting; however, the underlying molecular mechanisms remain poorly understood.

We have found that the C-terminal tail of the NMDA receptor subunit GluN2B undergoes liquid-liquid phase separation (LLPS) in vitro when in complex with PSD-95, the most abundant scaffolding protein of the PSD. Additionally, the complex's tendency to undergo LLPS can be regulated by changing the number of aromatic residues within the GluN2B C-terminal tail. Furthermore, using microscale thermophoresis we have identified a hitherto unmapped interaction between the GluN2B C-terminal tail and PSD-95.

To study the significance of the LLPS in vivo, we have designed GluN2B variants in which the tendency to undergo LLPS is either enhanced or disrupted. Subsequently, we are quantifying the synaptic targeting of GluN2B containing NMDA receptors by immunofluorescence microscopy of primary neuronal cultures.

In the end, our results will uncover whether LLPS is an underlying mechanism of synaptic targeting and clustering of NMDA receptors. Such molecular insights will bring us closer to understanding the mechanisms which function in maintaining the molecular architecture of the PSD.

BACK

A PDE2-PKA Dependent Regulation of NCLX is Mediating Neuronal Survival and Cognitive Function

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Impaired phosphodiesterase (PDE) function and mitochondrial Ca2+ ([Ca2+]m) signaling lead to ischemic damage and dysfunction in learning and memory. We monitored [Ca2+]m transients in hippocampal neurons evoked by caffeine and depolarization. We found that [Ca2+]m efflux is apparent in WT but diminished in neurons deficient the mitochondrial Na+/Ca2+-exchanger (NCLX). Depolarization-induced Ca2+ transients alone failed to evoke strong [Ca2+]m efflux in WT neurons. As caffeine is an inhibitor of PDE2, it can affect [Ca2+]m by inhibiting mitochondrial PDE2. We show that pretreatment with the selective PDE2 inhibitor Bay-60-7550 rescued [Ca2+]m efflux triggered by neuronal depolarization, as caffeine. Furthermore, inhibition of PDE2 acts by diminishing the Ca2+ dependent change of [cAMP]m thus promoting NCLX phosphorylation at its regulatory PKA S258-residue. Bay-60-7550 also enhanced [Ca2+]m efflux triggered by neuromodulators Vasopressin and Norepinephrine. We found that protection of neurons against excitotoxic insults, conferred by PDE2 inhibition, is strongly diminished in NCLX-KO neurons, thus is NCLX-dependent. Finally, administration of Bay-60-7550 enhanced learning and memory in object-recognition test in WT but not in NCLX-KO mice. Our results identify a link between PDE2 and [Ca2+]m signaling. [Ca2+]m dyshomeostasis is a prominent feature of multiple disorders, thus the link between NCLX and PDE2 may provide new therapeutic targets.

BACK

Structural Analysis of CaV1.2 Mediated PM-ER Contacts in The Postsynaptic Membrane by cryoEM

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In around 50% of dendritic spines the plasma membrane (PM) is in very close contact with the endoplasmic reticulum (ER) [1]. One essential function of the ER is cellular calcium homeostasis. Calcium plays a major role in neural networks, since it triggers changes in synaptic activity by induction of plasticity through calcium influx. The interaction of the ER-localised calcium sensor proteins stromal interaction molecules (STIM) and the PM-localised voltage gated calcium channel CaV1.2 stabilise PM-ER contacts [2]. It is currently unknown under which conditions these contacts are induced or stabilised and how the landscape of interacting STIM proteins and Cav1.2 is organised on the nanoscale. Here, we present our progress towards investigating the subcellular organisation of interacting proteins in the postsynaptic membrane using fluorescence and electron tomography under cryo conditions. Samples are native and unperturbed, prepared using thinning by focused-ion-beam (FIB) milling. Unveiling the nanoscale organization of interacting proteins in the postsynaptic membrane will be critical to understand synaptic plasticity and stability.

[1] Wu et al. (2017) Contacts between the endoplasmic reticulum and other membranes in neurons. PNAS 114(24):E4859-E4867.
[2] Liou et al. (2005) STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. Curr Biol. 15(13):1235-41.

BACK

NMDA Receptor C-terminal Domain Positive Residues Affect Receptor Function

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The NMDA receptor (NMDAR) is a tetraheteromeric, ligand-gated ion channel involved in memory and learning. Although the receptor has been subject of several structural and functional studies, the role of its long, disordered C-terminal domain (CTD) in channel function remains poorly understood. To study the role of the CTD, GluN1-1a mutants were designed, with particular focus on positive residues close to its transmembrane domain. We used the CTD-dependent NMDAR positive allosteric modulators pregnenolone sulfate and UBP684 to screen for relevant residues, and the effect they had on NMDAR function was measured using two-electrode voltage clamp on Xenopus oocytes. We found that a double mutant H840A/K841A completely inhibits UBP684 potentiation, similar to a complete CTD truncation. We also observed an increased glutamate-evoked response, and determined that this effect is, at least in part, due to an increase in the open probability by measuring the time constant of the decay when applying the open channel blocker MK-801, and by calculating the open ratio when applying the thiol-modifying agent MTSEA on A651C-GluN2B receptors. These studies provide a novel means of understanding the mechanisms that govern CTD-dependent NMDAR activity and advance our understanding of the molecular mechanisms that underlie synaptic plasticity.

BACK

Profiling of the Neuron-Specific Dystroglycan Interactome Reveals New Factors Contributing to Cobblestone Lissenchephaly and Neurodegeneration

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The brain complexity is generated by multiple types of neurons that connect to each other in a specialized manner, which often depends on selective cell adhesion. Importantly, multiple neurodevelopmental diseases are caused by deficiencies of cell adhesion molecules. We propose that differential cell adhesion is the final aftermath of differential neurogenesis, which suggests that timing and levels of cell adhesion protein expression must be precisely regulated. Dystroglycan (Dg) is a major non-integrin cell adhesion factor responsible for various processes relevant to total organism health. In humans, abnormal Dg functioning leads to dystroglycanopathies, a group of inherited disorders associated with severe brain and eye anomalies. Remarkably, among many cases of diagnosed dystroglycanopathies, only a small fraction can be linked directly to mutations in Dg or its regulatory enzymes, implying the involvement of other, not-yet-characterized, Dg-regulating factors. We used our previously established Drosophila dystroglycanopathy model to identify the neuron-specific Dg interactome. Currently, we are following up several novel and somewhat unexpected functional groups as potential new players contributing to dystroglycanopathies. In particular, we discovered that in the developing brain, the exocyst mediates Dg membrane trafficking to the membranes of differentiating neurons. Moreover, there are temporal changes in exocyst-Dg regulation, controlled by miRNAs.

BACK

The Developmental Changes in Intrinsic and Synaptic Properties of Prefrontal Neurons Enhance Local Network Activity from the Second to the Third Postnatal Weeks in Mice

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The prefrontal cortex (PFC) is characterized by protracted maturation. The cellular mechanisms controlling the early development of prefrontal circuits are still largely unknown. Our study delineates the developmental cellular processes in the mouse medial PFC (mPFC) during the second and third postnatal weeks and characterizes their contribution to changes in network activity. We show that spontaneous inhibitory postsynaptic currents (sIPSC) are increased, whereas spontaneous excitatory postsynaptic currents (sEPSC) are reduced from the second to the third postnatal week. Drug application suggested that the increased sEPSC frequency in mPFC in the second postnatal week is due to depolarizing GABA-A receptor function. To further validate this, perforated patch-clamp recordings were obtained and the expression levels of K–Cl cotransporter 2 (KCC2) protein were examined. The reversal potential of IPSCs in response to current stimulation was significantly more depolarized at P10 than P20 while KCC2 expression is decreased. Moreover, the number of parvalbumin-expressing GABAergic interneurons increases and their intrinsic electrophysiological properties significantly mature in the mPFC from P10 to P20. Using computational modeling, we show that the developmental changes in synaptic and intrinsic properties of mPFC neurons contribute to the enhanced network activity in the juvenile compared with neonatal mPFC.

BACK

Dissecting the Molecular Mechanisms of Neurodegeneration in a Drosophila SWS/NTE Neuropathy Model

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Mutations in human Neuropathy Target Esterase (NTE) gene cause motor neuron disease called spastic paraplegia, in which long spinal axons degenerate leading to limb weakness and paralysis. Drosophila Swiss cheese (SWS) is a highly conserved orthologue of vertebrate NTE, phospholipase that can degrade endoplasmic reticulum-associated phosphatidylcholine. Similar to humans, mutations of sws in Drosophila cause progressive neurodegeneration, shortened lifespan, and interestingly, activated innate immunity response. NTE/SWS is required for the glial wrapping of neurons; however, the role of SWS in glia is not clear. NTE/SWS is expressed in the glia, forming the blood-brain barrier (BBB) and its loss or downregulation affects BBB integrity. Importantly, glia-specific expression of Drosophila SWS or human NTE in sws mutant background fully rescues the organization of the surface glia and could partially rescue BBB permeability, suggesting a conserved function of NTE/SWS in glia. Moreover, the BBB phenotype can also be alleviated by anti-inflammatory agents. We hypothesize that abnormal phosphatidylcholine metabolism caused by the absence of NTE/SWS phospholipase results in abnormal amounts of unsaturated fatty acids. Understanding the molecular mechanisms of inflammation in neurodegenerative diseases may help to promote the use of anti-inflammatory therapy and dietary supplements for age-dependent neurodegeneration.

BACK

Lysine63 Polyubiquitination Regulates Synaptic Transmission and Protects from Age-Dependent Neurodegeneration in C. elegans

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Protein ubiquitination is a central coordinator of cellular physiology, regulating both protein turnover and signal transduction. Ubiquitin monomers are assembled into homotypic, heterotypic, mixed and branched polyubiquitin chains, which elicit unique biological outcomes for their protein substrates. Lysine63 (K63) polyubiquitination orchestrates proteasome-independent protein degradation, targeting proteins to the autophagy-lysosome pathway. Cylindromatosis (CYLD) functions as a deubiquitinating (DUB) enzyme with specificity towards K63-linked polyubiquitin chains. CYLD is highly expressed in neurons, where it determines the K63 polyubiquitination status of synaptic proteins, coordinating synaptic remodeling and plasticity. Our previous work has shown that autophagy contributes to synaptic plasticity via direct degradation of synaptic proteins, thereby regulating cognitive behaviors, such as learning and memory. Here we investigate the role of CYLD and K63 polyubiquitination in neuronal physiology. CYLD-1, the nematode CYLD homologue, is expressed in the C. elegans nervous system and regulates autophagy. Neuronal depletion of CYLD-1 perturbs neurotransmission, impairs learning and shortens lifespan. In line, transgenic animals paneuronally expressing recombinant ubiquitin capable of K63 conjugation only, phenocopy CYLD-1 deficiency. Additionally, CYLD-1 preserves motor neuron integrity and motility during ageing. Our findings highlight DUB enzymes as potential therapeutic targets to ameliorate age-associated neurodegeneration and cognitive decline.

BACK

Role of Developmental Regulators of Axonal Local Translation in Adult Axons

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Local mRNA translation (LT) is vital for axon development. Impaired LT is linked to the emergence of neurodevelopmental disorders. Also, LT is related to the intrinsic regenerative capacity of mature neurons. Axonal regeneration displays similarities with axon growth during development, exhibiting various cellular processes that require LT. Although mature PNS axons maintain the ability for LT, adult CNS axons exhibit a gradually decreasing LT potential, highly correlated with limited regenerative capacity. The regulatory mechanisms behind LT and its role in post-injury regeneration remain elusive. We have previously identified a ribonucleoprotein complex (Mena-RNP) that regulates LT in the developing brain. It consists of the cytoskeleton-associated protein Mena that interacts with known translation regulators (i.e. HnrnpK, PCBP1), controlling the translation of the RNP-bound mRNAs. We investigate the conservation of Mena-RNP in the adult nervous system, in an attempt to elucidate the role of Mena in axon regeneration. Employing both in vivo and ex vivo injury assays we observe that the Mena-RNP components differ between the developing and adult nervous system and in CNS vs PNS axons. We are currently exploring other molecules that function locally in axons and could potentially explain the differential capacity of adult CNS and PNS axons for regeneration.

BACK

Physiological Phosphorylation of tau at Disease-Relevant Sites Is Required for Dynamic Microtubule Interaction

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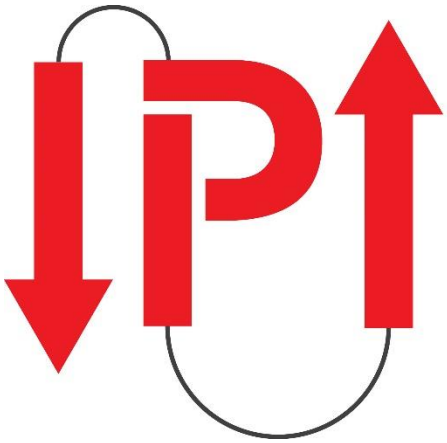
Ample evidence indicates that the hyperphosphorylation of microtubule-associated protein tau at selected sites is associated with the development of tauopathies. However, it is unclear whether physiological phosphorylation at the same sites is required for normal tau function in the cell.
To address this question, we produced and tested a phosphoblocking tau construct in which ten major phosphorylation sites, previously identified in paired helical filaments from Alzheimer's disease patients, were mutated from serine and threonine residues to alanine residues.
Blocking tau phosphorylation resulted in compartment-specific changes. In axon-like processes of model neurons, it increased tau-microtubule binding, reduced microtubule depolymerization, and increased the processivity of axonal transport. In contrast, in hippocampal neurons, tau phosphoblocking induced dendritic simplification.
Since GSK3β is a major tau kinase, we also tested the effect of its inhibition. We found that GSK3β inhibition increased tau-microtubule binding, similar to the effect of phosphoblocking mutations. Proteomics and phosphoproteomics analyses of model neurons subjected to GSK3β inhibition were carried out to identify the affected pathways.
The data indicate that physiological phosphorylation of tau at disease-relevant sites is required to ensure its dynamic interaction with microtubules and that its hypophosphorylation can have adverse effects.

BACK



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