Pennsylvania Convention Center, Ballroom A
Philadelphia, PA
Saturday, December 2, 2017
8:00 am – 5:30 pm
We thank ASCB President Pietro De Camilli for forming this Doorstep Meeting on the Cell Biology of Degeneration and Repair in the Nervous System, and for appointing its two organizers: Frank Bradke, German Center for Neurodegenerative Diseases (DZNE e.V.), and Kelsey C. Martin, UCLA David Geffen School of Medicine. Both are experts in this field and we appreciate their expertise.

Thanks to everyone for making this meeting happen!

Pietro De Camilli
Yale University School of Medicine/HHMI

Frank Bradke
German Center for Neurodegenerative Diseases (DZNE e.V.)

Kelsey C. Martin
University of California, Los Angeles, David Geffen School of Medicine

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Cell Biology of Degeneration and Repair in the Nervous System Doorstep Meeting

SCHEDULE

7:45 am  Registration Open
            Registration is located outside Ballroom A
            Do not go to the Registration Area for the 2017 ASCB|EMBO Meeting.

8:00 am  Breakfast

8:35 am  Welcome & Overview

8:45 am  Development and Reprogramming of Neuronal Diversity in the Neocortex
            Paola Arlotta, Harvard University

            The neocortex contains an unparalleled diversity of neuronal subtypes, each defined by distinct
            traits that are developmentally acquired under the control of several neuron subtype-specific
            and pan-neuronal genes. I will describe our recent work on the governing principles underlying
            developmental generation and postnatal maintenance of excitatory pyramidal neuron diversity
            in the cerebral cortex. I will also discuss how pyramidal neuron diversity impacts the behavior of
            other cell types during cortical development and discuss the critical effect on oligodendrocytes to
            guarantee generation of normal patterns of myelin distribution in different cortical layers. Once
            development is complete, it is well known that pyramidal neurons become permanently postmitotic
            and do not change their class-specific identity for the life span of the organism. I will show that
            during a defined window of postmitotic development (“critical window of nuclear plasticity”) pyramidal
            neurons can still change their class-specific identity in vivo and in turn reprogram the
            wiring of the afferent inhibitory circuit.

9:15 am  Saving the Synapse: MHC Class I and Synapse Pruning during Development and in Alzheimer’s Disease
            Carla Shatz, Stanford Bio-X, Stanford University

            Synaptic connections in adult brain are highly precise, but they do not start out that way. Precision
            emerges as connections are pruned or strengthened during a developmental process requiring
            neural activity. Activity also regulates neuronal gene expression. In an in vivo unbiased screen,
            Major Histocompatibility Class I (MHC1) genes were found to be regulated by activity, expressed in
            neurons, and located at synapses (Corriveau et al, 1998; Huh et al, 2000). To assess requirements for
MHCI in CNS, mutant mice lacking specific MHCI genes Kb and Db, were studied. Synapse pruning in the developing visual system fails, and ocular dominance (OD) plasticity in visual cortex is greater than in WT (Lee et al, 2014; Datwani et al, 2009). In a search for receptors for neuronal MHCI, PirB was found expressed in subsets of neurons throughout mouse CNS. In PirB KO mice, OD plasticity is enhanced (Syken et al., 2006), LTP and LTD are altered, and the pruning of dendritic spines on cortical pyramidal neurons is deficient not only during development in PirB KO mice (Djurisic et al, 2013; Vidal et al, 2016), but also when a soluble decoy receptor is infused in adult WT mice: Acute blockade of PirB in adult WT cortex triggers formation of new spines and functional synapses, and also restores visual function to an amblyopic eye (Bochner et al, 2014). Thus PirB, like MHCI, appears to regulate synapse pruning and to “brake” synaptic plasticity throughout life. The commonality of phenotypes in these mutant mice suggests a model in which PirB may bind and transduce signals from MHCI ligands in neurons. Moreover, without PirB, mice do not succumb to the devastating effects of Beta Amyloid—a main culprit for synapse and memory loss in Alzheimer’s disease (Kim et al, 2013). Together, results imply that these molecules, thought previously to function only in immunity, also act at neuronal synapses to regulate synapse pruning and plasticity in response to new experience. Changes in their function could contribute to developmental disorders such as Schizophrenia, and Alzheimer’s disease.

Supported by NIH Grants EY02858, MH071666, Mathers Charitable Foundation and Ruth K. Broad Biomedical Research Foundation

9:45 am  Gene Silencing Therapy for Neurodegenerative Disease  
*Don Cleveland, Ludwig Institute, University of California, San Diego*

Exciting discoveries in the genetics of human neurodegenerative disease have fueled multiple efforts for – at last – development of “on mechanism” therapeutics in the major neurodegenerative diseases. Currently, there is no disease-modifying therapies for any of the major diseases. I will cover how the combination of efforts using gene silencing with designer DNA drugs, adenoviral associated gene vectors, and genome editing mediated by site specific nucleases now raises the possibility of development of effective disease-modifying therapies.

10:15 am  Morning Break: Odd Posters & Networking

11:15 am  Dysfunction of Protein Translation in Neurodegeneration  
*Susan Ackerman, University of California, San Diego*

Neurodegenerative disorders affect many people, particularly in the aging population, yet the cause of these disorders is largely unknown. Using a forward genetic approach in mice, our lab has identified several novel molecular mechanisms that underlie CNS development and neuron death. Importantly, this phenotype-driven approach allows the identification, without a priori assumptions, of molecules critical to these processes. Furthermore, we use forward genetics to identify genes that modify the expressivity of the phenotype caused by the primary mutation. Together these approaches have identified new mechanisms of neurodegeneration and have demonstrated that dysfunction of protein translation greatly impacts neuronal homeostasis in the aging mammalian brain.
11:45 am  Dynamic RNA-protein Assemblies in Neurological Disease  
*J. Paul Taylor, St. Jude Children’s Research Hospital/HHMI*

Eukaryotic cells partition their contents into numerous specialized structures (organelles) that create microenvironments to facilitate specific functions. Membrane-less organelles such as RNA granules differ from classical membrane-delimited compartments in that they behave like liquid droplets that rapidly assemble and disassemble in response to changes in the cellular environment. Membrane-less organelles include nucleoli, Cajal bodies, speckles, paraspeckles, and PML bodies in the nucleus, as well as P bodies, stress granules, and RNA transport granules in the cytoplasm. Paradigm-shifting advances over the past year have revealed that diverse membrane-less organelles assemble via liquid-liquid phase separation (LLPS) of low sequence complexity domains that are particularly enriched in RNA-binding proteins (RBPs) such as TDP-43, hnRNPA1, hnRNPA2B1, TIA-1 and FUS. Importantly, mutations in these RBPs are causative of degenerative diseases, such as amyotrophic lateral sclerosis, frontotemporal dementia and inclusion body myopathy. We have hypothesized that the underlying basis of these diseases is disturbance of phase transitions that alters the dynamic properties of membrane-less organelles. I will present evidence that the mutations in disease-associated proteins alter the biophysical and material properties of these proteins in liquid assemblies and result in perturbed dynamics and/or functions of multiple membrane-less organelles.

12:15 pm  Lunch Break: The Kavli Foundation Talk, Roundtable Discussions & Networking

2:15 pm  Autophagy Dynamics in Neuronal Homeostasis and Neurodegeneration  
*Erika Holzbaur, University of Pennsylvania Perelman School of Medicine*

Neurons are highly polarized cells that are post-mitotic and must survive for decades in humans. These cells rely on homeostatic mechanisms to maintain cellular health, including autophagy and mitophagy. Deficits in autophagic flux lead to the accumulation of protein aggregates and dysfunctional mitochondria, and are characteristic of neurodegenerative diseases such as Parkinson’s, Huntington’s, and ALS. Live cell imaging of autophagy in neurons has revealed a dynamic pathway that is altered in both aging and disease.

2:45 pm  Chaperone Functions in Protein Quality Control and Implications in Neurodegenerative Disease  
*F. Ulrich Hartl, Max Planck Institute of Biochemistry*

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, we now know that in the crowded environment of cells newly synthesized polypeptides depend on molecular chaperone proteins to reach their folded states efficiently and at a biologically relevant time scale. Assistance of protein folding is provided by different types of chaperone which act to prevent misfolding and aggregation, often in an ATP-dependent mechanism. Once folded, many proteins continue to require chaperone surveillance to retain their functional states, especially under conditions of cell stress. Failure of the chaperone machinery to maintain proteostasis, i.e., the conformational integrity and balance of the cellular proteome, facilitates the manifestation of diseases in which proteins misfold and form toxic aggregates. These disorders include Parkinson’s, Huntington’s and Alzheimer’s disease.

I will discuss recent findings from model systems suggesting that toxic protein aggregation in neurodegenerative disease is both a symptom and a cause of proteostasis decline.

3:15 pm  Afternoon Break: Even Posters & Networking
4:15 pm  **Neuronal Mitostasis and Parkinson’s Disease**  
*Thomas L. Schwarz, Boston Children’s Hospital and Harvard Medical School*

Neurons last a lifetime, but proteins do not; proteins require constant turnover and synthesis. For mitochondrial proteins this is particularly true, because the reactive oxygen species formed by the electron transport chain are prone to damaging mitochondrial proteins. This presentation will review what we know about the synthesis and degradation of mitochondria in the special context of neuronal architecture and the problems posed by having mitochondria up to a meter away from the nuclear DNA encoding their proteins. Particular attention will be given to the evidence that Parkinson’s can be a mitochondrial disorder and to the role of PINK1 as a trigger of the mitophagic clearance of damaged mitochondria. PINK1 poses a particular problem for neurons because its normal half-life is just minutes; PINK1 mRNA transport on mitochondria and local translation can solve the problem of allowing a short-lived protein to support mitophagy throughout the axonal and dendritic arbors.

4:45 pm  **The Cell Biology of Protein Misfolding in Alzheimer’s and Parkinson’s Diseases**  
*Dennis Selkoe, Harvard Medical School/Brigham & Women’s Hospital*

Misfolding and progressive aggregation of specific proteins appears to be etiologic in several human neurodegenerative diseases. Many studies have examined the process of protein misfolding in vitro, resulting in valuable insights, but to what extent findings in pure in vitro mixtures reflect the situation in intact biological systems is unclear. Our laboratory has focused for over three decades on living neurons, animal models, and human brain tissue to learn more about the in vivo characteristics and bioactivities of endogenous forms of amyloid β-protein (Aβ), tau protein and α-synuclein (αSyn) in human neurodegeneration. Here, I will describe recent work on the effects of natural oligomers of Aβ isolated from Alzheimer’s disease cortex on iPSC-derived human neurons, including potent protection by certain monoclonal antibodies. I will then discuss our discovery that α-synuclein occurs physiologically in α-helical tetramers in intact neurons and that these rapidly disassemble upon cell lysis, yielding the “natively unfolded” monomers that have long been the focus of α-synuclein research. We have generated mice expressing “tетramer-abrogating” α-synuclein mutations; they develop nigrostriatal and cortical lesions, decreased tyrosine hydroxylase, and a progressive motor phenotype that includes tremor and gait defects which respond in part to L-DOPA administration. This recent work has implications for the initiation of Parkinson’s disease and other human synucleinopathies and suggests their potential prevention by compounds that stabilize physiological α-synuclein tetramers.

5:15 pm  **Wrap-Up**

6:00 pm  **Building Knowledge by Integrating Levels: Genes, Cells and Behavior**  
*Cori Bargmann, The Rockefeller University and Chan Zuckerberg Initiative*

2017 ASCB | EMBO Meeting, Fred Kavli Keynote Lecture
*(Admission to Keynote included with Doorstep Meeting registration)*

_all Doorstep Meeting attendees are invited to attend the Fred Kavli Keynote Lecture at 6:00 pm in Terrace Ballroom 3 of the Pennsylvania Convention Center, as well as the Opening Reception that follows the Keynote. If you are registered for the Annual Meeting and already have your Annual Meeting badge, you may wear that badge to gain entry. If you are not registered for the Annual Meeting or have not picked up your badge, please wear your Doorstep Meeting badge to gain entry._
Morning Poster Session Abstracts
10:15 am

1 Molecular Pathogenesis of Tubulin Disorders During Neural Development
Jayne E. Aiken, Emily Bates, and Jeffrey K. Moore

‘Tubulinopathies’ are severe human brain malformations associated with missense mutations in the tubulin genes. Despite the identification of many tubulin mutations in patients, we do not understand how these mutations impact the microtubule cytoskeleton, how the changes to microtubule function lead to brain malformations, or how different tubulin isotypes regulate microtubules to support normal neurodevelopment. TUBA1A α-tubulin is the most commonly affected tubulin isotype, with mutations linked to diverse cortical malformations including microlissencephaly, lissencephaly, pachygyria, and polymicrogyria. Here we focus on mutations affecting the conserved arginine at position 402 (R402), which is a hotspot accounting for 30% of all reported TUBA1A mutations in patients. We reveal that p.R402C and p.R402H mutations to Tuba1a dominantly disrupt cortical migration in the developing mouse brain, recapitulating the human lissencephaly phenotype. Further, using yeast mutants to mimic the R402C and R402H substitutions, we determine that the migrational defect is caused by disruption of microtubule motor dynein activity, which scales with abundance of mutant α-tubulin in the cell. Preliminary experiments in cortical neurons suggest that ectopic expression of R402C/H Tuba1a mutants is sufficient to disrupt microtubule function in a manner consistent with dynein impairment. Together, our results indicate that tubulinopathy mutations at p.R402 poison the microtubule network in young neurons by creating defective binding sites for dynein at the microtubule surface.

3 Dynamic Axonal Recruitment of the ESCRT Pathway to Promote Synaptic Vesicle Protein Turnover
Veronica Birdsall, Patty Sheehan, Clarissa L. Waites

Protein clearance mechanisms are essential for maintaining neuronal health via the elimination of damaged or misfolded proteins. Indeed, considerable evidence implicates dysfunction of degradative pathways, and in particular the endolysosomal pathway, in the etiology of neurodegenerative diseases such as frontotemporal dementia, ALS, and Alzheimer’s disease. However, little is known about the substrates and regulation of this pathway in neurons. Recent work in our lab has revealed that the endosomal sorting complex required for transport (ESCRT) machinery, a series of protein complexes in the endolysosomal pathway, is necessary for the degradation of synaptic vesicle (SV)-associated and other membrane proteins. Moreover, we find that elevated neuronal activity not only speeds SV protein degradation, but also stimulates the recruitment of ESCRT proteins into axons and presynaptic terminals. Using live imaging approaches, we have characterized the dynamic behavior of the initial ESCRT machinery that is required for cargo recruitment into the endolysosomal pathway. We find that these early ESCRT proteins undergo vesicular transport in axons, and that elevated neuronal activity induces an immediate, significant increase in vesicle motility as well as a change in directional movement. Furthermore, ESCRT-containing vesicles exhibit partial but not complete overlap with early endosome markers, indicating that they may represent degradative endosomes specialized for rapid mobilization in response to activity and other stimuli.

5 Parkinson Sac Domain Mutation in Synaptojanin 1 Impairs Clathrin Uncoating at Synapses and Triggers Dystrophic Changes in Dopaminergic Axons

Synaptojanin 1 (SJ1) is a major presynaptic phosphatase that couples synaptic vesicle endocytosis to the dephosphorylation of PI(4,5)P2, a reaction needed for the shedding of endocytic factors from their membranes. While the role of SJ1’s S-phosphatase module in this process is well established, the contribution of its Sac phosphatase domain, whose preferred substrate is PI4P, remains unclear. Recently a homozygous mutation in its Sac domain was identified in early-onset Parkinsonism patients. We show that mice carrying this mutation developed neurological manifestations similar to
those of human patients. Synapses of these mice displayed endocytic defects and a striking accumulation of clathrin coated intermediates strongly implicating Sac domain’s activity in endocytic protein dynamics. Mutant brains had elevated auxilin (PARK19) and parkin (PARK2) levels. Moreover, dystrophic axonal terminal changes were selectively observed in dopaminergic axons in the dorsal striatum. These results strengthen evidence for a link between synaptic endocytic dysfunction and Parkinson’s disease.

7
NRMT1 knockout mice have increased basal p53 levels in the brain
James Catlin and Christine Schaner Tooley

We have recently identified the first eukaryotic N-terminal methyltransferase, N-terminal RCC1 methyltransferase 1 (NRMT1). NRMT1 trimethylates the alpha-amino group on the N-terminus of proteins, and this methylation functions as a regulator of protein-DNA interactions. Specifically, it has been shown that N-terminal methylation of the substrate Regulator of chromatin condensation 1 (RCC1) is necessary for its association with chromatin and proper assembly of the mitotic spindle, and N-terminal methylation of the substrate DNA damage-binding protein 2 (DDB2) is necessary for its recruitment to sites of DNA damage. Given that NRMT1 has over 300 more predicted substrates, we were interested in determining the effect global NRMT1 knockout would have on organismal development and created the first NRMT1 knockout mouse. Initial characterization has shown these mice exhibit many phenotypes seen in mouse lines defective for DNA repair pathways, including reduced size, shortened lifespans, infertility, and altered liver metabolism. However, a comprehensive examination of how NRMT1 knockout specifically affects each organ remains to be completed. The objective of this study is to understand how NRMT1 loss affects the brain, as post-mitotic neurons are especially susceptible to DNA damage, which can promote neuronal apoptosis or senescence. We first harvested the brains from 6 month-old wild type control and NRMT1 knockout mice and used qRT-PCR and western blots to look at a key regulator of both apoptosis and senescence, p53. While transcript levels were similar between each group, p53 protein levels were significantly increased in all NRMT1 knockout mice as compared to controls. We then followed up this observation by looking at transcript and protein levels of downstream proteins involved in either apoptosis or senescence, and found that while senescence markers (p21, p16) remained mostly unchanged, the Bax/Bcl-2 ratio significantly increased. We are now looking to determine what exact apoptotic signaling pathway is being induced and what the mechanistic link is between NRMT1 loss and p53 upregulation.

9
Autophagolysosome disruption in a Drosophila model of ALS/FTD caused by C9orf72 expansion mutations
KM Cunningham, K Zhang, M Senturk, H Sung, Z Zuo, HJ Bellen, TE Lloyd

A GGGGCC hexanucleotide repeat expansion (G4C2 HRE) in the first intron of the C9orf72 gene has been identified as the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). One of the pathological hallmarks of C9-ALS is the presence of cytoplasmic protein aggregates colocalized with the autophagy receptor p62/SQSTM1. Interestingly, mutations in p62/SQSTM1 are also a rare genetic cause of ALS through an unclear mechanism. In a Drosophila model of C9-ALS expressing (G4C2)30, we have found that p62 is upregulated and forms large aggregates in motor neurons. p62 plays a key role in autophagy by binding ubiquitinated proteins and delivering them to the autophagosome for degradation via the lysosome. Surprisingly, we find that knockdown of p62 rescues degeneration in the fly eye and in motor neurons. Immunofluorescence and western blot analysis of autophagy and lysosome markers demonstrates an expansion of lysosomes and decreased delivery to and digestion of autophagic cargo in the lysosome. Using electron microscopy of the Drosophila eye, we observe a remarkable accumulation of expanded multilamellar bodies and autolysosomes that precedes neurodegeneration. We then tested genetic modifiers of autophagosome/lysosome function and find that Rab7, TRPML, and lysosomal V-ATPase overexpression rescue the neurodegenerative phenotypes in the Drosophila eye. Using electron microscopy of the Drosophila eye, we observe a remarkable accumulation of expanded multilamellar bodies and autolysosomes that precedes neurodegeneration. We then tested genetic modifiers of autophagosome/lysosome function and find that Rab7, TRPML, and lysosomal V-ATPase overexpression rescue the neurodegenerative phenotypes in the Drosophila eye. Furthermore, treatment with rapamycin and trehalose, pharmacological inducers of autophagy/lysosome pathway, rescue neurodegeneration in the Drosophila eye as well as the accumulation of p62 positive aggregates. We propose that C9orf72-HRE expression causes dysregulation of protein folding and degradation leading to cytotoxic protein aggregation, and that this is rescued by aggregate clearance through genetic and pharmacological upregulation of lysosomal function. This study suggests that drugs targeting lysosomal proteostasis pathways may have therapeutic potential for C9orf72-mediated ALS and FTD.
A role for the calcium-activated protease calpain in the regulation of netrin-1/DCC-mediated cortical axon outgrowth
Philippe M. Duquette, Doo Soon Im, David A Park, and Nathalie Lamarche-Vane

During embryonic development, neurons extend axons towards their appropriate synaptic targets to establish functional neuronal circuits. The growth cone, a highly motile structure at the axon tip, is capable of recognizing extracellular guidance cues and translating them into directed axon outgrowth through modulation of the actin cytoskeleton. The netrin family of guidance cues is vital for proper neuronal pathfinding. In particular, netrin-1 mediates its attractive function through the receptor deleted in colorectal cancer (DCC), which recruits proteins to mediate axon outgrowth and guidance. The calpain family of cysteine proteases is well known for its role in cleaving cytoskeletal proteins leading to cell death while playing a vital role in adhesion turnover during cell migration. Less is known about its role during brain development although some studies have highlighted its importance in the formation and maintenance of dendritic spines, and axon outgrowth. Here we identified DCC as a novel calpain substrate and we analyzed its role in the netrin-1/DCC signaling pathway during axon outgrowth. We found that calpain was able to cleave DCC in vitro. Calpain proteolysis of cytoskeletal targets is a mechanism of regulation of neurite consolidation and protrusive activity in neurons. We assessed calpain-specific spectrin cleavage, and found that netrin-1 activated calpain in embryonic cortical neurons in an Erk1/2-dependent manner. Furthermore, we demonstrated that netrin-1 stimulation promoted cleavage of DCC within the same time-frame of calpain and ERK1/2 activation. Interestingly, netrin-1-mediated Erk1/2 activation was abolished in calpain-1/2-deficient cortical neurons dissociated from Nestin-Cre;Capns1fl/fl embryos compared to control neurons. However, DCC expression was not unaffected in calpain-deficient cortical neurons. Using another calpain-specific substrate t-BOC that links calpain activity to fluorescence intensity in live cortical neurons, we showed that netrin-1 stimulated calpain activity in live cortical neurons. Interestingly, cortical neurons overexpressing calpastatin displayed longer neurites. Using an siRNA approach to diminish both calpain-1 and calpain-2 expression, neurons with reduced calpain expression also displayed longer axons and were unresponsive to netrin-1 stimulation. Altogether, we propose a novel model whereby netrin-1/DCC-mediated axon outgrowth is modulated by calpain-mediated proteolysis of DCC and cytoskeletal targets.

The Influence of Nato3 on LMX1 Gene Expression
Melina Frantzeskakis, Dayne Martinez, Merritt Taylor

Midbrain Dopamine neurons (mDA) can arise from the floor plate of the midbrain and are responsible for the symptoms of Parkinson's disease when they cease to function. mDA neurogenesis and maturation are regulated by multiple genes such as Shh, Foxa2, Lmx1a/b, Wnt1, among others. Genes that promote formation of mDA have potential to be used for clinical therapy development in Parkinson's disease. One gene involved in dopamine neurogenesis is the basic helix-loop-helix transcription factor Nato3. However, its mechanism of action is not well known. We hypothesized that Nato3 had the ability to upregulate the LMX1 markers in vivo and in vitro. Previous unpublished data produced by our lab using qPCR and immunostaining showed that overexpression of Nato3 upregulates LMX1b gene in vivo. These current data show upregulation of the LMX1a gene expression by Nato3 in the immortalized mouse midbrain SN4741 cell line, shown through qPCR data. Nato3 upregulation of Lmx1 genes indicate that Nato3 can influence the expression of genes known to drive dopamine neurogenesis, raising it as a potential target for therapeutic development.

Understanding programming logic of motor neurons from differentiated and undifferentiated cells
Görkem Garipler, Simon E. Vidal, Matthias Stadtfeld, Esteban O. Mazzoni

The safety concerns for transplantation of tissues derived from iPSCs/ESCs based differentiation protocols propose in vitro or in vivo transdifferentiation of somatic cells as a substitute for clinical applications. Although programming from somatic cells has the potential to be instrumental for cell replacement therapies, transdifferentiation is inefficient. The goal of this study is to understand how transcription factors (TFs) program rapidly-dividing pluripotent stem cells into post-mitotic motor neurons and then to apply this knowledge to efficiently program motor neurons from differentiated cell types.

Viral expression of 7 TFs (Ascl1, Brn2, Myt1l, Ngn2, Isl1, Lhx3 and Hb9) is able to transdifferentiate primary mouse embryonic fibroblasts (pMEFs) to spinal motor neurons (sMNs) with low efficiency (4%). Comparison of this approach
to an efficient (>90%) ESC based sMN programming protocol that expresses three transcription factors (Ngn2-Isl1-Lhx3, the NIL factors) lead to an increase of efficiency (20%) in pMEF based sMN programming with maintaining 1:1 stoichiometry between Isl1 and Lhx3. NIL factors induce secondary TFs, Ebf and OneCut families during programming in ESC and colocalize with these secondary TFs at sMN specific regulatory regions. Preliminary results demonstrate that NIL factors fail to induce Ebf and OneCut TFs in pMEFs, and thus, propose a potential mechanism causing inefficient sMN programming in pMEFs. To further improve the efficiency of transdifferentiation, I will investigate if forced expression of other TFs required for sMN differentiation improves the sMN programming efficiency from pMEFs. Rationally improving an inefficient transdifferentiation protocol by comparing to an efficient differentiation approach from ESC can lead to TF combinations that will be applicable to transdifferentiate other cell types. In the future, novel programming methods can be used to generate homogenous neuronal fates for in vitro disease modeling, drug studies and in vivo cell replacement therapies.

17
Study of Dendrite Regeneration using C. elegans Model
Harjot Kaur, Shirshendu Dey, Titash Mukherjee, Anindya Ghosh-Roy

Both dendrites and axons are vulnerable to physical insults during the life span of an individual. Several studies recently have focused on understanding the regenerative capacity of an injured axon. The p38 MAP kinase signaling cascade involving Dual Leucine zipper kinase DLK-1 is essential for axon regeneration. The cyclic AMP and mTOR signaling are limiting factors in axon regeneration. But less is known about dendrite regeneration. In Drosophila melanogaster, studies reveal that PTEN-Akt pathway plays an important role in dendrite regeneration while its independent to DLK-1 signaling. To understand the mechanisms of dendrite regeneration, we used PVD neuron in C.elegans, which has branched dendrites. The PVD neurons are responsible for harsh touch sensation. Using femtosecond laser, we severed the dendrites and axon initial segment (AIS) of this neuron. After the primary dendrite was severed near the cell body, we noticed sprouting of new branches from the cut site at 3-hour. By 24-hours the primary dendrite regrew, following similar trajectory and formed more complex branching patterns unlike the original menorah observed in uninjured PVD. These branches often lacked self-avoidance phenomenon. We quantified the regeneration pattern in two aspects - length of primary dendrite and number of branches. The primary dendrite regrew in length by 83.5±64.7 µm and 201.1±56.1 µm at 24-hour and 48-hour respectively. This was half the length of uncut dendrite. Severing the AIS led to complete retraction of the proximal axon after 3 hours. This was followed by the formation of a new process either from cell body or from sites of primary dendrites adjacent to the cell body. Eventually, these processes were guided to the ventral nerve cord. This response is reminiscent to the repolarization phenomenon observed in fly and vertebrates after cutting AIS. The extent of regrowth of the primary dendrite and the number of branches were not affected by the loss of dlk-1. This indicated that dendrite regeneration is independent of dlk-1 as seen in fly. Our future goal is to identify signaling mechanism that is important for the dendrite regrowth. Further, we want to correlate dendrite-remodeling responses with behavioral aspects of PVD neuron.

19
Altered Axonal lysosome transport contributes to Alzheimer’s disease pathology
Swetha Gowrishankar, Yumei Wu, Pietro De Camilli and Shawn Ferguson

Through a comprehensive analysis of organellar markers in mouse models of Alzheimer’s disease, we documented a massive and robust accumulation of lysosome-like organelles at amyloid plaques and establish that the majority of these organelles reside within swollen axons that contact the amyloid deposits. This close spatial relationship between axonal lysosome accumulation and extracellular amyloid aggregates was observed from the earliest stages on β-amyloid deposition. Notably, we discovered that lysosomes that accumulate in such axons are deficient in multiple soluble luminal proteases and thus predicted to be unable to efficiently degrade proteinaceous cargos. Of relevance to Alzheimer’s disease, β-secretase (BACE1), the protein that initiates amyloidogenic processing of the amyloid precursor protein (APP), is a substrate for these proteases and builds up at these sites. Furthermore, through a comparison between the axonal lysosome accumulations at amyloid plaques and neuronal lysosomes of the wildtype brain, we identified a similar, naturally occurring, population of lysosome-like organelles in neuronal processes that is also defined by its low luminal protease content. In conjunction with emerging evidence that the lysosomal maturation of endosomes and autophagosomes is coupled to their retrograde transport, our results suggested that extracellular β-amyloid deposits cause a local impairment in retrograde axonal transport, leading to the accumulation of lysosome precursors and a blockade in their further maturation.
In testing this hypothesis, we have identified JNK interacting protein 3 (JIP3) as an important regulator of both axonal lysosome abundance and maturation state. Interestingly, JIP3 KO neurons accumulate lysosomes within focal axonal swellings that closely resemble the dystrophic axons at amyloid plaques— including high levels of amyloid precursor protein (APP) processing enzymes (BACE1 and presenilin 2) and are accompanied by elevated Aβ peptide levels. We tested our hypothesis that these traffic jams of axonal lysosomes could thus potentially serve as disease-relevant sites of APP processing by depleting JIP3 in a mouse model of AD. JIP3 haploinsufficiency strongly increased both the abundance and size of amyloid plaques. These results establish a critical role for efficient axonal lysosome transport and maturation in protecting the brain from amyloid plaque pathology. Our study thus advances understanding of both Alzheimer’s disease brain pathology and provides new insights into the subcellular organization of neuronal lysosomes that may have broader relevance to other neurodegenerative diseases with a lysosomal component to their pathology.

21
Cellular aging potentiates neuronal beta-amyloid production by increasing APP endocytosis
Tatiana Burrinha, Ricardo Gomes, Claudia G. Almeida

β-Amyloid (Aβ) accumulation is a primary trigger for Alzheimer’s disease (AD). Exacerbated accumulation/oligomerization Aβ impacts synaptic function, with the eventual loss of synapses early on before the onset of the disease. Aging is the main risk factor for Alzheimer’s disease. Aβ can accumulate in the “normal” aging human brain and in aged mice. Defective clearance of Aβ has been postulated as responsible for Aβ accumulation in the late-onset AD, however, evidence indicates that the production of Aβ is also potentiated with aging. We set out to investigate whether cellular aging-driven alterations of intracellular trafficking potentiate Aβ production and thus cause synaptic decline. To dissect the endocytosis of the amyloid precursor protein (APP) and synapse changes with cellular aging we used an established a model of aging of primary mouse cortical neurons with time in culture. We confirmed that cortical neurons (E16) undergo in culture a stereotyped process of differentiation, maturation and aging in 28 days in vitro (DIV). At 28DIV, neurons accumulate the aging marker lipofuscin in lysosomes of the cell body. 28DIV neurons were previously shown to have higher processing of exogenous APP CTFs by γ-secretase. We now find that aged neurites evidence a dramatic rise in endogenous intracellular Aβ production. Indeed, APP becomes more localized to aged axons and dendrites. Since APP processing occurs at endosomes and defects in transferrin receptor endocytosis had been previously described in 28 DIV neurons, we measured APP endocytosis. Unexpectedly, we found an enhancement of APP endocytosis in aged neurons without altered APP levels. Accordingly, aged early endosomes were brighter and enlarged, suggestive of up-regulation of endocytosis. Importantly, in aged neurons, synapse number is decreased with prominent loss of spines. We thus asked if this synaptic decline could be reversed by inhibition of Aβ production. We found that both γ- and β-secretase inhibition can partially rescue aging-synaptic decline. Overall our data indicate that an increase in APP endocytosis contributes to the accumulation of Aβ with aging that impacts synaptic function even in normal neurons.

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ER-mitochondria tethering by PDZD8 regulates Ca2+ dynamics in mammalian neurons
Yusuke Hirabayashi, Seok-Kyu Kwon, Hunki Paek, Wolfgang M. Pernice, Ashleigh Raczkowski, Donald S. Petrey, Liza A. Pon and Franck Polleux

A network of contact sites between the membranes of different organelles are emerging as critical platforms for various forms of intracellular signaling. The interface between ER and mitochondria is of particular interest as a signaling hub because it is thought to play critical physiological functions ranging from Ca2+ exchange, to lipid biogenesis and regulation of mitochondria fission. In addition, changes in the number of these contacts have been observed in neurodegenerative mouse models and/or in the brains of patients presenting with Alzheimer’s disease (AD), Parkinson’s disease (PD), and Amyotrophic lateral sclerosis (ALS). However, despite the fact that multiple proteins are enriched at ER-mitochondria contacts sites, the molecular mechanisms underlying ER-mitochondria tethering are still largely unknown in metazoans.

Here, we will report the identification of PDZD8 as an ER protein present at ER-mitochondria contacts (Hirabayashi et al. Science 2017 in press). The SMP domain of PDZD8 is functionally orthologous to the SMP domain found in yeast Memm1, a member of the four proteins composing the ERMES complex identified in yeast by Peter Walter’s group as essential for ER-mitochondria contact formation in yeast (Kornmann et al. Science 2009). Using 3D FIB-SEM reconstructions, we demonstrate that PDZD8 is required for the formation of ER-mitochondria contacts in mammalian cells. Using a series of functional rescue experiments, we found that PDZD8-dependent ER-mitochondria contacts are required for proper function of neuronal Ca2+ homeostasis.
Ca2+ exchange between ER and mitochondria in mammalian cells. In neurons, PDZD8 is required for Ca2+ uptake by mitochondria following synaptically-induced Ca2+-release from ER and thereby regulated cytoplasmic Ca2+ dynamics. Thus, PDZD8 represents a critical ER-mitochondria tethering protein in metazoans. We suggest that ER-mitochondria coupling is involved in the regulation of dendritic Ca2+ dynamics in mammalian neurons.

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The ataxia disease gene VPS13D plays an essential role in mitochondrial morphology and transport in Drosophila neurons
Ryan Insolera, Eunju Seong, Luis M Rivera-Perez, David Lozano, Margit Burmeister, Catherine A Collins

The long term maintenance and survival of neurons is highly dependent on the proper functioning of mitochondria. This is evident by the disproportional representation of mitochondrial genes associated with neurodegenerative disorders. Originally discovered in yeast, vacuolar sorting protein 13 (vps13) has multiple variants in metazoans, three in Drosophila melanogaster (A, B, and D) and four in mammals (A through D). Human mutations in VPS13A, B, and C have all been linked to various forms of neurodegenerative diseases. Recent genetic studies in humans have associated mutations in VPS13D with ataxia. In order to understand the cell biology underlying the defects associated with dysfunction of the vsp13d gene in the nervous system, we are using Drosophila melanogaster as a model system. Loss-of-function mutations in vps13d lead to severe defects in mitochondrial morphology in multiple tissues, and early larval lethality. Targeted knockdown of vps13d in the nervous system circumvents this early lethality to allow further analysis, and reveals similar cell-autonomous mitochondrial defects in neurons. In larval motoneurons lacking vps13d, we observed defects in trafficking of mitochondria to axons and synaptic terminals. Loss of vps13d induces the formation of oversized, atypical mitochondria, some of which contain mitochondrial inner membrane proteins but lack markers targeted to the matrix. Most interestingly, targeted knockdown of vps13d in neurons leads to the accumulation of comparably atypical mitochondria in neighboring supportive glial cells. We are currently characterizing the nature of the cell-autonomous mitochondrial defects in neurons, and the origin of the non-cell autonomous effect on mitochondria in glia. An exciting possibility that we are testing is whether there is transfer of atypical mitochondria from neurons to glia. Altogether, these results suggest that vps13d plays an essential role in mitochondrial biology, and is required in neurons for the proper distribution of mitochondria to distal regions. In addition, vps13d disruption in neurons reveals a previously uncharacterized neuron-glia interaction which may be relevant for its roles in neurodegenerative disease.

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The role of a polyglutamine protein in developmental non-apoptotic cell death
Lena Kutscher, Mohammad Alam, and Shai Shaham

Cell death is an important cellular process in development and disease. While apoptotic cell death has been extensively studied, mutations blocking this process do not grossly perturb animal development. Likewise, mutations in murine apoptosis genes do not appear to block neuronal cell death in any neurodegeneration model. Thus, other cell death pathways are likely at play. Studies of the C. elegans linker cell provide strong evidence for the developmental relevance of non-apoptotic cell death. This male-specific gonadal leader cell dies during the L4-to-adult transition to allow formation of an open reproductive system. Molecular studies of LCD (Linker Cell-type Death) demonstrate that the process is caspase-independent. Instead, cell death appears to rely in part on a polyglutamine protein, PQN-41C, likely through a novel pro-death function of the heat shock factor protein, HSF-1. To investigate the function of PQN-41C further, we performed a protein interaction screen and identified HPK-1, a homeodomain protein kinase involved in the heat shock response. Mutations in hpk-1 suppress mutations in known linker cell death genes, suggesting a role in inhibiting linker cell death. As HPK-1 homologs have been shown to positively regulate HSF-1 upon heat shock in other systems, we hypothesized that HPK-1 promotes the heat shock function of HSF-1, thereby prohibiting HSF-1’s LCD-function. We found that in the absence of HPK-1, HSF-1 fails to translocate to the nucleus upon heat shock. Additionally, the heat-shock-related linker cell survival defect is abrogated in the hpk-1 null mutant. Together, our results suggest that PQN-41C negatively regulates HPK-1, preventing its activation of the HSF-1 heat shock function and thereby allowing the activation of the pro-LCD role.
of HSF-1. Future studies into the interplay of polyglutamine proteins and HSF-1 may shed light on non-apoptotic cell death and disease.

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Multi-Color Storm in Neurons Reveals Molecular Organization of the Lgi1 Synaptic Complex

Lara Laparra-Cuervo, Laurent Ladépêche, Jesús Planagumà, Joseph S. Borbely, Ángel Sandoval, Josep Dalmau, Melike Lakadamyali

Leucine rich glioma activated 1 (LGI1) is a neuronal protein that forms a trans-synaptic bridge linking pre- and postsynaptic transmembrane proteins (ADAM22 and ADAM23) and helps to organize a multimeric complex at the synapse including AMPAR and voltage-gated potassium channels [1]. LGI1 autoimmune encephalitis is a severe neuropsychiatric disorder related to epilepsy. It is an antibody-mediated pathogenesis where the patients produce autoantibodies against leucine rich glioma activated 1 (LGI1), which alter synaptic plasticity. However, the molecular mechanisms that lead to the observed problems in patients still remain largely unknown.

To elucidate early molecular changes at the synaptic level in LGI1 encephalitis, we used multi-color super-resolution microscopy (STORM) [2]. Using well-characterized synaptic markers (Homer and Bassoon) as molecular standards, we determined the positioning of LGI1 and 4 other related proteins (AMPA receptors, ADAMs and voltage-gated potassium channels) within the synaptic space at nanoscale resolution. Further, we compared this molecular architecture in healthy neurons versus neurons treated with antibodies from patients suffering from LGI1 autoimmune encephalitis. Our results show that LGI1 auto-antibodies impact the nanoscale organization of pre-synaptic proteins, while the post-synaptic protein organization is less affected. These results suggest a loss of LGI1 interaction with pre-synaptic proteins upon antibody binding and give further insight into early changes in pathology.


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Aβ Oligomers Mediate its Synaptotoxic Effects through AMPK-dependent Increase in Mitochondrial Fission in Pyramidal Neurons

Annie Lee, Chandana Kondapalli, Tommy Lewis, Georges Mairet-Coello, Franck Polleux

Alzheimer’s Disease (AD) is the most common form of dementia leading to socially devastating cognitive defects with currently no effective treatment. Current evidence shows that during the early stages of AD progression, oligomeric, soluble forms of Aβ42 peptide trigger the loss of excitatory synapses (synaptotoxicity) in cortical and hippocampal pyramidal neurons (PNs) even prior to the formation of insoluble Amyloid-β plaques in AD animal models. Previous work from our lab, as well as others, uncovered (1) that Aβ42 oligomers over-activate the stress-response AMP-activated kinase (AMPK) in a CAMKK2-dependent manner and (2) that pharmacological or genetic inhibition of CAMKK2 or AMPK protects hippocampal PNs from Aβ42-mediated loss of excitatory synapses observed in the hAPPSWE,IND transgenic AD mouse model (J20) in vivo (Mairet-Coello et al. Neuron 2013). However, the relevant downstream effectors and the cellular processes involved in AMPK-dependent synaptotoxicity are poorly understood. We recently identified that AMPK over-activation by Aβ42 oligomers triggers spatially restricted MFF-dependent mitochondrial fission and ULK2-dependent mitophagy in dendrites, resulting in both reduction of mitochondrial length and density. In the presence of oligomeric Aβ42, knockdown of MFF prevents mitochondrial fission, whereas genetic ablation of ULK2 still allows mitochondrial fission to occur without the subsequent degradation of mitochondria. Most importantly, both of these manipulations can prevent the synaptotoxic effects of Aβ42 oligomers, suggesting that the total volume of dendritic mitochondria plays a critical role in maintaining excitatory synapses. In parallel, we observe that a profound structural remodeling occurs specifically in the apical tufts of dendrites of CA1 PNs, a portion of the dendritic arbor where we also detect dramatic synaptic loss in the J20 AD mouse model. Overall, our project elucidates the cellular and molecular mechanisms mediating the effects of Aβ42 oligomers on mitochondrial defects, synaptic maintenance and their functional impact on hippocampal circuit in AD.
Ire1 RNase specificity separates transcriptional and post-transcriptional regulation of ER protein homeostasis
Weihan Li, Voytek Okreglak, Jirka Peschek, Philipp Kimmig, Peter Walter

The endoplasmic reticulum (ER) is the major folding compartment for most secretory and plasma membrane proteins in the cell. A conserved signaling pathway, the unfolded protein response (UPR), senses and modulates the folding capacity of the ER. To maintain ER protein homeostasis under ER stress conditions, the ER membrane embedded sensor, Ire1, binds unfolded proteins through its ER-lumenal domain and initiates two distinct mRNA processing programs through its cytoplasmic kinase/RNase domains. First, in both metazoans and S. cerevisiae, Ire1 catalyzes the unconventional cytoplasmic mRNA splicing of XBP1 (metazoans) or HAC1 (S. cerevisiae)—thereby initiating a transcriptional response that increases the ER folding capacity. Second, in metazoans and S. pombe, Ire1 selectively degrades ER-localized mRNAs—thereby post-transcriptionally reducing the ER’s protein folding burden through regulated Ire1-dependent mRNA decay. Thus, Ire1 homologs in S. cerevisiae and S. pombe are specialized to only one of the two functional outputs, while Ire1 in metazoans can perform both. To understand how Ire1 can regulate protein homeostasis through distinct RNA processing programs mechanistically, we characterized Ire1 from S. cerevisiae and S. pombe with in vivo and in vitro experiments. Surprisingly, despite relatively low sequence conservation in the lumenal domains, these domains share conserved ER-stress sensing mechanism. Conversely, despite high sequence conservation, Ire1 cytoplasmic domains recognize distinct RNA sequence and structural features, which leads to functional divergence in RNA processing. Finally, by applying our new findings, we successfully reconstituted unconventional mRNA splicing in S. pombe cells. Therefore, we engineered S. pombe into a metazoan-like Ire1 system, where unconventional mRNA splicing and selective mRNA decay co-exist. Our results provide new insights into a mechanistic understanding of Ire1 function and its interplay with RNA substrates.

Cytoskeletal Regulation of Neurodevelopment in an hIPSC-derived Autism Model
Taylor Rudisill, Brenna Kirk, Colin Johnson, Adrienne Orbita, Pranaya Pakala, Haroon Dar, Storm Davis, Rick Horwitz, Mike McConnell, Karen Litwa

Autism is one of the fastest growing developmental disabilities, currently affecting 1 in 68 children and rapidly increasing in prevalence. Emerging evidence suggests that altered neural connectivity, particularly at the level of synaptic connections, contributes to disease pathology. Dynamic rearrangements of the actomyosin cytoskeleton drive neural circuit formation, including neurite extension and the development of actin-enriched spines at excitatory synapses. Actomyosin regulatory pathways are also one of the major molecular mechanisms disrupted by both Autism-associated copy number variants (CNVs) and de novo mutations. Yet, it is still unknown how actomyosin regulation shapes developing cortical circuits and the impact of specific actomyosin regulatory pathways on Autism pathology. To understand the cytoskeletal mechanisms that lead to altered neural circuitry in Autism, we develop cortical brain organoids from patient induced pluripotent stem cells (iPSCs). Our Autism-derived cortical organoids exhibit increased excitatory synapse formation, similar to post-mortem patient samples and mouse models of Autism. To address whether myosin-II activity contributes to this observed increased in excitatory synapse area, we acutely treated 3-month old neurotypic cortical organoids with the RhoA kinase (ROCK) inhibitor, Y-27632. We confirmed that Y-27632 treatment reduced ROCK-driven myosin-II activation in the cortical organoids through reduced phosphorylation of the myosin regulatory light chain (RLC) at Ser19. Intriguingly, acute Y-27632 treatment mimicked Autism pathology by significantly increasing excitatory pre-synaptic surface area as labeled by vGlut-1. We hypothesize that a corresponding increase in synaptic Rac1 activity mediates this increase in excitatory synaptic surface area. In support of this hypothesis, we observe increased phosphorylation of the Rac1 downstream target, coflin, at Ser3 in Y-27632-treated cortical organoids. These results suggest that Rac1 activity dysregulation could be a driving mechanism underlying Autism synaptic pathology, leading us to investigate which Rac1 activity regulators are present during cortical development. In neurotypic cortical organoids, the Rac1 inactivator, ArhGAP23, is enriched at excitatory synapses. Through the use of a human cortical organoid model, we demonstrate that coordinated myosin-II and Rac1 activity underlie excitatory synapse development, and that alterations in the balance between these actomyosin pathways can promote Autism pathology.
A Non-Canonical Activation of CREB in Excitotoxicity-Induced Neuroprotection in C. elegans
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Excitotoxicity is a key degenerative process that occurs in brain ischemia and results primarily in necrotic neuronal death. During excitotoxicity, a complex network of signaling cascades leads from hyperactivation of Glutamate Receptors (GluRs) to cell death. However, intense cross-talk and redundancy in mammalian signaling cascades obscure our understanding of these mechanisms of cell demise and hinder the development of effective therapy. Surprisingly, GluRs also activate neuroprotective programs that mitigate neurodegeneration, a process where the transcription factor CREB has a key role. However, there is an unexplained contrast between the broad-spectrum nature of CREB's potential function (its ubiquitous expression, the hundreds of stimuli known to activate it, and its capacity to regulate many thousands of genes), and its ability to specifically control a defined subset of gene targets in excitotoxicity. To identify specific mechanisms of CREB activation that form the core of its ability to provide neuroprotection in excitotoxicity, we study this process in C. elegans, a model system that offers powerful research tools and highly conserved signaling pathways. We focus on comparing canonical modes of CREB activation (which involve CREB phosphorylation, as described in both mammals and nematodes), and non-canonical activation modes that involve the co-regulator CRTC. Using our nematode excitotoxicity model we find that the overall neuroprotective role of CREB is well conserved in excitotoxic necrosis. However, in contrast to studies of apoptosis, we find that in excitotoxic necrosis the conserved process of CREB-mediated neuroprotection is mediated by the non-canonical mode of activation, as it is dependent on CRTC but not on CREB phosphorylation. Finding conserved mechanisms that determine CREB's mode of activation, its co-factors, and the targets that are specifically regulated by this pathway during excitotoxicity might facilitate the development of novel therapeutic approaches to mitigate neuronal damage in brain ischemia.

Characterization of a MYO19 knockdown phenotype in a cultured neuron-like cell line
J.L. Bocanegra, J.L. Hawthorne, B.M. Fujita, A. Li, O.A. Quintero

Myosin-XIX (MYO19) is an actin-based motor protein implicated in normal mitochondrial dynamics and distribution. Mitochondrial movement and positioning is thought to be essential in order for energy intensive processes (such as cell division, cell motility, and morphogenesis) to occur efficiently. We hypothesized that MYO19 aids in mitochondrial dynamics and positioning during neuronal differentiation, a complex and energy intensive process resulting in long cellular extensions protruding from the cell body. During this cellular event MYO19 may be involved in supporting cell motility (growth cone or cell body motility) as well as overall morphogenesis (neurite establishment). In order to test this hypothesis, we used the murine, neuron-like CAD cell line and lentiviral infection to generate new lines stably-expressing short-hairpin RNA (shRNA) that target the MYO19 message. We generated multiple non-clonal CAD lines that each expressed a different shRNA, and verified knockdown via western blotting. We then induced differentiation, and wild type and shRNA cultures were imaged over a time-course of multiple days. Cultures were scored for the percentage of cells in the population that developed a visible neuron-like phenotype. Decreased MYO19 expression in the shRNA cell lines lead to a delay in differentiation compared to wild type CAD cells. shRNA-expressing CAD cell lines also showed a decrease in neurite length compared to wild-type cells. To determine whether decreased MYO19 expression led to motility defects, we analyzed cell body motility and neurite extension via quantitative time-lapse microscopy. Cell body velocity was approximately 40-70% slower in shRNA lines compared to wild type, depending on the shRNA line. Additionally, neurite extension velocity was approximately 40% slower than wild type. We then visualized mitochondria via time-lapse microscopy and analyzed their motility within neurites. Interestingly, mitochondrial velocity increased in shRNA lines by approximately 30-50% when compared to wild type, depending on the shRNA line. Although surprising, the observed increase in mitochondrial velocity is in agreement with previous work by Morris, Hollenbeck, and others. They demonstrated that in cultured neurons, axonal mitochondrial velocity increased when actin filaments were destabilized, thereby interfering with the linkage between actin and mitochondria. These results suggest that decreased levels of MYO19 may be influencing CAD cell differentiation by altering the cell's ability to move and position mitochondria via the actin network.
Synaptic vesicle clusters in nerve terminals: an example of liquid-liquid phase separation
Milovanovic Dragomir and Pietro De Camilli

Neurotransmitter containing synaptic vesicles (SVs) form tight clusters at chemical synapses. These clusters act as a reservoir from which SVs are drawn for exocytosis during strong and prolonged activity. Several components associated with synaptic vesicles and likely form such clusters, including synapsin 1, have been identified. However, it remains unclear how SV clustering is compatible with their motility, so that release sites can be rapidly replenished after vesicle fusion events. Recently, liquid-liquid phase separation was shown to be a mechanism through which components of the cytoplasm (protein and RNA) can assemble themselves into distinct compartments without a limiting membrane. A key feature of proteins that can undergo liquid-liquid phase separation is their ability for engaging in multivalent interactions through protein-protein interacting domains and/or low complexity amino acid regions. We are exploring the possibility that SV clusters may represent an example of liquid-liquid phase separation in which one component of the phase are SVs and the other component is represented by proteins of the intervening matrix, synapsin 1 in particular. Synapsin 1 is highly abundant proteins at the nerve terminals, it binds membranes, and it contains an extended low complexity region: all of these properties make synapsin 1 an ideal candidate for mediating the phase separation.

Basigin gene products associate with MCT1, MCT2, and MCT4 in neural tissues
Judith D. Ochrietor, Christopher J. Gilbert, and Joseph D. Fong

It is well known that neurons require large amounts of cellular energy to carry out their various functions. Although it was once thought that neurons solely rely on glucose as a substrate for cellular energy production, it is now known that small monocarboxylate molecules, like pyruvate, lactate, and ketone bodies, are also utilized. Monocarboxylates are transported across plasma membranes via facilitated diffusion using a family of transport proteins known as monocarboxylate transporters (MCTs). Four MCTs (MCT1, MCT2, MCT3, and MCT4) are expressed within neural tissues and cotransport a proton with the monocarboxylate molecule. Recently, these transporters have been linked to nerve regeneration and glioblastoma formation. Expression of the MCTs has been tied to co-expression of a cell adhesion molecule belonging to the Basigin subset of the immunoglobulin superfamily (IgSF). Basigin gene products are known to interact with MCT1 and MCT4 in the mammalian neural retina and this association is essential for the delivery of sufficient substrates to support the cellular energy needs of photoreceptors. An association between Basigin and MCT4 is present in developing glioblastomas, and disruption of the association can reverse glioblastoma growth, presumably by limiting cellular substrates. A previous study by this laboratory indicated that Basigin gene products use hydrophobic amino acids within specific regions of the transmembrane domain to interact with MCT1. We hypothesize that the same amino acids within the transmembrane domain are used to interact with MCT4, but that no association exists with MCT2, which typically interacts with a different member of the IgSF subset. Therefore, the purpose of the present study was to assess the association between Basigin gene products and MCT4, and with MCT2. Recombinant proteins corresponding to the transmembrane domain of Basigin gene products were used in in vitro binding assays with endogenous MCT2 and MCT4 from mouse brain protein lysates. Contrary to the hypothesis, it was determined that the transmembrane domain of Basigin gene products binds to both MCT2 and MCT4 in vitro. Different amino acids within the transmembrane domain of Basigin gene products are used for each association and the pattern is different from that used in the association with MCT1. The data suggest that Basigin, which is expressed in nervous tissues can influence MCT gene expression in those tissues and can associate with more than one MCT protein at the same time.

Nopo, the Drosophila ortholog of the microcephalic primordial dwarfism gene TRAIP, encodes a centrosomal E3 ubiquitin ligase specifically required for mushroom body development
O’Neill, R.S., Galletta, B.J., Fagerstrom, C.J., Rusan, N.M.

Primary microcephaly and microcephalic primordial dwarfism (MPD) are a spectrum of genetic disorders characterized by reduced brain size and, in MPD, reduced body size. Most known primary microcephaly and MPD genes function at centrosomes or in DNA damage response (DDR). While some functional connections between centrosomes and DDR have been established, it is likely that a deeper and more direct link is at play. We believe that primary microcephaly and MPD genes are opportune candidates for uncovering functional connections between centrosomes and DDR pathways.
and, more generally, how these functions impact neurodevelopment. As part of a large screen for neurodevelopmental defects, we homoed in on TRAIP, an MPD gene encoding an E3 ubiquitin ligase known to regulate DDR and apoptosis. Interestingly, the Drosophila melanogaster ortholog nopo (no poles) was named for its loss-of-function phenotype of acentrosomal spindles, suggesting that this DDR gene might function at centrosomes. We performed yeast two-hybrid analysis to reveal extensive interactions between Nopo and several core centrosome proteins, including Sas4, Ana2 and Plk4. Thus, we have established another potential link between a DDR gene and the centrosome. To explore the role of nopo in neurodevelopment, we analyzed both larval and adult brains from nopo mutant animals. We discovered that loss of nopo leads to defects in the mushroom body (MB), a brain region critical for memory formation. Nopo mutant α and β MB lobes are thin, fused, and are often missing. Axon guidance is also abnormal in nopo mutants as we find many misguided MB axons. Our studies of mutants for bendless, which encodes an E2 conjugating enzyme previously shown to interact with Nopo, reveal that 100% of MBs are fused, suggesting that Nopo functions with Bendless to ensure proper brain development and prevent MPD. We are currently focused on identifying the ubiquitination substrates of Nopo required for MB development; candidates include Nopo direct binding partners at the centrosome. Together, this work reveals an exciting new link between the DDR and the centrosome bridged by nopo. Furthermore, we have established D. melanogaster as a new model for understanding the role of TRAIP in neurodevelopment.

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Prion-like transmission of mutant huntingtin aggregates in Drosophila brains
Margaret M.P. Pearce, Kirby M. Donnelly, Weizhe Hong, Liqun Luo, Ron R. Kopito

Huntington’s disease (HD) is an inherited neurodegenerative disorder caused by expansion of a CAG repeat region in exon 1 of the huntingtin (Htt) gene. Htt proteins encoded by this mutant gene contain an expanded polyglutamine (polyQ) stretch near their N-termini and are prone to misfolding and aggregation. Accumulating evidence indicates that mutant Htt protein aggregates have prion-like properties—they spread from one cell to another and convert normally-folded Htt proteins into an aggregated state. We have recently shown in the intact Drosophila central nervous system (CNS) that mutant Htt aggregates in olfactory receptor neurons (ORNs) are cleared by neighboring phagocytic glial cells via Draper-dependent phagocytosis. Remarkably, a proportion of these phagocytosed neuronal Htt aggregates reach the glial cytoplasm and effect prion-like conversion of cytoplasmic, wild-type Htt into aggregates. We have also demonstrated that mutant Htt aggregates originating in ORNs can transfer into the cytoplasm of their post-synaptic partners, projection neurons (PNs) and there nucleate aggregation of wild-type Htt. Surprisingly, ORN-to-PN transmission of mutant Htt aggregates also requires Draper, suggesting that glial phagocytosis plays a central role in transferring aggregates between synaptically-connected neurons. Together, these findings demonstrate that pathogenic Htt aggregates can move between individual neuronal and glial cells in intact brains and suggest that phagocytic glia regulate both the clearance and spreading of aggregate neuropathology in the CNS.

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Proteomic Insights into Cytoskeletal Mechanisms of Neurodegeneration
Cynthia Carreon, Marcela Aguilera-Flores, Igor C. Almeida and Sukla Roychowdhury

Cytoskeletal defects are hallmark of several neurodegenerative diseases. Results from our laboratory indicate that Gbeta-gamma, a major component of G protein-coupled receptor (GPCR) signaling pathway, interacts with microtubules (MTs) and plays an important role in the neuronal differentiation of PC12 cells. Inhibitors of Gbeta-gamma disrupted MTs, blocked neurite outgrowth, and induced neuronal damage, suggesting a role of Gbeta-gamma in neurodegenerative processes. Gbeta-gamma has been shown earlier to promote MT assembly in vitro and in cultured PC12 and NIH3T3 cells. In an effort to understand the mechanism of cytoskeletal disruption and neurodegeneration, we carried out a high-resolution proteomic analysis of cytoskeletal fractions (CSKs) of NGF-differentiated PC12 cells treated with agent known to trigger CSK disruption and block neuronal differentiation. Using Scaffold perSPECTives software to analyze the proteomic data, we found that neuronal differentiation of PC12 cells dramatically altered the proteomic landscape of CSK. 4-Nonylphenol (4-NP), an endocrine disruptor likely to cause neuronal damage, significantly affected the protein composition/pattern of CSK, including the association with Gbeta-gamma. String Link and Gene Ontology enrichment analysis indicated that several biological pathways are affected by 4-NP, including pathways related to Alzheimer’s disease, Parkinson’s disease, and Huntington disease. This study has potential to identify new biomarkers and/or pathways involved in disruption of CSK and the development of neurodegenerative disorders.
Fic-mediated Adenylylation/AMPylation in Parkinson’s disease  
A. Sanyal, A. Chandran, S. Dutta, A.A. Koller, B. Watson, J. Rochet, S. Mattoo

During disease, cells experience various stresses that manifest as an accumulation of misfolded proteins and eventually lead to cell death. To combat this stress, cells activate a pathway called UPR (Unfolded Protein Response) that functions to maintain ER (endoplasmic reticulum) homeostasis and determines cell fate. We recently reported a hitherto unknown mechanism of regulating ER stress via a novel post-translational modification (PTM) called Adenylylation/AMPylation. Specifically, we showed that the human Fic protein, HYPE/FicD, catalyzes the addition of an AMP (adenosine monophosphate) to the ER chaperone, BiP, to alter the cell’s UPR response to misfolded proteins. Here, we report that we have now identified a role for HYPE in preventing aggregation of alpha-Synuclein (aSyn), an established cause of Parkinson’s disease (PD) neuropathy.

Modeling Protein Aggregation and the Heat Shock Response in ALS iPSC-derived Motor Neurons  
Emily R. Seminary, Samantha L. Sison, Allison D. Ebert

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder caused by the selective loss of the upper and lower motor neurons. Only 10% of all cases are caused by a mutation in one of the two dozen different identified genes, the most common of which are C9orf72, SOD1, and TDP-43, together responsible for at least 60% of familial ALS. The remaining 90% of cases are likely caused by a combination of as yet unidentified genetic and environmental factors. Remarkably, despite the large degree of heterogeneity, all cases of ALS have protein aggregates in the brain and spinal cord that are immunopositive for SOD1, TDP-43, OPTN, and/or p62. Protein inclusions are normally prevented and cleared by heat shock proteins (Hsps), suggesting that ALS motor neurons have an impaired ability to induce the heat shock response (HSR). Accordingly, there is evidence of decreased induction of Hsps in ALS mouse models and in human post-mortem samples compared to controls. However, the role of Hsps in protein accumulation in human motor neurons has not been fully elucidated. Here we generated motor neuron cultures from human induced pluripotent stem cell (iPSC) lines carrying mutations in SOD1, TDP-43, or C9orf72. We show that despite a lack of overt motor neuron loss, there is an accumulation of insoluble aggregation prone proteins in iPSC-derived motor neuron cultures but that content and levels vary with genetic background. Additionally, protein aggregation corresponds to an incomplete induction of the HSR and minimal stress granule formation. We therefore conclude that ALS iPSC-derived motor neurons recapitulate key early pathological features of the disease and fail to endogenously upregulate the HSR in response to increased protein burden. As such, we believe that iPSCs represent a valuable model to further study the role of the HSR in ALS.

Phagocytic Response in Microglia/Macrophages Correlates With Severity of Neurodegeneration and Behavioral Deficits in the NPC1 nmf164 Mutant Mouse  
Larisa Kavetsky, Kayla Green, Victoria Kuhnel and Ileana Soto

In Niemann Pick Type-C (NPC) disease, progressive and severe degeneration of neurons in different regions of the brain, but more severely in the cerebellum, is accompanied by neuroinflammation. Although neuroinflammation is considered a pathological hallmark of NPC, the temporal activation and its contribution to neuronal degeneration have not been elucidated. The aim of this study was to determine the sequence of neuroinflammation and its correlation with Purkinje cell loss and behavioral deficits in the NPC1 nmf164 mouse model of the disease. At early stages of NPC disease (4wk), when no signs of motor dysfunction were detected, microglia reactivity and activation were already evident by significant changes in IBA1+ cell number and morphology. Also, accumulation of CD68+ phagosomes in microglial cells was already evident at early stages of the disease. At stages of moderate (8wk, ~50%) and severe (12wk, ~80%) loss of Purkinje cells, the number of IBA1+ microglia/macrophages was significantly elevated and the morphology of this cells was found less ramified and more amoeboid shape. In addition, the accumulation of CD68+ phagosomes and autofluorescent material in these cells was remarkably higher when compared with younger NPC1 nmf164 mice, indicating increased phagocytic activity and lack of proper lysosomal function in these cells. We also found that the majority of these phagocytic cells were negative for the resident microglia marker TMEM119, suggesting that the majority of IBA1+ myeloid cells in the...
cerebellum at these stages of NPC disease are infiltrating monocytes. The increased neuroinflammation found in the late stages of NPC was directly correlated with the loss of Purkinje cells and motor deficits in the NPC1nmf164 mutant mouse. Our findings suggest that neuroinflammation in the cerebellum of NPC1nmf164 mutant mice is a pathological event that precedes the death of Purkinje cells. We are currently studying the correlation of microglia reactivity and the degeneration of Purkinje cell dendrites during early stages of the NPC disease when no loss of Purkinje cells is detected.

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Expression of WIPI2B counteracts age-related decline in autophagosome biogenesis in neurons
Andrea KH Stavoe, Erika LF Holzbaur

Autophagy defects are implicated in multiple late-onset neurodegenerative diseases. Since aging is the most common risk factor in neurodegeneration, we examined autophagy in aged neurons. Autophagosome biogenesis is known to occur preferentially at the distal tips of axons, and autophagosomes mature as they transit to the soma. We compared autophagosome biogenesis in neurons from young adult and aged mice, identifying a significant decrease in the biogenesis of autophagosomes during aging. While nucleation and initiation rates did not change during aging, we observed the frequent production of stalled Atg-13-positive, LC3-negative isolation membranes in neurons from aged mice. These stalled structures exhibited aberrant membrane morphology and failed to resolve into LC3-positive autophagosomes. Further, the majority of stalled autophagosomal structures were Atg9-positive, while autophagic vesicles that successfully recruited LC3 did not retain Atg9. To identify the underlying molecular defect, we queried expression levels of autophagy proteins and identified a specific reduction in the PI3P-binding protein WIPI2 in aged mouse brain. WIPI2 depletion in young neurons was sufficient to stall autophagosome biogenesis, phenocopying aged neurons. Importantly, reconstituting WIPI2 expression effectively restored autophagosome biogenesis in aged neurons. We additionally determined that the PI3P and Atg16L1 binding domains of WIPI2 were required for WIPI2-induced restoration of autophagosome biogenesis. Together, these data suggest a novel therapeutic target in age-associated neurodegeneration.

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The pivotal role of BAG3 in mediating autophagy and tau clearance in neurons
Maoping Tang, Gail VW Johnson

Phosphorylated tau shows an increased tendency for aggregation, and tau aggregation is a prominent feature of several neurodegenerative diseases, with the most notable being Alzheimer’s disease (AD). It’s also evident that pathological tau accumulation contributes to disease progression which is likely due in part to inefficient clearance mechanisms. Thus, approaches that facilitate tau clearance are being considered as therapeutic strategies for the treatment of AD. Autophagy plays a prominent role in the maintenance of neuronal proteostasis. Here, we report a novel mechanism of tau degradation by selective autophagy which is mediated by the co-chaperone Bcl2-associated anthanogene 3 (BAG3). BAG3 has been shown to be an essential component of a complex that targets substrates to the autophagy pathway for degradation. In rat primary neurons, we found that the proteasome inhibitor MG132 increases endogenous BAG3 expression and decreases phospho-tau levels. Further, knocking down BAG3 resulted in increases in the expression of the autophagy adaptor proteins SQSTM1/p62 and NBR1, as well as decreased LC3-II, concurrent with increases in the levels of phospho-tau species. Re-expression of BAG3 subsequent to knockdown rescued the effects of BAG3 deletion with decreases in phospho-tau levels. To analyze autophagic vacuole (AV) numbers and distribution neurons were transfected with a mCherry-LC3 reporter. AVs were analyzed in neurites and cell bodies separately and the data revealed that BAG3 deletion decreased the number of LC3 puncta in both compartments, but the size of AVs were larger, which indicated that BAG3 had a role in the autophagic process. Data from the proteinase K protection assay showed that BAG3 deletion increased phospho-tau and SQSTM1/p62 inside the completed autophagosomes, which suggests that autophagosomes were properly enclosed but the degradation process was blocked in the BAG3 depleted neurons, likely due to impaired fusion with lysosomes. The exact mechanisms by which BAG3 facilitates fusion of autophagosomes and lysosomes, and/or tau targeting and degradation in autolysosomes, will be determined in future studies. Taken together, these results indicate that BAG3 plays an important role in not only regulating tau clearance via macroautophagy, but also in the process of autophagic flux in neurons.
Palmitoylation-dependent Control of Myelination in Oligodendrocytes
Prasad Kanupathi, Dale D.O. Martin and Gareth M. Thomas

Myelination of neuronal axons is essential for rapid conduction of nerve impulses. Oligodendrocytes, the myelinating cells of the Central Nervous System, produce large amounts of myelin proteins, but how these proteins are trafficked to their appropriate destinations and arranged into compact myelin is unclear. Over 25 years ago, it was found that several major myelin proteins undergo palmitoylation, a protein-lipid modification that can dramatically affect protein targeting. However, since that time the functional role of myelin protein palmitoylation and the protein acyltransferases (PATs) that mediate this process have remained almost completely unaddressed. We found that a specific PAT, ZDHHC9, and its essential partner Golga7 are highly enriched in oligodendrocytes and that DHHC9/Golga7 co-operate to palmitoylate a novel site on Myelin Basic Protein (MBP), a major constituent of myelin. Interestingly, human ZDHHC9 mutations cause a form of Intellectual Disability associated with major decreases in white matter volume. These findings suggest that palmitoylation of MBP and perhaps other myelin proteins by ZDHHC9/Golga7 may be essential for normal myelination and higher brain function.

Induction of Activator of G-Protein Signaling 3 Puncta: Role of Serine/Threonine Residues in the G-Protein Regulatory Domain and Lysosomal Inhibition
Ali Vural, S. Sadik Oner, Dzwokai Ma, Stephen M. Lanier

Activator of G-protein Signaling 3 (AGS3), a receptor independent activator of G-protein signaling, contains 7 tetra-tricopeptide repeats (TPR) and 4 G-protein regulatory motifs (GPR) connected by a linker region. Disruption of TPR organization by point and deletion mutations induce the formation of punctate structures facilitating entrance into the aggresomal pathway. To further define regulatory factors affecting AGS3’s subcellular “positioning”, we addressed the role of potential sites of protein phosphorylation in the GPR domain. AGS3 is a phosphoprotein with putative phosphorylation sites in the GPR domain. We generated a series of AGS3 constructs with mutations of potential serine/threonine (S/T) phosphorylation sites in the GPR domain and transiently expressed the cDNA constructs in COS-7 and HEK-293 cells. Mutation of all 24 S/T residues in the GPR domain to alanine (AGS3-GFP-PM1) resulted in the distribution of AGS3 to cytosolic punctate structures in marked contrast to the distribution of WT AGS3 (cell cortex and diffuse cytosolic). Proteasome inhibition shifted AGS3-GFP-PM1 puncta to a perinuclear aggresome. The subcellular distribution of AGS3-GFP-PM1 to the pre-aggresomal punctate structures was also observed with mutation of a single residue (T602A) located between GPR-III and GPR-IV. Coexpression of Giα prevented the observed distribution of AGS3-GFP-T602A to the pre-aggresomal punctate structures, whereas cell treatment with the Giα antagonist gallein (10 µM) or with pertussis toxin (200 ng/ml) had no effect. Interestingly, the preaggresomal punctate distribution observed with AGS3-GFP-T602A was also observed with WT AGS3 upon cell treatment with the lysosome inhibitor (ammonium chloride - 25 mM, 24 hrs) and a protease inhibitor cocktail (Sigma; 1:100 dilution), but not with pharmacological manipulation of autophagy. These data are consistent with the hypothesis that the movement of AGS3 into or within the aggresomal pathway, and its potential functional role in this pathway, is a regulated process influenced by cell signaling mechanisms.

Elucidate the genetic architecture of age-dependent neurodegenerative diseases
Jinglin L. Xie, Sathvik Palakurty, Dan F. Jarosz

Neurodegenerative disease poses a major health problem in developed (and increasingly developing) countries. The most prevalent age-related NDs share a molecular link in the propensity for specific proteins to misfold and form aggregates in the brain. Previous work has established that neurodegenerative disease-associated human proteins can also aggregate and cause toxicity in the model yeast Saccharomyces cerevisiae. In fact, systematic deletion and overexpression screens in yeast have identified a number of genes that can modulate this toxicity. However, genetic variation consequential to human patients typically does not exist as precise deletion and overexpression constructs employed in genetic screens. As such, it is pertinent to identify the natural genetic variation that can modify the toxicity caused by neurodegenerative disease-associated proteins. Our preliminary study has demonstrated that closely-related wild yeast isolates show
varying degrees of resistance to this toxicity. By comparing the sequenced genomes and employing an approach we have recently pioneered to identify causal mutations at unprecedented resolution, I can uncover potential drug targets and biomarkers for various neurodegenerative diseases. To investigate the interplay between aging and genetic susceptibility to neurodegenerative disease, I will take advantage of a genetically-engineered yeast strain that allows for the enrichment of cells of different ages, and examine the effect of these candidate mutations in young and aged cells. This work is poised to shed light on the genetic basis of NDs in the context of aging, and uncover candidate mutations that are important for the detection and treatment of the most common neurodegenerative diseases.

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Par3 regulates retrograde trafficking of BACE1 and limits its convergence with APP
Miao Sun and Huaye Zhang

The cleavage of amyloid precursor protein (APP) by β-site APP cleaving enzyme 1 (BACE1) is the rate limiting step in the generation of β-amyloid (Aβ) during Alzheimer’s disease (AD) pathogenesis, making the physical convergence of APP and BACE1 a prerequisite for Aβ generation. However, the mechanisms regulating APP/BACE1 convergence remain unclear. Here we show that loss of the polarity protein Par3 reduces retrograde trafficking of BACE1 and increases its convergence with APP within the axon, which leads to increased intracellular Aβ accumulation. Par3 functions by interacting with BACE1 and promoting the phosphorylation of BACE1 at Ser498 through recruiting atypical PKC to the membrane. In human AD brains, there is a loss of both Par3 and Ser498 phosphorylated BACE1, suggesting that abnormalities in Par3-mediated BACE1 retrograde trafficking is important in the AD pathogenic process. Together, our studies provide mechanistic insight into a novel role for Par3 in regulating APP/BACE1 convergence and shed light on the mechanisms of AD pathogenesis.

Afternoon Poster Session Abstracts

3:15 am

2
Compromising microtubule stability leads to neurodegeneration in mice
Georgia Buscaglia, Jayne Aiken, Jeff Moore, Emily Bates

In neurons, microtubules help to establish the distinct architecture of the axons and dendrites, and facilitate protein trafficking. Mutations that disrupt TUBA1A, an alpha tubulin, cause developmental brain malformations. Indeed, we showed that an Asparagine to Aspartic acid substitution at residue 102 (Tuba1aND) results in developmental brain abnormalities and perinatal death in homozygous Tuba1aND mice. Data from yeast and mice showed that the Tuba1aND mutation compromises the stability of microtubules. Interestingly, mice heterozygous for this mutation (Tuba1aND/+) develop abnormal motor phenotypes with age, suggestive of neurodegeneration, despite apparently normal brain development. Microtubule networks are lost and disorganized in many neurodegenerative diseases, but it is not clear whether microtubule dysfunction causes neurodegeneration or is a result of loss of neuron health. Our data suggests that compromising microtubule function can cause neurodegeneration. The Tuba1aND/+ mutation is located adjacent to a magnesium (Mg2+) binding site in alpha tubulin and Mg2+ stabilizes tubulin heterodimers. We demonstrate that Mg2+ supplementation can partially attenuate the Tuba1aND/+ motor phenotypes. Therefore, we can manipulate the scale of microtubule dysfunction within the brain at different developmental and post-developmental time points using Mg2+. The Tuba1aND allele allows us to dissect the mechanism of microtubule function in neurodegeneration.

4
Changes in Cellular Prion Protein Sequence and Glycosylation Permit Propagation of Protein-only Conformer In Vitro
Cassandra Burke, Daniel Walsh, Geoffrey Noble, Umberto Agrimi, Michele Di Bari, Abigail Diack, Jean Manson, Surachai Supattapone

The protein-only hypothesis predicts that the abnormal conformer of the prion protein, PrPSc, is the sole component of infectious prions. However, achieving direct experimental support for this hypothesis has been difficult because multiple attempts to generate prions with significant levels of specific infectivity from pure prion protein have failed. As a step towards this goal, we sought to identify cellular prion protein (PrPC) substrates that are capable of propagating pure (protein-only) PrPSc molecules in vitro. We found that both bank vole (BV)PrPC and a mouse (Mo)PrPC mutant lacking
the second (C-terminal) glycosylation site (G2) are effective substrates for propagating protein-only recPrPSc molecules in vitro. Protein-only recPrPSc molecules potently seeded bank vole brain homogenate serial Protein Misfolding Cyclic Amplification (sPMCA) reactions at template concentrations <60 pg/mL, but failed to seed mouse sPMCA reactions at 100,000-fold higher concentrations. Additional analyses with chimeric substrates indicated that the ability of BVPrPC to propagate protein-only PrPSc requires BV-specific residues within the extreme C-terminal domain. In summary, we have identified the first PrPC substrates that can be seeded by protein-only PrPSc molecules and suggest novel experimental models to rigorously test the protein-only hypothesis.

6
Endogenous alpha-synuclein expression patterns revealed using a novel mouse model

Alpha synuclein (aSyn) is involved in synaptic vesicle trafficking and synaptic transmission, but is also strongly linked to Parkinson’s disease (PD) and other neurodegenerative disorders. In diseases where aSyn aggregates, or synucleinopathies, it accumulates in and spreads to different brain areas and peripheral organs. Although increased aSyn levels likely underlie familial PD with SNCA mutations thus implicating the protein in disease initiation, most synucleinopathies arise sporadically indicating that the expression of normal levels of wild type aSyn is sufficient for the development of disease. In spite of our increasing knowledge in the field, the physiological function of aSyn and its precise role in disease remain enigmatic urging the development of new tools for further investigations. Here, we report the development and characterization of a new mouse model expressing a GFP-aSyn fusion protein under the control of the endogenous Snca promoter. We describe the expression pattern of the fusion protein in the brain and peripheral organs and characterized its subcellular localization and trafficking in the brain. Primary neurons expressing GFP-aSyn were also successfully derived from this line. In addition, intracerebral injection of aSyn pre-formed fibrils induced formation of GFP-positive inclusions with a similar distribution pattern to that observed in wild type mice. We anticipate that this new mouse model will facilitate in vitro and in vivo studies that incorporate live imaging and detection of endogenous alpha synuclein, therefore providing new insights into aSyn function in health and disease.

8
The RNA binding protein Zfp106 protects against neurotoxicity caused by C9orf72 GGGGCC repeats
B Celona, J Von Dollen, SC Vatsavayai, R Kashima, JR Johnson, AA Tang, A Hata, BL Miller, EJ Huang, NJ Krogan, WW Seeley, BL Black

Expanded GGGGCC repeats in the first intron of the C9orf72 gene represent the most common cause of familial ALS, but the mechanisms underlying repeat-induced disease remain incompletely resolved. One proposed gain-of-function mechanism is that repeat-containing RNA forms aggregates that sequester RNA binding proteins, leading to altered RNA metabolism in motor neurons.

To identify new proteins associated with GGGGCC repeats, we performed RNA pulldown assays, followed by mass spectrometry and identified Zfp106 as a specific GGGGCC RNA repeat-binding protein. We found that Zfp106 binds directly to GGGGCC RNA repeats, and functionally interacts with the repeats in cultured neuronal cells. To gain additional insight into the molecular function of Zfp106, we used mass spectrometry to identify protein interactors of Zfp106. Remarkably, we found that Zfp106 interacts with multiple other RNA binding proteins, including the ALS-associated factors TDP-43 and FUS.

Zfp106 is highly expressed in skeletal muscle and motor neurons, and its human ortholog ZNF106 is located at chromosome 15q15.1, a locus associated with a familial recessive form of ALS. Therefore, we genetically inactivated the Zfp106 gene in mice. Zfp106 knockout mice develop severe motor neuron degeneration, which can be suppressed by transgenic restoration of Zfp106 specifically in motor neurons.

Finally, we used Drosophila as an in vivo gain-of-function model of C9orf72 neurodegeneration to test if the interaction between Zfp106 and GGGGCC repeats had a functional consequence on neurotoxicity. Expression of Zfp106 in glutamatergic neurons suppressed the loss of larval active zones at NMJs and pupal lethality caused by expression of 30 copies of GGGGCC. Moreover, Zfp106 co-expression partially suppressed the adult locomotor defect caused by expression of C9orf72 repeats. Therefore, Zfp106 is a potent suppressor of GGGGCC repeat-mediated neurotoxicity in a Drosophila model of C9orf72 ALS.
10 WITHDRAWN

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Tau protein disrupts nucleocytoplasmic transport in Alzheimer’s disease
Bahareh Eftekharzadeh, J. Gavin Daigle, Larisa E. Kapinos, Jeffrey D. Rothstein and Bradley T. Hyman

Tau protein, which normally functions to stabilize microtubules, is the major constituent of neurofibrillary tangles in Alzheimer’s disease (AD). The accumulation of tau in neuronal soma correlates with neuronal loss. However, the mechanism underlying tau associated neurodegeneration remains unclear. We now show that hyperphosphorylated tau can interact with nucleoporins (nups), protein constituents of the nuclear pore complex (NPC) and affect its functional integrity. Pathological tau leads to disruption of nuclear pore complex proteins, reduction of NPC complexes, cytoplasmic mislocalization of nups, and impairs nuclear export and import, in vitro, in tau overexpressing transgenic mouse models, and in human Alzheimer tissue. Correspondingly, nuclear pore component, Nup98, surprisingly colocalizes with neurofibrillary tangles in neuronal soma, and both in vivo and in vitro directly interacts with tau and facilitates its aggregation. These data support the hypothesis that phospho-tau directly interacts with nuclear pore complex constituents, leading to their mislocalization and to disruption of nuclear pore function, raising the possibility that nuclear pore dysfunction contributes to tau induced neurotoxicity in Alzheimer’s disease.

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The Golgi Outpost Protein TPPP Mediates Uniform Microtubule Polarity and Branching in Oligodendrocytes
Meng-meng Fu, Juan Oses-Prieto, Cheng-Yun Lee, Nay Saw, Rebecca Shi, Madhuri Nori, Mehrdad Shamloo, Al Burlingame, Ben A. Barres

Oligodendrocytes are specialized glial cells in the central nervous system that produce myelin, the fatty layers of insulation that wrap around axons to facilitate efficient action potential conduction. Unlike Schwann cells in the peripheral nervous system that ensheathe a single axonal segment, one oligodendrocyte can ensheathe multiple axonal segments and consequently extends multiple processes. These microtubule-rich processes are elaborate and highly branched, yet it is unclear how they are organized and how they form. We now show using live-cell imaging that microtubules in oligodendrocytes have uniform polarity, with growing EB3-labeled plus ends directed away from the cell body. Interestingly, though polarity is consistent throughout oligodendrocyte differentiation, speeds of polymerization vary at different developmental time points. In addition, we now show by immunostaining that oligodendrocyte processes contain Golgi outposts, which may act as a source of acentrosomal microtubule nucleation at sites that are far from the cell body. Previous experiments in Drosophila neurons have demonstrated roles for Golgi outposts in microtubule nucleation and dendrite branching. In order to screen for candidate Golgi outpost interactors, we used our lab’s RNA-Seq database to identify microtubule-associated proteins that are highly and specifically expressed in oligodendrocytes. We identify TPPP (tubulin polymerization promoting protein) and show that it selectively localizes to Golgi outposts but not to Golgi bodies in the cell body. Knockdown of TPPP results in aberrantly mixed microtubule polarity and increased branching in oligodendrocytes, but does not alter the speed of EB3-labeled plus ends, suggesting that contrary to its name, TPPP does not actually mediate microtubule polymerization in cells. An alternative possibility is that TPPP stabilizes microtubules by binding at the minus end and we are currently addressing this by using polarity marked microtubules and in vitro kinesin motility assays. In addition, we are using mass spectrometry to identify the Golgi outpost proteome, by purifying Golgi from rat pup brains followed by immunoprecipitation against TPPP. Finally, preliminary data from mice indicate that TPPP knockout may lead to sensorimotor deficits and anxiety-like behavior. Together, our data demonstrate that TPPP is required for uniform microtubule polarity and process branching in oligodendrocyte development.
Functional characterization of an in vitro generated 3-D nerve bundle using capillary alginate gel on a microelectrode array

**Dale S. George, Wesley A. Anderson, Alexander J. Bosak, Alicia R. Willenberg, Frank Sommerhage, Bradley J. Willenberg and Stephen Lambert**

Microelectrode arrays (MEAs) are a useful tool for monitoring the functional activity of electrically excitable cells. On an MEA, traditional 2-D neuronal cultures fail to provide detectable action potentials from individual axons, and instead, electrical readings are recorded largely from the neuronal cell body. Here, we show the functional characterization of an *in vitro* generated 3-D peripheral nerve bundle using a capillary alginate gel (Capgel™) from the dorsal root ganglion (DRG) of embryonic rats on an MEA. We demonstrate that, in the absence of neuronal cell bodies, bundling and outgrowth of axons within the gel lead to recordable action potentials. We also show that electrical readings can be recorded over several weeks and the well-wide mean firing rate of the 3-D nerve bundle is far superior to the 2-D control conditions. Further analysis of individual electrodes reveals the presence of multiple action potentials on the same electrode, indicating that several different axons within the same bundle are contributing to the electrical activity. This was further validated by treatment of the 3-D nerve bundles with capsaicin, a compound that elicits a response from nociceptive neurons. In response to capsaicin, we observe that some axons within the bundle have firing rates that are four times the basal readings, whereas other axons within the same bundle show no change in the firing rate. This supports the idea that the nerve bundles generated using Capgel™ are a heterogeneous population of axons which recapitulate the composition of in vivo nerve fascicles. Overall, we show here in the context of recording electrophysiological activity that a 3-D environment facilitates bundling of axons and yields enhanced recordable action potentials over longer periods of time. Clinically, hyperexcitability of the DRG is one of the important underlying mechanisms of neuropathic pain. We propose the use of this 3-D system for electrophysiological studies that may provide insight into cellular behaviors and serve as a platform for the development of new therapeutic targets.

Defining the role of primary cilia in neuronal function and neural degeneration

**Emily Bowie, Ryan Norris, Kathryn Anderson, and Sarah Goetz**

The serine-threonine kinase Tau tubulin kinase 2 (TTBK2) is required to initiate cilium assembly. It is also mutated in a dominantly inherited human ataxia: Spinocerebellar Ataxia Type 11 (SCA11). SCA11 is characterized by progressive cerebellar ataxia due in part to a loss of Purkinje neurons in the cerebellum. Four SCA11-associated mutations have been identified that cause similar truncations of TTBK2 following the kinase domain. The mechanisms by which these truncations cause disease remain unclear. We propose that ciliary dysfunction may be an underlying cause or contributing factor to neurodegenerative conditions such as SCA11.

To test this hypothesis, we are using a variety of mutant mouse lines including an allelic series of Ttbk2 as well as conditional alleles of Ttbk2 and other ciliary genes, as well as cell culture-based assays to test how the SCA11-associated mutations cause disease and examine the requirements for primary cilia within the developing and adult brain.

We find that the TTBK2 truncations associated with SCA11 are unable to mediate cilium assembly and that they also impair the function of WT TTBK2. We further show that conditional removal of Tbk2 throughout the brain of young adult mice leads to a rapid loss of cilia from post-mitotic neurons and the emergence of neurological phenotypes, including motor impairment and gait abnormalities. Our data are consistent with an important role for cilia in neural function and a link between ciliary dysfunction and neurodegenerative phenotypes. Ongoing studies in our lab focus on defining the specific cellular changes within the brain caused by loss of cilia and defining how ciliary signaling contributes to neural function.
Vesicular transporters heterogeneity regulates vesicle dynamics, localization and synaptic transmission in mouse central synapses
Laurent Guillaud, Abdelmonein Eshra, Dimitar Dimitrov, Tomoyuki Takahashi

At excitatory synapses, vesicular glutamate transporters (VGLUTs) are essentially thought to play a role in the refilling of neurotransmitter into synaptic vesicles (SVs). We have recently demonstrated that over-expression of 2 different isoforms, VGLUT1 and VGLUT2, can confer distinct dynamic properties to SVs in cultured giant presynaptic terminals. However, the functional significances of these different vesicle dynamics remained to be clarified. Here we show that upon co-expression of fluorescently labelled VGLUT1 and VGLUT2 in same terminals, SVs can be assorted into at least 2 different pools: VGLUT1-containing vesicles with high mobility and VGLUT2-containing vesicles with lower mobility. In physiological conditions, proximity ligation assay also revealed that VGLUT1-containing vesicles localized more efficiently to release sites than VGLUT2-containing vesicles. Electrophysiological recording and synapto-pHluorin imaging finally demonstrated that VGLUT1 over-expression increases synaptic transmission and vesicle recycling compared to VGLUT2 over-expression. Thus, we propose that VGLUTs heterogeneity might contribute to the regulation of synaptic transmission by modulating vesicle dynamics and trafficking, and controlling their accessibility to active zones.

Novel concepts of microtubule regulation during neuronal growth, maintenance and degeneration
Ines Hahn, Yue Qu, Jill Parkin, Meredith Lees, Andreas Prokop

Axons are the slender processes of neurons which electrically wire the nervous system which need to be maintained for decades. They are key lesion sites in trauma, neurodegenerative diseases and ageing. Parallel bundles of microtubules (MTs) form the structural backbones and life-sustaining transport highways of axons. Therefore, the formation and maintenance of ordered MT bundles is a key factor of axon development and longevity. However, the relevant underlying mechanisms are not known.

Using systematic combinatorial genetics of numerous actin and MT regulators, we developed the novel concept of “local axon homeostasis” which proposes that different mechanisms of MT regulation act jointly all along axons to maintain organised MT bundles. We previously reported one mechanism, mediated by the actin-MT linker Shot, which ensures that MTs are laid into parallel bundles. Here, we report two further contributing mechanisms: First, a cortical MT collapse factor provides a novel check point mechanism which can eliminate MTs that leave the bundled organisation and go off track escaping the guidance mechanism. When artificially detached from the membrane, this factor is able to deplete entire MT networks. Secondly, using a combination of super-resolution microscopy and genetics we found that evolutionary conserved periodic cortical actin rings in axons play important roles in sustaining the polymerisation of MTs, thus promoting MT bundles and counterbalancing MT collapse factor functions. To uncover the underlying mechanisms of this novel concept, we study functional links of cortical actin to MT-binding proteins which we identified as being essential for axonal MT polymerisation.

In conclusion, our local homeostasis concept provides a new framework for studying the processes of axon development, ageing, degeneration and regeneration.

A synthetic Hox locus to determine the epigenetic rules of neural differentiation
Sudarshan Pinglay, Jef Boeke, Liam Holt, Matthew Maurano and Esteban Mazzoni

Development requires the precise spatial and temporal control of gene expression. Hox genes have become models for this type of gene regulation due to their complex expression pattern. Mammalian Hox genes are organized into four chromosomal clusters (A, B, C and D), each of which harbors a subset of 13 paralogous Hox genes. In order to perform their evolutionarily conserved function of segmenting the body axis, they are expressed in a pattern that is colinear to their chromosomal organization. In vertebrates, certain subsets of Hox genes are expressed in specific tissues in order to control the formation of axial appendages such as limbs and the brain. This neo-functionalization of gene expression patterns in vertebrates has been most well studied in the developing limb bud of mice, and seems to be due to the action of distal enhancer elements. These enhancers associate with promoters of specific Hox genes and activate transcription. How these
enhancers selectively associate with their target genes at the required time remains largely unknown. In this project, we propose to utilize the power of megabase-scale DNA synthesis to construct synthetic mouse Hox (synHox) clusters with highly multiplexed, designer variations. Delivery of these highly variant, locus scale constructs to both endogenous and ectopic genomic loci will enable us to understand the mechanism of chromatin domain formation by identifying locus constituents that are necessary in the endogenous context and sufficient in the ectopic context to establish chromatin domains.

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Phagocytic Response in Microglia/Macrophages Correlates With Severity of Neurodegeneration and Behavioral Deficits in the NPC1nmf164 Mutant Mouse
Larisa Kavetsky, Kayla Green, Victoria Kuhnel and Ileana Soto

In Niemann Pick Type-C (NPC) disease, progressive and severe degeneration of neurons in different regions of the brain, but more severely in the cerebellum, is accompanied by neuroinflammation. Although neuroinflammation is considered a pathological hallmark of NPC, the temporal activation and its contribution to neuronal degeneration have not been elucidated. The aim of this study was to determine the sequence of neuroinflammation and its correlation with Purkinje cell loss and behavioral deficits in the NPC1nmf164 mouse model of the disease. At early stages of NPC disease (4wk), when no signs of motor dysfunction were detected, microglia reactivity and activation were already evident by significant changes in IBA1+ cell number and morphology. Also, accumulation of CD68+ phagosomes in microglial cells was already evident at early stages of the disease. At stages of moderate (8wk, ~50%) and severe (12wk, ~80%) loss of Purkinje cells, the number of IBA1+ microglia/macrophages was significantly elevated and the morphology of these cells was found less ramified and more amoeboid shape. In addition, the accumulation of CD68+ phagosomes and autofluorescent material in these cells was remarkably higher when compared with younger NPC1nmf164 mice, indicating increased phagocytic activity and lack of proper lysosomal function in these cells. We also found that the majority of these phagocytic cells were negative for the resident microglia marker TMEM119, suggesting that the majority of IBA1+ myeloid cells in the cerebellum at these stages of NPC disease are infiltrating monocytes. The increased neuroinflammation found in the late stages of NPC was directly correlated with the loss of Purkinje cells and motor deficits in the NPC1nmf164 mutant mouse. Our findings suggest that neuroinflammation in the cerebellum of NPC1nmf164 mutant mice is a pathological event that precedes the death of Purkinje cells. We are currently studying the correlation of microglia reactivity and the degeneration of Purkinje cell dendrites during early stages of the NPC disease when no loss of Purkinje cells is detected.

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Nanoscale redistribution of NMDA receptors subunits in anti-NMDA receptor autoimmune encephalitis

The anti-N-methyl-D-aspartate receptor (NMDAR) autoimmune encephalitis is a neuropsychiatric disorder mediated by NMDAR autoantibodies. Patients’ antibodies cause NMDAR internalization but the early events at the synaptic level that lead to the depletion of surface NMDARs are poorly understood. Here, using super-resolution microscopy and Monte Carlo simulations, we studied the effects of NMDAR autoantibodies on the nanoscale distribution of NMDAR subpopulations. Our results show an early, antibody-induced clustering of synaptic and extrasynaptic receptors. This clustering is subunit specific and mainly affects GluN2B-containing NMDARs. Following receptor internalization, the remaining surface NMDARs return to control clustering levels but are preferentially retained at the synapse. These results are recapitulated by Monte Carlo simulations if a model is considered by which antibodies induce NMDAR cross-linking and disruption of NMDAR-protein interactions within and outside the synapse. Finally, activation of ephrin-B2 receptor partially restores the nanoscale surface distribution of NMDARs.
Loss of dual leucine zipper kinase signaling is protective in the SOD1 mouse model of ALS

Hallmarks of chronic neurodegenerative disease include progressive synaptic loss and neuronal cell death, yet the cellular pathways that underlie these processes remain largely undefined. Here we provide evidence that Dual Leucine Zipper Kinase (DLK) is an essential regulator of neurodegeneration in Amyotrophic Lateral Sclerosis (ALS). We demonstrate that DLK/JNK pathway activity is increased in the SOD1 mouse model as well as sporadic ALS patients, and that genetic deletion of DLK protects against axon degeneration, neuronal loss, and functional decline in vivo. We have developed DLK inhibitors that preserve neuromuscular junction synapses upon chronic dosing in the SOD1 mouse. Furthermore, in an acute model of neurodegeneration, pharmacological inhibition of DLK pathway signaling is capable of reversing injury-related gene expression changes, suggesting that DLK inhibition could be a viable treatment option even after disease symptoms are already established. Finally, we have identified that pathological activation of DLK is a conserved mechanism that generalizes beyond ALS, regulating neurodegeneration in several disease models, thus making it an attractive target for therapeutic intervention in multiple neurodegenerative indications.

Loss of Lebercilin causes a severe alteration of RPE maturation and ciliary function in cellular and animal experimental models for LCA5
Lanfranco Leo, Jean Bennett, Jason Mills

Leber Congenital Amaurosis (LCA) is the most severe type of retinal dystrophies causing a very rapid and severe loss of vision in human and related experimental models. Like many of the other pathologies causing visual impairment, LCA causes an evident dysfunction at the photoreceptor level, leaving the other neuronal components in the vision network seemingly unaffected. There have been 18 identified genes associated with LCA, several of these encode for proteins related to ciliary function. We investigated an autosomal recessive form of LCA, Lebercilin (LC5), which is specifically involved in the regulation of the intraflagellar trafficking in ciliated cells. Lebercilin protein has been best characterized in photoreceptors, and has been shown to be critical for intracellular transport of rhodopsin through the connecting cilium to the outer segment (OS) layer. Loss of lebercilin results in excess protein accumulation in the ONL leading to cell death. Loss of photoreceptors can be document even before the outer segments have fully developed, suggesting that this early onset retinal dystrophy (EORD) could involve an additional cell compartment proceeding photoreceptor maturation. We hypothesize that ciliation in retinal pigmented epithelial (RPE) cells would be affected by loss of LC5 in murine and human experimental models leading to early pathologic consequences. Although it is known that RPE cells play an essential role in epithelial transport, protection against oxidative stress, secretion and phagocytosis for proper functionality and morphology of the photoreceptors, its role in retina degeneration, paradoxically, has been just recently appreciated. In order to investigate the pathophysiology of LC5 and understand how the RPE cells play a role in this EORD, we used patient iPSC-derived RPE cells and RPE flat mount from LC5 knockout mice. We observed a severe impairment of ciliogenesis and related alteration in RPE size, distribution and maturation. This new, so far unreported, phenotype could help to explain the severity of the LC5 depletion in the visual system, by affecting not only the retina directly but also by profoundly altering the physiology of its supporting cells. This complex mechanism of action, could lead to additional insight into the pathology of other EORDs where developmental impairments of the visual system effects neurodegeneration of the retina.
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Polyamine imbalance impairs autophagy-lysosomal system and causes oxidative stress in Snyder-Robinson syndrome
C Li, J Brazil, S Liu, C Bello, Y Zhu, M Morimoto, L Cascio, R Pauly, Z Diaz-Perez, M Malicdan, H Wang, L Boccuto, C Schwartz, W Gahl, C Boerkoel, RG Zhai

Polyamines are tightly regulated polycations essential for life. Loss-of-function mutations in spermine synthase (SMS), a polyamine biosynthesis enzyme, cause Snyder-Robinson syndrome (SRS), an X-linked intellectual disability syndrome; however, little is known about the neuropathogenesis. Here we show that loss of dSms in Drosophila recapitulates the pathological polyamine imbalance of SRS and causes survival defects and synaptic degeneration. SMS deficiency leads to excessive spermidine catabolism, which generates toxic metabolites that cause lysosomal defects and oxidative stress. Consequently, autophagy-lysosome flux and mitochondrial function are compromised in the Drosophila nervous system and SRS patient cells. Importantly, oxidative stress caused by loss of SMS is suppressed by genetically or pharmacologically enhanced antioxidant activity. Our findings uncover the mechanisms underlying the pathological consequences of abnormal polyamine metabolism in the nervous system and provide potential therapeutic targets for treating SRS and other polyamine-associated neurological disorders.

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Uncovering hyperactive arginine sensing pathways in tauopathies
Chao Ma, William Fraser, Jerry Hunt, Leslie Sandusky, Hans Osborne, Daniel Pedersen, Kevin Nash, Dave Morgan, Paula Bickford, Daniel Lee

Tauopathies including Alzheimer’s disease (AD) consist of age-associated neurodegenerative diseases for which no disease-modifying treatments exist. Our group has uncovered a unique interaction between the arginine metabolism and tauopathies. Arginine metabolism affects multiple biological processes that show considerable influence upon tau biology. We demonstrated in cells and animal models of tauopathy the benefits of increasing arginase 1 (Arg1) in reducing many aspects of the tau phenotype (hallmarks comprised of tau effects). Several seminal findings showed lysosomal and cytoplasmic arginine sensors that modulate mechanistic target of rapamycin complex 1 (mTORC1). The first identified arginine sensor is solute carrier family 38A9 (SLC38A9), a lysosomal amino acid transporter that signals arginine sufficiency to mTORC1 within the lysosome. The second arginine sensor is a cytoplasmic regulator known as cellular arginine sensor for mTORC1 (CASTOR) proteins. Other key studies revealed that G protein coupled receptor family C, group 6 member A (GPRC6A) binds arginine and may serve as an extracellular arginine sensor for mTORC1. Our data indicated increased activation of the arginine-sensing mTORC1 pathway in human AD brains and animal models of tauopathies. We discovered that arginine producing and metabolizing enzymes, arginine sensors (SLC38A9, CASTOR1, GPRC6A), and mTORC1 complexes all increased in the hippocampus of AD patients compared to control aged matched brains. We also found that tau increased total arginine levels in the mouse tauopathy brains by 35%, increased basal levels of extracellular arginine and arginine release following neuronal stimulation. Furthermore, genetic reduction of SLC38A9, CASTOR1, GPRC6A reduced tau expression, uncoupled mTORC1 signaling pathways in neuronal tauopathy cells. We posit that tauopathies cause impaired arginine metabolism and uncoupling of arginine-sensing mTORC1 signaling, which lead to hyper-mTORC1 activation creating a positive feed-forward loop to augment the tau phenotype. Therefore, arginine sensors can become novel therapeutic targets to modify tauopathies.

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Mimicking Phosphorylation of the Nato3 Transcription Factor Drives the Expression of Key Dopaminergic Neuron Markers In Vitro and In Vivo
Dayne Martinez, Melina Frantzeskakis, Dan Doyle, Nicholas Huisingh, Jordan Straight, Merritt Taylor

Development of midbrain dopaminergic (DA) neurons is interesting because it can inform the creation of disease models and novel therapies for Parkinson’s disease. Previous experiments demonstrated that overexpression of the Nato3 transcription factor in the developing chick is sufficient to drive an increase in the number of cells expressing DA neuron markers in the midbrain but not in other CNS regions. This raised the question of how Nato3 promotes an increase in cells expressing DA neuron markers in a regionally specific manner despite Nato3 being expressed more broadly. We hypothesized that Nato3 is regulated differently in distinct anatomical regions through phosphorylation. Here, we overexpressed several phosphomimetic Nato3 mutants in the developing chick using in ovo electroporation and analyzed
the subsequent number of cells expressing DA neuron markers using immunohistochemistry. We also overexpressed the phosphomimetic mutants in mouse mesencephalic SN4741 cells and monitored the expression of several genes important for generating and maintaining DA neuron identity using quantitative PCR. Multiple mutants drove ectopic expression of DA neuron lineage markers throughout the rostral chick CNS and increased the expression of key genes in SN4741 cells, suggesting that phosphorylation at some residues is important for the ability of Nato3 to drive DA neurogenesis.

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Participation of Reelin in PNS regeneration: Role in Schwann cell migration and axonal regeneration and outgrowth
Consuelo Pastén, Joaquin Cerda, Ignacio Jausoro and María-Paz Marzolo

ApoER2 and its ligand reelin have important roles in neuronal migration, polarization and differentiation during development in the central nervous system (CNS). Reelin triggers a neuronal cell signaling pathway that recently has been involved in the regulation of cytoskeletal dynamics and intracellular trafficking. Although Reelin is abundantly expressed in the CNS it has been also found in the peripheral nervous system (PNS), where its function has been less well studied. It is known that reelin is expressed in regenerating peripheral nerves and that reelin deficient mice (reeler) show decreased axonal regeneration in the PNS. However, the mechanism(s) by which reelin works in the PNS has not been addressed. We have found that Reelin and its receptor, apoER2, are expressed in sciatic nerve and their levels are increased upon injury. Reelin induces Schwann cell migration in a process that is dependent on the activation of Rac1 and the presence of Tiam1 and Par3. In addition, Reelin increases axonal outgrowth after axotomy of dorsal root ganglia (DRG) explants and regulates the activity of small GTPases of the Rho Family. Altogether, our results show that reelin signaling is present in cells from the PNS and therefore implying this pathway in the regeneration of this system. Funding: Fondecyt Regular Grant # 1150444 and MINREB.

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AnnexinA2 and A6 interact with the first exon of tau contributing to tau's axonal localization

During neuronal development, the microtubule-associated protein tau becomes enriched in the axon where it remains concentrated in the healthy brain. In the course of tauopathies such as Alzheimer’s disease, tau redistributes from the axon to the somatodendritic compartment, where it aggregates into filamentous structures, which form neurofibrillary tangles. The mechanisms that restrain tau in the axon in the healthy brain and which are involved in its redistribution during disease are not known. Previously, we identified the Ca2+-regulated plasma membrane-binding protein annexin A2 (AnxA2), a protein highly concentrated in the growth cone and axonal branches, as an interaction partner of tau (Gauthier-Kemper et al., 2011). In this study we mapped the interaction of tau with annexins using a heterologous yeast system. We identified the extreme N-terminus of tau as interaction site and demonstrated that the interaction is not affected by familial tau mutations in the first coding exon (E1) and tyrosine phosphorylation (Tyr-18). Tau interacts with the core domain of AnxA2 in Ca2+-induced open conformation and interacts also with AnxA6. Bioinformatic analysis identifies two conserved 8-amino-acid-long motifs within this region in mammals. Deletion of E1 leads to a moderately increased microtubule association. Using an in-cell competition assay, we provide evidence that the interaction via E1 is involved in tau’s axonal retention. Our data suggest a role of tau as a microtubule-plasma membrane linker, which contributes to axonal retention, a function acquired during the evolution of higher vertebrates.

Increasing glucose uptake suppresses age-dependent reductions in ATP levels in brain neurons and behavioral deficits in Drosophila

Mikiko Oka, Emiko Suzuki, Hiromi Imamura, Shin-ichi Hisanaga, Koichi M. Iijima, Kanae Ando

Aging has been associated with changes in metabolism at system and cellular levels. Brain neurons are highly energy demanding cells and require local energy supply due to their polarized structures. Disruption in energy metabolism in neurons is thought to cause age-related functional declines as well as neurological and neurodegenerative diseases. However, temporal and special information regarding age-dependent changes in ATP levels in brain neurons is limited. Using *Drosophila* as a model system, here we analyzed the age-dependent changes in ATP levels in the cell bodies and axons in brain neurons using FRET-based ATP biosensor. We focused on the mushroom body structure, in which the cell body region and the axonal region are easily distinguished. ATP levels in the axon were similar between young and aged flies. In contrast, we found that ATP levels in the cell body were significantly reduced during aging.

To gain insight into the mechanisms underlying age-dependent reduction in ATP levels in the cell bodies, we first compared the numbers and quality of mitochondria between young and aged flies via ultrastructural analyses. While the number of mitochondria was similar between young and aged fly brains, mitochondria with abnormal cristae were more often observed in aged brain neurons, suggesting that ratio of damaged mitochondria was increased during aging. Thus, increased ratio of damaged mitochondria was correlated with age-dependent reductions in ATP levels in the cell bodies. We next compared mRNA levels of glycolytic enzymes in young and aged fly brains. We found that mRNA levels of several rate-limiting enzymes in the glycolytic pathway were reduced during aging. Interestingly, flies with knockdown of pfk, one of the key glycolytic enzymes, did not show age-dependent reductions in ATP levels in the cell body, suggesting that reduced glycolysis also contributed to age-dependent reduction in ATP levels.

Interestingly, we also found that enhancement of glucose uptake by neuronal overexpression of glucose transporter (GLUT) can suppress age-dependent declines in ATP levels in the cell body. Moreover, neuronal overexpression of GLUT suppressed the age-dependent decline in locomotor functions in flies. Taken together, these results suggest that age-dependent reductions in ATP levels are due to mitochondrial damage and reduced glycolysis, while reduction in ATP levels as well as decline in neuronal functions can be suppressed by increasing GLUT into neurons. Further study of regulatory mechanisms underlying glucose uptake in brain neurons may lead to novel therapeutics against normal aging and age-related neurodegenerative diseases.

Enhanced Outgrowth and Regeneration in Adult Motor Neurons from Amyotrophic Lateral Sclerosis Mouse Models


Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by motor neuron cell death. Though ALS specifically affects motor neurons, certain groups of them are more resistant to disease progression. The majority of ALS studies have focused on the cellular mechanisms that cause loss of viability, less is known about the neurons that do survive. In this study, we address the latter by culturing spinal motor neurons from adult ALS mouse models at various states of disease progression. Surprisingly, we found that in comparison to age-matched controls, motor neurons cultured from SOD1{sp:G93A}{sp} ALS mouse models display increased outgrowth and branching. They also display an increase in the number and size of actin-based structures such as growth cones and filopodia. The most substantial increase in regeneration occurs in a SOD1{sp:G93A}{sp} ALS mouse model with a lower copy number of the transgene that yields delayed disease onset. This phenotype occurs independently of SOD1 enzymatic activity and is cell autonomous. Further, the enhanced outgrowth occurs before the mice become symptomatic, though the effect increases with disease progression. These results indicate that the surviving motor neurons in ALS are primed for regeneration well before an individual gets sick, in response to cellular stress. Understanding this mechanism of cellular resistance could result in new therapeutic targets for the treatment of ALS.
Glia and pioneer neurons direct hierarchical assembly of the C. elegans brain

Rapti Georgia, Li Chang, Shan Alan, Lu Yun, Shaham Shai

Faithful assembly of neural circuits requires a complex array of cellular interactions and molecular pathways guiding axon navigation. Circuit assembly begins when early neuronal processes extend over non-neuronal cells to form tracts that guide follower axons. Although axon guidance has been intensely studied for decades, major open questions remain. Among these are the molecular properties of pioneer axons, and the guidance interactions between pioneer axons and glia, which appose pioneer bundles. To determine how neurons and glia interact during CNS formation, we studied assembly of the C. elegans nerve ring (NR), a brain-like neuropil consisting of ~180 axons, and enveloped by four astrocyte-like CEPsh glia. Using time-lapse embryonic imaging, genetics, protein-interaction, cell ablations and functional studies, we uncovered the early events of NR assembly. We showed that the NR is populated in an orderly manner, with CEPsh glia playing key roles in assembly initiation. We identified a set of <10 pioneer neurons, with unique cellular, molecular, and growth properties that cooperate with glia to guide follower axons of diverse groups. Importantly, we demonstrate that CEPsh glia regulate pioneer and follower axon guidance using distinct signals, and identify a network of guidance cues acting in glia and pioneer neurons to drive assembly.

From a genetic screen, we isolated a novel mutant that spares axon-outgrowth initiation, but severely disrupts axon guidance, with more than 70% of axons of different subtypes failing to incorporate into the NR. Two mutations, in a Chimaerin (GTPase regulator) and a Furin (pro-hormone convertase), are causal for the mutant defects. These proteins act non-canonically in glia for pioneer-axon guidance and in both glia and pioneer neurons for follower-axon navigation. Importantly, we show that they together regulate guidance-cue trafficking. Single Chimaerin or Furin mutants, or in other axon guidance genes exhibit only mild defects in NR assembly, suggesting redundancy, a problem that has plagued genetic analysis of axon guidance in vertebrate and invertebrate settings. The double mutant we identified uncovers a genetic bottleneck that allowed us to genetically identify new, redundant axon-guidance genes, several of which appear to be previously unknown.

Taken together, our studies suggest a pivotal role for glia in initiation of CNS assembly, and open the door to uncovering new axon guidance genes. CEPsh glia are reminiscent of vertebrate radial assembly, whose molecular biology is not well understood. Moreover, mammalian homologs of some glial genes we identified have axon guidance roles in vertebrates, and are expressed in glia. Our studies, therefore, may reveal conserved glial mechanisms promoting CNS assembly.

Cytosolic proteostasis through importing of misfolded proteins into mitochondria

Linhao Ruan, Chuankai Zhou, Erli Jin, Andrei Kucharavy, Ying Zhang, Zhihui Wen, Laurence Florens & Rong Li

Loss of proteostasis underlies ageing and neurodegeneration characterized by the accumulation of protein aggregates and mitochondrial dysfunction. Although many neurodegenerativedisease-associated proteins can be found in mitochondria, it remains unclear how mitochondrial dysfunction and protein aggregation could be related. In dividing yeast cells, protein aggregates that form under stress or during ageing are preferentially retained by the mother cell, in part through tethering to mitochondria, while the disaggregase Hsp104 helps to dissociate aggregates and thereby enables refolding or degradation of misfolded proteins. Here we show that, in yeast, cytosolic proteins prone to aggregation are imported into mitochondria for degradation. Protein aggregates that form under heat shock contain both cytosolic and mitochondrial proteins and interact with the mitochondrial import complex. Many aggregation-prone proteins enter the mitochondrial intermembrane space and matrix after heat shock, and some do so even without stress. Timely dissolution of cytosolic aggregates requires the mitochondrial import machinery and proteases. Blocking mitochondrial import but not proteasome activity causes a marked delay in the degradation of aggregated proteins. Defects in cytosolic Hsp70s leads to enhanced entry of misfolded proteins into mitochondria and elevated mitochondrial stress. We term this mitochondria-mediated proteostasis mechanism MAGIC (mitochondria as guardian in cytosol) and provide evidence that it may exist in human cells.
Interphase localization of Abnormal Spindle to the nucleus is important for proper brain size

Autosomal recessive primary microcephaly (MCPH) is a neurodevelopmental disorder characterized by reduced brain size and life span. While the clinical aspects of the disorder are well characterized, the molecular mechanism remains poorly understood. Previous models favored cell division defects induced by mitotic spindle errors as the cause of the disorder, leading to reduced neuron/glia numbers and a smaller brain. The most commonly mutated gene in human MCPH patients, Abnormal Spindle-Like, Microcephaly Associated (ASPM) is known to be important for proper centrosome and mitotic spindle function during mitosis. However, our recent analysis of the Drosophila melanogaster ortholog, Abnormal Spindle (Asp), showed that mitotic spindle & cell division defects are not the primary cause of MCPH in Asp mutant animals, suggesting the current model needs to be revised. We now provide evidence that Asp contributes to proper brain size through a novel role in the interphase nucleus. Using a combination of transgenic rescue assays and high resolution microcomputed tomography (micro-CT) of intact animals, we have identified the minimal fragment of Asp’s N-terminus (AspNMF, 597 aa) required for proper brain size and morphology. Mutation of a highly conserved asparagine residue located within the ASH domain of the AspNMF fragment abolishes the rescue phenotype. Subcellular localization of AspNMF within the developing larval & adult brain revealed an unexpected localization to the interphase nucleus of distinct neural stem cell populations and mature neurons. Intriguingly, RNA-Seq analysis of Asp mutant brains revealed a significant downregulation of actin-related genes, including myosin heavy chain (Mhc) and the troponin complex member wupA, whose expression could be restored to wildtype levels in the AspNMF background. In accordance with Asp’s role as a nuclear protein, we identify and characterize a novel interaction between AspNMF & umbrea (HP6), a heterochromatin binding protein located exclusively in the interphase nucleus. Together, our data highlights the first interphase role for Asp and suggests that other MCPH genes may contribute to the disorder through non-canonical pathways that funnel through the nucleus.

Effects of Microtubule Drugs in Neurodevelopment and Injury
Yuyu Song, Timothy Mitchison

Microtubules (MTs) are structural components vital for important neuronal functions such as neurite outgrowth and maintenance, as well as for axonal trafficking and synaptic remodeling. Neuronal MTs are particularly stable compared with those in other cell types and often exist for years before they get degraded and recycled. They are mostly synthesized in the somatodendritic compartment and have to be transported along axons as much as a meter or more in humans to reach the terminals where synaptic proteins are delivered and synaptic transmission occurs. A significant portion of neuronal MTs stay polymerized when challenged with MT destabilizers in test tubes and various non-neuronal cell lines, suggesting that baseline MT stability is high in neurons. Such stability increases during development and maturation, but decreases with axonal injury and neurodegeneration. This leads to an intriguing question: can stabilizing MTs facilitate neurite outgrowth during early development or restore axonal integrity and function upon injury? To address these questions, we have characterized a group of MT stabilizing drugs (Epothilone D, Epothilone B, Ixabepilone, Taxol and Synstab) regarding their binding affinity to MTs in vitro using biochemical assays and fluorescent live imaging; and evaluated their effects on neurite growth during normal differentiation and regrowth after axotomy. Dose- and time- dependent drug treatments were performed on ReNcell VM cells (an immortalized human neural progenitor cell line), and differentiated VM cells that exhibit neuronal morphology and electrophysiology, followed by high-content live-cell imaging and automated imaging analysis. We found that these drugs showed bimodal effects on initial neurite extension and regeneration after injury. To understand the molecular mechanisms underlying regulation of MT stability by these drugs and their biological effects, we have used multiplex proteomics to study dose-dependent changes in signaling pathway components and MAPs over time. These results may provide useful information for understanding not only neuronal MT dynamics and stability in health and disease, but also for determining the therapeutic value of MT stabilizers in axonal injury and neurodegeneration where loss of neuronal MT integrity may exacerbate disease pathology.
A system to study alpha particle irradiation in cellular assays and its relevance to neurological pathologies
Fintan K. T. Stanley, N. Daniel Berger, Aaron A. Goodarzi

Low Dose ionizing radiation (LDIR) has been implicated in a variety of disease states, and it has been shown that neural stem cells and other cell populations are sensitized to this mode of radiation. However, the specific cellular effects of LDIR and its relation to disease remains poorly understood. In order to advance our understanding of the effects of LDIR on Myalgic Encephalomyelitis (ME), we have developed a medium-throughput system to analyze the cellular effects of alpha particle radiation. Radon represents the greatest single lifetime source of ionizing radiation (IR) exposure in humans and emits alpha particle radiation a form of high linear energy transfer (LET) IR. High LET IR is believed to cause significantly greater genomic instability than low LET IR such as x-rays and gamma-rays. We have developed a unique Am241 irradiation system to expose cells to alpha particles in a 96 well plate-based assay. Using this setup, we present a spatiotemporal study of alpha particle induced DNA damage responses and repair. We measure DNA double-strand breaks caused by alpha particles using a 3D analysis of the nuclear DNA damage γH2AX signaling response. Here, I present data from our system demonstrating a persistence in the DNA damage response from alpha radiation, as compared to gamma radiation. In addition, we report the cell type-specific effects of these types of radiation in different neural cell types. This work demonstrates that radon and alpha particle irradiation is a genuine public health concern, and legitimizes efforts to understand the consequences of LDIR exposure to human health.

The gap junction Nexus controls localization and mobility of neural proteins
Randy F. Stout, Jr., David C. Spray

Gap junctions connect astrocytes and oligodendrocytes, providing bidirectional exchange of nutrients, metabolites and intracellular signaling molecules. Each of these glial cell types expresses a distinct set of gap junction (GJ) proteins (astrocytes: Cx43, Cx30, & Cx26 oligodendrocytes: Cx32, Cx47 & Cx29) and each connexin forms a supramolecular complex (the GJ Nexus) made up of GJ channels and molecules that interact with GJs. Nexus composition is also determined by the posttranslational modification state of the Cx proteins and cell physiological state. GJs also have important non-channel functions including cell-cell adhesion and control of autophagy. We recently used live cell confocal microscopy and Fluorescence Recovery After Photobleaching (FRAP) to show that cysteine (Cys) residues within the cytoplasmic carboxyl-termini (CT) of Connexin 43 and Connexin 32 (Cx43 and Cx32) produce stably arranged orthogonal GJ channel arrays (GJ plaques) in the membranes of cells joined by those connexin isoforms whereas Cx30 & 26 (which have no Cys residues in the CT) form highly fluid GJ plaques. Here we report for the first time that Cx47 (contains 3 CT Cys residues) forms stably arranged GJs- thereby completing exploration of macroglial GJ stability characteristics (leaving the postulated xenotypic Cx29::Kv channel for continuing studies). Our discovery that mutation of Cys residues to alanine in the CT of Cx43 and Cx32 produces a switch from stable GJ Nexuses to a fluid structures allowed us to employ this as a new tool to test how Nexus stability affects mobility and localization of other membrane proteins- since known protein interaction sites are preserved in the Cys mutants. Here, we used live cell microscopy for four dimensional and multi-color FRAP to test how the GJ Nexus controls subcellular morphology near the GJ plaque and Cx43 mobility affects mobility of other Nexus components. We found that GJs excluded many membrane proteins from the GJ plaque membrane area. Some membrane proteins did infiltrate the Nexus and localization of the tight junction protein occludin (Ocldn) to the GJ plaque area was higher than other membrane areas. Cx43-CT mediated Nexus stability had a substantial effect on mobility of some membrane proteins (Cx30 and Ocldn) but a minor effect on other Nexus components. We examined localization and mobility of other proteins at the Nexus that are critical for function of specialized cellular compartments in astrocytes- where GJs are preferentially localized- such as peri-synaptic astrocyte processes (EAAT2b) and the perivascular endfeet (AQP4, Nectin-2, TJP1). We continue to explore ways the GJ Nexus acts as intercellular channels and membrane organizing nodes to determine astrocyte and oligodendrocyte cellular physiology.
Mutation of the Drosophila RNA-binding protein Muscleblind, causes accumulation of rhodopsin, ER stress and retinal degeneration

Izel Tekin, Jinfei D. Ni, Adishthi Gurav, Diego Acosta-Alvear and Craig Montell

Many inherited retinopathies result from the loss-of-function mutations in genes required for phototransduction, such as the gene encoding the light-sensitive receptor rhodopsin (Rh). Defects in the biosynthesis, folding, and/or trafficking of Rh result in insufficient protein levels in the photoreceptor cell membrane, which can lead to retinal degeneration and blindness. As in humans, mutations that reduce functional Rh levels in the fruit fly, Drosophila melanogaster, recapitulate clinical manifestations of retinopathies. Through a genetic screen for Drosophila genes required for phototransduction, we identified a mutation in one isoform of the evolutionarily conserved muscleblind-like (MBL) family of proteins. MBL is a member of a family of RNA-binding proteins that regulates the splicing, transport and stability of tissue-specific RNAs. In humans, the loss of function of either one of two isoforms of MBL (MBNL1/2) cause myotonic dystrophy. We found that mutations in one Drosophila Mbl isoform results in defects in phototransduction, and in progressive degeneration of the photoreceptor cells. The mutant flies also display a profound reduction in the levels of Rh1 and other phototransduction proteins, such as the TRP and TRPL cation channels, without discernible effects on their respective mRNA levels. We found that the Rh1 protein accumulated in the endoplasmic reticulum (ER) of the photoreceptor cells of mutant animals. Moreover, there was a significant increase in the mRNA levels of the transcription factor Xbp1, a major regulator of the ER stress response. Our results suggest that Mbl regulates the biosynthesis, folding, transport, or stability of Rh1. We suggest that the retention of Rh1 in the ER results in unmitigated ER stress, which consequently leads to photoreceptor cell death and retinal degeneration. We are currently conducting analyses aimed at revealing the specific mechanisms by which this Mbl mutation affects ER function in fly photoreceptor cells. By circumventing the developmental defects associated with the complete loss of Mbl, the mutation we identified allows us to dissect the homeostatic functions of Mbl in adult photoreceptor cells, and the specific molecular mechanisms that result in photoreceptor degeneration.

Neurodegenerative Disease Proteinopathies Are Connected To Distinct Histone Post-Translational Modifications

Chen, K., Bennett, S., Rana, N., Yosuf, H., Said M., Taaseen, S., Meltser, S., Mendo, N. and Torrente, M.P.

Amyotrophic Lateral Sclerosis (ALS) and Parkinson’s disease (PD) are devastating neurodegenerative diseases involving the progressive degeneration of neurons. No cure is available for patients diagnosed with these diseases. A prominent feature for both ALS and PD is the accumulation of protein inclusions in the cytoplasm of degenerating neurons; however, the particular protein comprising these inclusions varies. The RNA-binding proteins TDP-43 and FUS are most notable in ALS, while α-synuclein aggregates into Lewy bodies in PD. In both diseases, genetic causes fail to explain the occurrence of a large proportion of cases and, thus, both are considered mostly sporadic. We aim to understand the role of epigenetics in ALS and PD. In particular, we are interested in delineating histone post-translational modification profiles in both yeast and human ALS and PD models. Histones from cell models recapitulating FUS, TDP-43, and α-synuclein proteinopathies are probed for different histone modifications. Remarkably, we find distinctive changes in histone modification profiles for each proteinopathy model. We detect the most striking changes in the context of FUS aggregation: changes in several histone marks support a global decrease in gene transcription. We also detect more modest changes in cells overexpressing TDP-43 and α-synuclein. Our results highlight a great need for the inclusion of epigenetic mechanisms in the study of neurodegenerative disease. We hope our work will pave the way for discovery of more effective therapies to treat patients suffering from ALS, PD, and other neurodegenerative diseases.
Mitochondria-lysosome contacts regulate mitochondrial fission via Rab7 hydrolysis

Yvette C Wong, Dimitri Krainc

Both mitochondria and lysosomes are critical for maintaining cellular homeostasis, and dysfunction of both organelles has been observed in multiple diseases. Mitochondria are highly dynamic and undergo fission and fusion to maintain a functional mitochondrial network which drives cellular metabolism. Lysosomes similarly undergo constant dynamic regulation by Rab7 GTPase, which cycles from active GTP-bound state into inactive GDP-bound state upon GTP hydrolysis. Here, we investigated the regulation of mitochondria-lysosome membrane contact sites in living cells using high spatial and temporal microscopy. Mitochondria-lysosome contacts dynamically formed in healthy untreated cells and were distinct from damaged mitochondria targeted into lysosomes for degradation. Contact formation was regulated by active GTP-bound lysosomal Rab7, while contact untethering was mediated by Fis1 recruitment of TBC1D15/Rab7-GAP to mitochondria to drive Rab7 GTP hydrolysis to release contacts. Functionally, lysosomal contacts marked sites of mitochondrial fission allowing for lysosomal regulation of mitochondrial network dynamics, while conversely, mitochondrial contacts regulated lysosomal Rab7 hydrolysis via mitochondrial-localized TBC1D15. Mitochondria-lysosome contacts thus allow for bidirectional regulation of mitochondrial and lysosomal dynamics, and may explain the dysfunction observed in both organelles in various human diseases.

Stress granule assembly disrupts nucleocytoplasmic transport

K Zhang, JG Daigle, KM Cunningham, AN Coyne, K Ruan, JC Grima, KE Bowen, H Wadhwa, JD Rothstein, and TE Lloyd

Defects in nucleocytoplasmic transport (NCT) have been identified as a key pathogenic event in C9ORF72-mediated amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), the most common familial case of ALS and FTD. Furthermore, NCT disruption has also been implicated in other neurodegenerative diseases with protein aggregation, including Huntington’s disease, suggesting a shared mechanism by which protein stress disrupts NCT. Here, we show that cellular stress disrupts NCT via mislocalization of critical NCT components into stress granules, RNA/protein complexes that play a critical role in ALS pathogenesis. Importantly, inhibiting stress granule assembly suppresses NCT defects as well as neurodegeneration in C9ORF72-mediated ALS/FTD. Our findings identify a novel link between stress granule assembly and NCT, two fundamental cellular processes implicated in the pathogenesis of C9ORF72-mediated ALS/FTD and other neurodegenerative diseases.
Meeting Attendees at the Cell Biology of Degeneration and Repair in the Nervous System Doorstep Meeting*

*as of November 3, 2017

Susan Ackerman, Speaker
Max Adrian
Ueli Aebi
Zainab Afzal
Jayne Aiken
Rebecca Alvania
Paola Arlotta, Speaker
Adam Avery
Virginia Ayres
James Bamburg
Kelly Barford
Emily Bates
Lorena Benedetti
Seth Bennett
Xin Bian
Veronica Birdsall
George Bloom
Nicholas Boyer
Frank Bradke, Co-Organizer
Jennifer Brazill
Monika Brill
Gregory Brittingham
Katja Brose
Cassandra Burke
Mian Cao
Anna Caputo
Sydney Cason
Ana Castillio Orozco
James Catlin
Barbara Celona
Yi-Ju Chen
Wu Chien-Ting
Andrew Chisholm
Chia-Yu Chung
Don Cleveland, Speaker
Jonah Cool
Matt Cowan
Kathleen Cunningham
Pietro De Camilli
Alison Dell
Ozlem Dilek
Baojin Ding
Kirby Donnelly
Philippe Duquette
Bahareh Eftekharzadeh
Kelsie Eichel
Audrey Ettinger
Chantell Evans
Frances Evesson
Jill Falk
Shawn Ferguson
Melina Frantzeskakis
Meng-meng Fu
Görkem Garipler
Vladimir Gelfand
Dale George
Piya Ghose
Anindya Ghosh Roy
Samuel Gilberto
Sarah Goetz
Juliet Goldsmith
Swetha Gowrishankar
Kayla Green
Laurent Guillaud
Andrés Guillén Samander
Claudia Guimas Almeida
Ines Hahn
William Hancock-Cerutti
Olivia Harding
Jeffrey Harper
F. Ulrich Hartl, Speaker
David Hartmann
Thomas Hays
Ari Helenius
Sarah Hill
Yusuke Hirabayashi
Liam Holt
Erika Holzbaur, Speaker
Ryan Insolera
Sally Ishizaka
Meredith Jackrel
Katherine Jensen
Gail Johnson
Yilin Kang
Prasad Kanuparthi
Lukas Kapitein
Larisa Kavetsy
Lynn Kee
Philipp Kimmig
Lauren Klabonski
Helmut Kramer
Swathi Krishnan
Lena Kutscher
Laurent Ladepeche
Nathalie Lamarche-Vane
Sigrid Langhans
Lara Laparra Cuervo
Nina Latcheva
Claire Le Pichon
Annie Lee
Frances Lefcort
Stephane Lefrancois
Lanfranco Leo
Chelsea Leonce
Chong Li
Weihan Li
Shuo-Chien Ling
Karen Litwa
Yu-Huei Liu
Marcia Liz
Chao Ma
Itzhak Mano
Pallavi Manral
Kelsey C. Martin, Co-Organizer
Dayne Martinez
Jennifer Martinez-Bocanegra
Maria-Paz Marzolo
Hideko Matsumoto
Amber McCartney
Mirko Messa
Dragomir Milovanovic
Laurie Minamide
Michael Moenk
Amanda Neisch
Benedikt Niewidok
Jonathon Nixon-Abell
Christopher Obara
Judith Ochriector
Mikiko Oka
Eric Olson
Ryan O’Neill
Zachary Osking
Jun Hyun Park
Medha Pathak
Maggie Pearce
Richard Pellegrino
Kendall Perkins
K. Kevin Pfister
Jay Pieczynski
Laura Pontano Vaites
Bede Portz
Georgia Rapti
Tracy-ann Read
Kathryn Richmond
Sabrina Robertson
Avital Rodal
Eduardo Rosa-Molinar
Sukla Roychowdhury
Linhao Ruan
Stephanie Sansbury
Anwesha Sanyal
Delghir Sanzhikov
Lisa Satterwhite
Aleister Saunders
Gerald Schatten
Todd Schoborg
Nancy Schwartz
Thomas L. Schwarz, Speaker
Dennis Selkoe, Speaker
Emily Seminary
Ophir Shalem
Michal Sharoni
Carla Shatz, Speaker
Yari Sigal
Yuyu Song
Ileana Soto-Reyes
Sean Speese
Fintan Stanley
Andrea Stavoe
Randy Stout
Elizabeth Sun
Daria Svistunova
Sharan Swarup
Jason Swedlow
Katelyn Sweeney
Taketo Taguchi
Maoping Tang
Kandice Tanner
J. Paul Taylor, Speaker
Izel Tekin
Gareth Thomas
Jie Tian
Mariana Torrente
Kazuhito Toyouka
Chi Kwan Tsang
Jesse Vargas
Maria Vera
Eric Vitriol
Andre Voelzmann
Ali Vural
Clarissa Waites
Susan Walsh
Brittany Wheatley
Bettina Winckler
Benjamin Winter
Hetty Wong
Yao Liang Wong
Yvette Wong
Jing Lin Xie
Lihan Xie
Zhou Yu
Huaye Zhang
Ke Zhang
Linghua Zhang
Travel Awardees for the Cell Biology of Degeneration and Repair in the Nervous System Doorstep Meeting

Emily Bates
Mian Cao
Kathleen Cunningham
Meng-meng Fu
Anindya Ghosh Roy
Swetha Gowrishankar
Laurent Guillaud
Yusuke Hirabayashi
Ryan Insolera
Lara Laparra Cuervo
Annie Lee
Weihan Li
Dragomir Milovanovic
Mikiko Oka
Ryan O’Neill
Zachary Osking
Maggie Pearce
Georgia Rapti
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