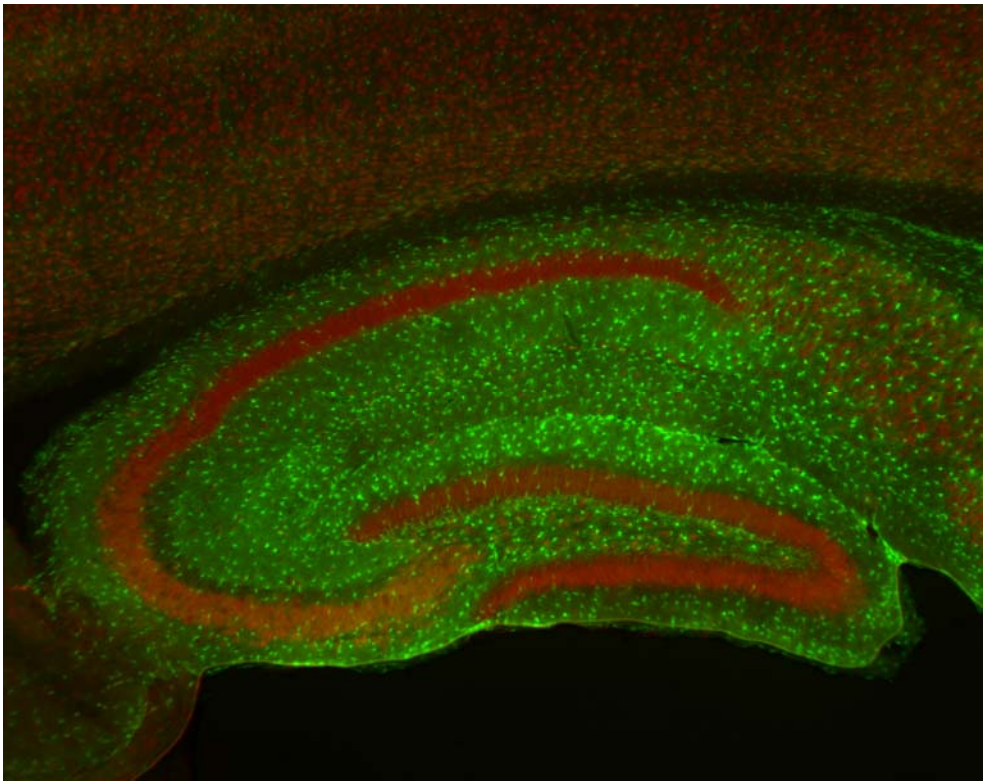
Abstracts of papers presented
at the 2010 meeting on

NEURODEGENERATIVE DISEASES: BIOLOGY & THERAPEUTICS

December 1–December 4, 2010



Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

NEURODEGENERATIVE DISEASES: BIOLOGY & THERAPEUTICS

December 1–December 4, 2010

Arranged by

Sam Gandy, *Mount Sinai School of Medicine*
Virginia Man-Yee Lee, *University of Pennsylvania*
Jeffrey Rothstein, *Johns Hopkins University School of Medicine*

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

This meeting was funded in part by **Merck Research Laboratories.**

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Cover: Rodent hippocampus.

NEURODEGENERATIVE DISEASES: BIOLOGY & THERAPEUTICS
Wednesday, December 1 – Saturday, December 4, 2010

Wednesday	7:30 pm	Welcome Remarks **1** Alzheimer's Clinical Trials Update
Thursday	9:00 am	**2** Biomarkers and Genetics
Thursday	2:00 pm	**3** RNA Metabolism in Neurodegenerative Disease
Thursday	4:15 pm	**4** Poster Session and Wine & Cheese Party
Thursday	7:30 pm	**5** Submitted Abstracts
Friday	9:00 am	**6** Protein Sorting and VPS10 Proteins in Neurodegeneration
Friday	2:00 pm	**7** Theme Stem Cells, Glia and Synaptic Factors
Friday	6:00 pm	Banquet
Saturday	9:00 am	**8** Drug Targets and Assays for Drugs

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

Printed on 100% recycled paper.

PROGRAM

WEDNESDAY, December 1—7:30 PM

SESSION 1 ALZHEIMER'S CLINICAL TRIALS UPDATE

Chairperson: **S. Gandy,** Mount Sinai School of Medicine, New York, New York

WELCOME

Jeff Rothstein
Johns Hopkins University School of Medicine

Bapineuzumab immunotherapy for Alzheimer's disease
Michael Grundman.
Presenter affiliation: Janssen Alzheimer Immunotherapy, South San Francisco, California. 1

Norm Relkin.
Presenter affiliation: Cornell University Medical College, New York, New York.

Andrew Protter.
Presenter affiliation: Medivation, Inc., San Francisco, California.

Therapy persistence with disease-modifying AD therapy—Impact on NNT and AD-free years in an asymptomatic population
Nicolas M. Furiak, Timothy M. Klein, Megha Bansal, Robert W. Klein.
Presenter affiliation: Medical Decision Modeling Inc, Indianapolis, Indiana. 2

THURSDAY, December 2—9:00 AM

SESSION 2 BIOMARKERS AND GENETICS

Chairperson: **V. Lee,** University of Pennsylvania School of Medicine, Philadelphia

Robert Dean.
Presenter affiliation: Eli Lilly and Company, Indianapolis, Indiana.

Radiopharmaceuticals for detection of pathological protein aggregates in neurodegenerative diseases
Franz Hefti, Chris M. Clark, Mark A. Mintun, Michael J. Pontecorvo, Daniel M. Skovronsky.
Presenter affiliation: Avid Radiopharmaceuticals, Philadelphia, Pennsylvania. 3

Perspectives for the future development of neuropathology—Biomarkers for Alzheimer's disease, Parkinson's disease and related disorders
John Q. Trojanowski.
Presenter affiliation: University of Pennsylvania, Philadelphia, Pennsylvania. 4

Gerald Schellenberg.
Presenter affiliation: University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

Andrew Singleton.
Presenter affiliation: National Institutes of Health, Bethesda, Maryland.

Genome-wide association study of frontotemporal lobar degeneration with TDP-43 inclusions
Vivianna Van Deerlin, Patrick Sleiman, Maria Martinez-Lage, Alice Chen-Plotkin, Li-San Wang, Gerard Schellenberg, Hakon Hakonarson, John Q. Trojanowski, Virginia M. Lee, and the International Collaboration on FTLD.
Presenter affiliation: University of Pennsylvania, Philadelphia, Pennsylvania. 5

THURSDAY, December 2—2:00 PM

SESSION 3 RNA METABOLISM IN NEURODEGNERATIVE DISEASE

Chairperson: **J. Rothstein,** Johns Hopkins University School of Medicine, Baltimore, Maryland

SESSION 4 POSTER SESSION and WINE & CHEESE PARTY

SESSION 5 SUBMITTED ABSTRACTS

Chairperson: **S. Gandy,** Mount Sinai School of Medicine, New York, New York

SESSION 6 PROTEIN SORTING AND VPS10 PROTEINS IN NEURODEGENERATION

Chairperson: **V. Lee,** University of Pennsylvania School of Medicine, Philadelphia

FRIDAY, December 3—2:00 PM

SESSION 7 THEME STEM CELLS, GLIA, AND SYNAPTIC FACTORS

Chairperson: **J. Rothstein,** Johns Hopkins University School of Medicine, Baltimore, Maryland

Using stem cells and reprogramming to study ALS
Francesco P. Di Giorgio, Gabriella Boulting, Evangelos Kiskinis, Alexandra S. de Boer, Sam Bobrowicz, Kevin C. Eggan.
Presenter affiliation: Harvard Stem Cell Institute, Cambridge, Massachusetts; Stowers Medical Institute, Kansas City, Missouri; Harvard University, Cambridge, Massachusetts. 80

Using embryonic stem cells to study motor neuron-glia interactions in ALS
Hemali P. Phatnani, Brad A. Friedman, Monica A. Carrasco, Flo Pauli, Tim Reddy, Michael Muratet, Rick Myers, Tom Maniatis.
Presenter affiliation: Columbia University Medical Center, New York, New York. 81

Effects of Nrf2 over-expression in mouse models of Alexander disease
Christine M. LaPash Daniels, Albee Messing.
Presenter affiliation: University of Wisconsin-Madison, Madison, Wisconsin. 82

FRIDAY, December 3

BANQUET

Cocktails 6:00 PM Dinner 6:45 PM

SATURDAY, December 4—9:00 AM

SESSION 8 DRUG TARGETS AND ASSAYS FOR DRUGS

A novel role of cytochrome C oxidase to rescue *pink1* toxicity in *Drosophila*
Ling-Yang Hao, Nancy M. Bonini.
Presenter affiliation: Howard Hughes Medical Institute, University of Pennsylvania, Philadelphia, Pennsylvania. 83

BAPINEUZUMAB IMMUNOTHERAPY FOR ALZHEIMER'S DISEASE

Michael Grundman

Janssen Alzheimer Immunotherapy, Clinical Development, South San Francisco, CA, 94080

Preclinical studies in transgenic mice that produce excess Aβ have shown that antibodies directed against the N-terminal of Aβ can reduce amyloid deposits in the brain parenchyma and cerebral blood vessels. Previous Aβ immunotherapy studies in humans utilizing active immunization with the full length Aβ42 peptide suggested potential clinical benefits. A one-year trial in immunized patients found that the subset of patients who were antibody responders showed improvement on a neuropsychological test battery. Additional follow-up of study subjects, approximately 4.5 years after immunization indicated that antibody responder patients showed less functional decline compared to placebo treated patients. Clinical testing of the full length Aβ42 peptide as a potential immunotherapy for AD was halted after meningoencephalitis developed in 6% of patients, a complication apparently due to a pro-inflammatory T-cell response against the Aβ peptide.

Bapineuzumab, an antibody targeted against the N-terminus of Aβ, is a passive Aβ immunotherapy currently being tested for AD. One phase 2 study explored the long term safety and efficacy of bapineuzumab in 234 patients randomly assigned to intravenous (IV) bapineuzumab or placebo in four ascending dose cohorts. While no significant differences were observed in the primary efficacy analysis, exploratory analyses showed potential treatment differences on cognitive and functional endpoints in study completers and apolipoprotein E (ApoE) ε4 non-carriers. Reversible vasogenic edema (VE) was detected on brain MRI in approximately 10% of bapineuzumab-treated patients and was more frequent in higher dose groups and ApoE ε4 carriers. In another, smaller (N=28) phase 2 study that utilized [^{11}C]PiB PET, bapineuzumab treated patients showed a reduction in [^{11}C]PiB retention at week 78 compared to placebo suggesting a reduction in cortical fibrillar Aβ burden. The treatment difference in [^{11}C]PiB retention tended to increase with longer treatment. A reduction in phospho-tau ($p<0.05$) was also observed in bapineuzumab vs. placebo treated patients when the CSF results in the two phase 2 studies were pooled.

These biomarker and clinical findings provide support for current phase 3 clinical trials with bapineuzumab. Different dose levels are being studied in carriers and non-carriers of ApoE4, and CSF, volumetric MRI, and amyloid imaging are employed to provide evidence of disease modification. Clinical efficacy measures combined with these biomarker results should help clarify the potential role of bapineuzumab as a disease modifying AD therapy.

(Current address for Michael Grundman: Global R&D Partners, LLC, San Diego, CA 92130; email: michael@grndpartners.com)

THERAPY PERSISTENCE WITH DISEASE-MODIFYING AD THERAPY – IMPACT ON NNT AND AD-FREE YEARS IN AN ASYMPTOMATIC POPULATION

Nicolas M Furiak, Timothy M Klein, Megha Bansal, Robert W Klein

Medical Decision Modeling Inc, Analytics, Indianapolis, IN, 46268

Objective: To describe how therapy persistence and rates of adverse events (AEs) for a hypothetical disease-modifying Alzheimer's disease (AD) therapy affect clinical outcomes when screening for AD neuropathology in a general asymptomatic population.
Background: Assuming the future availability of treatments that attenuate underlying disease pathology in AD, and the availability of disease-modifying therapies for AD, it will be important to evaluate the impact of AEs and therapy persistence on clinical outcomes from a societal perspective. A previously published AD screening model evaluating hypothetical therapy effectiveness and screening test characteristics demonstrated that a therapy increasing the time to clinical symptom onset by 50% significantly reduced incident dementia due to AD (DAD) cases. This study expanded the analysis to determine the impact of AEs and other discontinuation of therapy.
Design/Methods: Probabilistic sensitivity analysis (PSA) was performed on AE rates and first-year discontinuation of therapy for other causes. Based on data for drugs to prevent other serious medical conditions such as breast cancer recurrence, the mean first-year non-specific discontinuation rates were set at 17% in months 1-6, and 5% in months 7-12. The means for treatment-specific AE discontinuation rates in the first year were 10% and 2% per 5 years for remaining lifetime on therapy. The effectiveness of the hypothetical AD therapy was 50% and screening sensitivity and specificity were 0.87 and 0.78, respectively. PSA outcomes were summarized as acceptability curves showing the probability that an intervention reached thresholds of the number needed to treat (NNT) to achieve one AD-free year and avoid one case of DAD in 1000 cohorts of 1000 patients, each screened at age 70.
Results: The mean number of patients treated in each PSA cohort was 236 (range 191-275). Acceptability curves showed that with the screening and treatment characteristics, and discontinuation distribution specified there was a less than 1 in 1000 chance of the threshold for NNTs being greater than 5.5 patients to gain one AD-free year (median NNT 1.8) or 68.5 to avoid one DAD case.
Conclusions: Given the hypothetical screening and treatment characteristics examined, the maximum NNTs of 5.5 patients to gain one AD free year and 68.5 to avoid one AD case, verify that screening for targeted AD treatment can be extremely valuable. This study is limited in that cost outcomes and specific AE characteristics are unknown but acceptable NNT thresholds will depend on them.

RADIOPHARMACEUTICALS FOR DETECTION OF PATHOLOGICAL PROTEIN AGGREGATES IN NEURODEGENERATIVE DISEASES.

Franz Hefti, Chris M Clark, Mark A Mintun, Michael J Pontecorvo, Daniel M Skovronsky

Avid Radiopharmaceuticals, R&D, Philadelphia, PA, 19104

Brain imaging provides a non-invasive tool for the detection of the defining pathological protein aggregates of neurodegenerative diseases, including Aβ and tau aggregates in Alzheimer's disease (AD), α-synuclein aggregates in Parkinson's disease (PD) and dementia with Lewy Bodies (DLB), and huntingtin aggregates in Huntington's disease (HD). Radiopharmaceuticals selectively recognizing the individual aggregates are expected to be useful in the differential diagnosis of dementia and movement disorders, and for monitoring disease progression and the effects of disease-modifying drugs. Our efforts have focused on 18F-labeled radiopharmaceuticals for PET imaging, because of the high sensitivity and spatial resolution of the method and the possibility to make imaging diagnostics widely available.

Our radiopharmaceutical for β-amyloid, florbetapir F 18 (18F-AV-45), was selected after synthesis of several hundred candidate ligands and the evaluation of a select number in phase I human trials under exploratory INDs. In vitro studies with postmortem human brain tissue established that florbetapir F 18 binds with high affinity (Kd = 3 nM) to β-amyloid, and Phase I human studies showed kinetic properties desired for a PET imaging agent. The Phase II studies showed clear separation of an AD population from healthy controls and, furthermore, detection of β-amyloid aggregation in 38% of individuals with mild cognitive impairment and 14% of normal elderly individuals. Control subjects with positive florbetapir-PET images performed worse on cognitive testing than those with negative images, indicating that β-amyloid aggregation is associated with identifiable changes in cognitive function. Subjects carrying the ApoEε4 genotype had higher florbetapir-PET signals than non-carriers. A Phase III study definitively demonstrated the ability of florbetapir-PET to identify β-amyloid aggregation in the living brain. Correlations of both the visual and quantitative assessment of the florbetapir-PET image to β-amyloid aggregation found at autopsy were 0.78 and 0.75. Florbetapir had excellent reliability (test-retest variability <3%) and a short imaging time of 10 minutes.

PET imaging agents for tau and α-synuclein aggregates are currently at the discovery phase. Florbenazine F 18 (18F-AV-133), a radiopharmaceutical to quantify dopaminergic neuron degeneration is currently in clinical development. Updates of these programs will be presented at the meeting.

The studies were supported by SBIR grants R43AG030869, R43AG032206, and a grant by the Michael J. Fox Foundation.

PERSPECTIVES FOR THE FUTURE DEVELOPMENT OF NEUROPATHOLOGY: BIOMARKERS FOR ALZHEIMER'S DISEASE, PARKINSON'S DISEASE AND RELATED DISORDERS.

John Q Trojanowski

University of Pennsylvania, Pathology, Philadelphia, PA, 19104-4283

Informative biomarkers for Alzheimer's disease (AD), Parkinson's disease (PD) and related disorders are urgently needed to improve the accuracy of early diagnosis and increase the efficiency of clinical trials of disease modifying therapies for these disorders. Progress of the Penn Biomarker Core in the AD Neuroimaging Initiative (ADNI) towards developing a pathological cerebrospinal fluid (CSF) and plasma biomarker signature for mild AD as well as a biomarker profile that predicts conversion of mild cognitive impairment (MCI) and/or normal control (NC) subjects to AD is described. ADNI exemplifies the global effort to standardize and validate biomarkers of AD. Further, the Parkinson Progression Marker Initiative (PPMI) is a newly launched effort to do the same for Parkinson's disease (PD). Collaborative efforts to integrate data across diverse biomarker domains also is critical as exemplified by ADNI which has proposed a temporal ordering of changes in clinical measures, imaging data and chemical biomarkers that serve as mileposts and predictors of the conversion of NC to MCI as well as MCI to AD, and the progression of AD. For example, CSF studies by the ADNI Biomarker Core revealed a pathological CSF biomarker signature of AD defined by the combination of CSF levels of Aβ1-42 and tau that effectively delineates mild AD in the large multisite prospective clinical investigation conducted in ADNI. This signature appears to predict conversion from MCI to AD as well as cognitive decline in PD. Further, data fusion efforts across other ADNI Biomarker Cores generated a hypothetical model for the temporal ordering of AD biomarkers. This model suggests that Aβ amyloid biomarkers become abnormal first, followed by changes in neurodegenerative biomarkers (CSF tau, FDG-PET, MRI) and the onset of clinical symptoms. The timing of these changes varies between patients due to genetic and environmental factors that increase or decrease an individual's resilience in response to progressive accumulations of AD pathologies. Further studies in ADNI and PPMI will refine this model and render the biomarkers studied in ADNI more applicable to routine diagnosis and to clinical trials of disease modifying therapies, and PPMI work towards a similar goal for PD.
Supported by grants from the NIH and the Michael J. Fox Foundation.

GENOME-WIDE ASSOCIATION STUDY OF FRONTOTEMPORAL LOBAR DEGENERATION WITH TDP-43 INCLUSIONS

Vivianna Van Deerlin[1], Patrick Sleiman[3], Maria Martinez-Lage[1], Alice Chen-Plotkin[2,1], Li-San Wang[1], Gerard Schellenberg[1], Hakon Hakonarson[3], John Q Trojanowski[1], Virginia M Lee[1], and the International Collaboration on FTLD[1]

[1]University of Pennsylvania, Pathology and Laboratory Medicine, Philadelphia, PA, 19104, [2]University of Pennsylvania, Neurology, Philadelphia, PA, 19104, [3]Children's Hospital of Philadelphia, Center for Applied Genomics, Philadelphia, PA, 19104

Frontotemporal lobar degeneration (FTLD) is the second most common cause of presenile dementia. The predominant neuropathology is FTLD with TAR DNA binding protein (TDP-43) inclusions (FTLD-TDP). FTLD is commonly familial and mutations in progranulin (GRN) are identified in ~10-20%. A 45-site International Collaboration representing 11 countries was formed to collect FTLD-TDP cases to perform a genome-wide association study to identify susceptibility loci for FTLD-TDP. Over 600 FTLD-TDP cases confirmed to have TDP-43 pathology and/or GRN mutations were collected. The GRN gene was sequenced in most cases. 515 FTLD-TDP cases and 2,509 disease-free population controls were genotyped on Illumina HH550 or 610-Quad BeadChips. Cochran-Armitage trend test statistics were calculated at all markers following quality control filtering. Additional cases were used for replication by TaqMan SNP genotyping. To identify possible phenotype-associated differential expression TMEM106B gene expression was evaluated. Finally, the genetic and clinical characteristics of a subset of 97 cases with GRN mutations were assessed and compared to 453 cases of FTLD-TDP in which GRN mutations were excluded. FTLD-TDP associates with multiple SNPs mapping to a single linkage disequilibrium block on 7p21 that contains TMEM106B. Three SNPs retained genome-wide significance following Bonferroni correction (top SNP rs1990622, $P = 1.08 \times 10^{-11}$; odds ratio (OR) minor allele (C) 0.61, 95% CI 0.53-0.71). The association replicated in 89 FTLD-TDP cases (rs1990622; $P = 2 \times 10^{-4}$). Expression of TMEM106B was significantly correlated with TMEM106B genotype, with risk allele carriers showing higher expression. Age at onset, age at death, and presence of concomitant motor neuron disease differed between the GRN+ and GRN- groups. Variants in TMEM106B are implicated as a strong risk factor for FTLD-TDP, including in individuals with GRN mutations. TMEM106B variants may confer risk by increasing TMEM106B expression. GRN+ FTLD-TDP differs in key features from GRN- FTLD-TDP.

CORRECTION OF *SMN2* RNA SPLICING IN THE CNS AS A THERAPEUTIC STRATEGY FOR SPINAL MUSCULAR ATROPHY

Yimin Hua[1], Kentaro Sahashi[1], Gene Hung[2], Frank Rigo[2], Marco Passini[3], C Frank Bennett[2], Adrian R Krainer[1]

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Spinal Muscular Atrophy (SMA) is a genetic disease characterized by progressive degeneration of motor neurons in the anterior horn of the spinal cord, which in turn leads to severe muscle weakness and atrophy. SMA is caused by deletion or loss-of-function mutations in the *Survival-of-motor-neuron* (*SMN1*) gene. The paralogous *SMN2* gene, present in one or more copies in all SMA patients, attenuates the severity of SMA, but expresses only a low level of full-length SMN protein, due to alternative splicing that results in inefficient inclusion of exon 7. Increasing the extent of *SMN2* exon 7 inclusion to express more full-length, functional SMN protein in motor neurons is a promising approach to treat SMA. Previously, we identified an optimal 2'-*O*-(2-methoxyethyl) (MOE) 18mer antisense oligonucleotide (ASO) that targets an hnRNP A1 bipartite motif in an intron-7 splicing silencer (ISS-N1) and efficiently promotes *SMN2* exon 7 inclusion in liver and kidneys of transgenic mice after systemic administration. Because ASOs do not cross the blood-brain barrier, we explored direct delivery to the mouse central nervous system. Using a surgically implanted micro-osmotic pump, the ASO (dubbed ISIS-SMN_{Rx}) was delivered into cerebrospinal fluid through the right lateral ventricle in adult $Smn^{-/-}$ type-III SMA mice carrying a human *SMN2* transgene. Dose-response studies revealed that intracerebroventricular (ICV) infusion of the 18mer ASO increased *SMN2* exon 7 inclusion in spinal cord to ~90%, compared to ~10% in saline-treated mice. Western blotting and immunohistochemical analysis demonstrated a robust increase of the human transgenic SMN protein levels in spinal-cord motor neurons. We are using this and other ICV delivery methods, in combination with available SMA mouse models, to optimize the effectiveness of the ASO, characterize phenotypic improvement, and establish a time window for effective treatment. Our data show that ISIS-SMN_{Rx} is a promising drug candidate for SMA therapy.

RNA MAPS IN HUMAN NEURODEGENERATIVE DISEASE.

Robert B Darnell

Rockefeller University/HHMI, Molecular Neuro-Oncology, New York, NY, 10065

The brain has unique systems for regulating RNA metabolism, and these may play key roles in synaptic physiology and in degenerative human neurologic disease. Using studies of a neuron-specific RNA binding protein, Nova, as a platform, we have developed the HITS-CLIP method to identify protein-RNA interaction maps present in the living brain. Recently, we have expanded HITS-CLIP to the study of Argonaute-microRNA-mRNA ternary interactions, allowing us to precisely decode microRNA binding sites on a genome-wide scale. Overlaying these different sets of binding maps offers the potential to develop new insights into the dynamics of RNA regulation in the brain. At the same time, many robust protein-RNA and Ago-mRNA binding sites do not conform to current understanding of their function, offering a means for new discoveries of the role of RNA regulation in the normal and diseased brain. More recent insight into the role of these complexes in neurodegenerative disease has come from generating maps for RNA binding proteins implicated in motor neuron disease.

Ule J et al. Nature, 444, 580-86, 2006;
Licatalosi et al. Nature 456, 464-9, 2008.
Chi et al., Nature, 460:479-86, 2009.
Zhang et al., Science, 329:439-443, 2010

This work was funded by the NIH and HHMI

DECIPHERING THE ROLE OF TDP-43 IN ALS/FTLD AND OTHER NEURODEGENERATIVE DISORDERS

Robert H Baloh

Washington University in St. Louis, Neurology, St. Louis, MO, 63110

The identification of pathologic TDP-43 inclusions in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), followed by the discovery of dominantly inherited point mutations in both TDP-43 and another RNA binding protein (FUS/TLS) in familial ALS, have been critical insights for defining the molecular pathways underlying these neurodegenerative disorders.

TDP-43 transgenic animals recently generated by numerous labs appear promising, as they recapitulate some key features of ALS and FTLD pathology. Most develop progressive neurodegeneration with a strong threshold effect of transgene dosage. There is a notable predilection of TDP-43 toxicity for cortical layer V projection neurons and spinal motor neurons, mirroring at least in part the selective vulnerability seen in human ALS and FTLD. Although cytoplasmic TDP-43 inclusions are occasionally present in TDP-43 transgenic animals, surprisingly they are rare and do not appear to correlate with neurodegeneration. In contrast, other features observed in human pathologic samples are consistently found in TDP-43 overexpressing animals – cleavage into C-terminal fragments, and depletion from the nucleus prior to cell death, however whether these are key players in the pathogenesis remains to be determined. These studies suggest that the molecular pathways underlying selective vulnerability to TDP-43 toxicity are shared between humans and mice, supporting that they will provide useful tools for both translational and mechanistic studies.

While available transgenic rodent models recapitulate several features of ALS and FTLD, the biochemical basis of TDP-43 aggregation, the mechanism of how TDP-43 mutations lead to disease, and how neurodegeneration propagates through the vulnerable network remains enigmatic. In recent efforts to understand how TDP-43 alters its cellular localization in response to proteotoxic stress, we found that TDP-43 is sequestered into polyglutamine aggregates, which is mediated by a Q/N rich prion related domain in the C-terminal region of TDP-43. Interestingly, a similar domain was previously described in FUS/TLS. Ongoing efforts are aimed at exploring the importance of the prion related domain in TDP-43 normal function and pathologic aggregation.

RNA BINDING PROTEINS AND FRONTOTEMPORAL LOBAR DEGENERATION

Rocky Gao[1], Tanya Monahiem[1], Amar Kar[1], Kazuo Fushimi[1], Jane Y. Wu[1]
[1]Department of Neurology, Lurie Cancer Center, Center for Genetic Medicine, Northwestern University School of Medicine, Chicago, IL

Expression of genes critical for the formation and maintenance of the nervous system is controlled by complex networks of regulation at both transcriptional and post-transcriptional levels. RNA processing defects have been identified in a variety of neurodegenerative diseases and are emerging as important mechanisms underlying neurodegeneration. Genetic studies have uncovered both cis-acting mutations and trans-acting factors associated with neurodegenerative disorders, including tau-positive and ubiquitin-positive frontotemporal lobar degeneration (FTLD-tau and FTLD-u). Microtubule associated protein Tau is critical for microtubule stabilization and neuronal function. In the human brain, tau pre-mRNA splicing is regulated to maintain a delicate balance of different splicing isoforms, with the ratio of exon10-containing to exon 10-skipping transcripts as one. Aberrant tau exon 10 splicing has been found in a range of tauopathies, from progressive supranuclear palsy, cortical basal degeneration to FTLD-tau. Mutations in the human tau gene have been identified only in a small fraction of tauopathy cases with aberrant tau exon 10 splicing, suggesting the involvement of additional players in tauopathy. We have been systematically investigating molecular mechanisms regulating tau exon 10 alternative splicing. Our work has revealed both cis-acting splicing regulatory elements and trans-acting splicing regulators critical for tau exon 10 splicing. Using multiple approaches, including UV cross-linking, expression cloning, yeast RNA-protein interaction screening and tandem-affinity purification coupled mass-spectrometry, we have identified a number of RNA binding proteins that regulate tau exon 10 alternative splicing. These tau exon 10 splicing regulators include both activators and suppressors that interact with either intronic or exonic splicing regulatory elements. Experiments are underway to examine the significance of these RNA binding proteins in the pathogenesis of FTLD-tau. On the other hand, numerous studies have implicated two multi-function RNA binding proteins, TAR DNA binding protein-43 (TDP-43) and fused in sarcoma (FUS) in the pathogenesis of FTLD-u, including FTLD-TDP and FTLD-FUS. To understand molecular pathogenesis of FTLD-TDP, we have begun to examine molecular, biochemical and functional characteristics of TDP-43 in cultured cells and in animal models. Using the powerful Drosophila system, we have built an animal model for FTLD-TDP and begun to address a number of important issues, including whether increased expression of wild type TDP-43 may lead to neurodegeneration and whether loss of function or gain of neurotoxic function are the primary pathogenic mechanism(s). Our transgenic flies expressing human TDP-43 recapitulate important neuropathological and clinical features of human TDP-43 proteinopathy, providing a powerful animal model for this group of devastating diseases. Our data indicate that simply increasing TDP-43 expression is sufficient to cause neurotoxicity in vivo, suggesting that aberrant regulation of TDP-43 expression or decreased clearance of TDP-43 may contribute to the pathogenesis of TDP-43 proteinopathy. Using this fly model, we have begun to systematically compare effects of wild type and mutant TDP-43 in neurons and to search for genetic modifiers of FTLD-TDP. Implications of our findings will be further discussed. Taken together, RNA binding proteins play important roles in the pathogenesis of FTLD, including both FLTD-tau and FTLD-u. Aberrant gene regulation by RNA binding proteins and RNA processing defects represent a general theme in neurogenerative diseases.

DEFINING THE ROLE OF DBR1 AS A POTENT MODIFIER OF TDP-43 MEDIATED TOXICITY IN ALS

Maria Armakola, Aaron D Gitler

University of Pennsylvania, Department of Cell and Develpomental Biology, Philadelphia, PA, 19104

Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration with ubiquitin positive inclusions (FTLD-U) are a group of neurodegenerative disorders characterized by the presence of TDP-43 positive inclusions in the cytoplasm of degenerating neurons in the brain. These disorders are collectively known as TDP-43 proteinopathies. Mutations in the TDP-43 gene (*TARDBP*) have been found to be associated with familial cases of ALS and FTLD-U. TDP-43 is a ubiquitously expressed DNA/RNA binding protein that regulates gene expression in multiple levels, including transcription, RNA splicing and mRNA translation. The mechanism whereby TDP-43 contributes to pathogenesis is unknown. TDP-43 is normally a nuclear protein but pathological inclusions contain cytoplasmic TDP-43 suggesting that altered subcellular localization of the protein may be important for disease pathogenesis. Despite the central role of TDP-43 in the pathogenesis of ALS and FTLD-U, currently there is no TDP-43 directed therapy for the disease. To gain insight into disease pathogenesis we performed an unbiased screen to define modifiers of TDP-43 mediated toxicity using a yeast TDP-43 proteinopathy model. We identified a deletion of the *DBR1* gene as a potent suppressor of TDP-43 toxicity in yeast. *DBR1* encodes an RNA debranching enzyme that cleaves the 2'-5' phosphodiester bonds of the lariat introns formed during pre-mRNA splicing. Importantly, DBR1 is conserved from yeast to man. In our preliminary studies we found that deleting Dbr1 in yeast completely suppresses TDP-43 toxicity and that this rescue is specific to the loss of its debranching enzymatic activity, raising the intriguing possibility that this activity could be a promising therapeutic target. We are continuing to characterize the TDP-43/DBR1 interaction in mammalian cells and *Drosophila*. For this purpose we have developed a mammalian cell toxicity model of TDP-43 proteinopathy that will serve as a platform to determine the mechanism through which downregulation of DBR1 rescues TDP-43 mediated toxicity. To determine if DBR1 is a modulator of TDP-43 toxicity *in vivo* we are using a *Drosophila* model of TDP-43 proteinopathy. These studies will provide the foundation for determining if the enzymatic activity of Dbr1 is a promising therapeutic target for drug design.

DISEASE-LINKED MUTANT HUMAN TDP-43 ACCUMULATED TO MODERATE LEVELS CAUSES LATE ONSET MOTOR NEURON DEFICITS IN MICE

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In 2006, TAR DNA Binding Protein (TDP-43) was identified as the major protein component of ubiquitinated inclusions found the overlapping pathology in both frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) and Amyotrophic Lateral Sclerosis (ALS). Since 2008, thirty-eight mutations have been reported in familial and sporadic cases of ALS, suggesting that dysfunction of TDP-43 presents a common pathway of neurodegeneration linking familial ALS (accounting for 10% of all ALS cases) and sporadic ALS. To characterize the normal biological function of TDP-43 and how mutations in it produce disease, we generated multiple transgenic lines of mice expressing wild-type human TDP-43 as well as two disease-linked mutations, Q331K and M337V, which express myc-tagged human TDP-43 cDNA under the control of the mouse prion promoter (MoPrP). Three lines each of wild-type TDP-43, Q331K and M337V-expressing transgenic mice have been established. The highest expressing lines produce protein levels equal to the endogenous protein in the brain and spinal cord. Furthermore, biochemical, behavioral and pathological characterization of aging cohorts of wild-type, Q331K and M337V-expressing transgenic mice reveal an age-dependent development in mutant mice of markers of inflammation (astrogliosis and microgliosis) in the spinal cord accompanied by increased muscle activity identified by electromyography and a reduced number of motor axons.

A NON-ACTIVE SITE, FUNCTION-BLOCKING ANTI-BACE1 ANTIBODY THAT SIGNIFICANTLY REDUCES ABETA PRODUCTION *IN VIVO*

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Reducing Abeta production by direct inhibition of amyloid precursor protein (APP) secretases has been a major therapeutic strategy for treating Alzheimer's Disease. Development of small molecule inhibitors of beta-secretase (BACE1) or gamma-secretase has been met with many challenges. Progress has been limited by the difficulty of generating inhibitors that are truly selective and blood-brain barrier penetrant, thus raising concerns about both safety and efficacy. Here, we describe the characterization of a high affinity, phage-derived human antibody selectively targeting BACE1 (anti-BACE1). Anti-BACE1 inhibits activity of mouse and human BACE1 in APP enzymatic cleavage assays. Furthermore, anti-BACE1 inhibits endogenous BACE1 and Abeta production in human APP expressing cell lines and in primary neuron cultures. The activity of anti-BACE1 is highly selective, as shown by the complete lack of inhibitory effects on either CathepsinD or BACE2 activity, and by pharmacokinetic studies in wild-type versus BACE1 knockout mice. Systemic dosing of anti-BACE1 in vivo shows a strong reduction in peripheral Abeta levels, and a modest but significant reduction of CNS Abeta levels, correlating with anti-BACE1 antibody levels detected in brain. Finally, crystallography and competitive binding studies indicate that the mode of action for the anti-BACE1 antibody is non-competitive through binding to an exosite on BACE1. These data provide evidence that BACE1 may be therapeutically targeted in a highly selective manner via a passive immunological approach.

PROCASPASE-ACTIVATING COMPOUND 1 INDUCES A CASPASE-3-DEPENDENT CELL DEATH IN CEREBELLAR GRANULE NEURONS

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Procaspase-activating compound 1, PAC-1, has been introduced as a direct activator of procaspase-3 and suggested as a therapeutic agent against cancer. Its activation of procaspase-3 is dependent on the chelation of zinc. We have tested PAC-1 and an analogue of PAC-1 as zinc chelators in vitro as well as their ability to activate caspase-3 and induce cell death in chicken cerebellar granule neuron cultures. These neurons are non-dividing, primary cells with normal caspase-3. The results reported herein show that PAC-1 chelates zinc, activates procaspase-3, and leads to caspase-3-dependent cell death in neurons, as the specific caspase-3-inhibitor Ac-DEVD-cmk inhibited both the caspase-3 activity and cell death. Thus, chicken cerebellar granule neurons is a suitable model to study mechanisms of interference with apoptosis of PAC-1 and similar compounds. Furthermore, the present study also raises concern about potential neurotoxicity of PAC-1 if used in cancer therapy.

Key words: apoptosis, caspase-3, PAC-1, procaspase

VARIATION IN THE AGE AT ONSET OF MACHADO-JOSEPH DISEASE: THE INFLUENCE OF THE *APOE* POLYMORPHISM

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Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder of late onset (mean of 40.2 years), caused by a CAG expansion in the coding region of the *ATXN3* gene (14q32.1). Variation in age at onset is partially explained by the size of the CAG repeat tract in the expanded alleles. The remaining variation in onset should be the product of other factors, namely modifier genes. *APOE* genotypes have been described as a possible phenotype modifier factor in different neuropathological settings, namely Parkinson's disease and Huntington's disease. The main goal of this work was to investigate the putative modulating effect of *APOE* polymorphism on the age at onset of MJD. A series of 57 Azorean MJD patients was analyzed. Cases with the ε2/ε3 genotype presented an earlier mean age at onset than patients with the ε3/ε3 and the ε3/ε4 genotypes. When adding, in a generalized estimating equation model, the presence/absence of *APOE* ε2 allele to the number of CAG repeats, the explanation of the onset variance significantly increased from 68.2% to 71.6%. Furthermore, the presence of the *APOE* ε2 significantly decreases the age at onset by nearly 7 years. These preliminary results suggest that the *APOE* polymorphism may play a role as a modifier of the MJD phenotype.

MODELLING MOTOR NEURON DISEASE USING INDUCED PLURIPOTENT STEM CELLS

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The mechanistic basis underlying progressive and selective degeneration of motor neurons in motor neuron disease (MND) is poorly understood. The discovery that TDP-43 is the major protein within ubiquitinated cytoplasmic inclusions, the hallmark pathology in 90% of MND cases and the recent identification of causative TDP-43 mutations in 1-4% of familial and sporadic cases highlights the need to develop experimental models to address the consequences of mutant TDP-43. Recent discoveries in reprogramming and resulting derivation of human induced pluripotent stem cells (iPS) permit human-based cellular models to study the consequences of disease causing mutations. In order to establish a platform for in vitro disease modeling we sought in this study to first generate iPS lines from TDP43 M337V mutation carrying and control individuals and next optimize differentiation protocols for motor neurons. Generation of iPS cells from adult human fibroblasts was undertaken by viral transduction of Oct4, Sox2, Klf4 and Myc. Resulting clones were characterised and validated by standard criteria including expression of pluripotency markers, bisulphite sequencing and teratoma formation. Neural conversion of iPS cells was achieved by dual inhibition of SMAD-signalling and generation of functional MNs was assessed by IHC, PCR, electrophysiology and myoblast co-cultures following patterning with Shh and RA. TDP-43 mutant and control iPS lines both gave rise to HB9/ChAT (+) mature motor neurons that were electrophysiologically active and formed neuromuscular junctions in myoblast co-culture. Our results establish an in vitro human experimental platform to begin to address cellular autonomy of MN degeneration and the role of TDP-43.

REGULATION OF TDP-43 AGGREGATION BY PHOSPHORYLATION AND AUTOPHAGY PATHWAY

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TDP-43 proteinopathy has been linked to several neurodegenerative diseases, including Frontotemporal Lobar Degeneration with ubiquitin positive inclusions (FTLD-U) and Amyotrophic Lateral Sclerosis (ALS). Phosphorylated and ubiquitinated TDP-43 C-terminal fragments have been found in cytoplasmic inclusions in FTLD-U and ALS patients. However, the factors and pathways that regulate TDP-43 aggregation are still not clear. We found that the C-terminal 15kDa fragment of TDP-43 is sufficient to induce aggregation but the aggregation phenotype is modified by additional sequences. Aggregation is accompanied by phosphorylation at serine residues 409/410. Mutation of 409/410 to phosphomimetic aspartic acid residues significantly reduces aggregation and ubiquitination. Inhibition of autophagy dramatically increases TDP-43 aggregation. Furthermore, TDP-43 aggregates colocalize with markers of autophagy and the autophagy adaptor protein SQSTM1/p62. Overexpression of SQSTM1/p62 reduces TDP-43 aggregation. These studies suggest that aggregation of TDP-43 is regulated by phosphorylation events and the autophagy pathway.

PINK1 DEFICIENCY RESULTS IN ABNORMAL DOPAMINE HOMEOSTASIS AND AFFECTS INNATE IMMUNE AND CELL SURVIVAL SIGNALING

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Mutations in the mitochondrial kinase PINK1 are linked to recessive familial Parkinson's disease (PD) with onset in the third to fourth decade of life. In cultured cells PINK1 and Parkin (another recessive PD gene) maintain a healthy pool of mitochondria by promoting the degradation of functionally impaired mitochondria through autophagy. To further study how the loss of PINK1 affects mitochondrial function and other PD-relevant pathways *in vivo*, we generated and characterized a new line of PINK1-deficient ($PINK1^{-/-}$) mice. Compared to wildtype mice, Ficoll-gradient purified mitochondria from the brain of $PINK1^{-/-}$ mice displayed enhanced Ca^{2+}-induced permeability transition (rescued by cyclosporine A). Moreover, phosphorylated c-Jun accumulated in a subpopulation of dopaminergic neurons in young $PINK1^{-/-}$ mice, and PINK1-deficient mice aged 6 months and older showed reduced levels of dopamine associated with increased dopamine turnover in the striatum. In response to a peripheral inflammatory stimulus $PINK1^{-/-}$ mice had increased levels of select cytokines in the striatum. Quantitative transcriptional profiling showed abnormal striatal expression of genes that regulate innate immunity and apoptosis, mitogen-activated protein kinase signaling as well as axonal sprouting and regeneration. $PINK1^{-/-}$ embryonic fibroblasts showed reduced basal and inflammatory cytokine-induced NF-κB signaling and were severely impaired in the activation of the survival kinase Akt, which is required for the protection of dopamine neurons in various animal models of PD. Collectively, these results show multiple abnormalities in the nigrostriatal system of $PINK1^{-/-}$ mice and cells and implicate aberrant innate immune signaling and impaired activation of cell survival pathways as novel mechanisms involved in the pathogenesis of recessive Parkinsonism.

COVALENT MODIFICATION OF UBIQUITIN E1 ACTIVATING ENZYME BY N,N-DIETHYLDITHIOCARBAMATE PRODUCES ALTERED PROTEIN PROCESSING AND DOPAMINERGIC TOXICITY IN THE RAT

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Parkinson's Disease (PD) is the second-most common neurodegenerative disease in the United States, and is increasing in prevalence. PD is characterized by the loss of neurons in the nigrostriatal pathway and dyskinesis, as well as non-motor effects, such as cognitive deficits, dementia, and mortality. Sporadic idiopathic PD is thought to develop through interactions between age, genetics, and environment, but exact mechanisms are unknown. Loss of function mutations in Parkin and UCHL1 associated with familial forms of PD suggest that perturbations in the ubiquitin-proteasome system may contribute to idiopathic PD. The presence of reactive cysteines on E1 activating enzyme, which is at the apex of the ubiquitination cascade, may render it susceptible to inhibition through covalent modification by environmental agents that generate electrophiles. Because of its ability to modify proteins on cysteines, *we hypothesized that N,N-diethyldithiocarbamate (DEDC), a member of the dialkylthiocarbamate family of pesticides, could covalently modify the E1 activating enzyme, resulting in compromised protein processing and nigrostriatal toxicity*. To test our hypothesis, rats were exposed to 0.3 mmol/kg/day of DEDC for 8 weeks, and E1 function was assessed by measuring activated E1 and total ubiquinated protein levels, both of which were significantly decreased in brains of DEDC-treated rats as compared to non-treated controls. Covalent modifications of E1 assessed by shotgun LC/MS/MS of peptide digests revealed formation of an S-ethylaminocarbonyl adduct on a single cysteine residue (Cys234). Incubation of recombinant human E1 with ethyl isocyanate to form the S-ethylaminocarbonyl adduct significantly decreased E1 activity compared to non-treated E1, suggesting that this covalent modification is responsible for the inhibition observed in vivo. Total TH protein was significantly decreased in brains of DEDC-treated rats as compared to controls. There was no difference in dopamine levels, which may have been maintained through increased activation of TH via phosphorylation. Additionally, elevated levels of α-synuclein and phosphorylated tau proteins were observed in the DEDC-treated rats. These data suggest that inhibition of E1 by covalent modification at Cys234 by environmental agents can contribute to the development of PD particularly in individuals with SNPs in the *SNCA* and *MAPT* genes identified by genome wide analysis to increase the risk of sporadic PD.

TGF-β INDUCES TIAF1 SELF-AGGREGATION VIA TYPE II RECEPTOR-INDEPENDENT SIGNALING THAT LEADS TO GENERATION OF AMYLOID β PLAQUES IN ALZHEIMER'S DISEASE

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The role of a small TGF-β-induced TIAF1 in contributing to the pathogenesis of Alzheimer's disease (AD) was investigated. TIAF1 interacts with Smad4 in vivo, and blocks Smad-dependent promoter activation when overexpressed. Knockdown of TIAF1 by small interfering RNA resulted in spontaneous accumulation of Smad proteins in the nucleus and activation of the promoter governed by the Smad complex. TGF-β may rapidly induce TIAF1 self-aggregation that leads to apoptosis in a caspase-dependent manner in vitro. By filter retardation assay, we determined that TIAF1 aggregates are abundant (~55%) in the hippocampi of nondemented humans and AD patients. Total TIAF1-positive samples containing Aβ aggregates are 17% and 48%, respectively, in the nondemented and AD groups, suggesting that TIAF1 aggregation occurs preceding formation of Aβ plaques. TIAF1 aggregates might provide protection for Aβ plaque formation as determined in transgenic mouse models. TGF-β1 induces TIAF1 self-aggregation in a type II TGF-β receptor-independent manner in cells, and Smad4 interrupts the aggregation. TIAF1 physically binds Thr668-phosphorylated amyloid precursor protein (APP). TGF-β1 reduces the binding that allows TIAF1 aggregation and APP de-phosphorylation and fragmentation, Aβ production, and apoptosis. Together, TIAF1 self-aggregation occurs in nondemented humans and may facilitate the formation of Aβ plaques during progression toward AD.

EXOME SEQUENCING OF AN FTLD-TDP FAMILY TO IDENTIFY A NEW DISEASE GENE.

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Frontotemporal dementia (FTD) is the most common cause of early-onset dementia, and is characterized by behavioral and language dysfunction that may be accompanied by motor neuron disease. The neuropathological hallmark of FTD is the selective degeneration of the frontal and temporal lobes with the presence of intracellular protein aggregates, containing either the microtubule-associated protein tau (FTD-TAU) or the RNA binding protein TDP-43 (FTD-TDP). Familial FTD (accounting for about 50% of the cases) has been linked to mutations in several genes, including tau (MAPT), progranulin (GRN), valosin-containing protein (VCP) and charged multivesicular body protein 2B (CHMP2B). However, the cause of the disease in the majority of familial cases and in the common sporadic forms remains unknown. Exome capture (the massively parallel sequencing of enriched target exon sequences) allows an unbiased investigation of the complete protein-coding regions in the genome. In order to identify the causal gene underlying the disease in a FTD-TDP family (negative for tau and GRN mutations and showing an autosomal dominant pattern of disease inheritance), we captured and sequenced the exomes of 3 affected and 1 unaffected siblings. We achieved a mean coverage of 91X and sufficient depth (8X) to call variants at ~ 92% of each targeted exome. We identified an average of ~ 25500 variant (SNPs, indels or splice-site mutations) per sample, of which 1269 were common to the affected but absent in the unaffected siblings. Filtering against public SNP databases identified 23 novel variants. We were able to reduce the number of candidate genes to 8-9 by direct sequencing of the variants in an additional unaffected sibling. We are currently sequencing the variants in hundreds of unrelated controls to further narrow down the number of candidate genes. Exome sequencing of a small number of related affected and unaffected individuals in a pedigree, combined with sequencing of unrelated controls and functional studies of the identified variants, promises therefore to be a powerful, efficient strategy for identifying candidate genes underlying familial disorders, even in complex, heterogeneous diseases.

PROGRANULIN MODULATES NEURON DEVELOPMENT IN ZEBRAFISH

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Progranulin (PGRN) is a secreted protein that has been implicated in multiple physiological and disease processes. Autosomal dominant mutations within the progranulin gene (GRN) cause frontotemporal dementia with tau negative ubiquitin positive neuronal inclusions (FTLD-U). Almost all the mutations lead to PGRN haploinsufficiency. TAR DNA-binding protein 43 (TDP-43), encoded by the TARDBP gene, has been identified as the major pathological protein of FTLD-U and the presence of C-terminal TDP-43 in the ubiquitin inclusions is characteristic of GRN-mediated FTLD-U. PGRN is widely expressed by nerve cells, including many that appear unaffected in FTLD-U. Little is known of the mechanisms by which reduced PGRN expression leads ultimately to neuronal degeneration, and a fuller understanding the function of PGRN in neuronal cells should provide insight into this issue. Using motor neuronal cell lines with different levels of PGRN expression we have investigated the ability of PGRN to regulate neurite outgrowth, inhibit apoptosis, and regulate translocation of TDP-43 following apoptotic challenge. PGRN has a strong cytoprotective action, modulates the cytoskeletal architecture and promotes neurite-like outgrowth in vitro. We then employed zebrafish to study the role of PGRN in neurons in vivo. The zebrafish possesses four distinct GRN genes of which zfPGRN-A is the true orthologue of the single human gene. ZfPGRN-A is expressed in embryonic zebrafish neuronal tissue, including the primary motor neurons, which can be readily visualized in whole mount embryos. Ectopic over-expression of zfPGRN-A resulted in increased branching of the Caudal Primary (CaP) motor neurons by 48hrs. When ZfPGRN-A was knocked down using a morpholino antisense strategy we observed truncation of CaP motor neurons with premature branching and an impaired swimming response. Co-expression of zfPGRN-A mRNA rescued both the truncation and swimming defect. Two independent morpholinos were employed to exclude non-specific effects, and the reduction in zfPGRN-A protein levels was confirmed by Western blot analysis. The truncation and early branching phenotype observed by knockdown of zfPGRN-A are strikingly similar to those reported following morpholino knockdown of Survival of Motor Neuron-1 smn1 in zebrafish embryos, mutations of which cause Spinal Muscular Atrophy in humans. We therefore challenged smn1 knockdown embryos with zfPGRN-A mRNA and found a significant reversal of the smn1 knockdown phenotype. We conclude that PGRN expression is capable of modulating neuron survival, branching and development in vitro and in vivo.

RANBP2-MEDICATED NEUROPROTECTION CAUSES THE MODULATION OF PROTEIN-LIPID HOMEOSTASIS OF FUNCTIONALLY DIVERSE BUT LINKED PATHWAYS IN RESPONSE TO OXIDATIVE STRESS.

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Oxidative stress is associated with a plethora of human diseases, aging manifestations and metabolic deficits. Light-elicited oxidative stress is a deleterious risk factor linked to retinal dystrophies, such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP), which occur owing to the selective degeneration of photoreceptor neurons. The identification and characterization of factors and processes that suppress the deleterious effects of oxidative stress may provide insight into mechanisms of neuroprotection and uncover novel therapeutic targets by which the clinical manifestations of various diseases and aging can be modulated. The purpose of our work is to explore the molecular effects of insufficiency of RAN-binding protein-2 (RANBP2) in protecting photoreceptors and the supporting tissue, the retinal pigment epithelium (RPE), from degeneration induced by light-elicited oxidative stress. We contrasted the molecular, biochemical, cellular and subcellular phenotypes of photoreceptors and RPE of inbred wild-type mice and RANBP2-haploinsufficient mice in the absence or presence of light-elicited oxidative stress. We found that membrane biogenesis and the expression levels of a subset of functionally diverse and binding partners of RANBP2 – such as RAN GTPase, ubc9, subunits of the 26S proteasome and a set of orphan nuclear receptors – are differentially modulated by *RANBP2* haploinsufficiency and oxidative stress in the retina. The effects of oxidative stress are also accompanied in *RANBP2*-haploinsufficient mice by a decrease in the levels of ubiquitylated proteins, thus supporting that insufficiency of RANBP2 relieves its suppression on the activity of 26S proteasome in the presence of oxidative stress. Strikingly, the formation of lipid deposits induced by oxidative stress in the RPE – a hallmark of many neurodegenerative diseases and aging – is strongly suppressed by *RANBP2* haploinsufficiency. Conversely to the retina, the expression levels of most RANBP2-binding partners are not affected in the RPE. Thus, the data indicate that insufficiency of RANBP2 results in the cell-type-dependent downregulation of protein and lipid homeostasis, acting on functionally interconnected pathways in response to oxidative stress. These results provide a rationale for the neuroprotection from light damage of photosensory neurons by RANBP2 insufficiency and for the identification of novel therapeutic targets and approaches promoting neuroprotection.

NEURAL INDUCTION WITH A PRONEURAL GENE ENHANCE THE THERAPEUTIC FUNCTIONS OF MESENCHYMAL STEM CELLS IN ALS MOUSE MODEL

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Amyotrophic lateral sclerosis (ALS) is characterized by progressive dysfunction and degeneration of motor neurons in the central nervous system (CNS). In the absence of effective drug treatments for ALS, stem-cell treatment has emerged as a candidate therapy for this disease. Here, we investigated the effects of neural induction on the innate therapeutic potential of mesenchymal stem cells (MSCs). MSCs were neurally induced by transduction with the proneural transcription factor. MSCs transduced with proneural gene (Neu-MSCs) were next tested in mice carrying a high copy-number of the human mutant SOD1G93A transgene. After systemic injection, Neu-MSCs migrated to the spinal cord, where they protected ventral motor neurons but rarely became terminally differentiated neurons. Notably, Neu-MSCs, but not unprocessed MSCs, delayed disease onset if transplanted during pre-onset stages. If transplanted at onset ages, a single treatment with Neu-MSCs was sufficient to enhance motor functions during the symptomatic period (15–17 weeks). By contrast, unprocessed MSCs required repeated transplantation to achieve similar levels of motor function improvement. Our data indicate that systemically transplanted Neu-MSCs can migrate to the CNS and exert beneficial effects on host neural cells through paracrine effects for an extended period of time. Our findings strongly suggest a potential benefit of neural induction of MSCs for the long-term treatment of ALS.

BIOCHEMICAL CHARACTERIZATION OF FULL-LENGTH HUMAN RECOMBINANT LRRK1 AND LRRK2

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Leucine-rich repeat kinase 1 and 2 (LRRK1 and LRRK2) are large, complex multidomain proteins containing kinase and GTPase enzymatic activities and multiple protein-protein interaction domains. Rare mutations in LRRK2 are linked to autosomal dominant forms of Parkinson's disease (PD) and some common LRRK2 variants also increase the risk of PD in some populations. In contrast, LRRK1 mutations have not been reported yet and we have previously shown that pathological mutations in LRRK2 are more toxic than the equivalent mutations in LRRK1. Because LRRK2 is a kinase, this has led to the suggestion that kinase inhibitors might be a novel therapeutic opportunity for PD.

Starting from the idea that LRRK2 but not LRRK1 is associated with PD, we decided to characterize the two proteins in parallel to single out LRRK2 specific properties. At present, most biochemical studies have been performed using suspensions of protein bound to affinity resin. Reports on recombinant LRRK2 in solution refer to truncated fragments, often also containing large affinity tags (i.e. GST). Here, we produced highly pure recombinant LRRK1 and LRRK2 soluble proteins with a small affinity tag. Using a lentiviral expression vector, we have been able to express 3xFLAG tagged full-length LRRK1 and LRRK2 in HEK293T cells and purify them to >90% purity. The proteins obtained show autophosphorylation activity, although LRRK1 appears much less active than LRRK2, as we previously suggested with transient transfections. We also tested whether these proteins are functional GTPase and preliminary data suggest that both LRRK1 and LRRK2 display detectable GTPase activity in vitro. Both LRRK2 and LRRK1 are present in the cytoplasm and in the membrane fraction, suggesting that activated proteins might relocalize to different cellular compartment(s) in order to exert their biological function.

We expect that comparing the biochemical and structural properties of full-length LRRK2 versus LRRK1 could provide both, clues on the pathogenic mechanism of LRRK2 and the opportunity to design and test selective inhibitors of LRRK2 activity.

A YEAST FUNCTIONAL SCREEN PREDICTS NEW ALS DISEASE GENES

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Mutations in two related RNA-binding proteins, TDP-43 and FUS/TLS, have been linked to amyotrophic lateral sclerosis (ALS). Both proteins formed cytoplasmic inclusions and caused toxicity in yeast cells. Based on these properties, we designed a functional screen in yeast to identify new human genes associated with the pathogenesis of ALS. The human proteome contains at least 213 proteins harboring RNA recognition motifs (RRM proteins), including FUS/TLS and TDP-43. To find additional candidates with properties similar to TDP-43 and FUS/TLS, we expressed 132 human RRM proteins in yeast and identified 35 that formed cytoplasmic inclusions and were toxic. Sequence analysis of two of these genes, *EWSR1* (Ewing sarcoma breakpoint region 1) and *TAF15* (RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68kDa), in sporadic ALS patients identified two variants (G511A and P552L) in *EWSR1* and two variants in TAF15 (G391E and R408C). None of these variants were found in over 5,000 healthy control individuals. We also provide evidence that EWSR1 and TAF15 have similar in vitro and in vivo properties as TDP-43 and FUS, can confer neurodegeneration in *Drosophila*, and that disease-associated variants affect localization of each respective protein in motor neurons. Postmortem analysis of sporadic ALS cases revealed cytoplasmic mislocalization of EWSR1 and TAF15. Taken together, these data suggest that these newly identified variants are disease-associated mutations. The identification of mutations in two additional RRM proteins underscores a key role for RNA metabolism defects in ALS. Further, the analysis of additional genes identified in the yeast functional screen will facilitate ALS disease gene discovery and provide new insights into the pathogenic mechanisms of ALS, leading to more comprehensive treatment approaches.

SMALL MOLECULE ANTAGONISTS OF TOXIC B-AMYLOID ASSEMBLIES

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Alzheimer's disease, the most prevalent form of dementia and a leading cause of death for elderly individuals, is marked by the accumulation of neuritic plaques in the brain. Amyloid fibers of Aβ42 peptide comprise these plaques, and prefibrillar soluble oligomeric species of Aβ42 are also associated with brain impairment. There is currently no treatment to directly eradicate these assemblies. The small molecule 4,5-dianilinophthalimide (DAPH-1) has been found to possess anti-amyloid properties for Aβ42, so we sought to investigate analogs of DAPH-1 to determine if altering the constituent groups could enhance these effects. During assembly, DAPH-1 and analogs redirect Aβ42 oligomers into off-pathway aggregates. DAPHs also prevent amyloid fiber formation and can even remodel existing fibers. Certain analogs are more effective at antagonizing Aβ42 fibers, and, further, enhanced remodeling correlates with greater reduction of fiber toxicity. Further characterization of these analogs is required to determine their bioavailability and efficacy *in vivo*.

TDP-43 PATHOLOGY IN ALS: INVESTIGATING LOSS- AND GAIN-OF-FUNCTION IN *DROSOPHILA*.

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Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease with no effective treatments or cure. Recently TAR-DNA binding protein 43 (TDP-43) was identified as the major component of ALS inclusions. Moreover, mutations in TDP-43 have been associated with both sALS and fALS. To gain insight into the mechanisms by which mutations in TDP-43 lead to motorneuron degeneration we have generated *Drosophila* lines expressing wild type, mutant and human splice variants of TDP-43 under the GAL4/UAS system. We have also investigated the phenotype associated with loss of TDP-43 function using tissue specific RNAi knock-down of the two *Drosophila* homologues of TDP-43, TBPH and CG7804. We find clear differences in the cellular localization of the different TDP43 splice variants. Moreover, we find that it is the lack of nuclear TDP-43 rather than the presence of TDP-43 aggregates that is linked to lethality. Current studies are focused on studying the role of TDP-43 in neuronal development and degeneration in these *Drosophila* TDP-43 models of ALS pathogenesis.

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CHARACTERISATION OF THE IGG129-SAPORIN LESIONED RAT AS AN ANIMAL MODEL FOR CHOLINERGIC NEUROPATHOLOGY AND COGNITIVE DECLINE IN ALZHEIMER'S DISEASE.

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Loss of cholinergic neurons in the basal forebrain is one of the neuropathological hallmarks of Alzheimer's disease and is an important determinant of the cognitive decline in patients. In the present studies we characterised the IgG129-Saporin model as an animal model for the cholinergic neuropathology and cognitive symptomatology in Alzheimer's disease. This model is based on ablation of cholinergic neurons in the rat forebrain that express Nerve Growth Factor receptor (NGFr) with the neurotoxin IgG192-Saporin, a conjugate between IgG192, an antibody to the low affinity neurotrophin receptor ($p75^{NGFr}$), and the ribosome inactivating protein saporin. Bilateral, intracerebroventricular IgG192-Saporin injections caused a reproducible depletion of cholinergic neurons in the hippocampus and prefrontal cortex as measured by a 70 – 90% downregulation of choline acetyltransferase (ChAT) activity and a complete loss of NGFr expression in the basal forebrain as shown by immunohistochemistry. We assessed the integrity of two cognitive domains affected in Alzheimer's disease, namely executive function and episodic memory, by the attentional set-shifting and the Morris water maze tasks, respectively. In the attentional set-shifting task, which is dependent on the prefrontal cortex, IgG192-Saporin lesioned rats showed a distinct impairment in the extradimensional shift. In the water maze task, reflecting hippocampus-dependent visuospatial memory, IgG192-Saporin lesioned rats displayed a deficit in the acquisition phase. Studies are ongoing to characterise IgG192-Saporin lesioned rats over a broader range of cognitive domains. The present findings indicate that the IgG192-Saporin animal model, characterised by early cholinergic neuropathology and concurrent cognitive deficits, mimics some of the key hallmarks of Alzheimer's disease.

CELL AUTONOMOUS ALTERATIONS DUE TO MUTANT TDP-43 EXPRESSION IN MOTOR NEURONS DERIVED FROM MOUSE EMBRYONIC STEM CELLS

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Mutations in two DNA/RNA binding proteins, TAR DNA-binding protein (TDP-43) and Fused in Sarcoma (FUS) have been identified as primary causes of inherited Amyotrophic Lateral Sclerosis (ALS) and have led to what is likely to be a paradigm shift in efforts to understand the pathogenesis of ALS. The current evidence worldwide has produced the leading hypothesis that mutations in TDP-43 or FUS/TLS contribute to disease by causing aberrations in RNA processing. One key unanswered question underlying ALS pathogenesis is why motor neurons are exquisitely sensitive to mutation in either protein: how is motor neuron biology altered by either the loss of or mutation in either of these genes? The weight of current evidence strongly supports the view that there must be a component of motor neuron biology that selectively renders them sensitive to alterations in TDP-43 or FUS/TLS-dependent RNA processing. To determine the role(s) of these proteins in motor neurons, mutant expressing motor neurons have been obtained by differentiation of blastocyst-derived embryonic stem cell lines carrying transgenes encoding wild-type or mutant human TDP-43 (familial mutant M337V or sporadic mutant Q331K, each driven by the prion promoter) and GFP expressed under the control of the motor neuron specific Hb9 promoter. The resulting stem cells were then induced to differentiate into motor neurons (by exposure to retinoic acid and sonic hedgehog) and enriched populations of TDP-43-expressing motor neurons obtained by sorting of GFP-positive cells with flow cytometry. Alterations in RNA expression and splicing patterns have been analyzed. Furthermore, immunoprecipitation of myc-tagged TDP-43 from motor neurons has identified RNA targets that are differentially bound by wild-type or mutant TDP-43. Together, this study presents a novel approach for identifying cell-intrinsic properties of TDP-43 in motor neurons, one of the most relevant cell types for understanding ALS pathogenesis.

LOSS OF SORTILIN CAUSE INCREASED SURVIVAL OF RETINAL GANGLION CELLS IN A MOUSE MODEL OF TRANSIENTLY INCREASED INTRAOCULAR PRESSURE

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Nerve growth factor (NGF) signalling is known to be important to ensure normal development of the retina. Additionally, NGF signalling is involved in supporting survival of injured retinal ganglion cells (RGCs) in the adult eye following glaucoma. NGF is thought to exert its pro-survival effect by binding to a receptor complex consisting of specific tyrosine kinase A (TrkA) receptor and the p75NT receptor (p75NTR). However, NGF may also be released in a precursor form denoted proNGF. As opposed to its mature form, unprocessed proNGF acts as a death-inducing ligand when bound to a receptor complex comprising sortilin and the p75NTR. Elevated intraocular pressure (IOP)-induced retinal ganglion cell death is associated with an acute phase of necrosis and a delayed phase of apoptosis. It has previously been shown that expression of both sortilin and p75NTR is increased after IOP-induced ischemia, suggestive of active cell death signalling through this receptor complex.
We utilized sortilin knock-out (KO) mice to investigate whether loss of sortilin signalling would be neuroprotective in the IOP-model. We showed that, compared to wildtype controls, sortilin KO mice displayed significantly increased RGC survival two weeks following transient ischemia. Furthermore, in retinas isolated from injured eyes, expression of p75NTR and TrkA were increased and decreased, respectively, compared to uninjured control retinas, suggesting that pro-survival signalling through TrkA was reduced whereas death-signalling through p75NTR and sortilin was increased. These data indicate that sortilin signalling is involved in induction of RGC cell death following retinal ischemic injury.

A GENETIC SCREEN FOR DOMINANT SUPPRESSORS OF *PINK1* IN *DROSOPHILA MELANOGASTER*

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Parkinson's disease (PD) is one of the most common progressive neurodegenerative movement disorders, yet the pathogenesis of disease remains unclear. Several genes have been linked to hereditary forms of PD among which *pink1*. *pink1* encodes for a mitochondrial localized serine/threonine kinase. Recent data indicate that Drosophila loss of function mutations of *pink1* are viable, male sterile and show mitochondrial defects. These mitochondrial defects lead to muscle degeneration and reduced synaptic vesicle cycling in neurons. To further understand the relation between PINK1 and the mitochondria physiology we are isolating dominant suppressors and enhancers of *pink1*.
As *pink1* affects mitochondrial function and synaptic transmission, we are screening a collection of 500 mutants that affect neuronal communication, isolated in a forward EMS screen, for modifiers of *pink1*. We already identified one suppressor and one enhancer and we tested them for alleviation of or enhancement of the specific mitochondrial defects we observed in *pink1* mutants. We found that both these modifier genes impact ATP level and mitochondrial membrane potential defects of *pink1* mutant. These genetic modifiers will help us to elucidate the molecular pathways and interactions that control mitochondrial function especially as it impacts on the function and survival of neurons.

ASTROCYTIC REDOX REMODELING BY AMYLOID β PEPTIDE

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Although oxidative stress is a general characteristic of many neurodegenerative diseases including Alzheimer's disease (AD), the repertoire of disease-specific redox perturbations is poorly understood. Astrocytes play a critical role in redox homeostasis in brain and provide neurons with cysteine needed for glutathione (GSH) synthesis. In this study, we demonstrate that the signature of astrocytic redox responses provoked by amyloid β (Aβ) is distinct from that of a general oxidative insult (by the organic peroxide, tertiary-butylhydroperoxide (t-BuOOH)). Acute Aβ treatment increased levels of cystathionine β-synthase (CBS) and enhanced transsulfuration flux in contrast to repeated Aβ exposure, which decreased CBS and catalase protein expression level. Although t-BuOOH also increased transsulfuration flux, CBS levels were unaffected. The net effect of Aβ treatment was an oxidative shift in the intracellular GSH:glutathione disulfide redox potential in contrast to a reductive shift in response to peroxide treatment. In the extracellular compartment, Aβ treatment enhanced cystine uptake and cysteine accumulation, which was not observed with t-BuOOH and resulted in remodeling of the extracellular cysteine/cystine redox potential in the reductive direction. The redox changes elicited by Aβ but not peroxide were associated with enhanced DNA synthesis albeit without cell division, suggesting an S to G2/M phase block in the cell cycle. Our study suggests that the alterations in astrocytic redox status could compromise the neuroprotective potential of astrocytes and may be a potential new target for therapeutic intervention in AD.

LOSS OF ALS2/ALSIN AGGRAVATES MOTOR DYSFUNCTION IN A MUTANT SOD1-EXPRESSING MOUSE ALS MODEL BY DISTURBING THE ENDOLYSOSOMAL SYSTEM

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Loss of function mutations in the *ALS2* gene accounts for a number of juvenile motor neuron diseases, such as a juvenile recessive form of ALS (ALS2), a rare juvenile recessive form of primary lateral sclerosis (PLSJ), and an infantile-onset ascending hereditary spastic paralysis (IAHSP). Thus, its gene product ALS2/alsin may play an important role in maintenance and/or survival of motor neurons. ALS2 is a guanine nucleotide exchange factor for the small GTPase Rab5 and involves in macropinocytosis-associated endosome fusion and trafficking, anti-cytotoxicity, and neurite outgrowth. However, the physiological role of ALS2 and molecular mechanisms underlying motor dysfunction remain unclear. We here identified ALS2 as a novel regulator for endolysosomal protein degradation. Genetic ablation of *Als2* in $SOD1^{H46R}$ transgenic mice aggravated the mutant SOD1-associated disease symptoms such as body weight loss and motor dysfunction, leading to the earlier death. Light and electron microscopic examinations revealed the presence of degenerating and/or swollen spinal axons accumulating granular aggregates and autophagosome-like vesicles in early- and even pre-symptomatic $SOD1^{H46R}$ mice. Further, enhanced accumulation of insoluble high molecular weight SOD1, poly-ubiquitinated proteins, and macroautophagy-associated proteins such as polyubiquitin-binding protein p62/SQSTM1 and a lipidated form of light chain 3 (LC3-II), emerged in ALS2-deficient $SOD1^{H46R}$ mice. Intriguingly, ALS2 was colocalized with LC3 and p62, and partly with SOD1 on autophagosome/endosome hybrid compartments called amphisomes, and loss of ALS2 significantly lowered the lysosome-dependent clearance of LC3 and p62 in cultured cells. Based on these observations, disturbance of the endolysosomal system by ALS2 loss may exacerbate the $SOD1^{H46R}$-mediated neurotoxicity by accelerating the accumulation of immature vesicles and misfolded proteins in the spinal cord. We propose that ALS2 is implicated in endolysosomal trafficking through the fusion between endosomes and autophagosomes, thereby regulating endolysosomal protein degradation *in vivo*.

This work was supported by the Tokai University General Research Organization, JSPS, and MHLW (Japan).

INSIGHTS INTO TDP-43 PROTEINOPATHY USING DROSOPHILA MELANOGASTER AS A MODEL SYSTEM.

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TDP-43 (43 kDa TAR DNA-binding protein) is a major constituent of ubiquitin-positive cytosolic aggregates present in neurons of patients with amyotrophic lateral sclerosis (ALS) and ubiquitin-positive fronto-temporal lobar degeneration (FTLD-U). Inherited mutations in TDP-43 have been linked to familial forms of ALS, indicating a key role for TDP-43 in disease pathogenesis. We have utilized Drosophila melanogaster to develop an in vivo model of TDP-43 proteinopathy. Expression of wild-type human TDP-43 protein in Drosophila motor neurons led to motor dysfunction and dramatic reduction of lifespan; these phenotypes were enhanced by coexpression of Ubiquilin 1, a previously identified TDP-43-interacting protein. Interestingly, ectopically expressed TDP-43 was exclusively localized to motor neuron nuclei, suggesting that expression of wild-type TDP-43 alone is detrimental even in the absence of cytosolic aggregation. We are currently exploring the role that gene expression changes may play in TDP-43 toxicity. Using both constituitive and inducible systems, we have identified genes that are changed in vivo upon TDP-43 expression, with the goal of identifying genes or pathways whose modification can alleviate TDP-43-dependent phenotypes. Our findings demonstrate that TDP-43 exerts cell autonomous neurotoxicity in Drosophila and suggest that dose-dependent alterations of TDP-43 nuclear function underlie motor neuron death in ALS.

DEVELOPING APPLICATIONS FOR PRIMARY ASTROCYTES TO ENABLE DRUG DISCOVERY

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Astrocytes are a type of glial cell that play important roles in brain function. They regulate the extracellular ionic and chemical environment, respond to CNS injury, and are a major factor in the pathogenesis of ischemic neuronal death. Astrocytes remain as one of the most active research areas in neuroscience. Human astrocytes provide an excellent source of primary human cells and are the beginnings of a physiologically relevant system to use for a wide range of studies. However, the use of primary astrocytes has not been reported in high-throughput drug discovery settings. Life Technologies has developed human brain progenitor-derived astrocytes. To better enable our customers, we hypothesized that human astrocytes could be combined with BacMam-mediated gene expression and/or LanthaScreen cellular technology for high-throughput assays. To test susceptibility of human astrocytes to BacMam mediated gene expression, a series of transduction conditions were performed using BacMam virus for GFP-tagged p53. Cells were monitored for GFP expression using fluorescence microscopy for a period of 72 hours. To perform high-throughput assay feasibility, human astrocytes were seeded in a 384-well microplate and the astrocytes were transduced with BacMam virus expressing p53 under optimized conditions. Cells were treated with DNA damaging reagent to induce post-translational modifications of p53. Levels of post-translational modifications were quantitated using LanthaScreen cellular assay technology. To investigate potential for disease modeling, human astrocytes were transduced with BacMam viruses of Parkinsons-related disease target LRRK2 and it's putative substrates including alpha-synuclein. Over expression was confirmed by western blot and fluorescence microscopy. These studies have shown that human brain progenitor-derived astrocytes are amenable to BacMam delivery system and can be applied to high-throughput assays such as cellular LanthaScreen assays. This data shows that combining human astrocytes, BacMam technology and LanthaScreen cellular assays offers a physiologically relevant high-throughput compatible workflow for customers interested in neuroscience related disease modeling and drug screening.

CATHEPSIN B INHIBITION REDUCES BRAIN B-AMYLOID PEPTIDES WHILE INCREASING BRAIN BACE1 ACTIVITY IN ANIMAL MODELS EXPRESSING APP WITH THE NORMAL B-SECRETASE SITE SEQUENCE

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β-secretase inhibition is a potential disease-modifying therapy for Alzheimer's disease (AD). The cysteine protease, cathepsin B, is hypothesized to be a β-secretase, which cleaves the normal (wild-type) β-secretase site sequence in amyloid precursor protein (APP) to produce beta-amyloid peptides (Aβ). Evidence for cathepsin B being a β-secretase includes the fact that deleting the gene or chemically inhibiting the activity decreases brain Aβ and the carboxyterminal β-secretase fragment (CTFβ) in animal models. Moreover, inhibiting cathepsin B activity improves the memory deficit and reduces brain plaque in transgenic mice expressing APP containing the normal β-secretase site sequence. However, it is not clear if those effects are directly due to cathepsin B inhibition or an off-target inhibition of the aspartyl β-secretase, BACE1. To evaluate that issue, we studied the effect of oral administration of the potent cysteine protease inhibitor, E64d, to either guinea pigs, which are a model of normal human Aβ production, or to transgenic mice expressing human APP containing the normal β-secretase site and the London mutant γ-secretase site sequences, which overproduce Aβ and mimic AD behavior and pathology. Guinea pigs gavaged once–a-day for a week with E64d had a dose response reduction in brain, CSF and plasma Aβ(40) and Aβ(42), brain CTFβ and brain cathepsin B activity but increased brain BACE1 activity. Paired analysis from individual guinea pigs showed a strong positive correlation between brain Aβ(40) or Aβ(42) and brain cathepsin B activity but a weak negative correlation with brain BACE1 activity. Feeding young (6 month old) or old (12 month old) transgenic mice chow doped with E64d (10 mg/kg/day) for either 1 or 3 months also reduced brain, CSF and plasma Aβ(40) and Aβ(42), brain CTFβ and brain cathepsin B activity but increased brain BACE1 activity in both age groups. Paired analysis of individual mice showed a strong positive correlation between brain Aβ(40) or Aβ(42) and brain cathepsin B activity but only a weak negative correlation with brain BACE1 activity. Importantly, those feedings also improved the memory deficit and brain plaque in both young and old transgenic mice. These data show that the effects of inhibiting cathepsin B are not due to an off-target inhibition of BACE1. More importantly, these data support the potential of cathepsin B inhibitors generally and E64d specifically for treating AD since most AD patients express APP containing the normal β-secretase site sequence.

PHARMACOGENETIC FEATURES OF INHIBITORS TO CATHEPSIN B THAT IMPROVE MEMORY DEFICIT AND REDUCE BETA-AMYLOID RELATED TO ALZHEIMER'S DISEASE

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Beta-amyloid (Abeta) in brain is a major factor involved in Alzheimer's disease (AD) that results in severe memory deficit. Our recent studies demonstrate pharmacogenetic differences in the effects of inhibitors of cathepsin B to improve memory and reduce Abeta in different mouse models of AD. The inhibitors improve memory and reduce brain Abeta in mice expressing the wild-type (WT) beta-secretase site of human APP, expressed in most AD patients. However, these inhibitors have no effect in mice expressing the rare Swedish (Swe) mutant APP. Knockout of the cathepsin B decreased brain Abeta in mice expressing WT APP, validating cathepsin B as the target. The specificity of cathepsin B to cleave the WT beta-secretase site, but not the Swe mutant site, of APP for Abeta production explains the distinct inhibitor responses in the different AD mouse models. In contrast to cathepsin B, the BACE1 beta-secretase prefers to cleave the Swe mutant site. Discussion of BACE1 data in the field indicate that they do not preclude cathepsin B as also being a beta-secretase. Cathepsin B and BACE1 may participate jointly as beta-secretases for production of Abeta that is secreted from neurons and accumulates in AD brains. Significantly, the majority of AD patients express normal WT APP and, therefore, inhibitors of cathepsin B represent candidate drugs for AD.

GENOMIC PROFILE OF TOLL-LIKE RECEPTOR PATHWAYS IN TRAUMATICALLY BRAIN- INJURED MICE: EFFECT OF EXOGENOUS PROGESTERONE

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Background: Traumatic brain injury (TBI) causes acute inflammatory responses that result in an enduring cascade of secondary neuronal loss and behavioral impairments. It has been reported that progesterone (PROG) can inhibit the increase of some inflammatory cytokines and inflammation-related factors induced by TBI. Toll-like receptors (TLRs) play a critical role in the induction and regulation of immune/inflammatory responses. Therefore, in the present study, we examined the genomic profiles of TLR-mediated pathways in traumatically injured brain and PROG's effects on these genes. **Methods:** Bilateral cortical impact injury to the medial frontal cortex was induced in C56BL/6J mice. PROG was injected (i.p., 16mg/kg body weight) at 1 and 6 h after surgery. Twenty-four hours post-surgery, mice were killed and peri-contusional brain tissue was harvested for genomic detection. RT-PCR arrays were used to measure the mRNA of 84 genes in TLR-mediated pathways. **Results:** We found that 2 TLRs (TLR1 and 2), 5 adaptor/interacting proteins (CD14, MD-1, HSPA1a, PGRP and Ticam2) and 13 target genes (Ccl2, Csf3, IL1a, IL1b, IL1r1, IL6, IL-10, TNFa, Tnfrsf1a, Cebpb, Clec4e, Ptgs2 and Cxcl10) were significantly up-regulated after injury. Administration of PROG significantly down-regulated 3 of the 13 target genes after TBI (Ccl-2, IL-1b and Cxcl-10), but did not affect the expression of any of the detected TLRs or adaptor/interacting proteins. Rather, PROG up-regulated the expression of one TLR (TLR9), 5 adaptor/interacting proteins, 5 effectors and 10 downstream target genes. **Conclusion:** The results demonstrate that TBI can increase gene expression in TLR-mediated pathways. Moreover, PROG does not down-regulate the increased TLRs and their adaptor proteins in traumatically injured brain. The reduction of the observed inflammatory cytokines by PROG does not appear to be the result of inhibiting TLRs and their adaptors in the acute stage of TBI.

Key words: Toll-like receptors, Progesterone, traumatic brain injury, inflammation, neuroprotection, mouse

This work was supported by AHA Grant 0830481N to F.H., Emory University URC Grant (2009-002) to F.H., research funding from the Department of Pediatric Cardiovascular Surgery at the Children's Hospital of Atlanta and NIH 5R01NS048451 and 1R01HD061971 to D.G.S. A portion of this research was supported by a gift from BHR Pharma.

DETERMINING THE PHYSIOLOGICAL ROLE OF TDP-43 IN THE CENTRAL NERVOUS SYSTEM

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Tat activating regulatory DNA-binding protein (Tardbp or TDP-43), an essential RNA binding protein, has been associated with the pathophysiology of neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U). Understanding the biology of TDP-43 in the nervous system will be critical for clarifying the pathophysiology of this RNA binding protein in neurodegenerative diseases. Toward this end, we have begun to address the physiological role of TDP-43 and its downstream targets. Although our previous efforts revealed important roles of TDP-43 in regulation of body fat metabolism and formation of SMN-associated Gemini of coiled bodies, the physiological role of TDP-43 in the brain remains unknown. To examine the function of TDP-43 in forebrains of mice, we crossbred our conditional *TDP-43 knockout* mice with *CamKIIa-iCre* transgenic mice and we are currently analyzing the consequence of the lack of TDP-43 in this brain region and outcomes of these findings will be presented.

STRUCTURAL INSIGHTS INTO ALLOSTERIC INHIBITION OF GLUN2B *N*-METHYL-D-ASPARTATE (NMDA) RECEPTORS

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Majority of fast excitatory synaptic transmission in the mammalian brain is mediated by a class of molecules called ionotropic glutamate receptors, which include *N*-methyl-D-aspartate (NMDA) receptors. NMDA receptors are hetero-tetrameric ion channels that are composed of two GluN1 subunits and two GluN2 (A-D) subunits or GluN3 subunits. NMDA receptors play key roles in numbers of important processes including synaptic plasticity and development in normal state, whereas excessive activity of NMDA receptors is associated with ischemic brain injury and neurodegenerative diseases including Parkinson's disease, Alzheimer's disease and Huntington's disease. Activity of NMDA receptor is tightly controlled through multiple pathways. One such mechanism is allosteric modulation through binding of small molecules to the extracellular amino terminal domain (ATD) in a subtype specific manner, i.e. polyamines and protons bind GluN1, Zn^{2+} binds both GluN2A and GluN2B, and phenylethanolamine compounds bind GluN2B. To understand the molecular mechanism of the ATD-dependent allosteric modulation of NMDA receptors, we have solved the structures of GluN2B ATD in the zinc-bound and -free forms. The structures reveal an overall clamshell architecture with a unique domain orientation distinct from the non-NMDA receptor ATDs and molecular determinants for the zinc binding site, ion binding sites, and the architecture of the putative phenylethanolamine binding site.

WASP IS ACTIVATED BY PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE TO RESTRICT SYNAPSE GROWTH IN A PATHWAY PARALLEL TO BONE MORPHOGENETIC PROTEIN SIGNALING.

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A well organized nervous system is critical for accurate brain function as numerous psychiatric and neurological diseases are thought to arise from morphological defects in synaptic architecture and connectivity. We are therefore indentifying molecules and their effectors involved in regulating synaptic morphology. Phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) is a membrane lipid is involved in several important signaling pathways; and its metabolism is associated with neurological diseases including Alzheimer's Disease and Lowe Syndrome. However, the role of this lipid in the regulation of synapse morphology is ill-defined. Here we identify $PI(4,5)P_2$ as a gatekeeper of neuromuscular junction (NMJ) size and potentially also other synaptic types. We show that $PI(4,5)P_2$ levels in neurons are critical in restricting synaptic growth by localizing and activating presynaptic Wiscott-Aldrich Syndrome Protein (WSP). This function of WSP is independent of Bone Morphogenetic Protein (BMP) signaling but is dependent on Tweek, a novel neuronally expressed protein. Loss of $PI(4,5)P_2$-mediated WSP-activation results in increased formation of moesin-GFP patches that concentrate at sites of bouton growth. Based on pharmacological and genetic studies, moesin patches mark polymerized actin accumulations and correlate well with NMJ size. Taken together, we propose a model where $PI(4,5)P_2$ and WSP-mediated signaling at presynaptic termini controls actin-dependent synapse growth in a pathway at least in part in parallel to synaptic BMP signaling.

**T.M.K and R.L.H. contributed equally to this work.*

ALS-ASSOCIATED PROTEINS TDP-43 AND FUS/TLS FUNCTION IN A COMMON BIOCHEMICAL COMPLEX

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Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease that preferentially targets motor neurons. Dominant mutations in two related RNA-binding proteins, TDP-43 (43 kDa TAR DNA-binding domain protein) and FUS/TLS (fused in sarcoma/translated in liposarcoma) cause a subset of ALS. The convergent ALS phenotypes associated with TDP-43 and FUS/TLS mutations are suggestive of a functional relationship. Consistent with this idea, recently published studies from several laboratories, including ours, have demonstrated physical interaction between TDP-43 and FUS/TLS in mammalian cells. Our work demonstrated that TDP-43 and FUS/TLS selectively interact within the context of a □300 kDa TDP-43 complex in a manner that requires the TDP-43 Gly-rich domain, which is the target of most ALS-associated mutations in the protein. Cleavage products of TDP-43, which have received interest as disease determinants in ALS, cofractionated with the 300 kDa TDP-43 complex and retained interaction with FUS/TLS, but were excluded from a large (□2 MDa) TDP-43 ribonucleoprotein complex. Finally, both TDP-43 and FUS/TLS were required for maximal expression of HDAC6, a previously identified substrate of TDP-43, and both factors associated with HDAC6 mRNA in vitro and in cellulo. These findings support a model in which TDP-43 and FUS/TLS collaborate, potentially within the context of a core biochemical complex, to regulate a subset of RNA substrates. An implication of these findings is that participation of TDP-43 and FUS/TLS within common biochemical pathways may contribute to convergent ALS phenotypes associated with their respective gene mutations. Current studies are focused on establishing the impact of ALS-associated mutations on TDP-43 complex forming potential, including interaction with FUS/TLS, as well as further elucidation of TDP-43 core complexes.

ROLES OF BACE-1 AND CATHEPSIN B IN THE GENERATION OF AB PEPTIDE IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is an age-related neurodegenerative disorder that results in progressive loss of memory and accumulation of neurotoxic β-amyloid (Aβ) peptides in brain. The abnormal accumulation of Aβ peptides has been demonstrated as a major factor in the disease, based largely on studies of transgenic mouse models which overproduce Aβ, resulting in memory deficit and amyloid plaque brain pathology similar to that observed in AD patients. Aβ peptides are generated from the amyloid precursor protein (APP) by proteases known as β- and γ-secretases, which release the N- and C-termini of Aβ, respectively. The aspartyl protease BACE 1 is a β-secretase, but it is relatively inefficient at cleaving the wt β-secretase site. The cysteine protease cathepsin B (CatB) has been proposed as an alternative candidate β-secretase, which resides in the regulated secretory pathway of neurons, where it produces Aβ by efficient cleavage of the wt β-secretase site of APP. Inhibitors of cathepsin B significantly reduce brain levels of Aβ40 and Aβ42, and also reduce brain levels of C-terminal β-secretase fragment (CTFβ) derived from APP; notably, these inhibitors improve memory deficit in a AD mouse model expression human APP with wt β-secretase site. In this study, we have generated transgenic mice expressing either the wildtype (APP_{wtln}) or mutant ($APP_{mtswe/ln}$) β-secretase site form of APP and sufficient or deficient in either cathepsin B or BACE-1 to understand the mechanisms of Aβ generation. $APP_{wt}/CatB^{-/-}$ mice and $APP_{mt}/BACE^{-/-}$ mice showed decreases in Aβ peptide levels (both 1-40 and 1-42), decreases in CTFβ, increases in sAPPα, reduced levels of amyloid plaques and improved cognitive behavior compared to the sufficient animals. Other combinations of the mice ($APP_{mt}/CatB^{+/+}$, $APP_{mt}/CatB^{-/-}$ or $APP_{wt}/BACE^{+/+}$, $APP_{wt}/BACE^{-/-}$) did not have altered levels. These data suggest that both cathepsin B and BACE-1 are true β-secretases but that they function at different levels in the generation of Aβ peptides. These data strongly suggest that a therapeutically acceptable method of inhibiting Cat B can be developed to reduce brain Aβ in AD patients.

SONIC HEDGEHOG SIGNALING IS ESSENTIAL FOR THE MAINTENANCE OF CELLULAR HOMEOSTASIS WITHIN THE ADULT NIGRO-STRIATAL MICROCIRCUIT.

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The combinatory apposition of neuromodulatory, inhibitory and excitatory neurons within the same micro circuit greatly expands the repertoire of strategies available to the CNS for the homeostatic regulation of circuit performance. It also raises questions about how the circuit type specific relative proportion of the constituent neuronal subtypes, which display very different survival risks, is maintained throughout life. We tested the hypothesis that target derived survival factor signaling within microcircuits of the adult striatum plays a role in the homeostatic maintenance of circuit architecture.
In the striatum, the concerted action of the neuromodulators dopamine (DA), supplied by the ascending, mesencephalic dopamine system and acetylcholine (ACh), supplied by locally projecting, tonically active interneurons (TANS), together with gabaergic input from a set of local interneurons provides the means by which experience shapes the strength of the glutamatergic drive of medium spiny projection neurons (MSNs).
Using genetic gene expression - tracer and conditional ablation strategies together with acute pharmacological and neurotoxicological perturbations we found that the mature mesencephalic - striatal microcircuit embodies a reciprocal, trophic factor support loop with homeostatic properties that is absolutely required for the interdependent maintenance of TANS and fast spiking gabaergic interneurons (FS) in the striatum on one side and DA-neurons of the ventral midbrain on the other. One arm of this loop is provided by expression of the axonaly transported cell signaling factor sonic hedgehog (Shh) by all mesencephalic DA neurons, which signals to TANS and FS neurons throughout life and whose expression is repressed by signals emanating from TANS. The other arm is provided by the glia derived, dopaminotrophic factor (GDNF), which is expressed by all TANS throughout life and whose expression is repressed by DA – neuron produced Shh. The chronic, genetic interruption of trophic factor signaling causes late life onset, progressive loss of TANS, FS and DA neurons. Despite a correlated degeneration of TANS and DA neurons we find highly dynamic alterations in ACh and DA dependent neuronal signaling whose functional consequences on locomotion activity and gait dynamics can be ameliorated by DA substitution and anti cholinergic pharmacology of proven efficacy in the management of Parkinson's Disease.
Our work provides in vivo evidence for the maintenance of the relative proportions of the constituent members of neuronal assemblies through homeostatic trophic strategies and suggests that the maintenance of circuit specific, relative neuronal proportions trumps the importance of absolute neuronal survival.

TDP-43 PHOSPHORYLATION DRIVESS NEUROTOXICITY IN A C. ELEGANS MODEL OF TDP-43 PROTEINOPATHY

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Neurodegenerative disorders characterized by neuronal and glial lesions containing aggregated pathological TDP-43 protein in the cytoplasm, nucleus, or neurites are collectively referred to as TDP-43 proteinopathies. Lesions containing aggregated TDP-43 protein are a hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP). In addition, mutations in human TDP-43 cause ALS. We have developed a C. elegans model of TDP-43 proteinopathies to study the cellular, molecular, and genetic underpinnings of TDP-43 mediated neurotoxicity. Expression of normal human TDP-43 in all C. elegans neurons causes moderate motor defects, while ALS-mutant G290A, A315T, or M337V TDP-43 transgenes cause severe motor dysfunction. The model recapitulates some characteristic features of ALS and FTLD-TDP including age-induced decline in motor function, decreased lifespan, and degeneration of motor neurons accompanied by hyperphosphorylation, truncation, and ubiquitination of TDP-43 protein that accumulates in detergent insoluble protein deposits. In C. elegans, TDP-43 neurotoxicity is independent of activity of the cell death caspase CED-3. Furthermore, phosphorylation of TDP-43 at serine residues 409/410 drives mutant TDP-43 toxicity. This model provides a tractable system for further dissection of the cellular and molecular mechanisms underlying TDP-43 neuropathology.

INHIBITION OF EGFR IN AN ALS MOUSE MODEL DELAYS SYNAPSE ELIMINATION IN EARLY DISEASE

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting motor neurons. The G93A mutation in the Cu/Zn superoxide dismutase (SOD1) gene causes a version of familial ALS. The SOD1 G93A transgenic mouse harboring this human mutation is a well-characterized mouse model of ALS. Previous reports have shown that inhibition of epidermal growth factor receptor (EGFR) leads to neuroprotection of retinal ganglion cells in a model of glaucomatous optic neuropathy (Liu et al., 2006), and to improved recovery in a spinal cord injury model (Erschbamer et al., 2007). Furthermore, we found that EGFR inhibitors such as erlotinib (Tarceva□) protect neurons from axon loss in an NGF-dependent culture model of dorsal root ganglion (DRG) neurons. Thus, we assessed the therapeutic potential of this compound in the SOD1 mouse model in its ability to (1) slow motor neuron denervation at the neuromuscular junction, (2) delay motor neuron cell death, (3) delay disease onset, (4) improve motor behavior during disease progression, and (5) extend survival. Phospho-EGFR staining was reduced in the spinal cords of erlotinib-treated mice. Critically, erlotinib treatment slowed neuromuscular denervation and delayed disease onset in SOD1 mice. We are currently assessing whether erlotinib also extends lifespan and improves motor function in symptomatic SOD1 transgenic mice.

EVALUATING TRINUCLEOTIDE REPEAT EXPANSIONS IN ALS

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Amyotrophic lateral sclerosis (ALS) is a crippling neurodegenerative disease that attacks motor neurons. While mostly a sporadic disorder, mutations in several genes, such as RNA binding protein encoding gene TDP-43, have been identified in some ALS case. Aggregated TDP-43 is found in degenerating neurons of most ALS patients, and mutations in the TDP-43 gene have been linked to some rare familial and sporadic forms of ALS. Recently, we have found that ataxin 2, a polyglutamine (polyQ) protein, whose polyQ repeat expansions cause spinocerebellar ataxia type 2 (SCA2), is a potent modifier of TDP-43 toxicity in yeast and fly; expansions of 27-33Qs in the ataxin 2 gene (ATXN2) are significantly associated with increased risk for ALS. These data argue that ATXN2 is a new and relatively common ALS genetic risk factor. In addition to ataxin 2 and SCA2, there are other polyQ diseases caused by the CAG repeat within the coding region of the respective genes. Is the effect of polyQ expansions in ALS specific to ataxin 2 or do polyQ expansions in other genes also increase risk for ALS? If it is specific to ataxin 2 this suggests that the protein's normal function (i.e., regulation of RNA metabolism) is key. On the other hand, if polyQ expansions in other genes also occur, then it suggests that the expansion is key, perhaps by perturbing proteostasis networks in a general way. To test this, we choose four genes with polyQ expansions: ataxin 1, ataxin 3, ataxin 6, and Huntington. In addition, we analyzed two other genes, PABPC1 and PABPC3, which encode cytoplasmic polyA binding proteins. These proteins have been found to physically interact with ataxin 2 and also harbor trinucleotide repeats encoding alanine (polyA) instead of polyQ. We assessed the polyQ or polyA repeat lengths in these proteins by PCR and Genescan analysis of several hundred ALS and healthy controls. Other than ataxin 2, we did not identify a significant correlation between the incidence of ALS and trinucleotide repeat expansions in these other genes. Taken together, these data suggest that the effects of ataxin 2 polyQ expansions on ALS risk are likely to be rooted in the biology of ataxin 2 and/or ataxin 2-specific interactions rather than the presence of a polyQ repeat per se. These findings have important consequences for understanding of the role of ataxin 2 in ALS pathogenesis and provide a new framework for future mechanistic studies.

ELEVATED EXPRESSION OF WILD TYPE AND ALS-LINKED MUTATIONS IN FUS/TLS TRIGGERS NEURODEGENERATION IN MICE.

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are neurodegenerative diseases sharing overlapping clinical and pathological characteristics. Mutations in two wildly expressed nucleic-acid binding proteins, TDP-43 and FUS/TLS, cause an inherited form of ALS, and abnormal aggregations of these two proteins are present in both ALS and FTD independent of the mutations. To determine FUS/TLS pathogenic mechanisms, expression of wild type and two ALS-linked mutations (R514G and R521C) in FUS/TLS was achieved in the nervous system of mice (using a murine prion gene promoter). Lines of comparable wild type or mutant human FUS/TLS accumulation were established. Mutant FUS/TLS causes abnormal posture, clasping and hindlimb spread phenotypes. Aged wild type and mutant FUS/TLS expressing animals develop abnormal gait, accompanied by glia activation along with reduced numbers of motor and sensory axons. Both wild type and ALS-linked FUS/TLS accumulated in urea insoluble fractions and showed altered distribution, but do in different ways. Taken together, our results demonstrate that human wild type or ALS-linked mutations in FUS/TLS trigger neurodegeneration in mice and suggest that the underlying mechanisms for which wild type and ALS-linked mutants cause neurodgeneration are distinct, providing a molecular basis for FUS involvement in pathogenesis in the absence of mutation.

FORMATION OF AN RNA/DNA HYBRID R-LOOP AT THE HUMAN *FMR1* 5'UTR

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The human *FMR1* gene contains a $(CGG)_n$ trinucleotide repeat in the 5' untranslated region (5'UTR) that is responsible for a number of heritable disorders affecting both early neurodevelopment (FXS, OMIM #300624) and late-onset neurodegeneration (FXTAS, OMIM #300623). Research to date has revealed a basic understanding of the underlying cellular mechanisms of the *FMR1* repeat-expansion disorders, but many knowledge gaps remain in these mechanisms. Bioinformatic analysis of the sequence composition in and around the *FMR1* 5'UTR suggests that an RNA/DNA hybrid structure (R-Loop) forms following active transcription through the region. Non-denaturing bisulfite sequencing reveals the presence of extensive, continuous single-stranded DNA spanning the 5'UTR and the CGG repeats in genomic DNA harvested from cultured human skin fibroblasts from both "normal" (CGG<55) and "premutation" (55<200) repeat alleles. Elimination of this single-strandedness following RNase H digestion demonstrates that this feature is the result of an RNA/DNA hybrid forming and displacing the non-template strand of DNA. This data introduces a previously unknown molecular feature of this locus, with potential implications for the associated molecular pathologies.

GENETIC AND PHARMACOLOGIC MODULATION OF THE IIS AND HSF-1 PATHWAYS RESCUES MUTANT ATAXIN-3-MEDIATED PROTEOTOXICITY IN C. ELEGANS NEURONS

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The risk of developing neurodegenerative diseases increases with age. Although many of the molecular pathways regulating proteotoxic stress and longevity are well characterized, their contribution to disease susceptibility remains unclear. In this study, we developed a new Caenorhabditis elegans model of Machado-Joseph disease (MJD) pathogenesis. Pan-neuronal expression of mutant ATXN3 leads to neuron subtype-specific aggregation and neurological dysfunction. Analysis of specific neurons reveals that there is a very consistent pattern of neuronal susceptibility to the expression of both full-length and truncated forms of mutant ataxin-3. Importantly, when compared with the expression of polyQ-alone proteins, we have found a distinct vulnerability to pathogenic ATXN3 with dorsal nerve cord and certain sensory processes being highly affected, suggesting that ATXN3 protein flanking sequences and neuronal cell-intrinsic factors modulate polyQ-mediated pathogenesis.
Age strongly modifies disease manifestation and protein aggregation in our model. We have found that reduction of Insulin/Insulin Growth Factor (IGF)-1-like signaling and activation of the Heat Shock Factor-1 pathways, genetically or pharmacologically, protect against developing the disease, and this constitutes key information for the design of potential new therapies in MJD.

CHARACTERIZATION OF A NOVEL TRANSGENIC MOUSE MODEL OF MACHADO-JOSEPH DISEASE

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Machado-Joseph disease (MJD), also known as Spinocerebellar Ataxia type 3 (SCA3), is an autosomal dominant neurodegenerative disorder caused by the expansion of a polyglutamine tract (polyQ) in the C-terminus of the ATXN3 gene product, ataxin-3. Although ataxin-3 is ubiquitously expressed, only restricted neuronal populations of the central nervous system are affected in the disease. We have generated three lineages of transgenic mice expressing human ataxin-3 with an expanded CAG tract under the control of the CMV promoter: CMVMJD83, CMVMJD94 and CMVMJD135, carrying Q83, Q94 and Q135 polyQ stretches, respectively. Behavioral analysis revealed that transgenic CMVMJD83 mice did not manifest motor deficits during their lifetime (84 weeks), whereas CMVMJD94 animals developed a mild motor uncoordination phenotype starting at 16 weeks of age. Interestingly, CMVMJD135 transgenic animals developed a more aggressive motor uncoordination phenotype along with other neurological features such as gait impairment, body balance deficit, loss of limb clasping, grasping and muscular tonus. Immunohistochemistry revealed that ataxin-3 is located mainly in the perinuclear regions of neurons forming small-punctate aggregates in CMVMJD94 and CMVMJD135 mouse brains. In addition, in the CMVMJD135 model we observed large intranuclear inclusions, although not exclusively in the affected areas.
In summary, we have created two mouse models for MJD with different pathological and phenotypical features that may model different stages of the disease, which may contribute substantially for the understanding of MJD pathogenesis. The CMVMJD135 model is currently being used for therapeutical trials targeting different molecular pathways that might be involved in the disease, including autophagy.

A NEURODEGENERATIVE MUTATION IN CYTOPLASMIC DYNEIN REVEALS NOVEL REGULATION OF MOTOR ACTIVITY

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Cytoplasmic dynein is a minus-end directed microtubule motor protein responsible for many cellular functions including fast retrograde axonal transport. The Loa dynein mutant mouse was initially identified to develop progressive lower motor neuron degeneration similar to ALS; however, recent reports have found that these mice undergo severe sensory neuron loss. The Loa mutation is located within the tail domain of the dynein heavy chain, but no effects have been reported on dynein function. To test for defects in dynein function, we purified the motor protein from stockpiled brains of wild type and Loa/+ mice. The purified mutant and wild type preparations were indistinguishable in composition. Although basal ATPase activity was the same for both preparations of dynein, the mutant dynein exhibited a dramatic increase in the Km, but a normal Vmax, suggesting a decreased affinity for microtubules. This was confirmed by microtubule binding assays, which revealed decreased affinity of the mutant dynein in the presence, though not the absence, of ATP. We performed an extensive analysis of wild type and mutant dynein biochemically and at the single molecule level. Compared with wild type dynein, individual mutant dynein molecules associated with QD or polystyrene beads exhibited a 23% to 50% reduction in run length along microtubules due to significantly altered motor domain coordination. High resolution particle tracking using LysoTracker revealed a decrease in run length for lysosomes/late endosomes in mutant versus wild type hippocampal neurons, which could be quantitatively accounted for by computational modeling based on the processivity differences we had identified. These results provide the first indication that dynein tail mutations can affect motor activity. By impairing dynein's processivity, the Loa mutation likely compromises the ability of mutant dynein to efficiently transport cargo along the axon, leading to neurodegeneration in mice. Supported by RO1GM070676, AHA0825278F, GM47434, and the Columbia University Motor Neuron Center.

SMN-DEPENDENT U12 SPLICING DEFECTS CAUSE SYNAPTIC DYSFUNCTION IN A DROSOPHILA MODEL OF SPINAL MUSCULAR ATROPHY.

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Spinal muscular atrophy (SMA) is an inherited motor neuron disease caused by reduced levels of the survival motor neuron (SMN) protein. SMN is part of a macromolecular complex required for the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs)□the essential components of the pre-mRNA splicing machinery. We previously showed that, in mouse models of SMA, snRNP assembly defects correlate with disease severity and cause a preferential decrease in the levels of the snRNPs that are responsible for splicing a rare class of introns (U12-type). However, whether SMN-dependent splicing defects contribute to neuromuscular dysfunction remains to be determined. To address this fundamental issue, we carried out a genome-wide analysis of the consequences of SMN deficiency on the U12 splicing pathway and the relevance for neuromuscular function in Drosophila mutants of SMN. In this model of SMA, low SMN levels cause synaptic dysfunction at the neuromuscular junction (NMJ), decrease muscle size, and impair locomotion. We found that SMN deficiency affects the splicing of U12 introns and that expression of about 40% of all U12 intron-containing genes is decreased in Drosophila SMN mutants. Functional analysis of these SMN target genes led to the identification of a novel evolutionarily conserved transmembrane protein—which we named Stasimon—that is strongly expressed in neurons and essential for proper neurotransmitter release at the NMJ. Importantly, restoring neuronal expression of Stasimon in Drosophila mutants of SMN corrects synaptic dysfunction and improves muscle size and locomotion. These findings reveal that SMN-dependent U12 splicing events are essential for normal synaptic transmission in vivo and that SMN deficiency can cause neuromuscular dysfunction by affecting the splicing of genes critical for neuronal activity.

AN *IN VIVO* MODEL SYSTEM TO INVESTIGATE TAU HYPERPHOSPHORYLATION IN ALZHEIMER'S DISEASE

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Neurofibrillary tangles are a hallmark of Alzheimer's disease (AD) pathology. They are composed of hyperphosphorylated forms of the neuronal microtubule associated protein tau. Many kinases are capable of phosphorylating tau *in vitro*, but it is not clear which are important for the neurodegenerative process in AD. It is crucial to identify the kinases that regulate tau phosphorylation since they are potential therapeutic targets. *Drosophila melanogaster* is an excellent model system for studying human neurodegenerative diseases. Over-expression of human tau in *Drosophila* photoreceptor neurons results in neurodegeneration that bears many of the hallmarks of AD including presence of abnormally phosphorylated tau.

We are utilising *Drosophila* as *in vivo* animal assay to identify which human kinases and which phosphorylation events are responsible for the generation of toxic forms of tau. This model could be used to identify small molecules that prevent the neurodegenerative process in AD. With this aim, several *Drosophila* lines over-expressing human tau in photoreceptor neurons were established and the ability of human kinases to modulate tau-mediated neurodegeneration was tested.

As predicted, when human GSK3β was co-expressed with human tau in the photoreceptor neurons increased degeneration was observed similar to that previously reported with the *Drosophila* orthologue shaggy. This demonstrates that human kinases are functional in living *Drosophila*. Other kinases thought to be important in tau hyperphosphorylation are now being investigated, including the dual-specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A). In vitro DYRK1A phosphorylates only a small number of residues of tau. Since it is also highly expressed in Down syndrome patients as well as in AD brains, DYRK1A might play a potential important role in conferring tau toxicity during pathogenesis.

We show here that both DYRK1A and its *Drosophila* orthologue, minibrain, enhance the toxicity of tau in the fly eye. We are currently investigating which are the major phosphorylation events responsible for generating toxic forms of tau.

ELECTROPHYSIOLOGICAL CHARACTERISATION OF HUMAN EMBRYONIC STEM CELL AND HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS.

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The ability to generate subtype-specific populations of neurons from human embryonic stem cells (hESCs) and induced pluripotent stem cells (hIPSCs) presents an unrivalled model for the study of neurodegenerative disease in human cells. Neurodegenerative diseases often exhibit loss of region-specific neurons making the generation of neuronal subtypes key to the study of underlying mechanisms. Using whole-cell patch-clamp, hESC-derived neurons expressing regional markers for anterior, posterior, midbrain or motor neuron identity as well as hIPSC-derived motor neurons have been electrophysiologically analysed. Neurons from all populations fired TTX-sensitive action potentials in response to current injection. In addition, neurons displayed agonist-evoked currents indicating the expression of GABA, NMDA and AMPA type receptors. Finally, spontaneous excitatory post-synaptic currents were observed in older cultures signifying the capacity of these cells to form functional networks.

ANTIOXIDANTS HALT AXONAL DEGENERATION AND DISABILITY IN X-ADRENOLEUKODYSTROPHY

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Axonal degeneration is a main contributor to disability in progressive neurodegerative diseases associated to oxidative stress. Here we establish a direct link between oxidative stress and axonal damage in a mouse model of X-adrenoleukodystrophy, a lethal disease caused by loss of function of the ABCD1 peroxisomal transporter of very long-chain fatty acids (VLCFA). The mouse model for X-ALD exhibits a late-onset locomotor disability and axonal degeneration in spinal cords. We deliver conceptual proof of the capability of the antioxidants N-acetyl-cysteine, lipoic acid and alpha-tocopherol to scavenge VLCFA-dependent ROS generation. In a preclinical setting, the cocktail reversed: i) oxidative stress and lesions to proteins, ii) immunohistological signs of axonal degeneration and iii) locomotor impairment in bar cross and treadmill tests. These results warrant translation into clinical trials for X-adrenoleukodystrophy patients, and invite assessment of antioxidant strategies in other diseases with axonal degeneration in which oxidative damage might play a role.

5' REGULATORY REGIONS OF MACHADO-JOSEPH DISEASE GENE (*ATXN3*): SEQUENCE ANALYSIS IN PATIENTS AND CONTROLS

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Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder, with a mean onset in adulthood (around 40 years). Its causative mutation consists in an expansion of a (CAG)n tract in the coding region of the *ATXN3* gene (14q32.1), which encodes for ataxin-3. The inverse correlation between the number of CAG repeats in the expanded alleles and the age at onset accounts for only around 50 to 70% of onset variance, the remaining variation being dependent on factors currently unknown. Although ubiquitously expressed, differential levels of ataxin-3 may constitute one of the factors responsible for the clinical heterogeneity observed in MJD. The main goal of this work was to analyze the extent of the genetic variation upstream of the ATXN3 start codon, in its core promoter, as well as in its 5' untranslated region (UTR), which possibly influence gene expression levels and, therefore, may ultimately be associated with variance in the MJD's age at onset. Sequence analysis was performed in 59 MJD patients and 59 controls. The *ATXN3* core promoter region was shown to be highly conserved, with no polymorphisms being observed in the 236 analyzed chromosomes. In the 5'-UTR, one SNP (c.-31C>T) was found, both in MJD patients and controls. This SNP falls in a region which may affect CMYB transcription factor binding, as well as translation regulation and, therefore, may have a putative functional role.

THE RELATIONSHIP BETWEEN HIPPOCAMPAL AND PARAHIPPOCAMPAL GRAY AND WHITE MATTER IN CONTROLS, MILD COGNITIVE IMPAIRMENT, AND ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a complex disease process in which pathology plays a vital role in its diagnosis. For the past few years, neuroimaging, a non invasive in vivo procedure, has played an important role in the research and diagnosis of AD. Many studies have shown atrophy of the hippocampus (Hp) as well as the entorhinal cortex (EC) which also atrophies very early in the disease process. AD pathology starts in neither of those areas but in a transitional area called the transentorhinal area (TER) from which it spreads to the EC, Hp and the temporal isocortex and then throughout the entire brain. The diaschisis theory postulates that breaking neuronal ties between regions could also produce temporary impairments of function. Applying this theory on AD would show the altered white matter tracts in AD are due to their disrupted connections with the atrophied hippocampal formation. This study aims to compare the associations between the gray matter volumes and rates of atrophy EC, Hp, and TER with cingulum bundle in controls, amnestic MCI, and AD patients. The second aim of this study is, to determine the correlations of those three gray matter areas with memory scores specific for the EC and Hp such as immediate free recall and delayed free recall respectively. In this project, our hypothesis is that the TER is a key to a memory network and once disrupted affects the whole network. Therefore, correlating TER volumetric measurements with the other areas might be of particular interest. Also, using the diaschisis concept, the relation between gray matter atrophy and its associated white matter is evaluated with an expected positive correlation. Manual segmentation of the three areas Hp, EC, and TER is performed to obtain regions of interest and the volume measurements are extracted. A positive correlation is also expected between gray matter measurements and memory scores of the neuropsychological tests. Finally, TER atrophy is expected to correlate more with EC atrophy than with Hp.

SMALL QUINONE-TRYPTOPHAN MOLECULES RESCUE ALZHEIMER'S DISEASE MODEL

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The amyloid beta (Aβ) hypothesis proposes that increased levels of the Aβ protein and its consequent assembly and aggregation from soluble to oligomers and into fibrillar amyloid plaques in the brain are the primary events driving Alzheimer's disease (AD). The early soluble assemblies of Aβ, may be more pathogenic than the fibrillar structures. We and others have identified a key role of aromatic residues in the molecular recognition and self-assembly leading to the formation of various amyloid assemblies. Aromatic interactions are believed to provide selectivity as well as stability to the interacting molecules. Our strategy is to use small aromatic molecules that would bind the aromatic residues of the Aβ monomers thereby inhibit the early steps of the molecular recognition and structural transition of the monomers which lead to the formation of the toxic amyloid species. Quinones have been known as inhibitors of various metabolic pathways in the cell, serve as antibacterial, anti-viral, and anti-cancer agents and furthermore were shown to inhibitor several amyloidogenic peptides. We have synthesized a series of N-linked tryptophan-modified quinones and screened them for anti-Aβ activity. Two compounds, NQTrp and Cl-NQTrp, were most effective. They inhibit Aβ oligomerization and fibrillization in vitro and reduce the cytotoxic effect of Aβ oligomers towards cultured cells. NMR spectroscopy and molecular dynamics simulations provide a mechanistic basis for the activity of these compounds. When fed to Drosophila expressing Aβ in their nervous system, these compounds alleviated their Alzheimer's-related symptoms which include defective locomotion and reduced life span, while having no effect on control flies. Injection of Cl-NQTrp to 5xFAD transgenic AD mice resulted in significant improvement of their cognitive behavior, dramatic reduction in the level of both soluble and insoluble Aβ in their brain extracts and marked decrease in Aβ deposition in their brains. Thus, these Trp-modified naphthoquinones may be useful candidates as disease-modifying drugs for AD.

SMALL MOLECULE SCREEN OF YEAST TDP-43 PROTEINOPATHY MODEL IDENTIFIES BROADLY PROTECTIVE COMPOUNDS

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TDP-43 is an RNA binding protein that has recently been implicated in both Amyotropic lateral sclerosis (ALS) and Frontotemporal lobar degeneration with ubiquitin positive inclusions (FTLD-U). These two diseases affect different classes of neurons, yet share TDP-43 as the likely causative agent and make up a spectrum of disorders called TDP-43 proteinopathies. Modeling of TDP-43 proteinopathy is critical to gain an understanding of TDP-43's normal function, as well as its pathological mechanisms. We have used a recently developed yeast model of TDP-43 proteinopathy that recapitulates several relevant disease features to screen for compounds that rescue cellular toxicity. Yeast offers a tremendous opportunity for high throughput chemical screening where a simple return to growth identifies hit compounds. Such molecules are being employed as biological probes to further interrogate TDP-43 pathology in both yeast and neuronal models. The screen identified a number of small molecules, four of which are currently being investigated for modes of action in both yeast and neuronal systems. Surprisingly, identified compounds frequently display a broad protection over multiple yeast models – including α-synuclein and PolyQ – suggesting they may target fundamental protective pathways. The identification of compounds that rescue different models suggests that a particular druggable target may be exploited to potentially treat multiple diseases.

HUMAN GENETIC MODIFIERS OF MUTANT HUNTINGTIN AGGREGATION

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The appearance of protein aggregates in neurons is a common hallmark of many late-onset neurodegenerative disorders. In Huntington's disease, a trinucleotide-expansion in the disease gene leads to an enlarged glutamine-stretch in the Huntingtin-protein and its subsequent misfolding and oligomerization. How microscopically visible inclusions are formed via misfolding intermediates is incompletely understood. About 60% of the variation in the age of disease onset can be predicted by the length of the glutamine stretch. The other 40% is caused by environmental factors and other genetic factors. A better understanding of the aggregation-process could provide new means of therapeutic intervention.

In our lab, we use a small animal model for polyglutamine aggregation (the nematode *Caenorhabditis elegans*) to search for genetic modifiers of protein aggregation. By a genome-wide RNA-interference (RNAi)-screen (1) and chemical mutagenesis of polyglutamine-expressing worms (2), almost 200 genes were identified that positively or negatively affect protein aggregation. These genes included known regulators of protein aggregation, genes not previously linked to aggregation, and genes with unknown function.

To extrapolate these results to human disease, we examined the human homologs of these genes for a possible role as a genetic modifier of Huntington's disease. We designed siRNA-sequences for inhibition of protein expression, and used a polyglutamine-reporter system in cultured human cells to visualize aggregation. In addition to established modifiers of protein aggregation in mammalian cells (molecular chaperones, the proteasome system), also a number of genes that were not previously linked to this process showed to be involved in aggregation of mutant Huntingtin. We will report about these genes and their role in protein aggregation in more detail.

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SH3 DOMAINS FROM A SUBSET OF BAR-PROTEINS DEFINE A NOVEL UBL-BINDING MODULE AND IMPLICATE PARKIN IN SYNAPTIC UBIQUITINATION.

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Parkin, a gene responsible for a familial form of Parkinson's disease, encodes a RING-type E3 ubiquitin-ligase with an N-terminal ubiquitin-like domain (Ubl). By virtue of its similarity to ubiquitin, the parkin Ubl can interact with ubiquitin-binding domains (UBDs). However, almost nothing is known about the selectivity of parkin Ubl towards UBDs or its role in recruiting ubiquitination substrates. We report here that the SH3 domains of endophilin-A, syndapin and amphiphysin-II interact with parkin Ubl but not with ubiquitin or other Ubl's. These endocytotic SH3 proteins all have a BAR domain that binds and induces curvature in lipid bilayers. In synapses, parkin associates with endophilin-A1 in a phosphorylation-dependent manner and ubiquitinates SH3-associated proteins. The structure of the endophilin-A1 SH3 in complex with the Ubl was determined using NMR spectroscopy and showed that the Ubl C-terminal $PxRK^{73-76}$ motif, which is unique to parkin, folds upon binding the SH3 domain. This allows Arg75 to interact with an acidic loop specific to endophilin-A SH3, thus explaining the selectivity of the interaction. The Ubl binds the same SH3 surface as proline-rich domains (PRDs), effectively competing with the dynamin and synaptojanin PRDs. Thus our work establishes the SH3 domain as a novel Ubl-binding domain and implicates endophilin-A for the first time in synaptic ubiquitination and Parkinson's disease. Future studies will aim at identifying the ubiquitination substrates of parkin in the context of endophilin-A recruitment and the role this interaction might play in the regulation of vesicular transport.

COMPLEMENT 3/Aβ1-42 AND FACTOR H/Aβ1-42 IN HUMAN CEREBROSPINAL FLUID CORRELATE WITH PARKINSON DISEASE SEVERITY

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Background: There are currently no robust biomarkers to predict Parkinson disease (PD) progression, or to correlate with development of cognitive impairment in PD. In recent investigation, complement 3 (C3), factor H (FH), as well as amyloid beta peptide 1-42 (Aβ1-42), have been weakly associated with PD motor disability and/or the development of cognitive impairment in PD.

Methods: Re-analying C3, FH and Aβ1-42 in cerebrospinal fluid (CSF) from a well-characterized cohort of PD patients at various stages as determined by unified Parkinson's disease rating scale (UPDRS) motor scores and cognitive impairment states.

Findings: The C3/Aβ1-42 and FH/Aβ1-42 ratios not only correlated with PD severity, staged by UPDRS score but also aided in distinguishing PD with dementia (PD-D) from PD with no cognitive impairment (PD-NCI), and PD with cognitive impairment but no dementia (PD-CIND).

Interpretation: Though further validation is needed, these data suggested that component activation and a decrease in CSF Aβ1-42 are independent processes, and a combination of two markers might provide an effective panel of markers that could potentially useful in monitoring PD progression, including the development of PD dementia.

PARKINSON'S MUTATIONS OF LRRK2 REGULATE ITS AUTOPHOSPHORYLATION AND ACTIVITY

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Parkinson's disease (PD) is the second most common neurological disorder, affecting over 10 million people worldwide. PD patients suffer from a variety of symptoms including decreased olfactory acuity, tremor, and ultimately bradykinesia. Mutations in leucine-rich repeat kinase 2 (LRRK2) have been linked to both familial and sporadic PD, however the function of LRRK2 remains to be elucidated. Here we identify and characterize a LRRK2 autophosphorylation site that can be detected in cells and in transgenic mouse models. Importantly, LRRK2 mutations associated with PD modulate this autophosphorylation. This discovery offers a promising tool to identify LRRK2 inhibitors in cell-based assays and a potential biomarker for monitoring LRRK2 kinase activity *in vivo*.

A NOVEL SPLICE VARIANT OF TRF2 RETAINS REST IN THE CYTOPLASM AND PREVENTS EXCITOTOXIN-MEDIATED DYSREGULATION OF NEURONAL GENES

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Telomere repeat-binding factor 2 (TRF2) is essential for stabilizing the ends of chromosomes in dividing cells. However, we recently reported that TRF2 interacts with the transcriptional silencer REST at non-telomeric sites to regulate the expression of neuron-specific genes in neural progenitor and tumor cells. Here we identify a neuron-specific TRF2 alternative splicing isoform, TRF2-short (Sα) with a truncated C-terminus lacking Myb DNA binding domain and nuclear localization signal domains, and containing a novel nuclear export signal. A molecular switch from TRF2 to TRF2-Sα expression occurs during brain development coincident with the transition from proliferating neuronal precursors to mature postmitotic neurons. Unlike TRF2 which is concentrated in the nucleus of proliferating neural progenitor cells, TRF2-Sα is located in the cytoplasm where it binds REST. The association between TRF2-Sα and REST arises coincidentally with the maturation of Cajal-Retzius cells and pyramidal neurons in the developing neocortex. Overexpression of TRF2-Sα prevents nuclear accumulation of REST. Exposure cortical neurons to the glutamate receptor agonist kainate disrupts the interaction of TRF2-Sα with REST resulting in the nuclear translocation of REST and the repression of a subset of neuronal genes critical for neurite growth, neuron survival and synaptic plasticity. Overexpression of TRF2-Sα prevents nuclear translocation of REST and maintains the expression of neuronal plasticity genes. Our findings reveal a novel TRF2 isoform that serves a special function in the maintenance and modification of the mature differentiated state of neurons under adverse conditions such as excitotoxic stress.

LEUVEN MUTATION OF THE ALTERNATIVE β-SECRETASE CLEAVAGE SITE (β'-SITE) OF APP CAUSES INCREASED Aβ GENERATION AND IS LINKED TO ALZHEIMER'S DISEASE.

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BACE1 cleaves the amyloid precursor protein (APP) at Met671-Asp672 (β-site) to release the amino-terminus of the amyloid peptide Aβ. A secondary cleavage site of BACE1, at Tyr681-Glu682 (β'-site) is also known to occur in APP, but the quantitative importance as well as the functional significance of this cleavage remains elusive. A novel APP mutation, E682K, located at the β'-site, was identified in a patient with early-onset Alzheimer's disease (AD). In neurons expressing wild-type human APP, β'-site cleavage was found to be a relatively abundant event. The E682K mutation blocked the β'-site cleavage and shifted β-secretase cleavage of APP to the canonical β-site, causing increased generation of Aβ1-40/42, and explaining the pathogenic effect of the E682K mutation. Our work shows that processing at the β'-site is a major processing event of human APP in neurons and that β'-site cleavage has a 'protective effect' in APP metabolism. Disruption of the balance between β-site and β'-site cleavage by mutations or other effects will enhance the amyloidogenic processing of APP and may consequentially lead to AD, which should be taken into account when developing BACE-inhibitors.

GENOMIC STUDIES OF ALS: GENOME-WIDE ASSOCIATION STUDIES AND BEYOND

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The identification of genes underlying rare familial forms of amyotrophic lateral sclerosis (ALS) has provided fundamental insights into the pathogenesis of motor neuron degeneration, and has directly lead to animal models of the disease, as well as the promise of targeted therapeutics. Thus, it is not surprising that the discovery of new ALS genes is of great interest to the field.

Finland is an ideal location for performing genetics studies of ALS, because it has one of the highest incidences of the disease in the world, and because the population is known to be remarkably genetically homogeneous. To exploit this, we performed a genome wide association study of ALS in Finland to determine the genetic variants underlying disease in this population. Analysis revealed two highly significant association peaks in our GWAS, one located on chromosome 21 near the SOD1 gene, which is known to have a particularly high prevalence in the Finnish population, the other located on chromosome 9p21. Together, these two loci account for nearly the entire increased incidence of ALS in Finland, and represent the most significant “hit” reported to date in genome-wide association studies of this fatal neurodegenerative disease.

The next stage in the genetic dissection of ALS will be the rapid identification of mutations underlying familial disease using exome sequencing. This method allows us to enrich DNA samples for the regions of the genome that contain protein-coding exons, which is then sequenced using second-generation sequencing. The power of exome sequencing has already been demonstrated for rare monogenic disorders; what these studies elegantly show is that the process of identifying the underlying gene mutation in single gene diseases can now be performed in less than a month, as opposed to the years that a linkage and positional cloning project has traditionally taken. We will present the results of our exome sequencing of ALS families.

NEW RNA BINDING PROTEINS IN ALS

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Mutations in two related RNA-binding proteins, TDP-43 and FUS/TLS, have been linked to amyotrophic lateral sclerosis (ALS). We have been using yeast to define mechanisms of TDP-43 and FUS toxicity and to identify genetic modifiers. Using this approach, we identified the yeast homolog of human ataxin 2 as a potent modifier of TDP-43 toxicity. Ataxin 2 also mitigated toxicity of TDP-43 in Drosophila and the proteins interacted with each other in an RNA-dependent manner. Given these strong genetic and physical interactions between TDP-43 and ataxin 2, we analyzed the ataxin 2 gene for mutations in ALS patients. Ataxin 2 is a polyglutamine (polyQ) protein and long expansions of the polyQ repeat (>34 Qs) cause spinocerebellar ataxia type 2 (SCA2). We identified intermediate-length polyQ (27-33 Qs) expansions in ataxin 2 as a common genetic risk factor for ALS. This discovery underscores the extraordinary power of simple model systems like yeast and fly for uncovering novel insights into even complicated human diseases. Building on these studies, we have recently used yeast to explore the hypothesis that additional human RNA binding proteins, like FUS and TDP-43, might contribute to ALS. We screened all human RNA recognition motif (RRM)-containing proteins in yeast and identified 35 that aggregated and were toxic (including FUS and TDP-43). We are analyzing these genes for mutations in ALS patients and have recently identified several missense variants in two of the genes in ALS patients that are not in >5,500 healthy controls. We propose that TDP-43 and FUS might be the tip of an iceberg and many more aggregation-prone RNA binding proteins could contribute to ALS.

EXPERIMENTAL INDUCTION OF CEREBRAL Aβ DEPOSITION IN TRANSGENIC RODENTS

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The abnormal deposition of the protein fragment Aβ can be induced in Aβ-precursor protein (APP)-transgenic mice and rats by the intracerebral injection of brain extracts that are rich in aggregated Aβ. To assess potential similarities of this process with the propagation of prions in vivo, our laboratory and that of Mathias Jucker in Tübingen undertook a series of experiments that have shown that: 1) the seeding extract is effective only if it contains sufficient quantities of misfolded Aβ; 2) Aβ-rich brain extracts seed effectively whether the source of the extract is a human, aged monkey or genetically modified mouse; 3) the induction of Aβ aggregation by extracts from transgenic mice indicates that the phenomenon is not due to human-specific factors or a cross-species immune reaction; 4) the induction of Aβ deposition requires that the host generate human-sequence Aβ; 5) denaturation of the extract or specific immunoneutralization of Aβ inhibits seeding; 6) the seeded deposits appear only after a lag period, the length of which is governed by both the seed and the host; 7) Aβ seeds can spread from one brain region to another, possibly in part due to axonal transport; and 8) Aβ deposition can be seeded in an APP-transgenic rat model that normally does not develop Aβ-lesions, at least up until 30 months of age. Recent studies from the Jucker laboratory have shown that cerebral Aβ-amyloidosis can be seeded by the intraperitoneal infusion of Aβ-rich brain extract, indicating that the seed can be conveyed from the periphery to the brain. We are currently investigating the ability of heterogeneous agents to induce Aβ deposition, as well as putative strain-like variations in the pathogenicity of aggregated Aβ. Acknowledgments: Key collaborators on these studies include Mathias Jucker and Yvonne Eisele (U. Tübingen), Rebecca Rosen, Jeromy Dooyema, Amarallys Cintron, Eric Heuer, Brian Ciliax, Jason Fritz, Ranjita Betarbet and James Lah (Emory U.), and Harry LeVine III (U. Kentucky). Supported by NIH RR-00165, NIH P50AG025688 and the CART Foundation.

A CHEMICAL SCREEN IDENTIFIES DIVERSE PATHWAYS THAT SPATIALLY REGULATE AXON DEGENERATION

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Axon degeneration is a hallmark of both pruning during nervous system development and neurodegenerative disease. The molecular mechanisms regulating this active process are just beginning to be understood. In order to discover additional pathways regulating axon degeneration we conducted an unbiased small molecule screen and have identified modulators of various pathways that block axon degeneration following growth factor withdrawal. Notably, a number of kinases where identified as mediators of axon degeneration, and further mechanistic studies localized the function of distinct kinases to either the axonal or cell body compartments. Further investigation of these pathways in axon degeneration-specific assays led us to an unexpected finding: glycogen synthase kinase-3 (GSK-3) is a key regulator of a genetic axon degeneration program acting specifically in the cell body to regulate distal axon degeneration. To identify potential GSK-3-regulated axon degeneration genes, we performed time course microarray analysis on neurons selectively undergoing axon loss, with or without GSK-3 inhibition. Two candidate genes were identified - tbx6, a transcription factor, and dleu2, a long noncoding RNA. Consistent with our model, siRNA knockdown of tbx6 or dleu2 reduced axon degeneration *in vitro*. Additionally, these genes are both enriched in the hippocampus of clinical Alzheimer's disease samples, indicating that our proposed axon degeneration program may be active in human disease.

LACK OF COMPLEMENT RECEPTORS CR1 AND CR2 OR CR3 HAS DISTINCT EFFECTS ON AMYLOID PATHOLOGY IN MICE

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Alzheimer's disease (AD) is characterized by the accumulation of extracellular amyloid-β (Aβ) plaques and intracellular tangles. These disease hallmarks are accompanied by a strong neuro-inflammatory component including, but not limited to, activation of the complement system. Our lab and others have previously shown that inhibition of the complement system by overexpression of the endogenous C3 convertase inhibitor Crry or by deletion of the major complement component C3 leads to increased amyloid pathology and neurodegeneration. Our goal is to identify which receptors mediate this effect.
C3 and its cleavage products can be recognized by complement receptors 1 (CR1), 2 (CR2) and 3 (CR3). These receptors display differential expression patterns. CR1 is expressed on leukocytes, monocytes and B-cells, while CR2 is commonly described on B-cells. CR3, on the other hand, is expressed mainly on macrophages and microglia. In mice CR1 and CR2 are splice variants of the same gene, therefore deletion of the gene locus results in loss of both receptors. To identify the role of these receptors on cerebral amyloidosis we crossed mice lacking CR1/2 or CR3 with human amyloid precursor protein (APP) transgenic mice harboring the familial AD associated Swedish (KM670/671NL) and London (V717I) mutations.
Mice lacking and expressing CR3 were aged to 1, 3 and 12 months of age. Interestingly, analysis of mice at the pre-deposition ages (1 and 3 months) did not show changes in Aβ levels in Cortex or Hippocampus. In agreement with these findings we also did not observe any differences in the total number of microglia (Iba-1) or the number of activated microglia (CD68) in the brains of these animals. The analysis of the 12 months cohort is ongoing. In contrast, mice lacking CR1/2 display a significant increase in soluble Aβ at pre-deposition age (4-5 months) in both Cortex and Hippocampus. Unfortunately, this genetic deletion is associated with high lethality in APP trangenic mice and therefore we could not investigate the effect in older animals.
Surprisingly, our preliminary data shows a stronger effect of deletion of the more peripheral expressed CR1/2 than that of the microglial receptor CR3, at least at pre-deposition ages.

REGULATION OF ATXN1 PHOSPHORYLATION AT SER 776 BY PKA AND PP2A: AVENUES FOR SCA1 THERAPEUTIC DEVELOPMENT

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Spinocerebellar ataxia type 1 (SCA1) is a fatal autosomal dominant neurodegenerative disorder caused by expansion of a polyglutamine tract in ataxin-1 (ATXN1). Phosphorylation of the SCA1 encoded protein ATXN1 at Ser 776 modulates disease and its interaction with other cellular proteins. Thus, understanding the signaling pathway that regulates ATXN1 phosphorylation at S776 in the cerebellum is important for identifying targets for therapeutic development. In our previous studies, we showed that ATXN1 is phosphorylated in the cytoplasm and PKA is a strong candidate for the cerebellar S776-ATXN1 kinase. In this study, we showed that polyglutamine tract expansion renders S776-ATXN1 more susceptible to phosphorylation by PKA. In addition, we examined the dephosphorylation of phospho-S776-ATXN1 (pS776-ATXN1) in the cerebellum. We found that dephosphorylation of pS776-ATXN1 occurs in the nuclei of cerebellar cells, which are also the subcellular site where pS776-ATXN1 is enriched. Protein phosphatase 2A (PP2A), a major brain Ser/Thr phosphatase, interacts with and dephosphorylates pS776-ATXN1 in the cerebellum. Polyglutamine tract expansion seems to have no effect on dephosphorylation by PP2A. These results indicate that PKA and PP2A regulate cerebellar phosphorylation of ATXN1 at S776, thus, are target candidates for SCA1 therapeutic development.

WHAT CAN GENETICS TEACH US ABOUT TREATING ALZHEIMER'S DISEASE?

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Alzheimer's disease (AD) is strongly influenced by inheritance and genetic susceptibility as evidenced by numerous family and twin studies. Over the past two and a half decades, our laboratory has co-discovered the three early-onset familial AD genes, APP, PSEN1, and PSEN2, which can carry any of >200 fully penetrant mutations characterized by mendelian inheritance. For late-onset AD, the only well-established risk factor is the epsilon 4 variant of APOE, which increases risk by 3.7-fold in the heterozygous state and >10-fold when two copies are inherited. It has been estimated that 50-70% of the genetic variance of AD remains unexplained by the four established AD genes. We are engaged in two major efforts to identify the additional AD genes as part of our Alzheimer's Genome Project funded by the Cure Alzheimer's Fund. First, for published AD candidate genes, we have developed the AlzGene.org website, a comprehensive, online encyclopedia and database, which includes data on >650 AD candidate genes and >~3000 DNA variants that have been tested for association with AD. For all DNA variants tested in at least four independent samples (~300), AlzGene.org provides meta-analyses to determine the most promising AD candidate genes. These studies have led to over 40 candidate AD genes, including APOE, that yield significant results. However, the effects of these variants on risk are tiny compared to APOE (.75≤O.R.≤1.25). We are currently exploring whether these associations are being driven by rare late-onset AD mutations in linkage diequilibrium with the disease-associated common variants. We found this to be the case for the APP alpha-secretase gene, ADAM10, in which we discovered two rare mutations that severely impair ADAM10 cleavage of APP both in vitro and in transgenic mice. Second, in a parallel effort, we have carried out several genome-wide association studies on >800 well-characterized late-onset AD families (NIMH and NCRAD samples) using Affymetrix genotyping arrays containing either one million (6.0) or 500,000 (5.0) genomic single nucleotide polymorphisms (SNPs) as well as arrays containing 20,000 coding SNPs. We have previously reported four novel loci that achieved genome-wide significance (besides APOE) including a novel gene on chromosome 14q, GWA-14q, the ataxin 1 gene, the innate immune system lectin gene, CD33, and the synaptic gene, DLGAP1. Functional studies of the ATXN1 gene carried out both in vitro and in vivo in ATXN1 knockout mice show that ATXN1 can regulate Aβ levels via modulation of beta-secretase. We will also present new data implicating Aβ as an anti-microbial peptide in the innate immune system. These latter data in combination with several novel candidate genes emanating from the Alzheimer's Genome Project suggest that innate immune system in the brain may play a central role in the etiology and pathogenesis of AD, raising new possibilities for novel drug discovery.

DIABETES-ASSOCIATED SORCS1 REGULATES ALZHEIMER'S AMYLOID β METABOLISM: EVIDENCE FOR INVOLVEMENT OF SORL1 AND THE RETROMER COMPLEX

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SorCS1 and SorL1/SorLA/LR11 belong to the sortilin family of vacuolar protein sorting-10 (Vps10) domain-containing proteins. Both are genetically associated with Alzheimer's disease (AD), and SORL1 expression is decreased in the brains of patients suffering from AD. SORCS1 is also genetically associated with types 1 and 2 diabetes mellitus (T1DM, T2DM). We have undertaken a study of the possible role(s) for SorCS1 in metabolism of the Alzheimer's amyloid β peptide (Aβ) and the Aβ precursor protein (APP), to test the hypothesis that Sorcs1 deficiency might be a common genetic risk factor underlying the predisposition to AD that is associated with T2DM. Overexpression of SorCS1cβ-myc in cultured cells caused a reduction (p 0.002) in Aβ generation. Conversely, endogenous murine Aβ 40 and Aβ 42 levels were increased (Aβ 40, p 0.044; Aβ 42, p 0.007) in the brains of female Sorcs1 hypomorphic mice, possibly paralleling the sexual dimorphism that is characteristic of the genetic associations of SORCS1 with AD and DM. Since SorL1 directly interacts with Vps35 to modulate APP metabolism, we investigated the possibility that SorCS1cβ-myc interacts with APP, SorL1, and/or Vps35. We readily recovered SorCS1:APP, SorCS1:SorL1, and SorCS1:Vps35 complexes from nontransgenic mouse brain. Notably, total Vps35 protein levels were decreased by 49% (p 0.009) and total SorL1 protein levels were decreased by 29% (p 0.003) in the brains of female Sorcs1 hypomorphic mice. From these data, we propose that dysfunction of SorCS1 may contribute to both the APP/Aβ disturbance underlying AD and the insulin/glucose disturbance underlying DM.

DETERMINING THE PHYSIOLOGICAL ROLES OF TDP-43 AND ITS DOWNSTREAM TARGETS.

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Tat activating regulatory DNA-binding protein (Tardbp or TDP-43), an essential metazoan DNA/RNA binding protein thought to be involved in RNA transcription and splicing, has been associated with the pathophysiology of neurodegenerative diseases. However, neither the physiological role of TDP-43 nor its downstream targets are well defined. To begin to address these issues, we employed genetic approaches to determine the *in vivo* function of TDP-43 through analysis of *TDP-43* transgenic and conditional knockout mice as well as embryonic stem (ES) cell model system. To date, our transgenic efforts revealed that increased accumulation of human TDP-43 in neurons led to formation of abnormal intranuclear inclusions comprised of TDP-43 and fused in sarcoma/translocated in liposarcoma (FUS/TLS), and massive accumulation of mitochondria in TDP-43-negative cytoplasmic inclusions in motor neurons, lack of mitochondria in motor axon terminals, and immature neuromuscular junctions. Moreover, whereas an elevated level of TDP-43 disrupts the normal nuclear distribution of survival motor neuron (SMN)-associated Gemini of coiled bodies (GEMs) in motor neurons, its absence prevents the formation of GEMs in the nuclei of these cells. Interestingly, transcriptome-wide deep sequencing analysis revealed that a decrease in abundance of neurofilament transcripts contributed to the reduction of caliber of motor axons in TDP-43 mice. In parallel, our *TDP-43* knockout approach showed that postnatal deletion of *TDP-43* in mice caused dramatic loss of body fat. Importantly, our high-throughput DNA sequencing analysis on the transcriptome of ES cells lacking TDP-43 revealed a set of downstream targets of TDP-43 and showed that *Tbc1d1*, a gene known to mediate leanness and linked to obesity, is down-regulated in the absence of TDP-43. Collectively, our results established that TDP-43 is critical for fat metabolism and indicated that TDP-43 participates in pathways critical for motor neuron physiology, including those that regulate the normal distributions of SMN-associated GEMs in the nucleus and mitochondria in the cytoplasm.

REGULATION OF THE FRONTO-TEMPORAL DEMENTIA PROTEIN, PROGRANULIN, BY SORTILIN MEDIATED TRAFFICKING

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Mutations in the Progranulin (PGRN) gene, resulting in progranulin haplo-insufficiency, were recently found to be a major cause for Frontotemporal Lobar Degeneration with TDP-43 aggregates (FTLD-TDP). PGRN encodes a 68.5 kDa secreted glycoprotein that has been implicated in wound healing, inflammation, cell survival and tumorigenesis, with its normal function in the central nervous system (CNS) remaining to be defined. Here, we examined PGRN binding to the cell surface. PGRN binds to cortical neurons with high affinity via its C-terminus, and unbiased expression cloning identifies Sortilin (Sort1) as a binding site. Sortilin binds to progranulin with nanomolar affinity and Sort1-/- neurons showed significantly reduced binding to progranulin. Furthermore, Sortilin rapidly endocytoses and delivers PGRN to lysosomes. Mice lacking Sortilin have elevations in brain and serum PGRN levels of 2.5- to 5-fold. The 50% PGRN decrease causative in FTLD-TDP cases is mimicked in GRN+/- mice, and is fully normalized by Sort1 ablation. Sortilin mediated PGRN endocytosis is likely to play a central role in FTLD-TDP pathophysiology.

TREATMENTS FOR SMA AND FRDA: TARGETING MUTATED GENES THAT PRODUCE NON-MUTATED PROTEINS

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Spinal Muscular Atrophy (SMA) and Friedreich's ataxia (FRDA) each have unique genetic causes, neuropathologies, and disease presentations. However, these two diseases also share some striking similarities. They are both autosomal recessively inherited disorders that are caused by reduced expression of a single protein product. SMA is the consequence of mutation of the survival motor neuron 1 (SMN1) gene, reduced expression of full length SMN transcripts arising from a duplicate gene (SMN2), and consequently reduced levels of SMN protein. FRDA is caused by a GAA-CTT triplet repeat insertion in an intron of the frataxin gene that interferes with transcription elongation resulting in reduced amounts of the frataxin protein. An increase in gene expression can potentially be therapeutic since both SMN2 and mutant FXN would produce a native, fully functional protein. We are developing orally active, small molecules that can achieve gene expression changes and positive effects in animal models of disease. This presentation will summarize the pre-clinical characterization of the molecules and their targets and discuss issues that arise in advancing these experimental therapeutics through clinical development.

Frataxin expression can be increased in patient derived cells and transgenic mice with an HDAC inhibitor of the benzamide structural group. These molecules are class I HDAC specific and selective for HDAC3. While HDAC inhibitors alter expression of a number of genes, the frataxin gene expression is increased by these compounds only in the context of the mutant gene containing a triplet repeat insertion. Biomarkers of frataxin gene and protein changes have been developed to aid in assessing drug effects early in clinical research. Challenges also exist in how to determine optimal dosing to achieve target tissue changes of frataxin expression and how to measure treatment effects in FA patients.

A variety of targets are being evaluated to achieve increased expression of SMN protein to treat SMA including HDAC inhibitors, splicing modulators, and translation termination blockers. A screen for increasing SMN2 promoter activity using production of a reporter gene product identified a compound that appears to result in modest increases in SMN protein and prolonged survival in mouse models of SMA. The protein target of these compounds is the mRNA cap degrading enzyme DcpS. Characterization of a clinical candidate in vivo provides some insight into the challenges in clinical development and treatment of SMA in the course of normal disease presentation.

PATHOLOGICAL FEATURES OF ALS IN TRANSGENIC MICE EXPRESSING GENOMIC FRAGMENTS ENCODING ALS-LINKED TDP-43 MUTANTS

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Transactive response DNA-binding protein 43 (TDP-43) ubiquitinated inclusions are a hallmark feature of amyotrophic lateral sclerosis (ALS) and of frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U). Mutations in TARDBP, the gene encoding TDP-43, are associated only with 3% of sporadic and familial ALS, yet multiple neurodegenerative diseases exhibit TDP-43 pathology without known TARDBP mutations. To investigate pathogenic pathways associated with TDP-43 abnormalities, we generated transgenic mice expressing modest levels of human TDP-43 genomic fragments encoding WT TDP-43 or ALS-linked mutant forms of TDP-43 (A315T and G348C). The TDP-43G348C and TDP-43A315T transgenic mice and to a lesser extent TDP-43Wt mice exhibited during aging impaired learning and memory capabilities as well as motor dysfunction as monitored by the rotarod test. Analysis of the brain and spinal cord sections of the TDP-43G348C transgenic mice revealed ubiquitin positive TDP-43 inclusions in the cytosol of motor neurons during aging. Interestingly, prominent and progressive neuroinflammation was detected in the TDP-43 transgenic mice during aging with microgliosis and astrogliosis starting in the brain and later on in the spinal cord. TDP-43 mice exhibited other features of ALS with formation of peripherin accumulations and abnormal peripherin RNA splicing in the CNS. One such neurotoxic peripherin splice variant, called Per61, was upregulated in the TDP-43G348C mice whereas NF-L was downregulated. Even though TDP-43 transgenic mice do not develop paralysis and death, they exhibit many of the pathological changes of human ALS including TDP-43 inclusions, appearance of TDP-43 cleavage fragments, peripherin aggregates, NF-L donwregulation and neuroinflammation. Work is in progress to study why neuroinflammation develops in TDP-43 transgenic mice despite the absence of neurodegeneration.

RNA TARGETS FOR TDP-43: IDENTIFYING THE BASIS FOR NEURONAL VULNERABILITY IN ALS

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The identification of ALS-causing mutations in TDP-43 and FUS/TLS, two RNA/DNA binding proteins, combined with TDP-43 mislocalization in most incidences of sporadic ALS has initiated a paradigm shift in defining mechanisms underlying ALS. TDP-43 has been proposed to participate in several steps of RNA processing including alternative splicing regulation. A fundamental issue is the precise role(s) of TDP-43 in RNA metabolism regulation and how alterations in its properties may underlie neurodegeneration. We have now identified brain RNA targets for TDP-43 using cross-linking and immunoprecipitation coupled with high-throughput sequencing (known as HITS-CLIP or CLIP-seq). Furthermore, differences in adult brain mRNA levels and alternative splicing events affected by TDP-43 were comprehensively determined from more than two hundred million sequences (with a method known as RNA-seq) before and after antisense oligonucleotide-mediated depletion of TDP-43. A basis for neuronal vulnerability from TDP-43 mutation was uncovered from the RNA map of TDP-43 binding: TDP-43 has a predominant role in maintaining the abundance of brain-enriched RNA transcripts derived from genes with very long introns and which encode proteins involved in synaptic activity. Determination of the RNAs affected by TDP-43 also uncovered a broad role for TDP-43 in influencing splicing efficiencies of alternatively used exons and that TDP-43 alters expression of disease-related proteins, such as FUS/TLS and granulin. Lastly, TDP-43 was found to auto-regulate its own synthesis, providing a mechanism through which cytoplasmic aggregation will trigger runaway synthesis of TDP-43 that may in turn amplify the initiating aggregates.

USING STEM CELLS AND REPROGRAMMING TO STUDY ALS

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It has been proposed that human embryonic stem cells could be used to provide an inexhaustible supply of differentiated cell types for the study of disease processes. Although methods for differentiating embryonic stem cells into specific cell types have become increasingly sophisticated, the utility of the resulting cells for modeling disease has not been determined. We have asked whether specific neuronal subtypes produced from human embryonic stem cells and induced pluripotent stem cells can be used to investigate the mechanisms leading to neural degeneration in amyotrophic lateral sclerosis (ALS). We show that human spinal motor neurons, but not interneurons, are selectively sensitive to the toxic effect of glial cells carrying an ALS-causing mutation in the SOD1 gene. Our findings demonstrate the relevance of these non-cell-autonomous effects to human motor neurons and more broadly demonstrate the utility of human embryonic stem cells for studying disease and identifying potential therapeutics.

USING EMBRYONIC STEM CELLS TO STUDY MOTOR NEURON-GLIA INTERACTIONS IN ALS

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Amyotrophic Lateral Sclerosis (ALS) is a result of the selective dysfunction and progressive degeneration of motor neurons. Although the underlying disease mechanisms remain unknown, recent in vivo and cell culture experiments have implicated glial cells in motor neuron degeneration in ALS.

We have made use of the SOD1 mouse model of ALS to study the effect of glial cells bearing the mutant SOD1 transgene on motor neuron viability in cell culture. Specifically, we have studied the gene expression profiles of co-cultured mouse embryonic stem (ES) cell derived motor neurons and primary glia using the Illumina deep sequencing platform (RNAseq). In this study, we vary both the genotype of the motor neurons and glia, as well as time in culture as a means of examining both cell autonomous and non-cell autonomous effects of the mutant transgene. In addition, we carry out parallel studies with spinal cord samples from mutant and wildtype SOD1 mice, and compare both the in vivo and in vitro derived data sets with laser capture microdissection studies of both the ALS mouse model and human ALS patient samples.

We have detected significant cell autonomous and non-autonomous changes in gene expression in both motor neurons and glia, indicating that the two cell types profoundly affect each other's gene expression. In addition, we find a remarkable concordance between the different data sets mentioned above, thus validating the in vitro approach. We are currently analyzing these data sets to identify changes in the expression of specific genes and signaling pathways that may contribute to motor neuron degeneration in ALS.

EFFECTS OF NRF2 OVER-EXPRESSION IN MOUSE MODELS OF ALEXANDER DISEASE

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Alexander disease (AxD) is a fatal neurodegenerative disease caused by dominant mutations in the astrocyte intermediate filament protein glial fibrillary acidic protein (GFAP). The disease is characterized by cytoplasmic protein inclusions, called Rosenthal fibers (RFs), within the cell bodies and processes of astrocytes. Accumulation of total GFAP above an undefined toxic threshold, and activation of multiple stress pathways, are considered central elements in pathogenesis. However, some aspects of the stress response may be beneficial. Nrf2 is a transcription factor which binds to and activates a consensus antioxidant response element found in the promoter regions of multiple genes thought essential for protection against oxidative stress. Although Nrf2 is naturally elevated in AxD, we sought to test whether further elevation of Nrf2 expression in astrocytes above its naturally occurring levels would decrease GFAP and RFs in mouse models of AxD. We crossed transgenic mice over-expressing Nrf2 under the control of the GFAP promoter with knock-in mice expressing the R236H point mutation of GFAP (comparable to the R239H mutation in human GFAP). We found that Nrf2 over-expression in GFAP-R236H mice decreased GFAP protein levels in all six brain regions examined (olfactory bulb, hippocampus, cerebral cortex including underlying white matter, brain stem, cerebellum, and cervical spinal cord) at post-natal day 11 (P11) and 12 weeks of age, as measured by GFAP ELISA. Lower levels of GFAP were accompanied by fewer RFs in olfactory bulb and cortex at 12 weeks of age. Nrf2 over-expression also increased the body weight of GFAP-R236H mice to near wild-type levels. In contrast to GFAP-R236H mice, crossing Nrf2 over-expressing mice with transgenic mice over-expressing human wild-type GFAP (a more severe but less accurate model of AxD) led to an increase in GFAP and RFs, especially in brainstem, followed by early death between P11 and P18. Preliminary observations suggest death may be due to seizures. Our results suggest that pharmacological or genetic interventions aimed at increasing Nrf2 levels and activity may be a promising therapy for AxD, but that activation of stress pathways requires careful calibration to the degree of preexisting pathology.

A NOVEL ROLE OF CYTOCHROME C OXIDASE TO RESCUE *PINK1* TOXICITY IN *DROSOPHILA*

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Mutations in *Parkin/Park2*, *PINK1/Park6*, and *DJ-1/Park7* lead to autosomal recessive forms of Parkinson's disease. Deletions of these genes present with mitochondrial dysfunction in many model systems such as zebrafish, mammalian cells, and *Drosophila*. Genetic analysis has shown interactions between the three genes in *Drosophila*. To further elucidate mechanisms that lead to *pink1* associated mitochondrial dysfunction and degeneration, we used *Drosophila* mutant for *pink1* and performed an upregulation screen to identify suppressors of the muscle degeneration and germ line sterility defects found in the *pink1* mutants. One gene that consistently rescued *pink1* toxicity in the fly was *levy*, a nuclear encoded component of cytochrome c oxidase of the electron transport chain. Interestingly, *levy* null mutant flies display brain degeneration and early death, suggesting that this gene plays an important role in normal brain structure and function. We found that not only did upregulation of *levy* suppress *pink1* toxicity, but also reduced activity of *levy* rescued *pink1* toxicity. In addition to mitigating apoptosis of muscle and abnormal wing posture of *pink1* mutants, flies with reduced *levy* activity also show a dramatic increase in mitochondrial complex II and complex IV activities, along with rescue of lifespan. Moreover, normal wildtype flies with reduced *levy* also lived longer. These findings suggest that complex IV may be a potential target for mitigating toxicity of neurodegenerative disease genes and for promoting healthier lifespan.

AN INDUCIBLE CORTICOSTRIATAL NEURONAL CO-CULTURE ASSAY WITH COMPLEX PHENOTYPIC ENDPOINTS FOR DRUG DISCOVERY IN HUNTINGTON'S DISEASE

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A central challenge in the development of *in vitro* models for Huntington's disease (HD) is the recapitulation of the neuronal and glial complexity and interactions involved in the *in vivo* pathogenesis of the disease. Creating a primary neuronal-based model that is also amenable to moderate or high-throughput drug screening presents an even greater challenge.

Here we describe a mixed primary co-culture screening platform, including cortical and striatal neurons as well as glial cells, in which a tamoxifen-inducible Cre-lox system (Matsuda and Cepko, PNAS, 2007) is used to drive expression of a mutant HD-exon1 sequence encoding 73 glutamines (HttN90Q73). Co-cultures are co-transfected with a CRE driver, a loxP-HttN90Q73 sequence and fluorescent protein reporters, and then allowed to mature for one week before inducing HttN90Q73 expression with tamoxifen. Transfected neurons subsequently undergo progressive neurodegeneration over the second week in culture, which is rescuable by neuroprotective compounds previously characterized in *in vitro* and in HD animal models. An important feature of this screening assay is that different fluorescent reporters are used for striatal vs. cortical neurons, allowing their analysis as separate populations within these mixed and dense co-cultures using automated, high-content image analysis on the Cellomics ArrayScan VTI platform.

A key advantage of this new inducible co-culture model is that mutant Htt expression can be delayed until phenotypic differentiation of cortical and striatal neurons has progressed significantly further than in typical primary culture assays, following maturation of striatal and cortical phenotypes and after profuse, functional synaptic connectivity has been established. This approach thereby allows the use of more complex phenotypic endpoints, including image-based, high-content analysis of HD-related changes in dendritic morphology/extent and in synaptic numbers and function, and TR-FRET-based, high-throughput assays for levels and processing of soluble vs. oligomeric/aggregated Htt protein in the context of mature neurons and neuronal circuits.

The development of such high-content and high-throughput assay endpoints in more mature and complex neuronal culture platforms should support the identification of drug and drug target candidates with more predictive value for clinical benefit early in the drug discovery process.

Support: CHDI Foundation, Inc

DEFINING THE MECHANISTIC BASIS FOR TDP-43 AND FUS AGGREGATION.

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Amyotrophic lateral sclerosis (ALS) is a debilitating and fatal neurodegenerative disorder for which there is no cure. Burgeoning evidence suggests that RNA binding proteins play a critical role in pathogenesis. The RNA binding proteins, TDP-43 and FUS, are principal components of cytoplasmic inclusions found in the degenerating motor neurons of ALS patients. Moreover, mutations in TDP-43 and FUS are linked to familial and sporadic ALS. Here, I present our efforts to understand the mechanism of TDP-43 and FUS aggregation using pure proteins.

In the absence of other components, both TDP-43 and FUS spontaneously assemble into pore-shaped oligomers and ordered filamentous structures that bear striking ultrastructural resemblance to the cytoplasmic inclusions in degenerating motor neurons of ALS patients. The structures formed by pure TDP-43 or pure FUS are non-amyloid in nature, just like the aggregates in ALS. Importantly, under identical conditions numerous other RNA-binding proteins remain soluble, suggesting this ordered assembly process is not a ubiquitous property of RNA-binding proteins. Rather, the primary sequences of FUS and TDP-43 contain specific determinants that confer the ability to form self-organizing filamentous structures.

Indeed, we identified novel 'prion-like' domains in the N-terminal domain of FUS (residues 1-239) and in the C-terminal domain of TDP-43 (residues 277-414) (Cushman et al., 2010. J. Cell Sci. 123, 1191-1201.). Similar to prion domains found in yeast prion proteins such as Sup35, this domain is enriched in uncharged polar amino acids (especially asparagine, glutamine and tyrosine) and glycine. For TDP-43, the C-terminal prion-like domain plays a major role in driving aggregation. Indeed, the majority of TDP-43 mutations linked to ALS fall in the prion-like domain and accelerate the aggregation of pure TDP-43. For FUS, the N-terminal prion-like domain is also important for aggregation. However, additional C-terminal determinants, particularly the first RGG domain, are also critical, suggesting that FUS aggregates by a distinct mechanism to TDP-43. The majority of FUS mutations linked to ALS fall outside the prion-like domain and do not affect FUS aggregation kinetics. These key differences suggest that FUS and TDP-43, though similar RNA-binding proteins, likely aggregate and confer disease phenotypes via distinct mechanisms.

The pure protein aggregation assays we have established for FUS and TDP-43 provide a basic screening system to isolate specific molecules that antagonize and reverse their aggregation. As one example, we show that the Hsp104/Hsp70 chaperone system dissolves TDP-43 aggregates.

IMAGING NEURAL ACTIVITY WITH GENETICALLY ENCODED CALCIUM INDICATORS

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Understanding neural coding, learning and memory requires precise, simultaneous observation of multiple neurons in awake, behaving animals. These requirements can be met by optical recording of brain activity using genetically encoded calcium indicators (GECIs). Since they are encoded by DNA, GECIs can be delivered to the intact brain non-invasively, and targeted to defined populations of neurons and specific sub-cellular compartments for long-term, repeated, in vivo measurements. Over the last decade GECIs have been iteratively improved, and are now useful for quantitative imaging of neural activity in vivo. For example, GCaMP3 is being used to image large populations of neurons in behaving mice over months. Using GCaMP3 as an example, we describe the design and optimization of a GECI, including tuning indicators for specific applications. We will discuss the strengths and limitations of GCaMP3 in neural imaging, and propose strategies that might mitigate these limitations in next generation GECIs.

SENSITIVE SYSTEM FOR THE SELECTION OF GDNF AND ARTN MIMETICS – POTENTIAL FOR THE DISCOVERY OF DRUGS FOR THE TREATMENT OF NEUROLOGICAL DISORDERS.

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Glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs) comprise 4 proteins: GDNF, NRTN, ARTN and PSPN. They support survival and/or differentiation of distinct neuronal populations and protect them from injury. Therefore, GFLs are considered for the treatment of neurological disorders. GDNF and NRTN promote survival of dopaminergic neurons and thus have potential for the therapy of Parkinson's disease (PD). ARTN supports sensory and sympathetic neurons and holds promises for the management of chronic pain. PSPN may have impact in the therapy of stroke .

GFLs signal through the receptor complex consisting of transmembrane receptor tyrosine kinase RET, which is shared by all 4 GFLs, and a ligand-binding co-receptor GDNF family receptor α 1-4 (GFRα1-4) that is specific for each GFL. GFRα1 is specific for GDNF, GFRα2 - for NRTN, GFRα3 - for ARTN and GFRα4 - for PSPN. Some cross-talks also exist: GDNF can also bind to GFRα2, NRTN and ARTN to GFRα1.

GDNF and NRTN have been tested in successful phase 1 clinical trials for PD. However, a larger placebo-controlled study failed to demonstrate a clear benefit of GDNF. The failure of GDNF in the last trial can be partly explained by its bad pharmacokinetic properties: GDNF, as well as NRTN and ARTN, have high affinity for the extracellular matrix and, therefore, restricted distribution in tissues. Thus, the alternatives to these proteins, such as small molecule GFL mimetics, are still in high demand in clinic.

We developed the cell-based system for the selection of GFLs mimetics using luciferase as a reporter-gene. Luciferase expression in generated cells is controlled by the MAPK cascade which is in turn governed by GFRα1/RET or GFRα3/RET. To exclude molecules activating MAPK via other mechanisms we also made a RET-expressing cell line lacking any GFRα co-receptors. We proved the specificity and optimized the system for the high-throughput screening. We tested known agonists and antagonists of GFRα1/RET signalling to verify the applicability of the assay for the analysis of small molecules. Using our system we identified PSPN as a novel ligand for GFRα1/RET receptor complex. Since GFRα1/RET-dependent signaling promotes the survival of dopaminergic neurons, PSPN can be considered as a candidate for PD therapy. Thus, we developed high throughput assay for identification of GFLs' mimetics, which can facilitate discovery of drugs against PD.

MODULATING ER STRESS TO INHIBIT ANIMAL PARKINSON DISEASE PROGRESSION

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Parkinson Disease (PD) is a progressive neurodegenerative disorder that affects nearly 1% of the elderly population. The pathological hallmarks of PD include the loss of the dopaminergic neurons, the appearance of Lewy bodies and the activation of the unfolded protein response (UPR). Here, we present in vivo evidence that preconditioning the endoplasmic reticulum (ER) with mild stress response has strong neuroprotective effects in mice and flies PD models, and such effect is mediated through activation of autophagy We first show that intraperitoneal injection of tunicamycin, an inhibitor of protein glycosylation that induces UPR, results in the increase of XBP1 splicing and Bip expression in normal mouse brain, indicating that we have successfully administered a protective dose of tunicamycin that induces a mild ER stress response at a cellular level. We further observe that the tunicamycin treatment reduces dopaminergic neuron loss and ameliorates the Parkinson-like motor behaviour in mice treated with 6-hydroxydopamine. Moreover, we are able to obtain similar finding in two models of Parkinson disease in *Drosophila melanogaster*. Specifically, we show that feeding flies with tunicamycin protected the neurons against damages induced by paraquat treatment or through neuronal expression of α-Syn. Based on these findings, we conclude that mild ER stress promotes cellular survival mechanisms that inhibit apoptosis. Furthermore, we demonstrate that mild ER stress induces autophagy only in the presence of an apoptotic signal in both mouse and *Drosophila* models. We propose that the inhibition of apoptosis by mild ER stress activates autophagy. Together our findings show that mild ER stress is neuroprotective and suggest that ER conditioning is a promising therapeutic avenue in Parkinson disease.

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Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

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