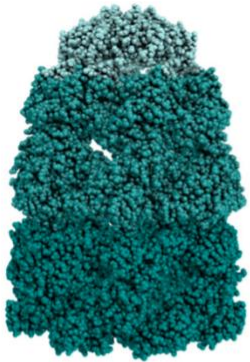


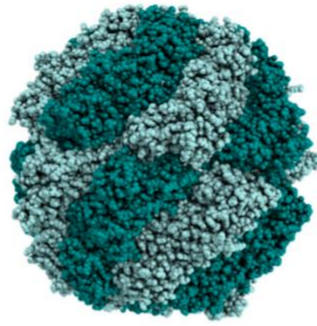


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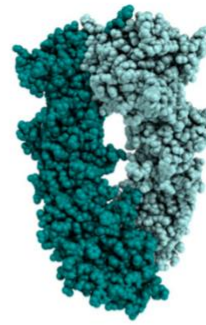
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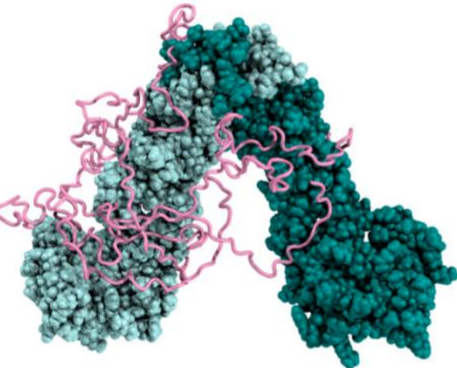
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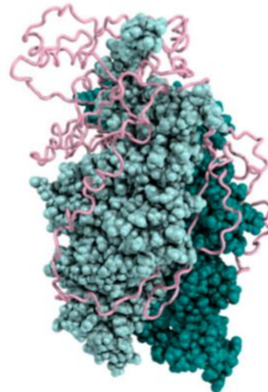
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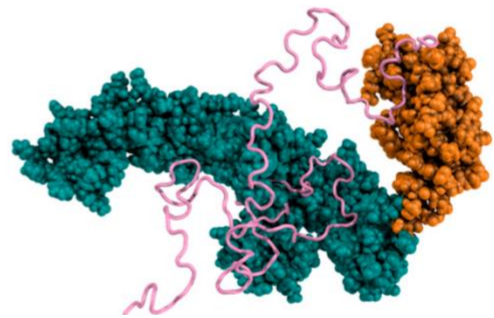
D Hsp40-PhoA



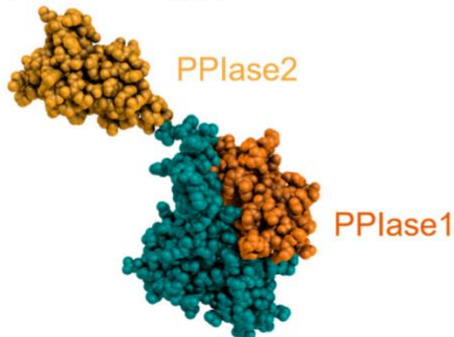
E SecB-PhoA



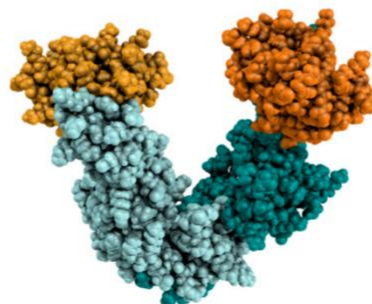
F TF-PhoA



G SurA



H PrsA

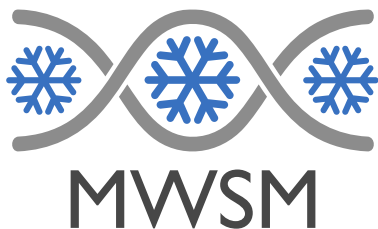


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Saturday, January 20th, 2024

James Allen Center
Northwestern University
Evanston, Illinois



29th Annual Midwest Stress Response and Molecular Chaperone Conference

*Saturday, January 20, 2024
Hosted by Northwestern University
James L. Allen Center
2211 Campus Drive
Evanston, Illinois 60208*

Program Chairs

Jill Johnson, *Department of Biological Sciences
University of Idaho*

Liming Li, *Department of Biochemistry and Molecular Genetics
Northwestern University*

Meeting Organizers

Rick Morimoto, *Department of Molecular Biosciences,
Northwestern University*

Rebecca Phend, *Department of Molecular Biosciences,
Northwestern University*

PROGRAM

All times in CST

7:30 - 9:00 AM

Continental Breakfast

9:00 - 9:05 AM

Opening remarks- Rick Morimoto, Northwestern University

Session I- Stress Responses and Proteostasis

Session Chair:

Randal Halfmann, Stowers Institute, Kansas City, MO

9:05 - 9:20 AM

The stress-activated ribosome-associated quality control pathway targets suboptimal codon-containing transcripts

Max Baymiller, Ben Dodd, **Stephanie Moon**
*Center for RNA Biomedicine, University of Michigan
Department of Human Genetics, University of Michigan Medical
School, Ann Arbor, MI*

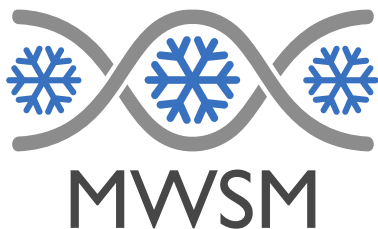
9:20 - 9:35 AM

VceC, a Brucella effector that manipulates the host ER stress response

Ben Watson, April Tsai, Nick Noinaj, Renee Tsohis, and Seema Mattoo
*Department of Biological Sciences, Purdue University, West
Lafayette, IN*

9:35 - 9:50 AM

Stress response prioritization revealed by spectral decomposition of single cell transcriptomes



29th Annual Midwest Stress Response and Molecular Chaperone Conference

Annissa Dea, Yongqing Lan, Valeryia Aksianiuk, Benjamin Doran, Arjun Raman, David Pincus

Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL

9:50 - 10:05 AM

A HSF1-JMJD6-HSP feedback circuit promotes cell adaptation to proteotoxic stress

Milad Alasady, Martina Koeva, Seesha Takagishi, David Amici, Roger Smith, Daniel Ansel, Susan Lindquist, Luke Whitesell, Elizabeth Bartom, Mikko Taipale, and Marc Mendillo

Department of Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, IL

Session II- Chaperone Function and Regulation

Session Chair:

Lukasz Joachimiak, UT Southwestern Medical Center, Dallas, TX

10:05 - 10:20 AM

Elucidating a direct role for molecular chaperones in regulating purine biosynthesis

Anthony Pedley, Jack Boylan, Zou Sha, Caitlyn Miller, Stephen Benkovic

Department of Chemistry, The Pennsylvania State University, University Park, PA

10:20 - 10:35 AM

Loss of FIC-1-mediated AMPylation engages the endoplasmic reticulum unfolded protein response to combat proteotoxic stress in *C. elegans* models of polyglutamine toxicity

Kate Van Pelt, Matthias Truttmann

Program in Cellular & Molecular Biology, University of Michigan, Ann Arbor, MI

10:35 - 10:50 AM

Brewing tumult in the buffer zone

Natalia Condic, Hatim Amiji, Dipak Patel, William Shropshire, Nejla Lermi, Youssef Sabha, Beryl John, Blake Hanson, **Georgios Karras**

Department of Genetics, Genetics and Epigenetics Graduate Program, The University of Texas MD Anderson Cancer Center, UTHealth Houston Graduate School of Biomedical Sciences; Houston, TX

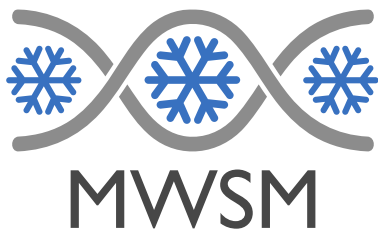
10:50 - 11:05 AM

Coffee Break

Session III- Protein Aggregation and Diseases

Session Chair:

Martin Duennwald, Western University, London, Ontario, Canada



29th Annual Midwest Stress Response and Molecular Chaperone Conference

11:05 - 11:20 AM

Determining the molecular interactions that drive fibril formation of hnRNPA2

Daniel Kieffer, Lukasz Joachimiak
*Center for Alzheimer's and Neurodegenerative Diseases/
Biochemistry, UT Southwestern Medical Center, Dallas, TX*

11:20 - 11:35 AM

Hsp40/70/110 chaperones limit human transthyretin protein aggregation

Claire Radtke, Adam Knier, Anita Manogaran
*Department of Biological Sciences, Marquette University,
Milwaukee, WI*

11:35 - 11:50 AM

Beyond heat shock: novel roles for HSF1 in synaptic protein regulation and its implications on cognitive functions

Nicholas Rozema, Nicole Zarate, Rachel Mansky, Nicolette Blomme, Erin Lind, Ying Zhang, Rocio Gomez-Pastor
Department of Neuroscience, Medical School, University of Minnesota, Minneapolis, MN

11:50 - 12:05 PM

Characterizing the interaction between α -synuclein and stress-inducible phosphoprotein 1 in Parkinson's Disease

Benjamin Rutledge, Justin Legleiter, Martin Duennwald, Marco Prado, Wing-Yiu Choy
Department of Biochemistry, Western University, London, Ontario, Canada

12:05 – 12:20 PM

Tribute to Betty Craig- Jill Johnson, University of Idaho

12:20 – 12:35 PM

Group picture

1:00 – 2:00 PM

Lunch

12:45 – 2:45 PM

Poster Session

Session IV- Protein Folding, Cellular Metabolism and Aging

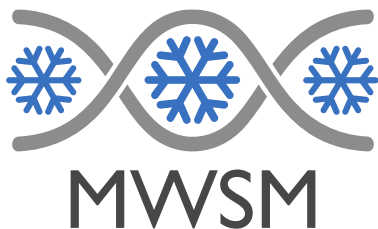
Session Chair:

Patricija van Oosten-Hawle, UNC Charlotte, Charlotte, NC

2:45 - 3:00 PM

Exploring unfolded protein response (UPR) interactions among organelles

Jeson Li, Nan Xin, Chunxia Yang, Larissa Tavizon, Ruth Hung, Travis Moore, Rebecca George Tharyan, Adam Antebi, **Hyun-Eui Kim**
Department of Integrative Biology and Pharmacology, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX



29th Annual Midwest Stress Response and Molecular Chaperone Conference

3:00 - 3:15 PM

Chaperoning huntingtin: investigating the sequestration of chaperones by huntingtin

Sarah Rolli, Emily Sontag
*Department of Biological Sciences, Marquette University,
Milwaukee, WI*

3:15 - 3:30 PM

Directed-evolution approach to nascent-protein folding and aggregation based on tuning the size of the ribosomal exit tunnel

Sofia Merrick Jinhong Jang, Silvia Cavagnero
*Departments of Biochemistry and Chemistry, University of
Wisconsin, Madison, WI*

3:30 - 3:45 PM

Identification of proteotoxic and proteoprotective bacteria that non-specifically affect proteins associated with neurodegenerative diseases.

Alyssa Walker, Rohan Bhargava, Michael Bucher, Amanda Brust, Cindy Voisine, Daniel M Czyz
*Department of Microbiology and Cell Science, University of
Florida, Gainesville, FL*

Plenary session

3:45 - 3:50 PM

Introduction of Plenary Speaker by Liming Li, Northwestern University

3:50 - 4:35 PM

SERF, an RNA G-quadruplex binding protein that promotes stress granule formation and protein aggregation

James Bardwell, Rowena G. Matthews Collegiate Professor of Molecular, Cellular, and Developmental Biology
Howard Hughes Medical Institute Investigator
University of Michigan, Ann Arbor, MI

4:35 - 4:50 PM

Q&A

4:50 - 5:00 PM

Closing Remarks by Jill Johnson, University of Idaho

5:00 - 6:30 PM

Reception w/food & beverages at the Allen Center

This annual meeting is generously supported by The Daniel F. and Ada L. Rice Institute for Biomedical Research.

We thank Sue Fox of Northwestern University for her assistance in planning and organizing this conference.



*29th Annual Midwest Stress Response and
Molecular Chaperone Conference*

ABSTRACTS: SPEAKERS



*29th Annual Midwest Stress Response and
Molecular Chaperone Conference*

SESSION I:

STRESS RESPONSES AND PROTEOSTASIS



The stress-activated ribosome-associated quality control pathway targets suboptimal codon-containing transcripts

Max Baymiller^{1,2}, Ben Dodd^{1,2}, [Stephanie L Moon](#)^{1,2}

¹Center for RNA Biomedicine, University of Michigan, Ann Arbor, MI

²Department of Human Genetics, University of Michigan Medical School

Disease-associated stresses (e.g., protein aggregates, inflammatory factors, heat, and pathogens) cause translational repression of mRNAs and their accumulation within condensates called stress granules. We previously observed that inhibition of ribosome-associated quality control (RQC) factors including valosin containing protein (VCP) and the proteasome traps certain mRNAs in the translating pool and prevents their localization to stress granules during arsenite or heat stress. We term this process the stress-activated RQC (saRQC) pathway. Because cellular stress, stress granule-like inclusion bodies, and the loss of VCP and proteasome activity are associated with aging and degenerative conditions, identifying the mechanisms of the saRQC holds promise for the development of novel therapeutic strategies to extend healthspan. The goal of this research is to identify the molecular features that cause some mRNAs to be released from polysomes by the saRQC. We propose that the saRQC senses and releases inefficiently translated mRNAs from the translating pool. We assessed the localization of transcripts encoding green fluorescent protein (GFP) using optimal or suboptimal codons in human cells. Transcripts that encode suboptimal codons are anticipated to have slower translation elongation rates due to the unavailability of the appropriate charged tRNAs. We expressed these transcripts in human U-2 OS cells and assessed their abundance at the mRNA and protein levels. We then assessed whether the inhibition of the saRQC impaired their localization to stress granules during stress. We made four key observations. First, codon suboptimal transcripts were less abundant than codon optimal transcripts, in line with past studies that suggest codon suboptimal transcripts are degraded more rapidly than codon optimal transcripts. Second, single cell analysis of GFP protein and GFP mRNA abundance revealed codon suboptimal transcripts have a lower protein:mRNA ratio than codon optimal transcripts, suggesting that codon suboptimal transcripts have lower translation rates. Third, treatment of stressed cells with VCP or proteasome inhibitors significantly reduced the localization of the codon suboptimal transcripts to stress granules, with no impact on stress granule localization of the codon optimal transcripts. Finally, puromycin rescued the localization of codon suboptimal transcripts to stress granules, suggesting these transcripts remain trapped within polysomes during arsenite stress upon saRQC inhibition. These results support the idea that the saRQC pathway releases transcripts from ribosomes that have slowed during elongation during arsenite or heat stress. Future studies will investigate whether and how dysfunctional release of inefficiently translated transcripts from the translating pool might contribute to neurodegeneration and aging.



29th Annual Midwest Stress Response and Molecular Chaperone Conference

VceC, a Brucella effector that manipulates the host ER stress response.

Ben G. Watson¹, April Y. Tsai², Nick Noinaj¹, Renee Tsois², Seema Mattoo^{1*}

¹Purdue University, Lafayette, IN

²University of California, Davis, Davis, CA

*Corresponding Author

The metazoan unfolded protein response (UPR) is an integrated signaling network initiated within the endoplasmic reticulum (ER) that regulates proteostasis in response to misfolded proteins. We previously reported the ER-luminal human FIC (filamentation induced by cyclic AMP) protein, HYPE (Huntingtin-yeast Partner E), as a critical regulator of UPR. HYPE reversibly adenylylates/AMPylates (adds an AMP moiety to) the HSP70 chaperone BiP/GRP78, thus regulating BiP's ATPase activity and protein folding capacity. Interestingly, infection by microbes often manifests as a signal for inducing UPR. Here, we report that the bacterial pathogen *Brucella abortus* grows in the ER lumen, where it secretes an effector protein VceC (VirB Coregulated Effector C) that activates the IRE1-mediated branch of the UPR. Further, VceC was found to be a membrane tethered protein that faces the ER lumen and binds with BiP in cellular pulldown assays. VceC is secreted via *Brucella*'s Type IV secretion system and is necessary for bacterial replication. However, its exact mechanism of action and the role of UPR activation during pathogenesis is unknown. To address this knowledge gap, we took a structure biology approach to elucidate VceC structure, function, and association with BiP and HYPE. Our data challenges the idea of a direct VceC-BiP interaction, and gave us interesting insights into the toxicity and stoichiometric states of VceC that may impact its role in manipulating ER stress.



29th Annual Midwest Stress Response and Molecular Chaperone Conference

Stress response prioritization revealed by spectral decomposition of single cell transcriptomes

Annisa Dea¹, Lan Yongqing², Aksianiuk Valeryia², Benjamin Doran³, Arjun Raman², David Pincus¹

¹Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL

²Department of Pathology, University of Chicago, Chicago, IL

³Department of Pathology, Pritzker School of Molecular Engineering, University of Chicago, Chicago, IL

Cells activate transcriptional stress response programs to adapt to fluctuating environments. While responses to individual perturbations, such as heat shock and osmotic stress, have been well characterized, responses to complex, multi-stress environments remain unpredictable. In the model eukaryote *S. cerevisiae*, there exists a dominant transcriptional signature common across a broad range of stressors, the Environmental Stress Response (ESR), that represses ribosome biogenesis and induce alternative metabolic programs. A prevailing model of adaptation is that cells are agnostic to the identity of each individual stressor and reduce the complexity of the environment to a generic representation of stress via ESR induction and growth rate. However, this model fails to account for genetic results demonstrating that specific factors are essential for responding to distinct stressors. Here we reconcile the transcriptomics with the genetics by demonstrating that environmental information is represented in a hierarchical manner in transcriptional variation. We exposed cells to a nested series of environments that captures changes in carbon source, temperature, osmotic pressure, and oxidative stress, and conducted single-cell RNA sequencing. We conducted spectral decomposition and mutual information across 10,000s of cells spanning 20 environments, and found that different environmental perturbations appear in semi-discrete channels of the principal component spectrum, suggesting a functional hierarchy and prioritization scheme by which cells integrate environmental information. Finally, we provide experimental support for this hierarchical organization by discovering a novel stress crosstalk mechanism whereby cells prioritize the response to hyperosmotic stress over the heat shock response when doubly stressed. Rather than compressing the environment into a single ESR parameter or maintaining a series of distinct stress responses, our results suggest that cells adapt to complex environments according to a prioritization hierarchy that presumably evolved to reflect the statistics of natural environmental fluctuations.



29th Annual Midwest Stress Response and Molecular Chaperone Conference

A HSF1-JMJD6-HSP feedback circuit promotes cell adaptation to proteotoxic stress

Milad J. Alasady^{1,2,3}, Martina Koeva^{4,5}, Seesha R. Takagishi^{1,2,3}, David R. Amici^{1,2,3}, Roger S. Smith^{1,2,3}, Daniel J. Ansel^{1,2,3}, Susan Lindquist^{4,5,6}, †, Luke Whitesel⁴, ‡, Elizabeth T. Bartom^{1,2,3}, Mikko Taipale⁷, Marc L. Mendillo^{1,2,3}

¹Department of Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, IL

²Simpson Querrey Center for Epigenetics, Northwestern University Feinberg School of Medicine, Chicago, IL

³Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL

⁴Whitehead Institute for Biomedical Research, Cambridge, MA

⁵Massachusetts Institute of Technology, Cambridge, MA

⁶Howard Hughes Medical Institute, Cambridge, MA

⁷Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, M5S 3E1, Canada.

†Deceased

‡Current address: Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada.

Heat Shock Factor 1 (HSF1) is best known as the master transcriptional regulator of the heat-shock response (HSR), a conserved adaptive mechanism critical for protein homeostasis (proteostasis). Combining a genome-wide RNAi library with an HSR reporter, we identified JMJD6 as an essential mediator of HSF1 activity. In follow-up studies, we found that JMJD6 is itself a non-canonical transcriptional target of HSF1 which acts as a critical regulator of proteostasis. In a positive feedback circuit, HSF1 binds and promotes JMJD6 expression, which in turn reduces HSP70 R469 monomethylation to disrupt HSP70-HSF1 repressive complexes resulting in enhanced HSF1 activation. Thus, JMJD6 is intricately wired into the proteostasis network where it plays a critical role for cell adaptation to the chronic proteotoxic stress associated with oncogenic transformation.



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SESSION II:

CHAPERONE FUNCTION AND REGULATION



29th Annual Midwest Stress Response and Molecular Chaperone Conference

Elucidating a Direct Role for Molecular Chaperones in Regulating Purine Biosynthesis

Anthony M. Pedley¹, Jack P. Boylan^{2,3}, Zou Sha¹, Caitlyn M. Miller^{3,4}, Stephen J. Benkovic¹

¹Department of Chemistry, The Pennsylvania State University, University Park, PA

²Molecular, Cellular, and Integrative Biosciences, The Pennsylvania State University, University Park, PA

³Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA

⁴Huck Institutes of the Life Sciences; The Pennsylvania State University, University Park, PA

Cell metabolism is the collection of biochemical transformations that convert nutrients into the biomass and energy needed for proliferation. In normal cells, these processes are regulated to restrict nutrient utilization to only what is needed; however, genetic alterations that emerge in cancers hijack these regulatory controls and result in a prolonged increase in nutrient uptake, often depleting the cell's microenvironment of the essential nutrients. This imparts a reorganization of metabolic networks to shunt resources around to meet the enhanced metabolic demands. While there is a basic understanding of the genetic events that result in this metabolic reprogramming, our appreciation of the post-translational changes to metabolic enzymes have been limited to substrate availability, allosteric modulation (i.e., feed forward activation and feedback inhibition mechanisms), and chemical modifications such as phosphorylation driven by aberrant cell signaling. Recently, we have shown that enzymes within de novo purine biosynthesis are also under the direct regulation of the molecular chaperone Hsp90 in cancer cells and that these interactions assist in preserving the liquid-liquid phase separation of pathway enzymes into biomolecular condensates called purinosomes. Further, we have discovered that those Hsp90 clients within the purine biosynthetic pathway all function as amidotransferases. Our current research efforts are focused on investigating the unknown actions (both structural and biochemical) of Hsp90 on amidotransferases and how these might regulate purinosome formation with the hopes of expanding our observations to other metabolic enzyme classes and gain a molecular basis for substrate recognition by Hsp90.



Loss of FIC-1-mediated AMPylation engages the endoplasmic reticulum unfolded protein response to combat proteotoxic stress in *C. elegans* models of polyglutamine toxicity

Kate M. Van Pelt^{1,2}, Matthias C. Truttmann^{2,3}

¹Program in Cellular & Molecular Biology, University of Michigan, Ann Arbor, MI

²Dept. of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI

³Geriatrics Center, University of Michigan, Ann Arbor, MI

Neurodegenerative diseases (NDs) are characterized by the failure of cellular proteostasis machinery, rendering key components of this network promising targets for attenuating pathological protein aggregation. Fic-type protein AMPylases are enzymes that catalyze the post-translational addition and removal of adenosine monophosphate (AMP) to serine/threonine residues of target proteins. Recent work from our lab and others has identified the human AMPylase, FICD, and its *C. elegans* ortholog, FIC-1, as potent regulators of the ER-resident HSP70 family chaperone, BiP, and its worm orthologs, HSP-3/4, respectively. Using *C. elegans* models of polyglutamine (polyQ) diseases, a family of NDs caused by the aberrant expansion of polyQ repeats, we previously reported that changes in FIC-1-mediated AMPylation directly alters polyQ aggregate number, size, solubility, and toxicity. In this study, we report that genetic deletion of *fic-1* rescues the developmental lethality of worms expressing aggregation-prone polyQs observed upon RNAi depletion of either *hsp-3* or *hsp-4*. Importantly, this protective effect continues with age, as *fic-1* loss is sufficient to extend the lifespan of polyQ-expressing worms when *hsp-3* or *hsp-4* are knocked-down during adulthood. Bulk RNA-sequencing (RNAseq) analysis of developing polyQ, *fic-1* KO worms (L4 larvae) on *hsp-3* or *hsp-4* RNAi revealed enriched gene signatures linked to the *ire-1* and *pek-1* arms of the endoplasmic reticulum unfolded protein response (UPRER), ER-nucleus signaling, and protein refolding. Indeed, we find that *pek-1* knock-down is sufficient to abolish the beneficial effect of *fic-1* loss during polyQ worm development. Taken together, our findings support a model in which the loss of FIC-1-mediated AMPylation bolsters the ER's capacity to respond to polyQ-associated proteotoxicity through activation of the UPRER and the subsequent upregulation of molecular chaperones and other stress-responsive genes.



29th Annual Midwest Stress Response and Molecular Chaperone Conference

Brewing tumult in the buffer zone

Natalia Condic^{1,†}, Hatim Amiji^{1,†}, Dipak Patel^{1,†}, William Shropshire^{3,4,5}, Nejla O. Lermi¹, Youssef Sabha¹, Beryl John¹, Blake Hanson^{3,4}, Georgios I. Karras^{1,2,*}

¹Department of Genetics, The University of Texas MD Anderson Cancer Center; Houston, TX

²Genetics and Epigenetics Graduate Program, The University of Texas MD Anderson Cancer Center, UTHealth Houston Graduate School of Biomedical Sciences; Houston, TX

³Center for Infectious Diseases, Department of Epidemiology, Human Genetics and Environmental Sciences, UTHealth School of Public Health at Houston, University of Texas Health Science Center, McGovern Medical School; Houston, TX

⁴Center for Antimicrobial Resistance and Microbial Genomics, University of Texas Health Science Center McGovern Medical School; Houston, TX

⁵The University of Texas MD Anderson Cancer Center; Houston, TX

[†]These authors contributed equally.

*Corresponding author.

Biological systems can tolerate many types of changes in their internal and external conditions, a characteristic known as robustness. Although robustness is widespread in living systems, its origins and causal buffering mechanisms remain enigmatic. Perhaps the best studied genes underlying buffering mechanisms encode members of the heat shock protein 90 (Hsp90) family. Hsp90 is constitutively expressed in excess over the cell's needs for growth and survival. This excess provides a protein-folding buffer that supports many cellular pathways and has been shown to influence the relationship between genotype and phenotype. Importantly, the Hsp90 buffering capacity of the cell is finite and can be exceeded by diverse proteotoxic challenges that lead to protein unfolding, titrating Hsp90 away from its normal clients and causing loss of their function. Thus, linking cellular pathways to this finite buffering capacity of Hsp90 by mutation renders the mutated pathways sensitive to compromise by proteotoxic stressors in the environment of the cell. However, the ecological Hsp90 stressors that reveal Hsp90-contingent variation and the role of such variation in the evolution of ecologically relevant traits remain elusive. To tackle our limited understanding of Hsp90's role in evolution, we focused on yeast carbohydrate metabolism. We employed Hsp90 inhibition as a proxy for proteotoxic environmental stress and compared the robustness of metabolic traits to Hsp90 inhibition across 711 yeast strains from diverse domesticated and wild ecological niches. Our results demonstrate that ecologically relevant metabolic traits in beer and bread yeasts have evolved robustness to stresses that exceed Hsp90's chaperone capacity. Specifically, gene duplications characteristic of beer and bread yeasts stabilize desirable metabolic traits against prevalent Hsp90 stressors associated with industrial fermentation. As a telling example, ethanol exerts selective pressure for gene duplications that underlie canalization. We propose that, during yeast domestication, metabolic traits relevant to beer and bread production have undergone genetic and environmental canalization through selection for robustness against niche-related Hsp90 stress. Our findings highlight the untapped potential of Hsp90-buffered variation as an important source of phantom heritability in ecologically relevant traits.



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SESSION III:

PROTEIN AGGREGATION AND DISEASE



29th Annual Midwest Stress Response and Molecular Chaperone Conference

Determining the molecular interactions that drive fibril formation of hnRNPA2

Daniel Kieffer, Lukasz Joachimiak

UT Southwestern Medical Center, Dallas, TX

The heterogeneous nuclear ribonucleoprotein (hnRNP) family, which includes TAR DNA-binding protein 43 (TDP-43), fused in sarcoma (FUS) and heterogeneous nuclear ribonucleoprotein A2 (hnRNPA2), regulates a broad range of RNA-based cellular processes including RNA splicing and transport. Under stress, these proteins help form ribonucleoprotein granules stabilized by RNA and hnRNP interactions. Failure to disassemble these granules leads to pathological conversion of hnRNPs into irreversible beta-sheet-rich amyloids that are linked to diseases including amyotrophic lateral sclerosis, frontotemporal dementia and multisystem proteinopathy. Mutations in the low complexity domain (LCD) of hnRNPs cause dominantly inherited diseases. Cryogenic Electron Microscopy (cryo-EM) structural analysis of disease-associated amyloids of hnRNPs have uncovered a diversity of structures whose amyloid core is comprised of the LCD. It is unknown how these structures are formed in disease. Here we used cryo-EM structural analysis, computational modeling, and peptide systems to uncover the biophysical rules for hnRNPA2 amyloid assembly. We validated that hnRNPA2 forms monomorphic fibrils by reproducing a cryo-EM fibril structure of the wild-type hnRNPA2 LCD to a resolution of 2.0Å. We employed computational modeling to define the energetic hotspots that stabilize this fold. Derived from these analyses, we use peptide systems to determine key interactions and features of the fibril to adopt this unique fold. Utilizing our peptide system, we were able to reduce the fibril to the minimal fold necessary for fibril formation. These data set the stage to manipulate the folding of hnRNPA2 into defined shapes to begin to design diagnostic and therapeutic approaches to treat this family of diseases.



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Hsp40/70/110 Chaperones Limit Human Transthyretin Protein Aggregation

Claire M. Radtke*, Adam S. Knier*, Anita L. Manogaran

Department of Biological Sciences, Marquette University, Milwaukee, WI

*co-first authors

The molecular chaperone network plays an essential role in maintaining protein homeostasis but can lose efficiency with age, leading to the accumulation of disease related protein aggregates. Therefore, it is critical to understand chaperones' individual functions and their interactions with co-chaperones to maintain proteostasis. Here, we use the human aggregating transthyretin (TTR) protein, associated with the fatal disease transthyretin amyloidosis, to better understand the in vivo relationship between molecular chaperones and protein aggregation. We have developed a tractable yeast-based system expressing human TTR, which forms both SDS-resistant oligomers and SDS-sensitive large molecular weight complexes. Our lab identified that while the yeast specific disaggregase, Hsp104, has no direct effect on TTR aggregation, overexpression of a middle-domain Hsp104 mutant possibly unbalances the chaperone network by titrating away unknown factors responsible for maintaining smaller TTR aggregate populations. To identify chaperones that directly limit TTR aggregation and may be prone to chaperone titration, we used a candidate-based approach and targeted the Hsp40/70/110 chaperone network because of its known protein disaggregation activity. We find the loss of Sse1 (Hsp110), Ssa1/Ssa2 (Hsp70) or Sis1 (Hsp40) all independently result in the loss of small TTR aggregate populations, and an increase in larger sized aggregates. Interestingly, we find the overexpression of the human Hsp40, DnaJB1 in place of Sis1, increases the population of smaller TTR aggregates. Taken together, our data implies that the Hsp40/70/110 chaperone network may provide context to potential therapeutics for protein aggregation related diseases.



Beyond Heat Shock: Novel roles for HSF1 in synaptic protein regulation and its implications on cognitive functions

Nicholas B. Rozema¹, Nicole Zarate¹, Rachel Mansky¹, Nicolette Blomme¹, Erin Lind², Ying Zhang³, Rocio Gomez-Pastor¹

¹Department of Neuroscience, Medical School, University of Minnesota, Minneapolis, MN

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Heat Shock Transcription Factor 1 (HSF1) is a stress protective transcription factor canonically known for its role in cellular stress responses and the regulation of protein quality control systems. However, any role HSF1 may play under non-stressful, physiological conditions within the central nervous system has been understudied. Importantly, HSF1 levels decline during aging and this decline is exacerbated in various neurodegenerative diseases. Recent evidence has demonstrated that HSF1 also participates in the regulation of synaptic genes within different contexts and brain regions, highlighting non-canonical roles for HSF1 beyond the regulation of protein quality control systems. Here, we showed that HSF1 regulates cognitive flexibility, a core executive function that declines during aging. HSF1 directly controls the expression of synaptic proteins, essential in maintaining excitatory synapse stability and neurotransmission, within the mouse striatum, a brain region that controls movement and some forms of cognition. Striatal synaptic connections decline during aging and contribute to alterations in synaptic dysfunction and behavioral deficits, but whether HSF1 is responsible for age-dependent loss of these synapses and its associated cognitive deficits has remained elusive. We have utilized i) a model of chronic HSF1 reduction across the mouse lifespan through genetic ablation of a single *Hsf1* allele (*Hsf1*^{+/-} mice) and ii) a model of acute HSF1 reduction via adeno-associated viruses (AAVs) to spatially and temporally restrict HSF1 reduction to the striatum. Through chromatin immunoprecipitation sequencing in wildtype mouse striatum and RNA-sequencing, we have demonstrated HSF1 primarily controls the expression of key synaptic genes specifically within the postsynaptic compartment within the mature striatum, but not chaperones. Chronic HSF1 depletion also resulted in a specific reduction in excitatory thalamo-striatal (T-S) synapses, an important synaptic circuit involved in cognitive functions such as goal-directed learning, action selection, and flexible control of behavior. This suggests that depletion of HSF1 may contribute to memory-related deficits associated with aging. In support of this hypothesis, acute reduction of HSF1 in the striatum via AAVs resulted in apparent disruption of spatial working memory and cognitive flexibility of associative learning. Additionally, we are currently making strides towards understanding the role of HSF1 at the synapse within the context of individual excitatory pathways within the striatum. These results demonstrate an emerging role for HSF1 in synaptic gene regulation that has important implications in synapse maintenance and memory during aging and that can result in effective therapeutic interventions to ameliorate deficits in cognitive flexibility in the aging brain.



29th Annual Midwest Stress Response and Molecular Chaperone Conference

Characterizing the interaction between α -synuclein and stress-inducible phosphoprotein 1 in Parkinson's Disease

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The aggregation of alpha-synuclein (α -Syn), a presynaptic neuronal protein, is a hallmark of Parkinson's disease (PD). α -Syn lacks a well-defined structure and behaves as an intrinsically disordered protein (IDP), sampling an ensemble of conformations. Under pathological conditions, α -Syn forms toxic polymorphic oligomers and subsequently cellular inclusions comprised of fibrils, called Lewy bodies (LBs).

Molecular chaperones, such as Hsp90 and Hsp70, maintain the solubility of many neurodegeneration-associated IDPs, including α -Syn. Stress-inducible Phosphoprotein 1 (STI1), also known as Hsp-organizing protein (HOP) in humans, is a major co-chaperone to both Hsp90 and Hsp70. In two mouse models of α -Syn misfolding, STI1 co-immunoprecipitated α -Syn, and co-deposited with Hsp90 and Hsp70 in insoluble protein fractions. Overexpression of STI1 in PD mouse models exacerbates behavioural phenotypes when injected with α -Syn pre-formed fibrils. These findings reveal that STI1 modulates the generation and accumulation of toxic α -Syn conformers in mouse models of α -Syn misfolding.

Nuclear Magnetic Resonance (NMR) analyses revealed a dynamic binding mechanism between STI1 and α -Syn, mediated by the STI1 TPR2A domain and two independent binding motifs in the C-terminal of α -Syn. Both binding motifs of α -Syn interact with TPR2A and compete for a single binding interface, which can be effectively blocked by Hsp90 peptides. The direct interaction between STI1 and α -Syn alters the aggregations kinetics of α -Syn to favour the formation of cytotoxic oligomers. The modulation of α -Syn aggregation by STI1 represents an ATP-independent holdase mechanism separate from its canonical co-chaperone function. The influences of STI1 in α -Syn aggregation demonstrates a corruption of the proteostasis pathway, facilitating PD development.



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SESSION IV:

PROTEIN FOLDING, CELLULAR METABOLISM AND AGING



29th Annual Midwest Stress Response and Molecular Chaperone Conference

Exploring Unfolded Protein Response (UPR) interactions among organelles

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Our research delineates a novel Mitochondria to ER Stress Response (MERSR) essential for maintaining protein homeostasis under proteotoxic stress. Triggered by perturbations in mitochondrial proteostasis, MERSR selectively inhibits the IRE1 branch of the unfolded protein response in the endoplasmic reticulum (UPRER) and concurrently upregulates the PERK-eIF2 α signaling pathway. This intricate regulation not only modulates stress response but also bolsters the cellular capacity to manage proteostatic challenges. The significance of MERSR is highlighted in *C. elegans* models harboring poly-glutamine and amyloid-beta aggregates, where its activation is associated with a reduction in aggregate burden and improved disease outcomes. These findings emphasize the complexities of inter-organelle communication in maintaining cellular homeostasis and underscore the critical role of intercompartmental signaling pathways in modulating diseases.



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Chaperoning Huntingtin: Investigating the sequestration of chaperones by huntingtin

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Huntington's disease (HD) is a neurodegenerative disease caused by a CAG repeat expansion within the huntingtin gene. Mutant huntingtin protein (HTTex1p) created by this expansion forms aggregates in the brain. Previous literature suggests that these protein deposits are both protective and toxic to the cells. Chaperones are sequestered into HTTex1p aggregates, possibly as part of protective mechanisms. It is hypothesized that proteostasis breakdown in HD depletes chaperones therefore they are no longer present at high enough levels to maintain proteostasis. We find that Hsp40, Hsp70, and Hsp90 alter the aggregation and toxicity of HTTex1p in a yeast model. Chaperone knockout increases the toxicity of HTTex1p and increases the amount of insoluble protein. Additionally, depletion of Hsp40 induces toxicity of normally non-toxic HTTex1p species. Preliminary data suggests that co-chaperone HOP is linked to HTTex1p toxicity. We are investigating the direct binding sites between HTTex1p and chaperones to better understand how they interact.



**Directed-Evolution Approach to Nascent-Protein Folding and Aggregation
Based on Tuning the Size of the Ribosomal Exit Tunnel**

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Optimization of protein folding, and elimination of protein aggregation are important for large-scale production of protein-based pharmaceuticals including interferon and insulin. In addition, prevention of protein aggregation is a key step in the cure of misfolding-related diseases including deadly neuro- and cardio-myopathies. Production of protein-based pharmaceuticals by recombinant-DNA technologies is more convenient and affordable in prokaryotic host organisms (e.g., *E. coli*), rather than in eukaryotes (e.g., baculovirus, Chinese hamster ovary cells). Yet, prior research shows that prokaryotic *in-vivo* protein overexpression often suffers from the formation of aggregates known as inclusion bodies. While it is presently unknown why bacterial cells are more likely to yield insoluble overexpressed proteins, the extremely fast release of fully synthesized nascent chains from the ribosome is a likely culprit. We hypothesize that fast ejection from the ribosomal tunnel in *E. coli* hampers the ability of newly synthesized proteins to attain their native fold before aggregation takes over. In this work, we take advantage of the selective pressure imposed by macrolide antibiotics, which enables the generation of bacterial strains bearing mutated ribosomal proteins (r-proteins). We hypothesize that these mutations affect the structure of the ribosomal exit tunnel, given that they are typically located across the L4 and L22 r-proteins. We tested the ability of the mutant strains to increase soluble-protein formation upon release from the ribosome in bacteria. The mutant strains show slower growth rate and variable gene-product solubility. Colony PCR followed by genome sequencing serves to confirm the identity and nature of the mutations. Overall, this study targets the improvement of protein-folding quality upon release from the ribosome for biotechnology and pharmaceutical purposes. In the future, we plan to extend our strategy to the large-scale production of a variety of other targets, including antibodies and other proteins of biomedical and pharmaceutical relevance.



Identification of proteotoxic and proteoprotective bacteria that non-specifically affect proteins associated with neurodegenerative diseases.

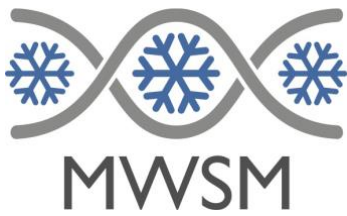
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Neurodegenerative protein conformational diseases (PCDs), such as Alzheimer's, Parkinson's, and Huntington's, are a leading cause of death and disability worldwide and have no known cures or effective treatments. Emerging evidence suggests a role for the gut microbiota in the pathogenesis of neurodegenerative PCDs; however, the influence of specific bacteria on the culprit proteins associated with each of these diseases remains elusive, primarily due to the complexity of the microbiota. In the present study, we employed a single-strain screening approach to determine the effect of 229 unique bacterial isolates from the Human Microbiome Project on the enhancement or suppression of disease-associated protein aggregation and the associated toxicity. Aggregation and the associated toxicity was assessed in *Caenorhabditis elegans* expressing human tau, A β 1-42, α -synuclein, and polyglutamine tracts. Here, we present the first comprehensive characterization of the effect of the human microbiome on proteins associated with neurodegenerative diseases. Our results indicate that bacteria affect the aggregation of metastable proteins by modulating host proteostasis rather than selectively targeting specific disease-associated proteins. These results reveal bacteria that potentially influence the pathogenesis of PCDs and open new promising prevention and treatment opportunities by altering the abundance of beneficial and detrimental microbes.

ABSTRACTS: POSTERS



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Tight regulation of a nuclear HAPSTR1-HUWE1 pathway essential for mammalian life

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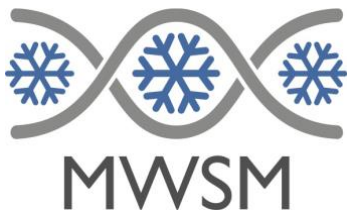
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The recently discovered HAPSTR1 protein broadly oversees cellular stress response signaling. HAPSTR1's function requires HUWE1, a ubiquitin ligase which paradoxically marks HAPSTR1 for degradation. Yet, the biochemical nature and physiological significance of the HAPSTR1-HUWE1 pathway remain unclear. Here, we demonstrate that HAPSTR1 enables nuclear localization of HUWE1 with pleiotropic effects on cellular signaling. We further identify the ubiquitin ligase TRIP12 and deubiquitinase USP7 as upstream regulators which critically titrate HAPSTR1 stability and protein levels for optimal cell fitness. Finally, we generate conditional Hapstr1-knockout mice, finding that Hapstr1-null mice are perinatal lethal, adult mice depleted of Hapstr1 have diminished fitness, and primary cells explanted from Hapstr1-null animals falter in culture coincident with HUWE1 mislocalization and failure to suppress p53. Altogether, we identify novel components and functional insights into the conserved HAPSTR1-HUWE1 pathway and demonstrate its requirement for mammalian life.



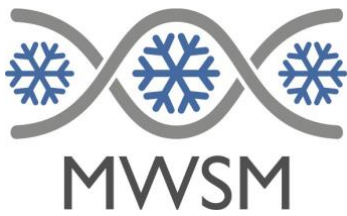
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Role of HSP90 in aging

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As a cell ages, its ability to regulate protein homeostasis (proteostasis) deteriorates with a hallmark being the accumulation of irreparably damaged and dysfunctional proteins. An imbalance in proteostasis correlates with various diseases from Alzheimer's and Huntington's to cardiovascular impairments, to type-2 diabetes, and cancers. A well-coordinated network comprising molecular chaperones and the ubiquitin-proteasome system operates in healthy cells to maintain proteostasis. Hsp90 is an essential evolutionary conserved molecular chaperone that supports protein health by preventing aggregation and phase separation of metastable proteins. There is mounting evidence that the proteostasis network breaks down with age and aging pathways can directly regulate elements of the proteostasis network to extend a healthy lifespan. Although previous studies show that imbalances in Hsp90 levels and activity can lead to variations in lifespan, little is known about the role of Hsp90 in maintaining proteostasis during aging. Our goal is to decipher how Hsp90 interacts with its cochaperones and clients to regulate the proteostasis network in budding yeast during chronological aging using cell biology and chemical biology approaches followed by mass spectrometry.



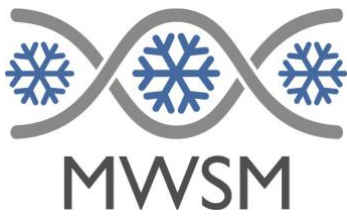
Distinguishing Amyloid Variations with Innovative Biosensor Cells

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Across many neurodegenerative diseases, including Alzheimer's, Parkinson's, and Amyotrophic Lateral Sclerosis (ALS), the most prominent biochemical hallmark and potential cause of disease is the ordered aggregation of precursor peptides into amyloid fibrils. Familial, or genetically inherited, forms of these diseases often arise from mutations in the precursor peptides (such as amyloid- β , tau, α -synuclein, and TDP-43). However, most cases are sporadic, and amyloid deposition is identified later in life after symptom onset. While genetic testing can be used to diagnose familial tauopathies, diagnosing sporadic forms relies on assessing the present symptoms and biomarkers, which often overlap between diseases. Using innovative biosensor cells, we hope to discriminate between neurodegenerative diseases by identifying patterns in disease-specific amyloid properties. Recent findings suggest that amyloids in these diseases are structurally unique, propagating through self-templating "seeds" that maintain the conformation of the original amyloid. We are investigating this templating activity using biosensor cells that express amyloidogenic monomers tagged with the photoconvertible fluorophore mEos3.2. Upon addition of seeds to these cells, the tagged monomers bind to the seeds and assemble into amyloid fibrils. Cells containing amyloids are detected through Distributed Amphifluoric FRET (DAmFRET), a flow cytometry assay that quantifies *in vivo* protein self-assembly. Our preliminary data for tau and TDP-43 biosensors demonstrate that these cells provide a reliable measurement of intracellular seeding activity in a conformation-specific manner. The addition of structurally distinct TDP-43 amyloids to wild-type or mutant TDP-43 biosensors resulted in unique assembly profiles. Additionally, no assembly was seen in cross-seeding experiments, where tau or TDP-43 amyloids were added to biosensor cells expressing the opposite protein. This suggests that the biosensor cells are selective to the protein expressed, rather than general amyloid structure. Expansion of these biosensor cells to include other amyloids will aid in identifying the factors that differentiate neurodegenerative diseases at the molecular level, improving the understanding of their disease mechanisms and allowing for the development of specialized therapeutics.



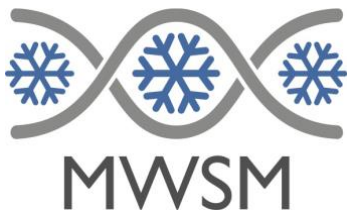
Limb Girdle Muscular Dystrophy (LGMD) D1: Disease mutations in two adjacent domains showed comparable mechanistic characteristics; implication for identical therapeutics.

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The intracellular homeostasis machinery plays a critical role in maintaining the fine balance between protein synthesis, proper folding, and degradation. Flaws in any of these processes may lead to protein misfolding and aggregation often associated with protein conformational disorders affecting a variety of organs, including the brain (Alzheimer's disease) and muscles (Limb Girdle Muscular Dystrophies), among others. Molecular chaperones, known as heat shock proteins, protect against protein misfolding and aggregation. DNAJB6, a ubiquitously expressed type II Hsp40 co-chaperone is one such protein. Historically, mutations within a 12 amino-acid region of the DNAJB6 G/F domain have been associated with the dominantly inherited muscle disease limb-girdle muscular dystrophy type D1 (LGMDD1). Recently, our collaborators discovered novel LGMDD1-associated mutations in the J domain of DNAJB6. No treatments are currently available and the major hindrance in understanding the disease pathogenesis is the inability to point out a specific client protein for DNAJB6 in skeletal muscle. The yeast type II Hsp40 co-chaperone, Sis1, is homologous to DNAJB6 and has an important role in yeast for the propagation of two yeast prions, [RNQ+] and [PSI+]. Previously, we have shown that the LGMDD1 mutants in Sis1 not only show substrate specificity but also substrate-conformer-specific effects. Further, we showed that the deleterious effect of the G/F domain of DNAJB6 mutants was HSP70-dependent. Here we aimed to elucidate whether these novel J domain mutations affect chaperone function in a manner similar to that of previously identified and characterized LGMDD1-associated G/F domain mutations. We found that novel disease-associated variants in the Hsp40 J-domain lead to aberrant chaperone function and altered protein homeostasis in a client and conformer-specific manner both in yeast and in vitro. In fact, the J-domain mutants showed variability in substrate processing in a manner identical to that of the G/F domain mutants. The binding affinity of the mutants to Hsp70 (Ssa1) was significantly reduced both in the presence and the absence of the client proteins. The stimulation of the Ssa1 ATP hydrolysis rate was significantly reduced with the mutants, albeit this was again a substrate-conformer-specific phenomenon. Moreover, we identified a novel client-dependent viability defect when one of the J-domain mutants is expressed. This is the first time, to our knowledge, that steady-state levels of a mutated chaperone have been shown to be dependent on stabilization by a client. Lastly, we performed simulation studies to back up our functional mechanistic data. We identified possible residues responsible for the change in protein-protein interaction with respect to the mutants of LGMDD1. This body of work enables direct comparisons between disease-associated mutants in different domains so that we may begin to not only understand how LGMDD1 mutants could impact disease severity and pathogenesis but also whether similar therapeutic avenues could be explored to treat patients with different mutations in the future.

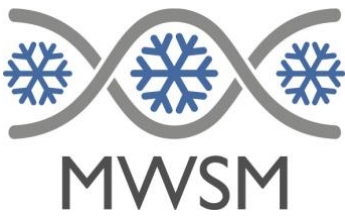


Synonymous codon substitutions enhance transcription and translation of an upstream gene in *E. coli*

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Synonymous codon substitutions are genetic mutations that do not change the encoded protein sequence. Although often assumed to be phenotypically silent, synonymous codon substitutions can cause diverse effects on mRNA transcription and protein translation. Yet, the full range of effects of synonymous codon substitutions on the production and maintenance of a functional proteome remains poorly understood. We are using the well characterized Tet ON/Tet OFF divergent expression system to broadly test the effects of synonymous codon substitutions on gene expression. In Tet ON/Tet OFF, the expression level of TetR, encoded upstream of a gene of interest (GOI), regulates expression of both the GOI and tetR, by binding to and repressing a divergent promoter between the GOI and tetR. We found that synonymous mutations within a GOI can enhance transcription from a transcriptional start site (TSS) identified within the GOI. The effects of specific codon substitutions on transcription from this intragenic TSS are poorly predicted by existing prediction algorithms. Surprisingly, we found that transcription from this TSS bypasses canonical repression of tetR transcription. As a result, TetR protein accumulation increases, repressing GOI expression. This mechanism of transcription enhancement was corroborated by inserting an antisense terminator downstream of the intragenic TSS, which led to the expected reduction in tetR mRNA level. Even more surprisingly, although the mRNA produced from the intragenic TSS constitutes a minor fraction of tetR-encoding RNA, its level is predictive of TetR abundance, whereas total tetR mRNA level is not. We are currently testing whether this unexpected correlation between TetR and the minor fraction of tetR mRNA originating from the intragenic TSS is due to enhanced translational efficiency of the longer mRNA, or whether the longer RNA serves as a regulator of translational efficiency of canonical tetR mRNA. Collectively, these results demonstrate that synonymous codon substitutions can enhance intragenic RNA transcription to regulate upstream gene expression.



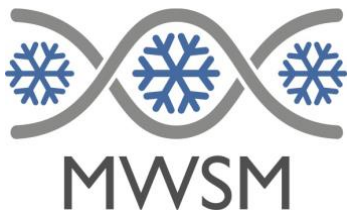
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Chaperone tug of war: stress granules vs. amyloid during heat stress recovery

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Protein aggregation is a cellular phenomenon that can be initiated by the age-related decline of proteostasis networks and stress. In response to environmental stress, proteins and RNA can undergo liquid-liquid phase separation to form transient biomolecular condensates called stress granules (SGs). Following stress, SGs must be faithfully disassembled by molecular chaperones for protected proteins to return to normal function. Importantly, it has been shown that the persistence of proteins within these condensates can foster solidification into irreversible aggregates or amyloid. While it appears that SGs may advance amyloid formation, it is unclear how amyloids influence condensate dynamics. Our lab has established a yeast-based system to study the relationship between SGs and amyloid. Interestingly, we found that the presence of yeast amyloids ([PSI⁺] or [PIN⁺]) or a human amyloid (transthyretin) severely delays heat-induced SG disassembly. Our data suggests that both aggregating species may compete for chaperone availability as chaperones are responsible for disassembling SGs and fragmenting amyloid. Indeed, the SG disassembly delay can be rescued upon Hsp104 or Hsp40 overexpression, suggesting the chaperone system is burdened when both amyloid and SGs are present. However, it is unlikely the SG recovery defects are due to chaperone titration alone. Biochemical analyses show that SG components, chaperones, and amyloid co-sediment, suggesting that the aggregating species may associate with each other. Taken together, these data raise the possibility that amyloid dysregulates SG disassembly not only by sequestering chaperones, but also by incorporating SG components into stable complexes.



Enhancing Protein Homeostasis in Alveolar Type II Cells: Nrf1-Mediated Transcription and ER-Associated Degradation (ERAD) Modulate Stress Response to Surfactant Protein C Aggregates

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Rationale: The dysfunction of epithelial cells has emerged as a central element in the pathophysiology of diffuse parenchymal diseases, notably pulmonary fibrosis (PF). Among these cells, alveolar type II (AT2) cells play a pivotal role in surfactant biosynthesis and the maintenance of alveolar homeostasis. In cases of familial PF, more than 50 surfactant protein C (SP-C) mutations have been identified, with a substantial number occurring within the Bri2 chondromodulin-1 domain (BRICHOS) domain. Previous in vitro studies from our laboratory revealed that BRICHOS SFTPC mutations lead to the production of endoplasmic reticulum (ER) retained and aggregation-prone conformers. This aberrant protein folding activates two crucial components of the proteostasis network—ER-associated Degradation (ERAD) and the Unfolded Protein Response (UPR) comprising of three branches: Protein kinase R-like ER kinase (PERK), the Inositol-Requiring kinase 1 (IRE1), and Activating Transcription Factor 6 (ATF6). While detailed studies have explored the transcriptional programs governing the individual UPR pathways, the manner in which these pathways are transcriptionally integrated to compensate for deficiencies remains unclear. Furthermore, ancillary to the UPR, the ubiquitin-proteasome system (UPS) is a pivotal pathway transcriptionally regulated by resident ER-transmembrane protein Nuclear factor E2-related factor 1 (Nrf1). Using SP-C BRICHOS as a model substrate in both in-vivo and in-vitro murine models, we characterized proteasome homeostasis and UPR responses in AT2.

Methods: Our inducible in vivo model demonstrates expression of a BRICHOS SP-C mutation after two sequential doses of tamoxifen and develops spontaneous lung fibrosis after 3-4 weeks. To study quality control signaling that may underlie epithelial cell dysfunction during the initial injury phase (week 1-2) of the model, AT2s were isolated at Day 7 and Day 14 to assess markers of ER-stress and proteasome upregulation. AT2 cells and single cell suspensions prepared from the lungs of SP-CBRICHOS murine PF models were subjected to population and single cell RNA-seq respectively. In-vitro experiments were performed using stable doxycycline-inducible mouse lung epithelial (MLE-12) cell lines expressing either wildtype SP-C (Dox-SP-CWT) or Dox-SP-CBRICHOS interrogated using cycloheximide chase and / or treatments with activators or inhibitors of ATF6, IRE1a, PERK, ERAD (VCP/p97), or autophagy (Bafilomycin A1). Additionally, stable XBP1 and IRE1 KO variants of Dox-SP-CWT and Dox-SP-CBRICHOS lines were generated to validate small molecule inhibitor studies. Readouts included qPCR, luciferase reporter assays, and immunoblotting to assess UPR signaling and apoptosis, coupled with fluorescence microscopy to localize SP-C expression.

Results: Bioinformatic analysis of AT2 isolated after in-vivo induction of SP-CBRICHOS demonstrated biphasic expression patterns for multiple proteasomal subunits accompanied by NRF1 pathway activation. Western blots of AT2BRICHOS cell lysates confirmed the presence of mutant SP-C aggregates, accumulation of polyubiquitinated substrates, and increased active Nrf1 splicing. Inhibition of ERAD in Dox-SP-CBRICHOS increased ER stress

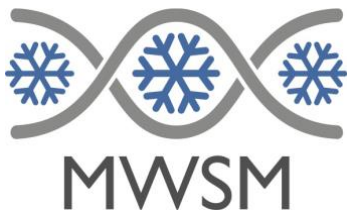


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accompanied by elevations in proteasome subunit gene expression. ATF6 or IRE1a activation during ERAD inhibition accelerated aggregate SP-C degradation. Knockout of XBP1 in Dox-SP-CBRICHOS MLE12 resulted in decreased expression of proteasomal subunits while IRE1a KO increased C/EBP Homologous Protein (CHOP) suggesting a cytoprotective role for IRE1a RNase activity in UPR-active AT2.

Conclusion: Taken together these data demonstrate a primary role for ERAD in the proteostatic response to SP-C aggregates and suggest a potential for ERAD as an AT2 quality control susceptibility hub during IPF. Furthermore, the observation that Nrf1, a known regulator of proteasome transcription, is activated by SP-CBRICHOS suggests Nrf1 may be a novel target for intervention in PF.



Understanding a novel interaction between HSF1 and ERR α in breast cancer chemotherapy

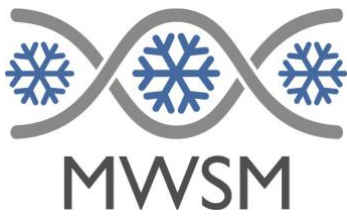
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Breast cancer, affecting 1 in 8 women, is marked by oncogenic mutations causing transcriptional dysregulation fostering cancer cell survival benefits such as treatment resistance. Cancer cells adeptly counteract stress conditions by exploiting stress response pathways. ERR α activity has been shown to be associated with poor outcomes in breast cancer patients. Moreover, in endometrial and esophageal cancer, ERR α confers resistant phenotypes by modulating mitochondrial homeostasis. In oncogenesis, HSF1, a key regulator of stress response, is implicated in promoting cell proliferation and invasion. Interestingly, HSF1 has also been known to modulate cellular metabolism to lead to chemoresistance in pancreatic cancer. Despite these insights, any interplay between ERR α and HSF1 remains unexplored. Analyses of ChIP Seq from three cancer cell lines revealed a substantial amount of overlapping binding peaks and shared target genes between HSF1 and ERR α . Gene ontology enrichment revealed these shared target genes are enriched for pathways frequently dysregulated in cancer, such as cell junction assembly, cell proliferation and cell signaling pathways. Co-Immunoprecipitation of either HSF1 or ERR α results in detection of the other indicating a possible transcriptional complex with HSF1 and ERR α . We also observed that inhibition of ERR α inhibitor (XCT790) led to reduction of ERR α and HSF1 protein levels while inhibition of HSF1 (DTHIB) caused a reduction in both HSF1 and ERR α protein levels, further suggesting some biological interplay between these proteins. Gemcitabine is an FDA-approved chemotherapeutic drug used in breast cancer patients. Treatment with Gemcitabine increases Reactive Oxygen Species (ROS) generation and induces metabolic reprogramming via changes in mitochondrial metabolism. Interestingly, computational analyses using expression data for HSF1 and ERR α indicate a high positive correlation between IC50 for Gemcitabine and HSF1-ERR α expression in breast cancer cell lines, suggesting a role of HSF1 and ERR α in influencing response to this chemotherapy. Future studies will aim to understand the role of this interaction on oncogenesis and progression of cancer and probable therapeutic strategies exploiting this interaction, leading to improved chemotherapeutic response in patients.



Cryo-EM Structure and Molecular Dynamic Simulations Provide Explanations for the Enhanced Structural Stability and ATP Activity of the Pathologically Associated Human Mitochondrial Chaperonin Mutant

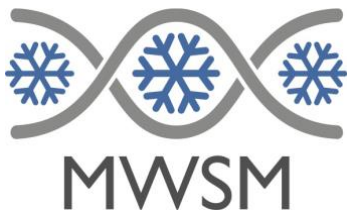
Aiza Syed¹, Jihang Zhai², Baolin Guo², Yuan Zhao², Joseph Che-Yen Wang³, and Chen, Lingling¹

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Chaperonins Hsp60s are conserved among three domains of life and are required for cellular vitality. Chaperonins assist folding of cellular proteins using an ATP-dependent mechanism. The well investigated molecular mechanism of E coli chaperonin GroEL has been generally applied to other chaperonins. However, human mitochondrial chaperonin mHsp60 exhibits molecular characteristics distinct from GroEL, suggesting a different mechanism. A notable characteristic of mHsp60 is its dynamic subunit association, shown by the rapid dissociation from the native heptamer to lower oligomers, and another is its reduced ATPase activity in comparison to GroEL. We previously found that the pathological mutant mHsp60V72I exhibits enhanced subunit association stability and ATPase activity, however, V72 is not located in the inter-subunit interface or the ATP-binding site. To provide structural explanations for the V72I allostery, here we determined a cryo-EM structure of mHsp60V72I. Our structural analysis combined with molecular dynamic simulations showed that compared to wildtype the mutant was associated with the larger inter-subunit interface, more binding free energy, higher dissociation force, and smaller radius of gyration, all contributing to the mutant's enhanced subunit association stability. We also showed that the gate to the nucleotide-binding (NB) site in mHsp60V72I mimicked the open conformation in the nucleotide-bound state and that an open channel leading to the NB site was found in mHsp60V72I, both promoting the mutant's ATPase activity. Our studies not only highlight the importance of mHsp60's distinct characteristics in its biological function but also suggest potential molecular pathways for the evolution of chaperonins at the molecular level.



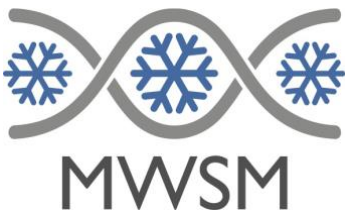
29th Annual Midwest Stress Response and Molecular Chaperone Conference

Understanding the Role of Chaperone Phosphorylation in the Activation of the Oncogenic Src Kinase

Choi, Ashley, Mitchem, Megan, Truman, Andrew

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"The v-Src kinase is encoded by Rous Sarcoma Virus (RSV) and causes cancer by promoting uncontrolled cell division. Previous studies determined that the Hsp70 and Hsp90 molecular chaperones are key for the folding and activity of v-Src. Although substantial research has gone into identifying inhibitors of chaperones to treat cancer, these have failed in clinical trials due to patient toxicity. Our lab has been a central proponent of the idea of the "Chaperone Code and establishing that chaperone activity can be fine-tuned through post-translational modifications such as phosphorylation. In this study, we set out to determine if phosphorylation of Hsp70 is important for v-Src activity. To achieve this goal, we use the model organism budding yeast, in which expression of v-Src protein is toxic and results in cell death. We inducibly express v-Src in yeast containing mutations in 73 Hsp70 phosphorylation sites to either non-phosphorylatable or constitutively phosphorylated forms (146 mutants total). In contrast to wild-type yeast, we identified 9 phosphorylation site mutants in Hsp70 that allowed yeast to grow in the presence of v-Src. We are currently mapping these mutations to the structure of Hsp70 to determine regions of the chaperone responsible for v-Src activity. Going forward, we will determine the molecular cause for immunity to v-Src toxicity in both yeast and mammalian cells. Although still in the early stages, our data suggest that manipulation of chaperone phosphorylation may provide a novel anticancer strategy.



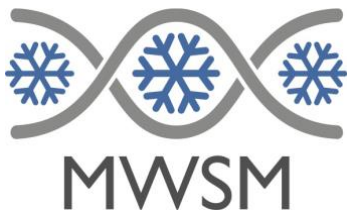
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ESCRTing Misfolded Proteins within the Nucleus

Craig, Sara and Sontag, Emily Mitchell

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Cells encounter stress in their environments that they must overcome to survive. Stress can cause protein misfolding, leading to disruption of most cellular functions. To respond, the cell has a network of proteins to act upon stress-induced protein misfolding. These pathways make up the proteostasis network and act to refold, sequester, or degrade misfolded proteins so that the cell can continue to function. The nucleus creates the intranuclear quality control compartment (INQ) to sequester misfolded proteins within the organelle. Our previous work showed that the INQ is cleared in an ESCRT-dependent manner through budding into the vacuole. Typically, E3 ligase Rsp5 ubiquitinates misfolded proteins and they are shuttled to the proteasome by ubiquitin protein Dsk2. ESCRT-I protein Vps23 has been shown to block polyubiquitination by Rsp5, leading to the possibility that Vps23 is intertwined with UPS clearance. Vps23 is also a component of the ESCRT-dependent clearance pathway for the INQ. We hypothesize that Vps23 is targeting ubiquitinated cargo to vacuole, acting as an alternative pathway for Rsp5 substrates. Deletion of either Dsk2 or Vps23 leads to an accumulation of NLS-LucITs in the cytoplasm, like what was seen in cells unable to perform ESCRT-mediated clearance of the INQ.



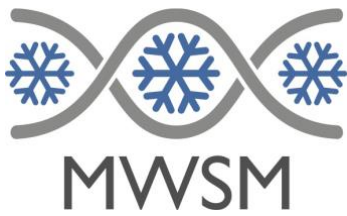
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Detecting Contributions of Translational Efficiency and Degradation on Protein Homeostasis

Diehl, Jacob, Clark, Patricia L.

Department of Chemistry and Biochemistry University of Notre Dame, South Bend, IN

Statistically, amino acid mutations are more likely to destabilize rather than stabilize a protein structure. Likewise, many mutations lead to adverse effects on protein folding pathways, increasing the propensity that a protein will misfold and aggregate, rather than fold to its native structure. In the cell, impaired stability and/or folding can increase the likelihood that a protein will be degraded by cellular proteases. However, currently it is difficult to dissect the impact of mutations on protein degradation from other mechanisms that regulate steady-state protein levels in the cell, including transcription level and translational efficiency. We are developing a fluorescent reporter system to measure the impact of synonymous mutations and amino acid substitutions on transcription level and translational efficiency and distinguish these effects from effects on folding efficiency. The abundance of the fluorescent reporter protein (measured by fluorescence emission spectroscopy) is compared to the abundance of a protein of interest and its mutants (measured by western blotting). To develop this approach, we selected Staphylococcal nuclease (SNase) as the protein of interest, taking advantage of the large number of SNase mutations that have been characterized for their effects on native structure stability. If successful, this reporter system will enable us to develop a more comprehensive understanding of the effects of mutations on all fundamental mechanisms that regulate protein homeostasis.

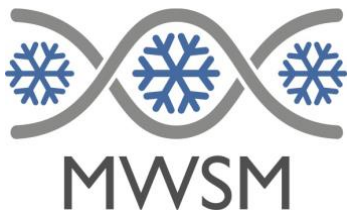


Fluorescence and Single-Particle Cryo-Em Analysis of Ribosome/Nascent-Globin Complexes: Protein-Protein Interactions and Pharmaceutical Implications

Guan, Hongqing, Masse, Meranda M., Hutchinson, Rachel, Cavagnero, Silvia

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Ribosomes facilitate cotranslational protein folding by confining nascent chain motions and by allowing interactions between ribosome-bound nascent chains (RNCs) and the ribosomal surface close to the exit tunnel. Once a protein is released from the ribosome, it is kinetically channeled and remains trapped in either the native or aggregated states. The major deleterious consequences of protein aggregation include neurodegenerative diseases in humans, as well sub-optimal production of protein-based therapeutics in bacteria. Developing a better understanding of ribosome-RNC interactions will enable the rationally refinement of strategies to guide cotranslational protein folding towards the desired native state. Previous studies identified interactions between RNCs of an intrinsically disordered protein and L23 and L29 ribosomal proteins (r-protein), but no information has been available about the nature of the interactions between RNCs and the outer ribosomal surface. In this study, we address the structure of apomyoglobin-RNC by fluorescence anisotropy decays and single-particle cryo-EM. Hydrophobic interactions between RNCs and non-polar regions of the L23 r-protein are observed on the outer ribosomal surface, suggesting that the ribosome exhibits a chaperone-like behavior. Frequency-modulated fluorescence anisotropy decays identified a compact N-terminal RNC region comprising ca. 60-80 residues having a rotational correlation time characteristic of a folded domain. Further, L23 also harbors a docking site for the trigger factor (TF) chaperone. No prior research has established whether L23 interactions with nascent chains and/or TF are mutually exclusive or how these interactions facilitate cotranslational protein folding. We are in the process of addressing the above questions by investigating structure of the RNC-TF complex by fluorescence anisotropy decays and single-particle cryo-EM. The results of our studies will provide invaluable information on RNC structure, dynamics and folding kinetics. In addition, they will reveal specific avenues to reprogram cotranslational protein folding and promote more widespread solubility of newly synthesized proteins.

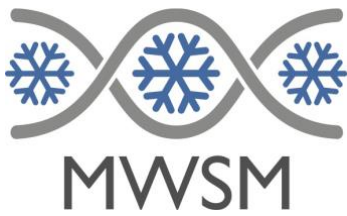


Analysis of stress-induced gene regulation by stress granules

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Center for RNA Biomedicine, University of Michigan, Ann Arbor, MI

The mechanisms of how cells adapt to stress are poorly understood, yet have major implications for human health. Many stressors activate the integrated stress response causing translation suppression, stress granule (SG) formation, and stress-induced gene expression. SGs are condensates of proteins and non-translating RNAs. Stress-induced gene mRNAs evade translational suppression during stress. A gap in knowledge is what role (if any) SGs play in stress-induced gene regulation and cell survival. We hypothesized that stress-induced gene mRNAs evade SGs to permit their translation during stress. We used single molecule fluorescence in situ hybridization of endogenous stress-induced gene mRNAs to measure mRNA abundance and localization. A subset of stress-induced gene mRNAs increased in abundance in U-2 OS cells, supporting the idea that these mRNAs have different modes of transcriptional and translational regulation. Next, we found the degree of stress-induced gene mRNA localization to SGs was variable across genes, with most being largely excluded, while JUN and EPRS1 were enriched in SGs. This suggests SGs negatively regulate translation of a subset of mRNAs. Using translation inhibitors that release or prevent mRNAs from being in ribosomes only increased the localization of ATF4 and GADD34 mRNA to SGs. Conversely, trapping mRNAs on mono- or polysomes decreased SG localization of all mRNAs, suggesting that translation of mRNAs prevents SG localization. These findings give valuable insight into the kinetics of stress-induced gene translation during stress. Finally, we found SG-deficient U-2 OS cells had reduced viability upon arsenite stress compared to wild type cells, suggesting the hypothesis that stress-induced gene regulation by SGs could contribute to cellular resilience to stress. Together, these findings highlight unique mechanisms of stress-induced gene regulation and suggest that SGs play a crucial role in this regulation to promote cell viability.



Protein aggregation promotes HSF1 activity enhancing cell survival during metastatic breast cancer colonization

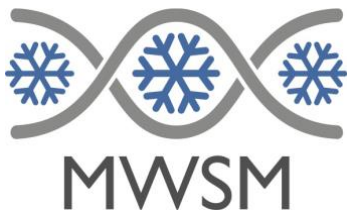
Hockaden, Nataha¹, Leriger, Gabi¹, Wang, John², Ray, Haimanti³, Carpenter, Dr. Richard¹

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Breast cancer is the most commonly diagnosed cancer in women and is the second leading cause of cancer-related deaths in women. Approximately 20-30% of patients will develop metastases which are responsible for greater than 90% of breast cancer deaths. Metastasis is a complex process in which the cells combat many forces to survive and colonize to different areas of the body. Colonization is the rate-limiting step of metastasis because most cells die and only a small fraction of those that survive can form metastases. Utilizing an HSF1 activity signature, we found that patients with high HSF1 activity have significantly worse metastasis-free survival. The physiological function of HSF1 is the master regulator of the heat shock response wherein it upregulates chaperone proteins under stress conditions. Because of these functions and the fact that the process of metastatic colonization is known to involve the stem cell population and incur external stressors, we hypothesized that HSF1 may function in metastatic colonization. In vivo studies showed that mice receiving cells intracardiacally with knockdown of HSF1 had a significantly reduced metastatic burden, indicating HSF1 is necessary for the completion of metastasis and colonization. Consistent with these findings, bone metastatic tumor specimens from patients show increased HSF1 activation compared to their matched primary breast tumors. We observed increased protein amyloid aggregates that correlate with an increase in HSF1 activity during mammosphere formation, suggesting that colonization induces aggregation leading to HSF1 activation that promotes a cell survival response. Overall, my results indicate that HSF1 activity increased during breast cancer metastasis and HSF1 is necessary for colonization. Going forward, I want to understand what controls HSF1 activation during metastasis, which I hypothesize that protein aggregation is increased leading to HSF1 activation.



Targeting aging and neurodegenerative diseases by neuromodulators

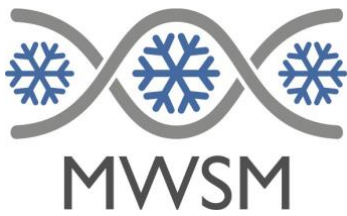
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Aging is the primary risk factor for multiple neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). It has been shown that the same genetic or pharmacological interventions that extend lifespan are also protective against neurodegenerative diseases across species. However, the common neurons and signaling pathways shared by aging and neurodegenerative diseases are not known. Since age-related decline of proteostasis is a common cause of AD, PD, and HD, and at the same time a major target of multiple longevity pathways including mitochondrial UPR, ER UPR and the heat shock response, it is likely that aging and neurodegenerative diseases share common mechanisms that can be targeted by drugs benefiting both models.

Here we utilize a longevity gene surrogate reporter to examine neuromodulator drugs that extend lifespan and improve neurodegenerative disease models in *C. elegans*. *C. elegans* is a highly genetic tractable and optical transparent model with a short lifespan of around 30 days. AD, PD, and HD *C. elegans* models have severe motility defects and short lifespans (~10 days) and are great models for fast screening of drugs. We have identified 7 drugs that induce the longevity gene, and 3 drugs that improve motility and extend lifespan of *C. elegans*. We will utilize these drug hits as tools to interrogate the common genes and mechanisms responsible for the health benefits in delaying aging and improving neurodegenerative diseases.



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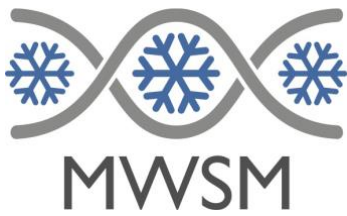
Directed-Evolution Approach to Nascent-Protein Folding and Aggregation Based on Tuning the Size of the Ribosomal Exit Tunnel

Merrick, Sofia K.* , [Jang, Jinoh*](#) , Cavagnero, Silvia

University of Wisconsin, Madison, Madison, WI

* Equal contributors

Optimization of protein folding, and elimination of protein aggregation are important for large-scale production of protein-based pharmaceuticals including interferon and insulin. In addition, prevention of protein aggregation is a key step in the cure of misfolding-related diseases including deadly neuro- and cardio-myopathies. Production of protein-based pharmaceuticals by recombinant-DNA technologies is more convenient and affordable in prokaryotic host organisms (e.g., *E. coli*), rather than in eukaryotes (e.g., baculovirus, Chinese hamster ovary cells). Yet, prior research shows that prokaryotic *in-vivo* protein overexpression often suffers from the formation of aggregates known as inclusion bodies. While it is presently unknown why bacterial cells are more likely to yield insoluble overexpressed proteins, the extremely fast release of fully synthesized nascent chains from the ribosome is a likely culprit. We hypothesize that fast ejection from the ribosomal tunnel in *E. coli* hampers the ability of newly synthesized proteins to attain their native fold before aggregation takes over. In this work, we take advantage of the selective pressure imposed by macrolide antibiotics, which enables the generation of bacterial strains bearing mutated ribosomal proteins (r-proteins). We hypothesize that these mutations affect the structure of the ribosomal exit tunnel, given that they are typically located across the L4 and L22 r-proteins. We tested the ability of the mutant strains to increase soluble-protein formation upon release from the ribosome in bacteria. The mutant strains show slower growth rate and variable gene-product solubility. Colony PCR followed by genome sequencing serves to confirm the identity and nature of the mutations. Overall, this study targets the improvement of protein-folding quality upon release from the ribosome for biotechnology and pharmaceutical purposes. In the future, we plan to extend our strategy to the large-scale production of a variety of other targets, including antibodies and other proteins of biomedical and pharmaceutical relevance.



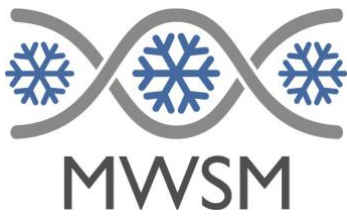
Untangling Two Mechano-Sensing Pathways with Hypermatrices

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Epithelial cells counteract cyclic, uni-axial stress by reorienting their nuclei orthogonal to the direction of stress. This reorientation is accomplished via two distinct pathways. There is a rapid nuclear response that is H3K9me3-mediated and calcium-dependent. There is also a slower-acting tissue response that is mediated by cell-cell contacts. We present a non-negative tensor model that can differentiate between these two distinct pathways. Analyzing multi-omic data using tensor methods offers several advantages over matrix-based approaches. Multi-omic data encompasses many biological data types, including methylation, histone modifications, and transcriptomics, each adding an additional dimension to the dataset. When such data is treated as a collection of matrices, however, critical information is lost. This is analogous to the fact that the eigenvalues of a matrix are lost when the entries of the matrix are reorganized as a vector. Here, we employ a non-negative tensor model for multi-omics to untangle two mechano-protective responses that epithelial cells use to counteract cyclic, uni-axial stress. Our non-negative tensor model supports the hypothesis that the tissue-level pathway inhibits the nuclear pathway and identifies differentially expressed genes associated with each pathway.



Developing a *D. melanogaster* model of hypomorphic PERK to investigate its role in neurodegeneration

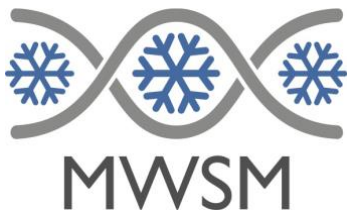
Keren, Ido, Perhacs, Julia, Gidalevitz, Tali

Drexel University, Philadelphia, PA

Tauopathies such as Alzheimer's disease and progressive supranuclear palsy are debilitating neurodegenerative diseases. The endoplasmic reticulum stress sensor PERK has been identified as a genetic risk factor for tauopathy in humans, but the mechanism by which it modifies pathology is not fully understood. Different tau models have suggested that both increased and decreased PERK activity may contribute to pathology. However, human risk factor PERK variants were shown to be hypomorphs.

To investigate whether decreased PERK activity causes or exacerbates tau-related neurodegeneration, we are developing a model for hypomorphic PERK in *Drosophila melanogaster*. The genetic tractability of fruit flies and the complexity of their central nervous systems provide a variety of scorable phenotypes ideal for this study. We are characterizing the degree of decreased PERK activity in two hypomorph fly lines via molecular and phenotypic readouts. In addition to confirming increased mortality under ER stress, we observe that these flies are inherently deficient in their ability to pupate and eclose. This suggests a developmental impairment dependent on PERK.

We will use PERK hypomorph lines to test if decreased PERK levels alone are detrimental to neuronal function or development, or if a genetic predisposition to neurodegeneration is necessary for PERK-dependent tauopathy risk. Furthermore, we will test whether that risk is contingent upon ER stress or a separate functional pathway of PERK. Understanding this mechanism will provide insight for therapeutic strategies against tauopathy, resolving whether they should aim to enhance or inhibit PERK activity in carriers of risk alleles.

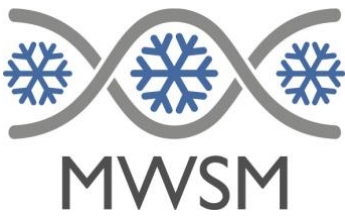


Delineating the cellular proteostasis network using a comprehensive chemical-genetic approach

Klein, Austin, Smith, Roger, Wong, Josiah, Mubarak, Hannah, Amici, David, Mendillo, Marc

Department of Biochemistry and Molecular Genetics, Northwestern University, Chicago, IL

The proteostasis network (PN) comprises molecular chaperones, co-chaperones, and a variety of additional support proteins that are integral for the proper synthesis, folding, stability and degradation of the cellular proteome. Dysfunction of the PN has been implicated in numerous diseases ranging from cancer to Alzheimer's to Parkinson's. Thus, understanding the regulation and function of the PN is critically important in understanding its role in health and disease, and in improving therapeutic strategies. We have recently developed a computational pipeline to analyze genome scale fitness screening data from a large set of cancer cell lines to identify a network of genes with vital functions spanning diverse stress contexts. While this network contained both known and novel factors with critical roles in proteostasis regulation, it remains incomplete. Here, we pursue an integrated approach combining our mammalian chemical-genetic screening platform with our computational pipeline to identify components, function, and regulatory nodes within the PN. We assembled a comprehensive library of sgRNAs that target 1,500 PN genes including canonical chaperones and co-chaperones. Immortalized mammalian cells were infected with our sgRNA PN library and individually treated with a diverse collection of compounds that broadly modulate the proteostasis milieu to identify genes critical for growth in these stressed cells. Our data reveal canonical functions of well-studied components within the PN along with functions of those whose activity were previously unknown. In addition, we define functional relationships between chaperones and their regulators, along with implications for disease-relevant contexts in which their function becomes critical to cell fitness.



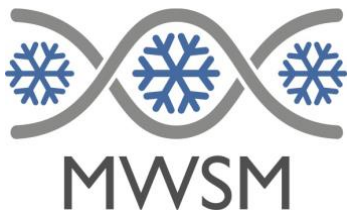
29th Annual Midwest Stress Response and Molecular Chaperone Conference

ERAD co-chaperone Sgt2 alters spatial sequestration of mutant huntingtin

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Huntington's disease (HD) is an inherited fatal neurodegenerative disease that degenerates the basal ganglia impairing voluntary movement and cognitive function. A mutation in the huntingtin gene causes the formation of protein deposits of mutant huntingtin (mHTT) in the striatum and cortex of the brain. There is evidence of a breakdown in protein quality control (PQC) mechanisms, but how HD causes a breakdown in proteostasis remains unclear. One mechanism of PQC, called spatial sequestration, sequesters misfolded proteins into spatially distinct membrane-less compartments. Chaperones are well known for sorting misfolded proteins, soluble oligomers, and insoluble aggregates into these compartments. However, the role of co-chaperones in spatial sequestration remains unclear. Using a yeast model, we are investigating the role of co-chaperone Sgt2 in spatial sequestration. Sgt2 has been identified as a key player in a variety of PQC mechanisms for its ability to bind hydrophobic substrates in the cytosol and for its ability to interact with subunits of the 26S proteasome and heat shock proteins through its tetratricopeptide repeat (TPR) domain. We have found deletion of Sgt2 in our mHTT yeast system exacerbates toxicity and forms small distinct inclusions compared to wild type cells which are more prone to form larger coalesced inclusions. We hypothesize Sgt2 is required for the coalescence of Q-bodies into a singular, spatially distinct compartment. Experiments are underway to test this hypothesis.



Single-Molecule Study of Disaggregation Kinetics of Biomolecular Condensates by Heat Shock Proteins

Lin, Diangen¹, Lin, Kyle M.², Drummond, D. Allan³, Squires, Allison H.⁴

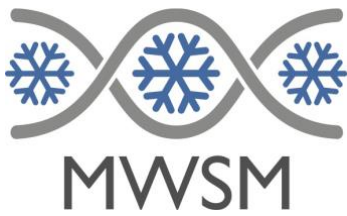
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Heat shock and other cellular stresses cause the formation of protein aggregates and the production of heat shock proteins (Hsps), several of which are molecular chaperones that help restore cellular homeostasis by dispersing stress-induced aggregates. Such aggregates are frequently assumed to be the products of toxic protein misfolding. However, mounting evidence shows that endogenous heat-induced protein aggregates form by adaptive processes of biomolecular condensation, such as liquid-liquid phase separation, rather than misfolding. One such protein, poly(A)-binding protein (Pab1) from yeast, forms condensates that are much more efficient substrates for yeast Hsps than misfolded protein aggregates. This discovery has reopened fundamental questions about Hsp-driven disaggregation: How are non-misfolded, endogenous biomolecular condensates dispersed by Hsps? Quantitative molecular-scale information on the kinetics of condensate disaggregation remains lacking. Here, we employ single-molecule total internal reflection fluorescence microscopy (TIRFM) to quantify the rate of disaggregation of Pab1 condensates. We directly image Pab1 condensates in the presence of Hsps shown to be necessary and sufficient for rapid and complete condensate dispersal. We measure the condensates' change in fluorescence intensity at discrete intervals over time. To determine the effect of condensate size on the rate of disaggregation, we further classify individual Pab1 condensates based on their initial sizes, estimated by their initial fluorescence intensities and extrapolated intensities of single Pab1 molecules. Our work using a single-molecule imaging approach will illuminate how condensates of varying sizes may be disaggregated differently, information normally obscured by bulk biochemical measurements of condensate disaggregation. Furthermore, characterization of the kinetics of condensate disaggregation will inform future investigations of Hsp binding and stoichiometries and contribute to fundamental understanding of molecular-scale interactions between heat shock proteins and biomolecular condensates.



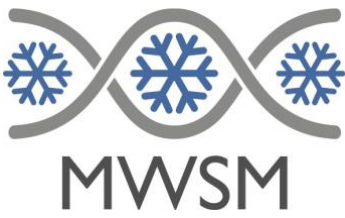
Single-molecule imaging to reveal how chaperones engage native condensates

Lin, Kyle M.¹, Lin, Diangen¹, Yoo, Haneul^{1,2}, Drummond, D. Allan¹, Squires, Allison H.¹

¹University of Chicago, Chicago, IL

²Max Planck Institute of Epigenetics, Freiburg, Germany

Heat shock and other cellular stresses trigger protein aggregation, which is resolved by the action of stress-induced molecular chaperones. These aggregates are often presumed to be the result of unregulated protein misfolding. However, emerging work shows that many stress-induced protein aggregates form by dynamic, non-misfolding processes of biomolecular condensation. Our group recently showed that one such biomolecular condensate, heat induced aggregates of poly-A binding protein (Pab1) from yeast, is a far more efficient substrate for yeast molecular chaperones than misfolded protein aggregates. Here, we measured the molecular-scale dynamics of chaperone recruitment to Pab1 biomolecular condensates to learn how these dynamics may differ between condensates and misfolded aggregates. A single-molecule imaging approach provides the most direct access to visualizing how protein condensates recruit and engage chaperones. We developed a custom two-color total internal reflection fluorescence microscopy (TIRFM) platform to track interactions between purified, fluorescently labeled chaperones and labeled Pab1 condensates in vitro. Using a fluorescence anisotropy-based assay to measure disaggregation, we show that fluorescently labeled chaperones and fluorescently labeled Pab1 condensates are both disaggregation-competent in vitro. Using our two-color imaging platform, we characterize the single-particle brightnesses of chaperones and Pab1 condensates, and we observe the colocalization of Pab1 condensates with Hsp40, a class of chaperone required for Pab1 condensate dispersal. Building on these results, we will compare the recruitment of different classes of chaperones (Hsp40s) to Pab1 condensates and misfolded protein aggregates as model substrates, in order to reveal the molecular-scale dynamics of chaperone-condensate interactions.



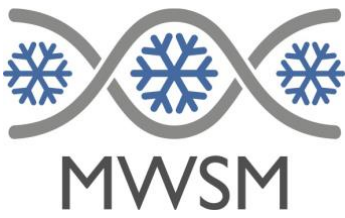
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Impact of insulin signaling on neurotoxicity in *c. elegans* expressing *tdp-43*, an ALS associated protein

Lotesto, Vincent, Voisine, Cindy

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Amyotrophic Lateral Sclerosis (ALS) is an age-related neurodegenerative disease affecting motor neurons. A pathological feature shared among neurodegenerative diseases is the aggregation of proteins such as the ALS associated Tar DNA-binding protein 43 (TDP-43). Proteotoxic stress induced by TDP-43 aggregates leads to inappropriate signaling at the neuromuscular junction. This causes muscular atrophy and the progressive loss of voluntary muscle movement. Growing evidence suggests that these aggregates also influence sensory function. For example, many ALS patients have reduced ability to identify common odorants. Manipulation of signaling pathways that regulate cellular stress responses may offer therapeutic interventions for these motor and sensory pathologies. Mutations that alter insulin-like signaling (ILS) are known to increase cellular stress resistance. We are using the nematode *Caenorhabditis elegans* to determine the impact of the ILS pathway on ameliorating the neurotoxicity of TDP-43. Using well-characterized behavioral assays, we have shown that transgenic *C. elegans* expressing TDP-43 pan-neuronally exhibit reduced motor and chemosensory neuron functionality. Our lab sought to determine whether mutations in the insulin-like receptor, *daf-2*, would restore neuron function in TDP-43 expressing animals. Our data show *C. elegans* expressing TDP-43 in the *daf-2* mutant background exhibit a significant increase in motor neuron activity. We are currently exploring whether the *daf-2* mutation also restores the ability to detect volatile odorants. We hypothesize that *C. elegans* expressing TDP-43 in the *daf-2* mutant background will exhibit recovery of chemosensation. Understanding ways to address proteotoxicity and improve neuronal functionality may assist in improving the prognosis of individuals afflicted with ALS.



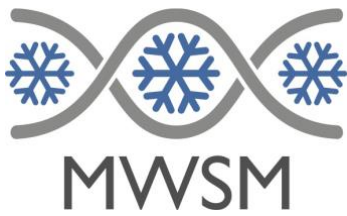
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Hsp90 mediates an alternative pathway for tail-anchored protein membrane insertion

Mankovich, Anna, Kolhe, Janhavi, Freeman, Brian

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The biogenesis of Tail Anchored (TA) membrane proteins requires collaboration between molecular chaperones, TA transporters, and TA translocases in the Guided Entry of Tail-anchored protein (GET) pathway. Many TAs perform essential cellular functions yet, intriguingly, the individual components of the GET pathway are not required for cell viability. Hence, cells must have an alternative mechanism to the GET pathway to ensure essential TAs are inserted into their membranes. Through a mass spectrometry based cross-linking screen that has expanded the Heat shock protein 90 (Hsp90) interactome, we have identified that Hsp90 interacts with 34 different TAs, the cochaperone Sgt2, and the TA translocase of the GET pathway. Using fluorescently tagged TAs and Hsp104 in yeast, we have demonstrated that TAs begin to aggregate when both Hsp90 is inactivated and the TA transporter, Get3, is knocked out which, is indicative of disrupted TA insertion. This disruption is further evident in pulse-chase experiments where a decrease in TA insertion into the ER membrane is observed following inhibition of Hsp90. These results suggest that Hsp90 has a role in TA membrane insertion by driving an alternative route to the GET pathway. This defines a novel function for Hsp90 in TA biogenesis and further develops the role of Hsp90 in maintaining cellular proteostasis under non-stress conditions.



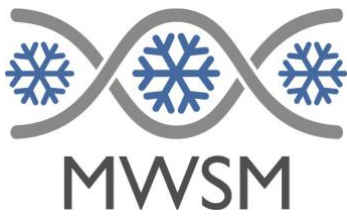
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Monitoring Transthyretin Protein Aggregation using yTRAP

Martin, Sean, Radtke, Claire, Knier, Adam, Kuborn, Abigail, Dorweiler, Jane, Manogaran, Anita

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Transthyretin amyloidosis (ATTR) is a fatal disease associated with aggregation of the transthyretin (TTR) protein. Clinical and mouse model research show that there is a strong link between TTR aggregation and molecular chaperone response. For example, knock out of HSF1 in transgenic mouse models is associated with increased accumulation of intracellular TTR aggregates. However, the relationship between specific chaperones and TTR aggregation is poorly defined. To begin to understand this relationship, our lab has developed a novel yeast-based system to rapidly detect TTR aggregation as a tool to test the relationship between TTR aggregation and chaperones. We modified a previously established high-throughput system developed for the Sup35 prion, called the Yeast Transcriptional Reporter of Aggregating Proteins (yTRAP). This system relies on TTR fused to a synthetic transcriptional activator (TTR-synTA), which can bind to a transcription activation site upstream of a mNeonGreen reporter. As expected, when TTR is not aggregated, mNeonGreen levels are high. However, when aggregation is driven by high overexpression of a TTR-TAP tag fusion protein, the TTR-synTA is cross-seeded into the aggregate resulting in lower expression of the reporter gene. Therefore, this system creates an indirect, inverse relationship between TTR aggregation and mNeonGreen fluorescence. Our previous work has shown that altering levels of Hsp104 can influence the size of TTR aggregates. We will present work that shows how TTR yTRAP readouts relate to biochemical analysis. This work will provide validation of the system and allow us to explore how other molecular chaperones act on TTR aggregation.

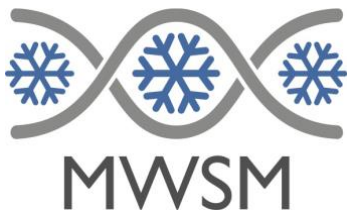


**Proteostasis collapse and disruption of Integrated Stress Response pathway in aging
and neurodegenerative disorders**

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Proteasome function decreases with age as measured by proteasome activity and accumulation of ubiquitinated proteins in human tissues and many model organisms. Proteasome dysfunction is associated with ubiquitin-containing inclusion bodies in neurodegenerative diseases. This project aims to understand the molecular mechanisms of proteostasis collapse, a hallmark of aging and neurodegeneration. Proteostasis is the coordinated synthesis, processing, and degradation of proteins. The Ubiquitin Proteasome System (UPS) is the primary protein degradation pathway. Proteasome collapse is associated with activating the Integrated Stress Response (ISR) signaling pathway. During the ISR, exposure to stressful conditions leads to mRNA translation inhibition, and coalescing of mRNAs and RNA-binding proteins into biomolecular condensates called Stress Granules (SGs). Defects in the ISR pathway and persistent SGs are also associated with aging and neurodegenerative diseases. The correlation between ISR and UPS is apparent, but the process of SG accumulation during proteasome inhibition remains unclear. Our lab will use biochemical assays, fluorescent microscopy, and live cell imaging to determine the effects of proteasome inhibition on translation activity and stress granule accumulation upon stress. By using fluorescent microscopy and western blot we found that proteasome inhibition activates the ISR but does not form SGs. Overloading the UPS with proteasome inhibitors and puromycin, which releases defective ribosomal products from translation, causes SG formation. Preliminary data suggest that p62, a receptor protein that delivers ubiquitinated proteins to autophagosomes for degradation, increases upon proteasome inhibition. These results suggest three possible mechanisms of aberrant SG formation upon catastrophic proteasome inhibition: 1) translation initiation and elongation are inhibited, 2) ubiquitinated proteins seed SGs, or 3) p62 may drive the degradation of ubiquitinated proteins in the absence of the UPS and prevent SG accumulation. Understanding how proteasome overload is connected to ISR dysfunction at the molecular level will help us identify potential targets for aging and neurodegeneration.

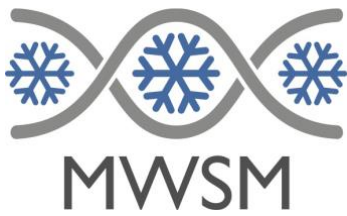


Hsp70 phosphorylation Modulates Ribonucleotide Reductase Subunit abundance in Yeast

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Yeast cells respond to genotoxic stress by activating a well-conserved signaling cascade that comprises of Mec1, Rad53 and Dun1. A major effector of this pathway is transcription and activation of the Ribonucleotide Reductase (RNR) complex which is made up of four subunits, Rnr1, 2, 3 and 4. RNR activity is critical for the synthesis of new DNA bases and is required for both DNA replication and repair. Previous studies from our lab have demonstrated that RNR is client of the molecular chaperone Hsp70 in yeast and mammalian cells. There is growing evidence that phosphorylation of molecular chaperones (the chaperone code) impact chaperone function by altering client binding, ATPase activity and co-chaperone interaction. In this study we queried the impact of Hsp70 phosphorylation on the RNR complex in yeast. We screened 146 yeast strains expressing mutations in Hsp70 phosphorylation sites (73 phospho-mutants and 73 phospho-mimics) for sensitivity of well characterized RNR inhibitor Hydroxyurea (HU) and identified 13 phosphorylation sites important for the cellular resistance to HU. To determine the impact of these sites on the RNR complex, we examined RNR subunit abundance via Western Blotting. Interestingly, the steady state levels of RNR3 and RNR1 were substantially decreased in 80% of the mutants screened. Our current working model is that many of the Hsp70 phosphorylation sites lie on the interaction surface between RNR and Hsp70 and may represent a way to regulate for cells to rapidly alter RNR activity. We hope to test this theory in yeast and mammalian cells using cross-linking mass spectrometry (XL-MS). These studies may provide a novel anticancer therapeutic strategy based on controlling Hsp70 phosphorylation.



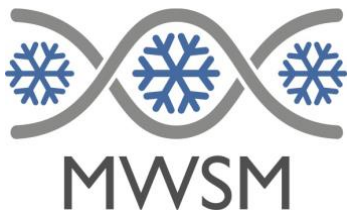
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Homomeric Protein Assembly *In Vivo*

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Proper folding is a prerequisite for protein function. Most proteins are multimeric, meaning that folding includes the additional step of assembling individual subunits together to form the native multimeric structure. Although little is known about multimeric protein folding, failing to form proper subunit interactions can lead to loss of protein function, aggregation and/or degradation. During protein synthesis, individual subunits of homomeric proteins are close to one other on neighboring ribosomes and may start to assemble co-translationally (“co-co” assembly). Alternatively, nascent chains may interact co-translationally with a full-length subunit (“co-post” assembly). Work from the Bukau and Kramer labs indicates that co-co interactions between nascent chains enhance the efficiency of homomeric protein folding and assembly (Bertolini et al. (2021) *Science*). Here we present results from a novel assay designed to test the extent to which co-translational assembly occurs during homomultimeric protein assembly and at which point during translation. As an initial model, we used the *E. coli* homotrimer chloramphenicol acetyltransferase (CAT). The CAT native trimer structure is thermostable to 80°C and shows no evidence of subunit exchange over the lifetime of *E. coli*. CAT does not refold to its native structure after dilution from a chemical denaturant, indicating that the “pioneer round” of CAT folding, potentially including co-translational folding and/or assembly, is particularly important for achieving the native CAT structure. However, we and others have shown that CAT native structure formation requires the presence of the CAT C-terminal residues (Van de Schueren et al. (1996) *JMB*). To resolve this conundrum, we designed a novel fluorescence reporter assay to help identify co-translational assembly mechanisms. Crucially, this assay should prove useful for testing the contribution of co-translational assembly to the folding of any multimeric protein, including heteromeric multimers.



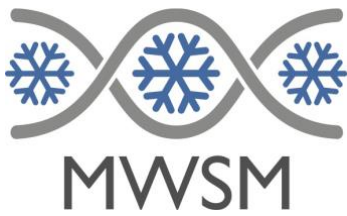
Effects of hydrophobic sequence patterns on disordered protein conformational ensembles

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²University of Chicago, Chicago, IL

Intrinsically disordered proteins (IDPs) and regions (IDRs) are known to play important roles in signaling and other key cellular functions. Although disordered proteins do not adopt a well-defined, folded 3D structure, their conformational ensembles can range from highly expanded to collapsed, molten globule-like ensembles. Although much attention has been paid to IDPs with a high fraction of charged residues, the sequence composition of some IDPs is indistinguishable from well-folded proteins, including a significant fraction of hydrophobic residues. We have previously shown that PNt, the N-terminal segment of pertactin, a *Bordetella pertussis* virulence protein, has a highly expanded conformational ensemble despite having the sequence composition of a well-folded protein. In contrast, the pertactin C-terminal segment (PCt), which has a similar amino acid composition, is stably folded in isolation. Our previous studies demonstrated that PNt has unusually low clustering of its hydrophobic residues. Interestingly, increasing hydrophobic clustering in PNt is sufficient to significantly contract its conformational ensemble. Moreover, we have shown that the sequences of water-soluble proteins have been selected to have low hydrophobic clustering, potentially to minimize self-associations that can lead to phase separation and/or aggregation. To test the impact of hydrophobic clustering on folding, disorder and self-association, we asked whether reducing hydrophobic clustering is sufficient to not only destabilize a folded protein structure but also generate an expanded disordered ensemble. To do this, we constructed a series of PCt “sequence swap” mutants, preserving the sequence composition of wild type PCt but lowering its hydrophobic residue clustering. These swap sequences preserve the positions of all charged residues as in wild type PCt. Using a combination of small-angle X-ray scattering, circular dichroism spectroscopy, and biochemical assays, we show that altering hydrophobic patterning is sufficient to both unfold and expand the conformational ensemble of PCt, past the “danger zone” of self-association that leads to aggregation.



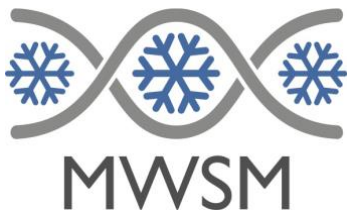
**Investigating Potential Alternative Redox Partners of the Yeast Thioredoxin Reductase
Trr1**

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During oxidative stress, organisms employ a conserved protein disulfide reduction system consisting of thioredoxin and its partner enzyme thioredoxin reductase to mitigate the adverse effects of oxidative protein damage. Despite established partnering between thioredoxin and thioredoxin reductase to protect against oxidative stress, several lines of evidence point to thioredoxin-independent roles for thioredoxin reductase. Using the *Saccharomyces cerevisiae* thioredoxin system as a model, we report that yeast lacking the cytosolic thioredoxin reductase Trr1 exhibit a more pronounced slow-growth phenotype than yeast lacking the corresponding thioredoxins (Trx1 and Trx2). Here, we use a chemical cross-linking approach coupled with mass spectrometry-based proteomics to identify proteins associated with Trr1 in thioredoxin-deficient cells. The results from these studies and subsequent co-immunoprecipitation experiments reveal a new set of thioredoxin reductase interaction partners that may serve as alternative redox partners. We are currently developing a reconstitution system to determine if the oxidized forms of the new Trr1 interaction partners can function as redox partners with Trr1 *in vitro*. Collectively, our results suggest that Trr1 may partner with other proteins in addition to thioredoxins, potentially accounting for the exacerbated phenotypes in Trr1-deficient yeast.



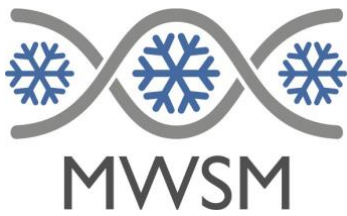
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Impact of extracellular scl gene family in *C. elegans* expressing tdp-43: unraveling neurotoxicity mechanisms

Parikh, Krishna, Voisine, Cindy

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that primarily affects nerve cells responsible for controlling voluntary muscles. Degeneration and eventual death of neurons in the brain and spinal cord, result in muscle weakness, atrophy, and ultimately paralysis. TAR DNA-binding protein 43, TDP-43, is a nuclear protein that plays a role in regulating gene expression and RNA processing. However, in ALS and other neurodegenerative diseases, TDP-43 forms abnormal aggregates in the cytoplasm of affected neurons. We are using the nematode *C. elegans*, to investigate TDP-43 related neurotoxicity. *C. elegans* are transparent worms with a simple nervous system and are amenable to genetic manipulation. A ribosome profiling experiment on young adults that express human TDP-43 pan-neuronally and wild type animals revealed a set of differentially translated genes belonging to the scl gene family. To understand how these genes modulate TDP-43 related neurotoxicity, I will be performing genetic crosses to introduce specific scl gene deletions into TDP-43 transgenic animals. Many members of the scl gene family are not only upregulated more than 50 times in ribosome profiling data sets but specific members are also responsible for longevity and stress resistance. Manipulating scl gene family members might reveal potential therapeutic approaches to treat ALS pathology targeting TDP-43 neurotoxicity.

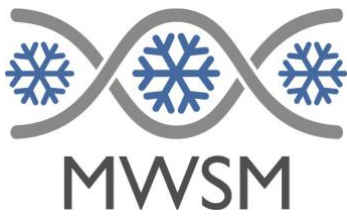


Do Charged Residues in Grp94 Facilitate Client Binding?: Insights into Chaperone Function

Paul, Kylie, Unruh, Erin, Nancy Rotich, Audrey McCamish, Andrea Kravats

Cell, Molecular and Structural Biology Program, Miami University, Oxford, OH

The Hsp90 family of chaperones functions to target misfolded proteins, prevent aggregate formation and bind to specific substrates for proper folding. Paralogs of the Hsp90 family exist in the cytoplasm (Hsp90), in the mitochondria (TRAP1) and in the endoplasmic reticulum (Grp94). The ER paralog Grp94 is of particular interest to this investigation since it is understudied and is implicated in a variety of diseases, including cardiovascular disease, neurodegenerative diseases, and cancer. Grp94 is a homodimer comprised of an N-terminal domain (NTD) that is responsible for nucleotide hydrolysis and peptide binding, a charged linker region responsible for binding calcium, the middle domain (MD) that is involved in client binding, and the C-terminal domain (CTD) that participates in client binding and dimerization. Depending on cellular conditions, Grp94 can act as a holdase to prevent protein aggregation or as a foldase to directly remodel clients. The foldase activity of Grp94 is mediated by the chaperone BiP (ER paralog of Hsp70) that modulates protein refolding. The holdase activity of Grp94 does not result in refolding of the misfolded protein, but rather misfolded proteins are bound by Grp94 and segregated from the cellular environment to avoid further aggregation. Previous research in our lab investigated the prevention of aggregation activity of Grp94. Studies with individual Grp94 domains indicated that model misfolded clients bind at the NTD of Grp94. Previous literature identified the site His146 on the NTD that significantly reduced peptide binding in comparison to wild-type (WT) Grp94. Interestingly, the peptide binding mutant located in the NTD, H146D, resulted in decreased aggregation of two model substrates (luciferase and citrate synthase), possibly due to increased electrostatic interactions between the charged residue and substrate. This was an interesting finding, because client remodeling and peptide binding were thought to be distinct and occur in different regions of Grp94. In this work we investigate how point mutations in the NTD of Grp94 affect substrate binding using fluorescently labeled peptides. We have created various mutants within the general peptide binding region of Grp94. We are assessing peptide binding affinity with WT Grp94 and mutants. Together, this information will provide insight into charged interactions between client and chaperone and enable us to understand how Grp94's NTD functions in binding potentially harmful protein aggregates.



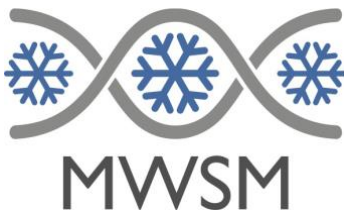
Nuclear Type I Myosins are Involved in Genome Organization and Nuclear Structure

Peng, Audrey, Freeman, Brian

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Nuclear myosins are involved in several nuclear processes including transcription, chromatin remodeling and DNA repair. Although its presence as an associated factor with multiple nuclear complexes has been established, how it is used as a molecular motor in the nucleus, in particular for genome organization, has only recently been probed and a causal relationship is still lacking. Here, we show the importance and role of myosins in the nucleus and how it might relate to DNA organization. Using an Anchor-away strategy to deplete Type I Myosins from the nucleus in budding yeast, we show that Type I Myosins in the nucleus are essential for cell survival and its depletion causes a collapse in nucleus structure. To assess if such a collapse is due to changes in genome organization, we subjected cells depleted of nuclear Type I Myosins to Hi-C and found a decrease in contact frequency globally despite a strengthening of contact domains as well as inter-molecular contacts at telomeres.

Furthermore, there is a striking increase in interaction across the long arm of Chromosome XII, where the rDNA is. Overall, we have discovered novel roles for Type I Myosins in the maintenance of nuclear processes and seek to further elucidate its roles in genome re-organization through each round of cell division.



ER stress sensor PERK functions in localization of neuronal growth factors through CaMKII

Perhacs, Julia¹, Klabonski, Lauren², Bond Newton, Sarah³, Argon, Yair⁴, Gidalevitz, Tali⁵

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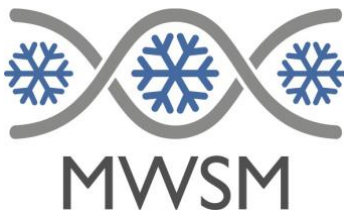
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Neuronal function depends on the precise localization and release of neuromodulators, such as growth factors, in response to stimuli. For example, IGF and TGF- β are released locally in the brain, are neuroprotective, and critical for neuronal development, yet it is unknown how they are sorted and targeted to release sites in neurons. Our lab recently identified an unexpected, but exciting role for ER stress sensor, PERK, in neuronal growth factor localization. PERK has not been identified in the context of maintaining neuronal polarity, however several genetic variants of PERK are linked to the tauopathies Alzheimer's Disease (AD) and Progressive Supranuclear Palsy (PSP). Some of the variants have decreased kinase activity, but it is unknown how decreased PERK activity sensitizes neurons to pathology. Understanding PERK's role in localizing growth factors may provide a molecular link to neurodegeneration, as well as provide insight into the unknown in fundamental neurobiology.

Using our in vivo *C. elegans* model with functional, fluorescently-tagged growth factors, we observed that in the absence of PERK, a dendritic TGF- β -like growth factor is mistargeted to the axons, while an axonal IGF-like protein is retained in the cell body. My preliminary data show that PERK's role is kinase dependent, and because it is unclear how PERK may directly mediate growth factor localization, I asked whether PERK functions through one of its known downstream pathways. Strikingly, I found that CaMKII, a kinase whose function is critical for neural plasticity and memory, is genetically downstream of PERK. Importantly, CaMKII has a known function in axonal targeting of neuropeptides, which has the potential to explain the connection to PERK. Defining how PERK and CaMKII interact to control axonal-dendritic targeting of select neuroprotective growth factors could aid in understanding how known PERK variants sensitize individuals to tauopathy-based pathologies.



Identification of a CHIP/CHMP2B complex disrupted in SCA48 and FTD

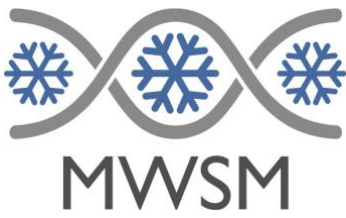
Rodriguez, Ryan¹, Anna Umana¹, Ran Ming¹, Jamie Scaglione¹, Matt Scaglione^{1,2,3}

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C-terminal of HSC-70 interacting protein (CHIP) is an E3 ubiquitin ligase that plays a critical role in maintaining cellular proteostasis. CHIP helps maintain proteostasis by recruiting chaperones via a N-terminal tetratricopeptide repeat (TPR) domain that directly binds a C-terminal VD motif of chaperones. This results in the ubiquitination and degradation of chaperone-bound client proteins. More recently, mutations in CHIP have been found to cause spinocerebellar ataxia type 48 (SCA48). Many of the SCA48 causing mutations map to the TPR domain of CHIP and impair TPR function. To clarify how mutations in CHIP disrupt function, we performed a proximity labeling experiment and found that TPR mutations disrupted the interaction of CHIP with many proteins that terminate in a VD motif. Among these proteins we identified Charged Multivesicular Body Protein 2B (CHMP2B), as a protein that competes with chaperones for binding to the TPR domain of CHIP via a C-terminal VD motif. Interestingly, C-terminal truncation mutations in CHMP2B cause an aberrant endosomal phenotype and frontotemporal dementia (FTD) consistent with a critical role for CHIP in regulating CHMP2B function. Here we have characterized the CHIP/CHMP2B interaction and demonstrated that similar to CHMP2B mutations that cause FTD, SCA48 mutations in CHIP result in defective endosomal processing. Together our data identify a novel biological role for CHIP in endocytosis. Our data also suggest that cognitive defects associated with SCA48 may be caused by a similar mechanism to that of CHMP2B mutations in FTD.



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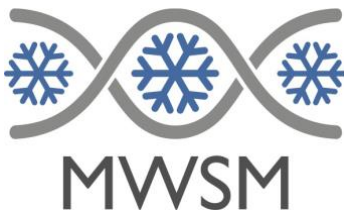
Resource allocation and growth control in complex environments

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²University of Chicago, Chicago, IL

In biology, it is difficult to predict cellular physiology amongst different environments due to the significant heterogeneity of possible responses. Understanding the connection between the environment, gene expression, and their combined effect on physiology, such as growth rate, is integral to understand cells. Finding a general, simple quantitative theory to understand how cellular growth rate is determined is necessary. Here, we studied two prevailing theories for growth control: (1) resource allocation in the context of *E. coli* and (2) the coordination of *S. Cerevisiae* environmental stress response (ESR) with growth. As a case study, we use RNA sequencing and proteomics data from *S. pombe* exposed to different forms of nitrogen. We find that resource allocation and ESR patterns are partially seen in *S. pombe* gene expression, but not in their protein abundances. We determine the similarities and differences between the two theories in attempt to understand what is needed to quantitatively understand growth regulation. In the future, a comprehensive theory will be useful to quantify abnormal cellular growth, such as cancer and aging.



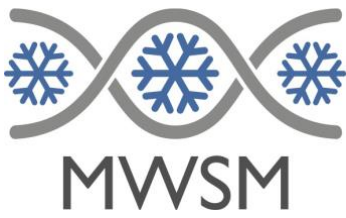
Investigating the Role of ERp29 in rescuing CFTR from degradation in yeast

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Mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene can cause its abnormal trafficking to the plasma membrane or its early degradation, resulting in insufficient chloride transport and mucociliary clearance, a characteristic of Cystic Fibrosis (CF). The most common mutation of CF, F508del is characterized by a misfolded protein that cannot escape the ER and is degraded via ubiquitination and ER-associated degradation (ERAD). We have previously demonstrated that overexpression of ERp29, a novel 29 KDa ER luminal chaperone, increases expression of F508del at the surface of epithelial cells, while depletion of ERp29 causes reduced expression and maturation of WT-CFTR. These data support the hypothesis that ERp29 is a key player in promoting CFTR folding and maturation en route to Golgi and finally the plasma membrane. Interestingly, the yeast *S. cerevisiae* does not express ERp29 or a homolog of ERp29, and both WT and F508del CFTR are retained in the ER and undergo ERAD in this yeast. We therefore tested whether ERp29 could rescue CFTR from degradation in yeast by co-expressing CFTR (WT or F508del) and ERp29 in yeast, with either constitutive or methionine-repressible expression of ERp29. Preliminary results from cycloheximide chase protein degradation studies suggest that WT-CFTR has a half-life of ~60 minutes in yeast, and that co-expression of ERp29 stabilizes the protein and increases this half-life. These data suggest that ERp29 stabilizes CFTR and slows its degradation in *S. cerevisiae*, perhaps via a direct interaction or as part of a larger complex. As ERp29 has a -KEEL ER retention motif, while the KDEL Receptor homolog in *S. cerevisiae* is the HDEL receptor, we also constructed ERp29-HEEL and ERp29-HDEL that we posited would better allow interaction of a CFTR-ERp29 complex with the HDEL receptor and promote the exit of CFTR from the ER via COP II vesicles. These experiments are currently in progress and will further our understanding of the mechanism(s) by which ER luminal chaperones such as ERp29 may promote the biogenesis and trafficking of transmembrane proteins such as CFTR.



Nascent Chains Derived from a Foldable Protein Sequence Interact with Specific Ribosomal Surface Sites near the Exit Tunnel

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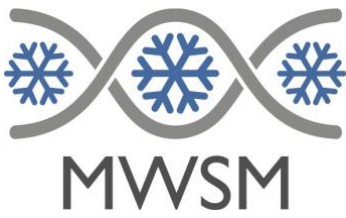
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⁷PPD/Thermo Fisher Scientific, Madison, WI

In order to become bioactive, proteins must be translated and protected from aggregation during biosynthesis. The ribosome and molecular chaperones play a key role in this process. Ribosome-bound nascent chains (RNCs) of intrinsically disordered proteins and RNCs bearing a signal/arrest sequence are known to interact with ribosomal proteins. However, in the case of RNCs bearing foldable protein sequences, no direct information is available on these interactions. Here, via a combination of chemical crosslinking and time-resolved fluorescence-anisotropy, we find that nascent chains of the foldable globin apoHmp1-140 interact with the E. coli ribosomal protein L23 and have a freely-tumbling non-interacting N-terminal compact region comprising 63- 94 residues. Longer RNCs (apoHmp1-189) also interact with an additional yet unidentified ribosomal protein, as well as with the trigger factor molecular chaperone. Surprisingly, the apparent strength of RNC/r-protein interactions does not depend on nascent-chain sequence. Overall, foldable nascent chains establish and expand interactions with selected ribosomal proteins and chaperones, as they get longer. These data are significant because they reveal the interplay between independent conformational sampling and nascent-protein interactions with the ribosomal surface.



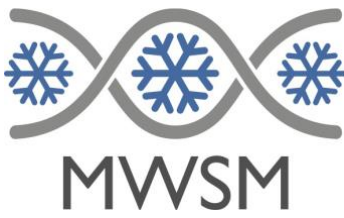
29th Annual Midwest Stress Response and Molecular Chaperone Conference

Oxidation of a Conserved C-Terminal Cysteine in Microbial Thioredoxin Reductases

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The enzyme thioredoxin reductase partners with thioredoxin, a protein disulfide reductase, to regulate various metabolic processes and confer oxidant defense. Microbial thioredoxin reductase employs two active site cysteines to reduce thioredoxin, yet there is also a semiconserved C-terminal cysteine that is found in the enzyme in fungi and Gram-negative bacteria. This site along with the active site cysteine pair is targeted by the thiol-reactive cross-linker divinyl sulfone in the baker's yeast thioredoxin reductase Trr1. To explore whether this C-terminal cysteine has a role in redox chemistry, we conducted coupled-activity assays with Trr1, thioredoxin, and a peroxiredoxin, finding that the active site cysteines, but not the C-terminal cysteine, of Trr1 are required for reduction of thioredoxin. However, treatment of Trr1 with the disulfide donor DTNB or with strong thiol oxidants (e.g., hypochlorite, diamide) suggested that the C-terminal cysteine in Trr1 is oxidizable, undergoing a molecular weight shift on non-reducing SDS-PAGE indicative of intrasubunit disulfide bond formation. We tested whether this site is susceptible to oxidation in thioredoxin reductases from other species, finding that it undergoes molecular weight shifts consistent with oxidation in many cases. Forthcoming experiments include mapping this disulfide in Trr1 by mass spectrometry and determining if this site in Trr1 is oxidizable *in vivo*. Our results to date suggest that the C-terminal cysteine in microbial thioredoxin reductases is subject to oxidation. Given its reactivity, this site could be targeted in future antimicrobial drug design.

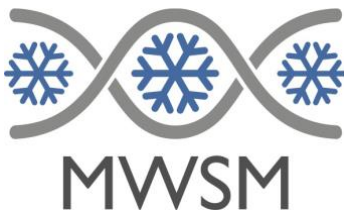


Hippocampal neurons demonstrate resilience to mutant Huntingtin aggregation and toxicity in a new mouse model of Huntington's disease.

Solem, Melissa, Rozema, Nicholas B, Pelzel, Ross, Brown, Taylor, Reid, Emma, Mansky, Rachel, Blomme, Nicolette, Gomez-Pastor, Rocio

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Huntington's disease (HD) is a neurodegenerative disease resulting in devastating motor, cognitive, and psychiatric deficits. HD is caused by a poly-glutamine expansion of exon 1 of the Htt gene resulting in misfolding and aggregation of the mutant HTT protein (mHTT). The striatum, a brain region involved in motor control, is the region most significantly impacted in HD and is the focus of most HD studies. However, despite well-documented deficits in learning and memory in HD, knowledge of the potential implication of the hippocampus remains limited due to the lack of hippocampal pathology observed in various models of HD. We now report enhanced hippocampal mHTT aggregation by modifying the commonly used knock-in HD model zQ175, which expresses a human exon 1 of Htt containing ~188 CAG repeats under the endogenous mouse Htt promoter. We show that after removing a PGK-Neomycin cassette expressed in zQ175 mice using the Cre recombinase system, the newly generated zQ175 Δ Neo mice exhibit significantly enhanced mHTT aggregation in the hippocampus, a region with very limited mHTT aggregation in the original zQ175 model. Importantly, zQ175 Δ Neo mice also exhibit enhanced mHTT aggregation in the striatum and increased neuropathology shown by decreased expression of striatal neuron markers compared to zQ175. Intriguingly, despite increased mHTT aggregation in the hippocampus, no markers of hippocampal pathology were observed through analyses investigating neuroinflammation, cortical layer thinning, chaperone expression, and synaptic density. Our findings imply that hippocampal neurons are resilient to mHTT toxicity therefore suggesting that cognitive deficits in HD might not be caused by hippocampal degeneration. Our data further demonstrates the selective vulnerability of striatal neurons in HD and suggests that the zQ175 Δ Neo mouse model may serve as a valuable tool to understand the fundamental susceptibility differences to mHTT toxicity between different neuronal subtypes.



HDX-MS finds that partial unfolding with sequential domain activation controls condensation of a cellular stress marker.

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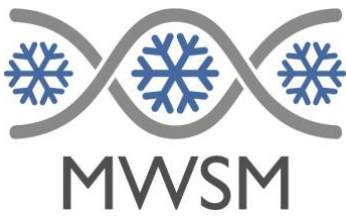
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Eukaryotic cells form biomolecular condensates to sense and adapt to their environment. Poly(A)-binding protein (Pab1), a canonical stress granule marker, condenses upon heat shock or starvation, promoting adaptation (5). The molecular basis of condensation has remained elusive due to a dearth of techniques to probe structure directly in condensates. Here we apply Hydrogen-Deuterium Exchange/Mass Spectrometry (HDX-MS) to investigate the molecular mechanism of Pab1's condensation. We find that Pab1's four RNA recognition motifs (RRMs) undergo different levels of partial unfolding upon condensation, and the changes are similar for thermal and pH stresses. Although structural heterogeneity is observed, the ability of MS to describe individual populations allows us to identify which regions contribute to the condensate's interaction network. Our data yield a picture of Pab1's stress-triggered condensation, which we term sequential activation, wherein each RRM becomes activated at a temperature where it partially unfolds and associates with other likewise activated RRM to form the condensate. Subsequent association is dictated more by the underlying free energy surface than specific interactions, an effect we refer to as thermodynamic specificity. Our study represents a methodological advance for elucidating the interactions that drive biomolecular condensation. Furthermore, our findings demonstrate how condensation can use thermodynamic specificity to perform an acute response to multiple stresses, a potentially general mechanism for stress-responsive proteins.

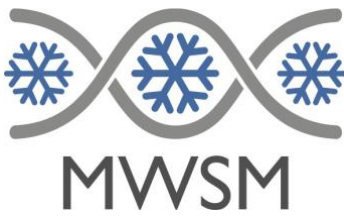


Recapitulating Vectorial Appearance of Polypeptide Chains *In Vitro* Enhances Folding to the Native Structure

Soto-Santarriaga, Iker F., Clark, Patricia L.

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Typical *in vitro* protein refolding experiments enable refolding to begin with interactions formed between any portions of the polypeptide chain. In contrast, protein folding in the cell proceeds vectorially, in a specific direction determined by protein synthesis (N- to C-terminus) or secretion (either N- to C- or C- to N-terminus, depending on the secretion mechanism). The vectorial appearance of the polypeptide chain allows proteins to begin folding by exploring a simple energy landscape that increases in size and complexity as the amino acid chain lengthens. Here we explore the hypothesis that *in vitro* refolding of large or otherwise complex proteins is prone to misfolding and aggregation in part because existing refolding experiments do not replicate the gradual introduction to the folding energy landscape produced by vectorial appearance. Currently, no simple method exists to test the impact of vectorial appearance on protein folding mechanisms. In this study, we adapted the ring-shaped AAA+ translocase ClpX to induce vectorial protein folding *in vitro* via translocation through the central nanometer-sized pore of ClpX from either N- to C- or C- to N-terminus. We used this approach to study the impact of vectorial folding of two diverse fluorescent proteins. We show that refolding is enhanced by vectorial appearance and that the folding outcome is affected by folding direction. Additionally, we show that vectorial appearance exerts the largest effects at early stages of the folding process.

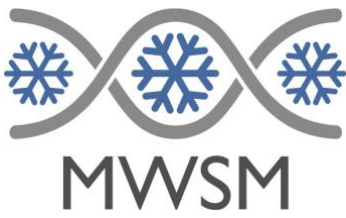


The effect of environmental stress on proteostasis

Studivant, Jeremiah; Womble, Hannah; Baldonado, Mc Noriel; Woodward, Ellen; Talbott, Hannah; Kikis, Elise A.

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One of the hallmarks of aging is the declining ability to maintain a healthy proteome. While even “normal” (non-disease) proteins tend to misfold with age, this is especially pronounced for proteins associated with progressive neurodegenerative diseases, such as Huntington’s disease and Alzheimer’s disease. These disorders are characterized by the misfolding, aggregation, and neurotoxicity of specific proteins. Disease-associated protein misfolding, like age-dependent normal protein misfolding, can be exacerbated in genetic backgrounds with an overabundance of metastable proteins or genetic backgrounds harboring specific genetic risk factors, such as APOE4 for Alzheimer’s disease. Although important advances have been made in recent years to identify such genetic risk factors for neurodegenerative diseases, we still know relatively little about how exposure to environmental risk factors, such as traffic derived air pollution, affects the progression of neurodegenerative disease. Exposure to nano-sized particulate air pollution (nPM), collected from an urban interstate, has been shown to trigger the accumulation of misfolded and oligomerized amyloid beta in mice. Likewise, we have shown that nPM increases the aggregation and toxicity of Huntington’s disease- and Alzheimer’s disease-associated proteins in *C. elegans*. Here, we demonstrate that other nano-particles including those derived from diesel exhaust, nano-plastics such as polystyrene, and even nano-gold, can likewise impact the proteostasis balance. However, different chemical compositions of nanoparticles have differing effects. Namely, nano-polystyrene and nano-gold significantly increase the aggregation of disease-associated metastable protein, leading to increased proteotoxicity. On the other hand, nanoparticulate matter derived from diesel exhaust appears to be generally toxic to *C. elegans* but not in a manner dependent on protein aggregation. Altogether, these findings represent an important survey of the effects of environmental stress on proteostasis and open the door to studies of their molecular mechanisms of action.



The Functional Proteome Landscape Reveals Adaptive Regulation of the Proteasome in Aging

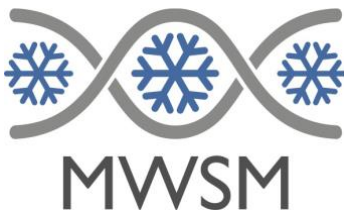
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The conformational stability of the proteome has tremendous implications for the health of the cell and its capacity to determine longevity or susceptibility to age-associated degenerative diseases. For humans, this question of proteome conformational stability has the additional complexity from non-synonymous mutations in thousands of protein coding genes challenging the capacity of the proteostasis network to properly fold, transport, assemble and degrade proteins. Here, we quantify the proteome-wide capacity to such challenges using the isogenic organism *Caenorhabditis elegans* by examining the dynamics of global proteome conformational stability in animals expressing different temperature-sensitive (ts) proteins or short polyglutamine (polyQ) expansions in the context of biological aging and upon heat shock. Using limited proteolysis of native extracts together with tandem mass tag-based quantitative proteomics, we found that different mutant proteins elicit distinct proteome metastability patterns in a cell autonomous and non-autonomous manner. The most dramatic effects occur in aging and heat shock with one-third of the proteome undergoing conformational changes in early adulthood. These age-dependent metastable proteins overlap substantially with ts proteins and polyQ; moreover, expression of polyQ accelerates the aging phenotype. Proteasome remodeling mediated by its regulators may serve as a general adaptive response to accumulated metastable proteins in stress and aging. Together, these results reveal that the proteome responds to misfolding one-at-a-time to generate a metastable sub-proteome network with features of a fingerprint for which aging is the dominant determinant of proteome metastability.



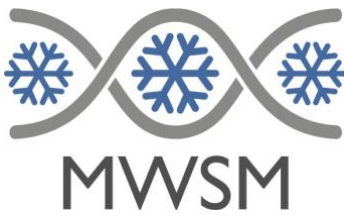
Non-cell-autonomous regulation of germline proteostasis by insulin/IGF-1 signaling via the intestinal peptide transporter PEPT-1

Tahir, Muhammad¹, Stacey L. Edwards², Allison C. Morphis², Mary V. Johnson¹, Vitor De Oliveira¹, Siyan Liu¹, Tuong Ngoc-Gia Nguyen¹, Tomasz Chamera², Jian Li¹

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Gametogenesis involves active protein synthesis and heavily relies on proteome integrity. How animals regulate germline proteostasis at the organismal level is still poorly understood. Our recent work in *C. elegans* indicates that germline development requires coordinated activities between insulin/IGF-1 signaling and HSF-1, the transcriptional regulator of many molecular chaperones in both stress and physiological conditions. In this study, we show that HSF-1 is important for germline proteostasis at ambient temperature. Depletion of HSF-1 protein from germ cells impairs chaperone gene expression, causing protein degradation and aggregation, and consequently declines in fecundity and gamete quality. Reduced insulin/IGF-1 signaling confers germ cells' tolerance to limited protein folding capacity by lowering ribosome biogenesis and translation. Interestingly, regulation of germline proteostasis by insulin/IGF-1 signaling occurs non-cell-autonomously. Our data suggest that insulin/IGF-1 signaling controls the expression of the evolutionarily conserved intestinal peptide transporter PEPT-1 via its downstream transcription factor FOXO/DAF-16, therefore allowing dietary proteins to be incorporated into an amino acid pool that fuels ribosomal biogenesis and translation in the germline. We propose that this pathway has a critical role in regulating germline protein synthesis, which needs to be at a fine balance with HSF-1-dependent protein folding to achieve proteostasis in gametogenesis.



Heat stress and SCA48 mutations reveal a role for CHIP in regulating RNA metabolism

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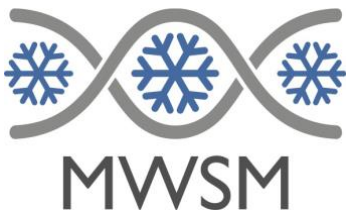
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The E3 ubiquitin ligase, C-terminus of Hsc70 Interacting Protein (CHIP), is a critical regulator of cellular proteostasis that when mutated causes spinocerebellar ataxia type 48 (SCA48). CHIP binds chaperones and targets misfolded proteins for degradation during stress. In vivo, CHIP is critical to mount a proper response to cellular stress and deletion of CHIP results in a loss of viability in response to heat stress. More recently a genetic interaction between CHIP and TATA-Binding Protein (TBP) has been identified in patients suggesting that CHIP plays a role in regulating RNA metabolism. Here we performed a series of studies that identified a reversible structural change in CHIP that is induced by heat stress and by SCA48 mutations in the TPR domain of CHIP. We also found that similar with heat stress, SCA48 mutants accumulate in the nucleus. To gain insight into the function of CHIP in the nucleus we performed a proximity labeling experiment and found that CHIP binds the Transcription Factor IIA (TFIIA) in a TPR dependent manner promoting the TFIIA/TBP interaction. We further show that CHIP is essential for proper regulation of RNA metabolism under conditions of cellular stress. Together these findings identify a novel role for CHIP in regulating RNA metabolism and suggest that stabilizing the TPR domain of CHIP is one potential way to treat SCA48.



Grp94 Protects Citrate Synthase from Heat Induced Aggregation and Inactivation

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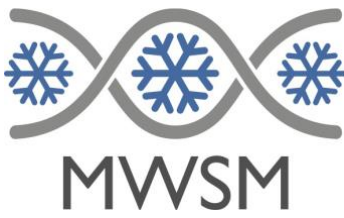
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Grp94 is comprised of 3 domains: the N-terminal domain (NTD), the middle domain (MD) and the C-terminal domain (CTD). Grp94 has been shown to function in active remodeling of client proteins and in protection from formation of aggregates. This work explores Grp94's ability to protect against aggregation by binding misfolded or denatured client conformations. We investigated Grp94's aggregation prevention potential using two model substrates, citrate synthase and luciferase. In vitro fixed angle light scattering assays were used to identify which domain(s) of Grp94 could prevent protein aggregation. We found that only the N-terminal domain of Grp94 is responsible for aggregation prevention. Dynamic light scattering (DLS) was used to characterize the size of aggregates and Grp94 was observed to reduce the size of citrate synthase aggregates by forming Grp94-citrate synthase complexes. To test whether Grp94 was able to refold and reactivate citrate synthase, we monitored the activity of the enzyme after heat denaturation. Interestingly, all domains of Grp94 protected the enzyme against thermal inactivation. The refolding and prevention of aggregation functions of Grp94 are independent of one another since all domains could support an active conformation of citrate synthase while only the NTD could prevent aggregation. In studies from other labs, the MD and CTD were responsible for aggregation prevention with specific clients while the NTD accelerated aggregation of a client. Additional studies with biologically relevant clients are needed to determine if these interactions are client specific. Together this information can lead to a better understanding of Grp94 chaperoning mechanisms and client recognition, and in the future could lead to interventions for neurodegenerative diseases caused by protein misfolding and aggregation.

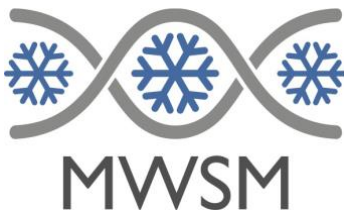


Hsp90-Mediated Gut-Brain Signaling Unveils a Role in Lipid Metabolism to enhance proteostasis in *C. elegans* Models of Neurodegenerative Diseases

Uvarova, Valeria, Beaumont, Jonathan, van Oosten-Hawle, Patricija

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Heat shock protein 90 (Hsp90) is a conserved and essential molecular chaperone that is involved in the maintenance of cellular proteostasis. In metazoans such as *C. elegans*, Hsp90 functions as an integrator of the organismal stress response across different cell types and tissues, known as transcellular chaperone signaling. Our lab has previously shown that modulating Hsp90 expression levels in the *C. elegans* intestine or the nervous system has consequences for the animal's ability to respond to environmental challenges, aging as well as age-associated neurodegenerative diseases. Here, we show that altering Hsp90 expression levels in the *C. elegans* gut, a major fat storage organ, triggers changes in lipid metabolism resulting in reduced triglyceride storage. Immunoprecipitation of Hsp90 overexpressed in gut cells reveals that Hsp90 directly contributes to this remodeling of lipid metabolism via interacting with lipid catabolic enzymes. Using *C. elegans* models of Alzheimer's and Huntington's Disease, our data demonstrates that gut-specific Hsp90 overexpression prevents the age-dependent neurotoxicity associated with these human neurodegenerative diseases and extends lifespan. This shows that Hsp90 is involved in the gut-brain signaling axis benefitting neuronal health. We hypothesize that as a consequence of the Hsp90-dependent rewired fat metabolism, free fatty acids are released from the gut. Interestingly, both poly-unsaturated fatty acids and mono-unsaturated fatty acids can act as signaling molecules and are implicated in longevity interventions. Current efforts are aimed at determining the differential lipid profiles in our strains to identify specific fatty acids that could serve as intracellular signaling molecules to mitigate neurotoxicity and extend health span. We are hoping for our work to lead to potential translational implications that mitigate the age-associated progression of human neurodegenerative diseases.



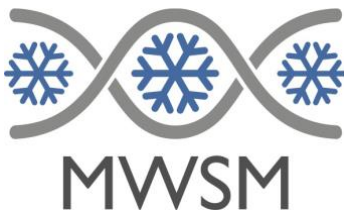
AlphaFold-Guided Discovery of Novel Proteopathies: Unveiling Uncharacterized Protein Misfolding Disorders

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The use of deep learning to predict protein structure accurately and efficiently has enabled researchers to query complex biological questions that were previously not possible. AlphaFold is a highly accurate protein structure prediction algorithm, but its value for understanding the effects of pathogenic protein mutations is yet to be fully explored. Using AlphaFold2, we present the wild type and mutant structure predictions of all clinically pathogenic single missense mutations from the ClinVar archive of proteins under 400 amino acids in length. Root mean square deviation (RMSD) and predicted local difference distance test (pLDDT) were calculated globally and locally for all structures and leveraged to screen for previously uncharacterized proteopathies. Specifically, we identified mutations that hypothetically increased aggregation propensity, either by destabilizing the native structure, stabilizing putative alternative structures, or increasing predicted tendencies for amyloid formation. We hope to identify novel correlations between structural prediction metrics (pLDDT and RMSD) with genetic and molecular characteristics, including inheritance patterns, mutation impacts (gain or loss of function), expression levels, tissue-specific expression, subcellular localization, and traditional amyloid prediction algorithms. Employing Distributed Amphifluoric FRET (DAmFRET), a flow cytometry assay to quantify in vivo protein self-assembly, we will bridge our computational predictions with empirical findings. Specifically, this methodology will be applied to both the human ORFeome (hORF) and human disease ORFeome (hdORF) to determine whether the computationally predicted amyloids correspond to the experimentally determined aggregates in vivo. This research not only advances our understanding of protein aggregation mechanisms in human diseases but also showcases the integrative use of computational and experimental techniques to decode complex biological phenomena.



Investigating the Role of Prion Protein Ortholog ERF3 in the Dictyostelium Stress Response

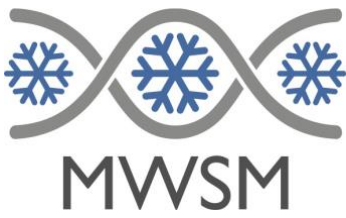
Williams, Felicia¹, Travis, Kanasha¹, Haver, Holly¹, Wu, Yumei¹, Scaglione, Matthew^{1,2,3}

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Proteins containing repetitive, low-complexity, or prion-like domains are particularly prone to aggregation. While protein aggregation has been widely associated with adverse cellular impacts, this phenomenon has also been positively implicated in response to environmental stressors, suggesting that there may be a beneficial role to this process. The proteome of the social amoeba *Dictyostelium discoideum* encodes a large number of proteins predicted to be aggregation prone, with nearly one-fourth of proteins predicted to be prion-forming. Despite this unusually large number of predicted prions encoded in the *Dictyostelium* genome, few studies have investigated the potential roles and regulation of prion-like proteins in this organism. Here, we have begun investigating the behavior of known prion orthologs in *Dictyostelium*. We began our studies by investigating ERF3, the *Dictyostelium* ortholog of the yeast prion protein SUP35. Unlike in yeast, ERF3 lacks the Q/N-rich domain required for prion formation by Sup35, raising the question of whether or not it behaved as a prion in *Dictyostelium*. Here, we have found that ERF3 undergoes reversible protein aggregation during cellular stress. We further show that ERF3 aggregation is mediated by its C-terminal GTPase domain in a manner independent of the Q/N rich domain found in yeast SUP35. Moreover, we find that ERF3 aggregation is induced during *Dictyostelium* development suggesting that regulated protein aggregation plays a role in the *Dictyostelium* developmental cycle. Together, these findings suggest that *Dictyostelium* is not immune to protein aggregation, and that regulated protein aggregation may have beneficial effects during cellular stress and during *Dictyostelium* development.



29th Annual Midwest Stress Response and Molecular Chaperone Conference

Transcriptome-wide mRNA clustering precedes stress granule formation and excludes stress-induced transcripts

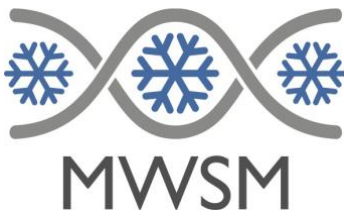
Wong Hickernell, Caitlin*, Glauninger, Hendrik*, Bard, Jared A.M.*, Wallace, Edward, Drummond, D. Allan

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*denotes equal contribution

When cells encounter environmental stress, they experience a number of conserved molecular changes including the formation of biomolecular condensates, clusters of protein and RNA. These clusters, known as stress granules when visible by microscopy, are consistently present across different species and stress conditions. While it has long been speculated that stress granules may play an adaptive role in a cell's stress response, their precise function has remained elusive. Out of the many proposed functions for stress granules, most do not require the large size of stress granules, raising the question of whether stress granules themselves are functionally important or merely easy to see.

Here, our investigations of stress-induced mRNA condensation reveal that submicroscopic molecular clusters form in functionally coherent ways even when stress granules are not visible or are pharmacologically blocked. Using sedimentation, we observe that most RNA transcripts condense during stress, while stress-induced transcripts are excluded from condensates. This exclusion is not due to specific sequence features or membership in specific regulon, but due to the timing of transcription. Additionally, we find that perturbing the translational state of transcripts affects the magnitude of condensation, connecting RNA condensation to translation initiation. Our results suggest a model in which RNA blocked at the translation initiation step condenses, while translationally competent transcripts escape condensation. Condensation may function to sequester most mRNA during stress, promoting the translation of specific stress transcripts.



Investigating Proteostasis Imbalances in *C. elegans* expressing TDP-43, an Amyotrophic Lateral Sclerosis disease associated protein

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized primarily by degeneration of motor neurons leading to muscle atrophy and paralysis. Mutations in TAR-DNA protein of 43 kDa (TDP-43) have been linked to familial cases of ALS. TDP-43 is an RNA binding protein that shuttles mRNA between the nucleus and cytosol. In ALS, TDP-43 accumulates in the cytosol of affected neurons as hyperphosphorylated and ubiquitinated aggregates. Imbalances in key proteostasis pathways, such as protein clearance, have been implicated in many neurodegenerative diseases. Proper targeting of misfolded proteins for degradation is critical for cellular health. To learn more about the connection between proteostasis imbalances and TDP-43 neurotoxicity, we are taking advantage of the nematode *C. elegans* simple nervous system and well characterized behavioral assays. Using a ribosome profiling dataset of *C. elegans* expressing TDP-43 pan-neuronally, I identified three upregulated genes, *usp-33*, *asp-17*, and *T28H10.3* that are involved in protein clearance. Currently, I am conducting a genetic cross to introduce deletions of these genes into TDP-43 expressing worms. Using molecular genotyping, I will identify F3 TDP-43 expressing worms that are homozygous for the deletions. Once the new strains are generated, I will perform a thrashing assay to evaluate motor neuron defects. I expect *C. elegans* expressing neuronal TDP-43 with a deletion of the *usp-33* gene, as well as strains with *asp-17* and *T28H10.3* gene deletions, will exhibit fewer body thrashes. The enhanced motor neuron deficits in TDP-43 animals along with upregulation of these genes suggests that neurons may be activating mechanisms to clear TDP-43 aggregates. In addition, I will conduct a western blot to determine the level of TDP-43 in the newly generated strains. I expect *C. elegans* expressing neuronal TDP-43 and harboring deletions of *usp-33*, *asp-17*, and *T28H10.3* genes will exhibit an altered level of TDP-43, demonstrating a role for these genes in TDP-43 clearance. Understanding the mechanisms neurons employ to clear TDP-43 aggregates may reveal therapeutic targets to alleviate ALS pathology.