Annual Midwest Stress Response and Molecular Chaperone Meeting

MWSM

Saturday January 18th 2025

Northwestern University
Pancoe Abbott Auditorium
Evanston, Illinois

30th Anniversary Midwest Stress Response and Molecular Chaperone Conference

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Saturday, January 18, 2025
Northwestern University
Pancoe Abbott Auditorium & Cafe
2200 Campus Drive
Evanston, Illinois 60208

Program Chairs
Jeff Brodsky, *Department of Biological Sciences University of Pittsburgh*

Sue Fox, *Department of Molecular Biosciences Northwestern University*

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

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

PROGRAM

8:00 - 8:55 AM	**Continental Breakfast**
8:55 - 9:00 AM	**Opening remarks** Rick Morimoto, Northwestern University, Evanston, IL
9:00 – 10:00 AM	**~50 years of Hsp70s and J-domain proteins** Betty Craig, University of Wisconsin-Madison, Madison, WI
Session I **Co-Chairs**:	**Marc Mendillo**, Northwestern University, Feinberg School of Medicine, Chicago, IL **Emily Sontag**, Marquette University, Milwaukee, WI
10:00 - 10:20 AM	***Mining the Hsp90 interactomes for functional roles*** Brian Freeman, University of Illinois Urbana-Champaign, Urbana, IL
10:20 - 10:40 AM	***Transcriptome-wide mRNA condensation in unstressed cells*** D. Allan Drummond, The University of Chicago, Chicago, IL
10:40 - 11:00 AM	***Off to a great start: New tools to study the earliest steps of protein folding*** Patricia Clark, Notre Dame, South Bend, IN
11:00 - 11:25 AM	**Coffee Break**
Session II **Co-Chairs**:	**Liming Li**, Northwestern University, Feinberg School of Medicine, Chicago, IL **Andrew Truman**, University of North Carolina, Charlotte, NC
11:25 - 11:45 AM	***Role of polyphosphate in amyloidogenic processes*** Ursula Jakob, University of Michigan, Ann Arbor, MI

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11:45 – 12:05 PM ***Redox modulation of proteostasis***
Kevin Morano, University of Texas Health Science Center, Houston, TX

12:05 – 12:25 PM ***A cache of endo-siRNAs regulate the C. elegans heat shock response***
Veena Prahlad, Roswell Park, Buffalo, NY

12:25 – 12:30 PM **Group picture**

12:30 – 1:30 PM **Lunch** in the Pancoe Cafe

Session III- Flash Talks
Co-Chairs: **Tali Gidalevitz**, Drexel University, Philadelphia, PA
Anat Ben Zvi, Ben-Gurion University of the Negev, Be'er Sheva, Israel

1:30–1:55 PM

Protein engineering approach towards controlling tau aggregation
Chad Dashnaw, UT Southwestern Medical Center, Dallas, TX

Class-specific chaperone behavior in biomolecular condensate dispersal
Kyle Lin, The University of Chicago, Chicago, IL

Mistranslation impairs distinct branches of proteostasis
Donovan McDonald, Western University, London, Ontario, Canada

Activation of the integrated stress response by loss of proteasome activity
Arya Menon, University of Michigan, Ann Arbor, MI

Almost FAIMous: Mechanistic insights into the aggregation inhibition and disaggregation activity of FAIM
Joshua Mitchell, Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, MI

A novel role for ER stress sensor, PERK, in axonal-dendritic targeting of neuronal growth factors
Julia Perhacs, Drexel University, Philadelphia, PA

Targeting FKBP51 with antisense oligonucleotides: A therapeutic approach for tauopathies
Ahmed Ramadan, University of South Florida, Tampa, FL

Characterization of a new Hsp110 inhibitor as a potential antifungal
Cancan Sun, Virginia Commonwealth University, Richmond, VA

E3 ligase recruitment by UBQLN2 protects substrates from proteasomal degradation
Sachini Thanthirige, University of Pittsburgh, Pittsburgh, PA

Hsp90-induced metabolic rewiring in the intestine regulates neuronal health and longevity
Valeria Uvarova, University of North Carolina, Charlotte, NC

Session IV- Poster Session Pancoe Café
1:55 – 3:10 PM

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Session V

Co-Chairs:
James West, The College of Wooster, Wooster, OH
Elise Kikis, The University of the South, Sewanee, TN

3:10 - 3:30 PM ***Identifying rare variants linked to ERAD and protein conformational disease***
Jeff Brodsky, University of Pittsburgh, Pittsburgh, PA

3:30 – 3:50 PM ***Critical beginnings: Tuning protein folding and aggregation pathways at birth***
Silvia Cavagnero, University of Wisconsin, Madison, WI

3:50 – 4:10 PM **Roles of RNA quadruplexes and SERF proteins in folding**
Jim Bardwell, University of Michigan, Ann Arbor, MI

Session VI

Co-Chairs:
Daniel Czyz, University of Florida, Gainesville, FL
Cindy Voisine, Northeastern Illinois University, Chicago, IL

4:10 - 4:30 PM ***Identification of yeast Hsp90 mutants with differing effects on client proteins***
Jill Johnson, University of Idaho, Moscow, ID

4:30 - 4:50 PM **Organismal proteostasis**
Rick Morimoto, Northwestern University, Evanston, IL

Closing Remarks and Reception

4:50 - 5:00 PM **Closing Remarks**

5:00 – 6:15 PM **Reception w/appetizers & beverages in the Pancoe Café**

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

SPEAKER ABSTRACTS

SESSION I

Mining the Hsp90 interactomes for functional roles

Zlata Gvozdenov[1,2], Audrey Yi Tyan Peng[1], Anusmita Biswas[1], Zeno Barcutean[1], Janhavi Kolhe[1], Daniel Gestaut[3], Judith Frydman[3], Kevin Struhl[2], Brian Freeman[1]

[1]Department of Cell and Developmental Biology, School of Molecular and Cellular Biology, University of Illinois-Urbana-Champaign, Urbana, IL
[2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA
[3]Department of Biology, Stanford University, Stanford, CA

Molecular chaperones govern proteome health to support cell homeostasis. Hsp90 is one of the most abundant and conserved eukaryotic molecular chaperones, yet the proteins that directly physically interact with this central chaperone had been underappreciated. Using a chemical-biology approach we mapped the associations within the Hsp90 physical interactome under various physiological conditions including cell aging. In addition to changes in client protein interactions, Hsp90 shifts its connections with various other molecular chaperones including the Tailless complex polypeptide 1 Ring Complex (TRiC) or Chaperonin Containing Tailless complex polypeptide 1 (CCT). As our understanding of TRiC/CCT function in eukaryotes is limited to cytosolic events, we explored the role of TRiC/CCT within the nucleus to comprehend how this chaperonin connects to the Hsp90 molecular chaperone in this central eukaryotic cell compartment.

Transcriptome-wide mRNA condensation in unstressed cells

D. Allan Drummond

Department of Biochemistry & Molecular Biology and Genetic Medicine, The University of Chicago, Chicago, IL

During stress, many eukaryotic mRNAs and proteins condense into large cytosolic clusters called stress granules, structures whose function(s) have remained unclear. We recently showed that prior to stress granule formation, most pre-stress mRNAs condense into translationally silent clusters, while new transcripts--such as those induced during stress--escape condensation. As a consequence, stressed cells shift their translational activity from old messages to new, stress-relevant messages. Perhaps strangely, we discovered that even under non-stress conditions, many messages are in condensates, apparently as a result of transient pauses in translation initiation. I will discuss what we believe is happening: that eukaryotic cells use condensation under all conditions to synchronize transcriptional and translational activity, solving a general problem created by the presence of a nuclear membrane.

Off to a great start: New tools to study the earliest steps of protein folding (talk title)
Vectorial appearance induces conformational biases that alter protein folding yield
Iker Soto Santarriaga, Patricia Clark

University of Notre Dame, South Bend, IN

Every protein in every organism is synthesized from N- to C-terminus on a ribosome and can start to f fold during synthesis. Folding during vectorial appearance is therefore the physiological environment under which amino acid sequence evolution has occurred, yet we still know very little about the conformations adopted by partially synthesized, N-terminal ribosome-bound nascent chains while they await their C-terminal interaction partners, and to what extent co-translational folding influences subsequent post-translational protein folding, binding, degradation and other events. The complexities of co-translational folding reactions can make it difficult to unambiguously assign how folding is impacted by vectorial appearance of the nascent polypeptide chain, versus (as examples) interactions with the ribosome surface or a chaperone. Can vectorial appearance lead to conformational differences that affect protein folding mechanisms, including the partitioning between native folding and misfolding? We have developed a novel, simple experimental system to explicitly and unambiguously test the impact of vectorial protein appearance on protein folding rate and yield.

SESSION II

Role of polyphosphate in amyloidogenic processes

Ursula Jakob

University of Michigan, Ann Arbor, MI

Inorganic polyphosphate (polyP), one of the first high-energy compounds on earth, defies its extreme compositional and structural simplicity with an astoundingly wide array of biological activities across all domains of life. Recent studies demonstrated that this simple polyanion stabilizes protein folding intermediates and scaffolds select native proteins, allowing it to act as molecular chaperone that protects cells against protein aggregation, pro-amyloidogenic factor that accelerates both physiological and disease-associated amyloid formation, and as a modulator of liquid-liquid phase separation processes. These activities help to explain polyP's known roles in stress responses and pathogenicity, provide the mechanistic foundation for its potential role in human neurodegenerative diseases and cancer, and open a new direction regarding its influence on cell migration through condensate formation. The talk will highlight critical unanswered questions and point out potential directions that will help to further understand the pleiotropic functions of this ancient and ubiquitous biopolymer.

Redox modulation of proteostasis

Kevin Morano

University of Texas Health Science Center, Houston, TX

Cells employ multiple systems to maintain homeostasis when experiencing environmental stress. For example, the folding of nascent polypeptides is exquisitely sensitive to proteotoxic stressors including heat, pH and oxidative stress, and is safeguarded by a network of protein chaperones that concentrate potentially toxic misfolded proteins into transient assemblies to promote folding or degradation. The redox environment itself is buffered by both cytosolic and organellar thioredoxin and glutathione pathways. How these systems are linked is poorly understood. Here, we determine that specific disruption of the cytosolic thioredoxin system resulted in constitutive activation of the heat shock response in *Saccharomyces cerevisiae* and accumulation of the sequestrase Hsp42 into an exaggerated and persistent juxtanuclear quality control (JUNQ) compartment. Terminally misfolded proteins also accumulated in this compartment in thioredoxin reductase (*TRR1*)-deficient cells, despite apparently normal formation and dissolution of transient cytoplasmic quality control (CytoQ) bodies during heat shock. Notably, cells lacking *TRR1* and *HSP42* exhibited severe synthetic slow growth exacerbated by oxidative stress, signifying a critical role for Hsp42 under redox-challenged conditions. Hsp42 localization patterns in *trr1*Δ cells mimic those observed in chronically aging and glucose-starved cells, linking nutrient depletion and redox imbalance with management of misfolded proteins via a process of long-term sequestration. Thiol-sensitive higher molecular weight multimers accumulate in *trr1*Δ cells that appear to form via the sole cysteine at position 137 in the Hsp42 intrinsically disordered domain, and preliminary data indicate that interprotein disulfide bonds between Hsp42 monomers may promote the exaggerated JUNQ compartment via the amino-terminal prion-like domain.

A cache of endo-siRNAs regulate the *C. elegans* heat shock response

Veena Prahlad

Roswell Park Comprehensive Cancer Center, Buffalo, NY

The accurate response to heat shock requires the coordination of transcription and translation of the protective heat shock proteins (hsp) with the proteotoxic challenges to be managed. We have recently found that in response to heat shock, *C. elegans* upregulates not only protective hsp genes but also synthesizes small double-stranded RNAs, called 22G RNA, which are antisense to the induced hsp genes. These 22G RNAs regulate the amounts of hsp mRNA available for translation *by protecting hsp pre-mRNA from premature splicing and maturation*. In mutants defective in synthesizing 22G RNAs (*prde-1, drh-3*), *hsp* pre-mRNA is spliced prematurely during heat shock instead of later, leading to the untimely accumulation of HSP mRNA and protein. As has been shown in seminal early work on the heat shock response, this leads to the premature resumption of protein synthesis in animals that have downregulated protein synthesis due to heat shock, sterility, and embryonic death. These defects can be rescued by injecting 22G RNA into prde-1 mutants. Intriguingly, our preliminary data also suggest that ds22G RNA modulate organism wide hsp mRNA accumulation through the sid-1 transmembrane protein that exchanges dsRNA between cells and tissues. We speculate that the mechanisms utilized by the 22G RNAs in regulating pre-mRNA maturation could resemble those used by the U1 snRNPs in protecting pre-mRNA from early maturation. These studies reveal a new mechanism of control over hsp mRNA through endogenously generated antisense dsRNA.

SESSION III:

FLASH TALKS

Protein engineering approach towards controlling tau aggregation

Chad Dashnaw, Simran Rastogi, Lukasz Joachimiak

University of Texas Southwestern Medical Center, Dallas, TX

Tau amyloid formation is implicated in a range of neurodegenerative diseases termed tauopathies. Advances in cryogenic-electron microscopy (cryo-EM) suggests a link between amyloid structure (i.e., polymorph) and disease phenotype. Our inability to direct tau folding towards a specific structural polymorph inhibits our capacity to characterize this relationship and leverage these amyloid structures in novel diagnostic and therapeutic strategies. We utilize computational methods to generate structural and molecular fingerprints for each of the tauopathy structural polymorphs to uncover the residues that dictate the thermodynamics of each disease state. From this work, we employ protein design to engineer tau variants that preferentially stabilize tau amyloid conformations linked to chronic traumatic encephalopathy. In vitro and cell-based aggregation assays validate increased stability of designed tau variants. Cryo-EM microscopy suggests that pairwise-mutations are sufficient to alter the aggregation pathways available to tau towards defined disease states. These methods are easily adaptable to all amyloidogenic proteins and can be used to understand the sequence-structure relationship of amyloid toward the design of reagents that can diagnose disease based on amyloid conformation.

Class-specific chaperone behavior in biomolecular condensate dispersal

Kyle Lin[1,2], Diangen Lin[3,4], Estefania Cuevas-Zepeda[1], Kavya Vaidya[4], Ayesha Ejaz[5], Haneul Yoo[6,7], D. Allan Drummond[7,8,9], Allison Squires [4,9,10]

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[7] Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL
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[9] Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL
[10] Chan-Zuckerberg Biohub Chicago, Chicago,IL

Environmental signals, such as a sudden increase in temperature, cause cellular proteins to cluster into biomolecular condensates that recruit the molecular chaperone Hsp70 and its J-domain protein (JDP) counterparts for dispersal. At the molecular scale, much is unknown about how JDPs and Hsp70 participate in dispersal—including which classes of JDP directly recognize condensates as substrates. Two major types of JDP, Class A and B (Ydj1 and Sis1 in budding yeast), both directly bind misfolded proteins and independently support the dispersal of misfolded protein aggregates with Hsp70. However, biomolecular condensates of yeast poly (A)-binding protein (Pab1) require specifically the Class B JDP, Sis1, for their dispersal. Not only is the Class A JDP Ydj1 unable to support Pab1 dispersal, but it slows dispersal in a concentration-dependent manner when Sis1 is present. Why do JDP classes behave differently in condensate dispersal? We propose two general models: either Ydj1 fails to recognize condensates as a substrate, while interacting with Hsp70 and slowing its recruitment to condensates (an “off-substrate” model); or Ydj1 binds condensates in competition with Sis1, but Ydj1 fails to recruit Hsp70 or handoff substrate to Hsp70 effectively (an “on-substrate” model). To test these models, we use a combination of experimental approaches: total internal reflection fluorescence (TIRF) single-molecule microscopy to visualize the direct interactions of Sis1, Ydj1, Hsp70, and Pab1 condensates; biochemical bulk assays to assess chaperone activity on Pab1 and luciferase substrates; and generation of a mutant (EEVD-less) Hsp70 which is defective in collaboration with Sis1. Our results so far support an “on-substrate” model for Ydj1 inhibition of condensate dispersal, in which Ydj1 is able to recognize the condensate as a substrate, but it fails to coordinate with Hsp70 in recruitment or substrate handoff. These and future experiments planned will help elucidate class-specific differences in JDP function and paint a novel, molecular-scale picture of how chaperones engage biomolecular condensates as physiological substrates.

Mistranslation impairs distinct branches of proteostasis

Donovan McDonald, Rebecca Dib, Christopher De Luca, Martin Duennwald

Western University, London, Ontario, Canada

Protein function relies on their accurate biosynthesis during translation. Despite the requirement for faithful translation, variants of tRNA genes that induce amino acid misincoporation are prevalent in human populations. Mistranslation has been studied in many model systems, and induces pleiotropic effects on proteostasis, ranging from protein misfolding to impaired protein biosynthesis. We hypothesize that different types of mistranslation influence different branches of proteostasis. We employed a tractable yeast model that facilitates thorough and quantitative analysis of specific proteostasis pathways impaired by mistranslating tRNA variants. We tested two mistranslating tRNASer variants, each inducing either proline to serine (P>S) or arginine to serine (R>S) misincorporation. We show that P>S misincorporation impairs fitness and sensitizes cells to misfolding of cytosolic and nuclear proteins more than R>S misincorporation. Of note, we also show that, despite both tRNA variants inducing misincorporation of serine, they induce the accumulation of misfolded proteins that stem from distinct defects in proteostasis and have unique biochemical properties. Specifically, we are the first to show that R>S misincorporation reduces that association of Hsp70 with misfolded proteins, while P>S misincorporation impairs degradation of nascent polypeptides. These findings provide strong contributions to our understanding of how different mistranslating tRNA variants impair proteostasis.

Activation of the integrated stress response by loss of proteasome activity

Arya Menon, Ben Dodd, Stephanie Moon

Program in Chemical Biology, Department of Human Genetics, Center for RNA Biomedicine, University of Michigan, Ann Arbor, MI

Proteasome function decreases with age as measured by proteasome activity and accumulation of ubiquitinated and oxidized proteins in human tissues and many model organisms. The Ubiquitin Proteasome System (UPS) regulates protein homeostasis by degrading nascent peptides, regulatory proteins, and misfolded or damaged proteins. Integrated Stress Response (ISR) is a signaling pathway that regulates mRNA translation and protein synthesis during stress. Upon stress, phosphorylation of the translation initiation factor eIF2α by any of four kinases (GCN2, PERK, PKR, or HRI) activates the ISR and inhibits mRNA translation. Defects in the ISR pathway and hyperactive ISR kinases are also associated with aging and neurodegenerative diseases. The relationship between ISR and UPS during proteasome suppression remains unclear. We are applying genetics, biochemical assays, and fluorescence microscopy to determine the effects of proteasome inhibition on ISR. First, we established a cell line system to study chronic lower-level proteasome function associated with aging by using sublethal nanomolar concentrations of proteasome inhibitor, bortezomib, for 24 hours. Low levels of bortezomib inhibited the proteasome and resulted in the accumulation of ubiquitinated proteins, p62, protein aggregates, and aggresomes. Next, we found that chronic proteasome inhibition induced phosphorylated eIF2α to levels similar to acute stresses. Stress-induced genes ATF4 and GADD34 were also regulated by proteasome inhibition. Together, these results suggest that ISR is activated during chronic proteasome suppression. To determine the mechanism of eIF2α phosphorylation and identify how proteasome suppression is sensed as stress, we used CRISPR/Cas9 to perform a single knockout of PERK, GCN2, and/or HRI. GCN2 is activated by amino acid deprivation, uncharged tRNAs, and ribosomal collisions; HRI activation occurs via iron depletion, mitochondrial and/or oxidative stresses, and PERK is activated during the unfolded protein response. Strikingly, we found neither GCN2 nor PERK knockout could reduce P-eIF2α upon chronic proteasome inhibition, while P-eIF2α levels were restored to unstressed levels in cell lines devoid of HRI kinase. Because heme is a known inhibitor of HRI, we tested the hypothesis that reduced heme led to HRI activation and observed heme supplementation did not limit eIF2α phosphorylation. This result suggests that HRI is activated independently of heme levels during proteasome suppression. To further explore the mechanism of HRI activation we did quantitative proteomics and transcriptomics of low nanomolar bortezomib-treated cell lines. Our proteomics and transcriptomics data show 1) upregulation of HRI kinase, 2) upregulation of genes involved in cellular, mitochondrial, and oxidative stress, and 3) upregulation of various heat shock proteins. Future work will determine the mechanism of HRI activation in the context of chronic proteasome suppression. Understanding how proteasome overload is connected to persistent ISR activation at the molecular level will likely identify therapeutic targets for aging and neurodegeneration.

Almost FAIMous: mechanistic insights into the aggregation inhibition and disaggregation activity of FAIM

Joshua Mitchell, Tom Rothstein

Western Michigan University, Homer Stryker M.D. School of Medicine, Kalamazoo, MI

Proteostasis is a prerequisite for cellular function and comprises a network of biological processes involved with folding, chaperoning, and degrading proteins. Dysregulation of any of these processes can result in deleterious effects across the cellular environment, from initiation of the unfolded protein response to the generation of protein aggregate (amyloid). Amyloid formation disrupts normal cellular processes and is a hallmark of many diseases such as Alzheimer's Disease, Parkinson's Disease, Type 2 Diabetes and cardiac amyloidosis (CA). The Rothstein lab has previously discovered a protein, Fas Apoptosis Inhibitory Molecule (FAIM), that is able to inhibit protein aggregation and disaggregate mature amyloid fibrils. FAIM is highly evolutionarily conserved, has no known fold, and the mechanism of action is not understood. We have sought to elucidate FAIM's mechanism of action to improve our understanding of proteostasis, especially in respect to misfolding and aggregation, to develop novel therapeutic approaches to these diseases.

We investigated whether additional aggregating proteins are susceptible to FAIM's ability to inhibit and disaggregate proteins, or whether FAIM has a narrow substrate profile. We found that other aggregating proteins, including shorter polypeptides and larger proteins, are susceptible to inhibition of aggregation and disaggregation by FAIM, suggesting that FAIM's mechanism has broader substrate recognition that previously thought. Recent work in the lab has shown that FAIM can enhance the recovery of denatured luciferase, suggesting that FAIM is altering protein folding dynamics, and that this chaperoning activity may be the mechanism that acts as both an inhibitor to aggregation, and a means to disassemble protein aggregate. Further investigation of this protein and its function will reveal the mechanistic insights of FAIM's activity, as well as guide our understanding of previously unknown proteostatic mechanisms, to aid in the development of novel therapeutics to treat protein aggregation diseases.

A novel role for ER stress sensor, PERK, in axonal-dendritic targeting of neuronal growth factors

Julia Perhacs[1], Lauren Klabonski[2], Sarah Bond-Newton[3,4], Kelly Jordan-Sciutto[3], Yair Argon[5], Tali Gidalevitz[1]

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[4]Department of Oral Medicine, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA
[5]Division of Cell Pathology, Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA

Genetic variants of PERK, an endoplasmic reticulum stress sensor, are genetically linked to tauopathies including Alzheimer's Disease (AD) and Progressive Supranuclear Palsy (PSP). Recent findings in human IPSC derived neurons suggest a decreased kinase activity as a likely mechanism. Moreover, our recent data show that PERK activity is required for the normal function of sensory neurons in vivo. While prolonged activation of PERK's canonical response can exacerbate late stages of neurodegeneration, it is unknown how PERK supports neuronal function in healthy individuals, and how the decreased kinase activity of the identified risk variants sensitizes neurons to pathology during an individual's life.

Our lab recently uncovered a novel role for PERK in the localization of neuroprotective IGF-like and TGF-β-like growth factors. Surprisingly, while precise localization and release of neuromodulators and growth factors is essential for the normal neuronal function and communication, it is yet unknown how these soluble proteins are sorted and targeted to their release sites in either axons or dendrites. Using our in vivo *C. elegans* model, we initially observed that in the absence of PERK, the dendritic TGF-β-like growth factor is mistargeted to the axons, while an axonal IGF-like protein is retained in the cell body. This effect is specific, as PERK deletion does not disrupt the axonal/dendritic polarization of other tested proteins and organelles.To understand how PERK's function affects localization, we tested its canonical ER stress pathway, as well as its kinase dependence. We found that PERK's novel role is kinase-dependent, but but does not depend on its canonical kinase target, eIF2α. Because it is not immediately obvious how the resident ER protein could directly orchestrate polarized sorting at the trans-Golgi, we probed putative downstream candidates. We found that CaMKII, a protein integral to neuronal plasticity and memory, is genetically downstream of PERK. In *C. elegans*, published evidence shows that CaMKII affects axonal targeting of neuronal peptides, supporting the proposed connection between CaMKII and PERK's role in growth factor localization. Further investigation of the PERK-CaMKII pathway will aid in understanding the fundamental question of axonal-dendritic targeting of soluble proteins, as well as suggest a potential link to neuronal susceptibility in degenerative disease.

Targeting FKBP51 with antisense oligonucleotides: A therapeutic approach for tauopathies

Ahmed Ramadan[1,2], Niat Gebru[1,2], Laura Blair[1,2,3]

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[3]Research and Development, James A. Haley Veterans Hospital, Tampa, FL

The 90 kDa heat shock protein, Hsp90, plays a central role in protein homeostasis, contributing to the folding of nascent proteins and refolding or degrading misfolded proteins. One of the main Hsp90 clients is the microtubule-associated protein, tau, which supports neuronal structure and function. Tau is observed to be hyperphosphorylated and aggregated in a wide range of neurodegenerative diseases, such as Alzheimer's disease, collectively called tauopathies. Under normal physiological conditions, Hsp90 in conjunction with other co-chaperones regulates and initiates the degradation of the aberrant tau. However, under pathogenic conditions, our group and others have reported upregulation of some of these co-chaperones, including FK506 binding protein 51 (FKBP51). Interestingly, FKBP51 levels are also significantly increased with aging. We also showed that overexpression of FKBP51 in tau transgenic mice promoted the accumulation of neurotoxic tau oligomers. Mice lacking FKBP51 are viable and show resilience toward stress-induced phenotypes. Knockdown of FKBP51 in vitro lowers phospho-tau accumulation. Taken together, this suggests that lowering FKBP51 levels may be beneficial for the treatment of tauopathies.

Antisense oligonucleotides (ASOs) have increasingly emerged as a successful drug class during the last decade, especially in neurological disorders. ASOs are short, synthetic oligonucleotides that bind to a specific mRNA of a protein-coding gene in a Watson-Crick manner. ASOs have a wide variety of chemical modifications and types, one of the most widely used and established is the Phosphorothioate (PS) backbone, which initiates the RNase H1 medicated degradation of the mRNA.

Here, we hypothesize that FKBP51 ASOs will provide resilience in the brain and reduce aberrant tau levels. Using proteomic profiling in brain tissue from aged wild-type and FKBP51 KO mice compared to young wild-type mice, we found that the protein profile of the aged FKBP51 KO is maintained in a young-like state. We also identified that only three proteins are uniquely and consistently upregulated in the brains of male and female FKBP51 KO mice (Mycbp2, Spryd3, and Fbxo45). These three proteins were reported to participate in forming a protein complex that functions as a unique type of ubiquitin E3 ligase complex, and this complex is crucial for neuronal development and function. We predict that the upregulation of these proteins, as a result of knocking out FKBP51, contributes to the resilience in this model. Next, we worked to develop effective FKBP51 ASOs to have a therapy with translational potential. To do this, we tested GapmeR ASOs in vitro, which were highly effective at lowering FKBP51 levels. In a pilot in vivo study, we measured a significant, but modest decrease in FKBP51 levels in brain tissue from wild-type mice. Efforts to identify more potent FKBP51 ASOs are currently underway. We expect to test these ASOs for their effects on tau, after they are optimized. Overall, this work has identified new targets that may mediate the resilience of FKBP51 mice, while developing tools to lower FKBP51 levels in vivo.

Characterization of a new Hsp110 inhibitor as a potential antifungal

Cancan Sun[1], Yi Li[2], Justin Kidd[1], Jizhong Han[2], Liangliang Ding[2], Aaron May[3], Lei Zhou[2], Qinglian Liu[1]

[1]Department of Physiology and Biophysics, School of Medicine, Virginia Commonwealth University, Richmond, VA
[2]Shenzhen Bay Laboratory, Institute of Molecular Physiology, Shenzhen, Guangdong Prov., China
[3]Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, VA

Fungal infections present a significant global health challenge, prompting ongoing research to discover innovative antifungal agents. The 110 kDa heat shock proteins (Hsp110s) are molecular chaperones essential for maintaining cellular protein homeostasis in eukaryotes. Fungal Hsp110s have emerged as a promising target for innovative antifungal strategies. Notably, 2H stands out as a promising candidate in the endeavor to target Hsp110s and combat fungal infections. Our study reveals that 2H exhibits broad-spectrum antifungal activity, effectively disrupting the in vitro chaperone activity of Hsp110 from Candida auris and inhibiting the growth of Cryptococcus neoformans. Pharmacokinetic analysis indicates that oral administration of 2H may offer en-hanced efficacy compared to intravenous delivery, emphasizing the importance of optimizing the AUC/MIC ratio for advancing its clinical therapy.

E3 ligase recruitment by UBQLN2 protects substrates from proteasomal degradation

Sachini Thanthirige[1], Ashley Scheutzow[2], Gracie Siffer[3], Matthew Wohlever[1]

[1]University of Pittsburgh, Pittsburgh, PA
[2]University of Michigan, Ann Arbor, MI
[3]Northwestern University, Evanston, IL

Ubiquilins are a family of proteins critical to cellular proteostasis that are also linked to neurodegenerative diseases, with specific mutations in UBQLN2 causing dominant, X-linked ALS. Despite an initial characterization as proteasomal shuttle factors, Ubiquilins have paradoxically been reported to stabilize numerous substrates. The basis of this triage decision remains enigmatic, as does the physiological significance of Ubiquilin phase separation, the unique role of each Ubiquilin paralog, and the mechanistic defects of ALS causing mutations. To address these questions, we utilized a library of triple knockout (TKO) rescue cell lines with physiological expression of single Ubiquilin paralogs or disease mutants in an isogenic background. Our findings reveal that UBQLN2 has a unique ability to protect substrates from proteasomal degradation and ALS causing mutations reduce substrate stabilization. In vitro and cellular studies demonstrate that substrate stabilization correlates with the recruitment of multiple E3 ligases, including SCFbxo7. Imaging studies reveal that substrate and UBQLN2 puncta co-localize in cells and that puncta formation is dramatically reduced with UBQLN2 mutants that cannot recruit E3 ligases. As previous work has demonstrated that Ubiquilin phase separation is modulated by polyubiquitin chains, we propose that E3 ligase recruitment by UBQLN2 promotes the formation of biomolecular condensates, which protects substrates from proteasomal degradation. This proposal unifies many existing observations in the field and presents a new paradigm for understanding Ubiquilin function in health and disease.

Hsp90-induced metabolic rewiring in the intestine regulates neuronal health and longevity

Valeria Uvarova, Emani Foster, Patricija van Oosten-Hawle

Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC

Hsp90 is a highly conserved and essential molecular chaperone that regulates stress responses and maintains cellular proteostasis. Our lab has previously shown that tissue-specific modulation of Hsp90 expression levels in the gut and the nervous system induces a protective inter-tissue stress signaling response regulating organismal proteostasis. Here, we investigate how Hsp90 actively coordinates gut-to-neuron signaling to regulate aging. Our results show that constitutive overexpression of Hsp90 in the *C. elegans* gut correlates with decreased toxicity of age-associated protein aggregates in the nervous system, such as amyloid-beta and polyglutamine (polyQ40) proteins, and extends the lifespan of these neurodegenerative disease models. Interestingly, the protective effects on neuronal proteostasis and longevity are more pronounced when Hsp90 is overexpressed in the gut than in the nervous system itself. This suggests an intercellular gut-to-brain signaling mechanism that is initiated by intestinal Hsp90. Using transcriptomic and metabolic profiling, we demonstrate that gut-specific Hsp90 overexpression induces metabolic changes. These included reduced levels of triglycerides, increased lipase activity, and increased concentrations of odd-chain free fatty acids, particularly pentadecanoic acid (PA), an essential odd-chain fatty acid commonly found in dairy products. We hypothesize that Hsp90 interaction with specific client proteins in the gut underlies the rewired lipid metabolism leading to gut-to-neuron signaling and that free fatty acids may serve as intercellular signals. Supporting this hypothesis, dietary supplementation with PA extended both the lifespan and healthspan of *C. elegans*. Our results suggest that Hsp90-induced rewiring of lipid metabolism contributes to its downstream effect on neuronal proteostasis and aging

SESSION V

Identifying rare variants linked to ERAD and protein conformational disease

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To maintain protein homeostasis, eukaryotes evolved a hierarchy of protein quality control checkpoints along the secretory pathway, including endoplasmic reticulum associated degradation (ERAD) and post-ER quality control. To date, >70 human diseases have been linked to the ERAD pathway. Many of the identified disease-associated aberrant proteins emerged as a result of studies of well-established mutations in genes linked to specific human diseases. In addition, many of the corresponding gene products are membrane proteins, which must surmount more formidable folding barriers compared to soluble proteins. Although these biased analyses have proved vital to define how aberrant membrane proteins are selected, delivered, and degraded by the proteasome, numerous uncharacterized single nucleotide variants likely exist in the human genome that similarly compromise membrane protein biogenesis and engage the ERAD machinery. These rare variants, which are most likely present in heterozygosity, may predispose an individual to the disease or could be passed onto an individual's progeny.

To identify rare single nucleotide variants in membrane protein-encoding genes that reduce folding fidelity, we employed parallel strategies. First, phenotypic data in the UK Biobank were analyzed using a computational/experimental pipeline we developed to identify individuals with variants in the KCNJ1 gene, which encodes the renal outer medullary potassium channel (ROMK). Because some mutant forms of ROMK are associated with a catastrophic disease, known as type 2 Bartter syndrome, we focused on individuals who harbor single nucleotide variants and exhibit clinical outcomes consistent with a partial loss of ROMK function, i.e., people who are heterozygous at the KCNJ1 locus. Second, we surveyed genomic databases and used computational methods to predict mutation pathogenicity and disease severity. Consistent with prior work, in which we identified Bartter syndrome-associated ROMK mutants selected for ERAD, several of the newly uncovered mutant proteins from both strategies were similarly ERAD-prone. In contrast, other mutant proteins folded and trafficked efficiently to the plasma membrane, yet lacked channel activity. These combined results indicate that rare single nucleotide variants that affect ion channel folding or activity can now be identified in an unbiased manner, thus significantly expanding the number of ERAD-associated substrates amenable to emerging therapeutics. Current efforts are building upon these analyses to other disease-associated ion channels, one of which is linked to autosomal dominant polycystic kidney disease (ADPKD).

Critical beginnings: Tuning protein folding and aggregation pathways at birth

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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. In our study, we employed a combination of single-particle cryo-EM and time-resolved fluorescence-anisotropy, low-pH gels and Western blotting to analyze protein-protein interactions experienced by nascent chains in bacteria. We found that ribosome-bound nascent chains (RNCs) of single-domain proteins establish and expand noncovalent contacts with selected ribosomal proteins and chaperones, as they get longer. On the outer surface of the ribosome, RNCs interact specifically with a highly conserved nonpolar patch of the L23 r-protein. Some of the examined RNCs also comprise an independently compact and dynamic N-terminal region lacking contacts with the ribosome. In all, nascent proteins traverse the ribosome and interact with it via their C-terminal regions, while N-terminal residues tend to sample conformational space and form a compact subdomain. The data reveal a peculiar interplay between RNC independent conformational sampling and interactions with the ribosomal surface and with chaperones, at birth. Strategies to further favor co- and immediately post-translational folding and discourage aggregation will also be discussed.

Roles of RNA quadruplexes and SERF proteins in folding (talk title)
An RNA g4 quadruplex binding protein promotes stress granule formation

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Factors that affect the folding of proteins are not limited to classical protein-based chaperones, like GroEL and the HSPs; other cellular components can also influence folding. G4 quadruplex RNAs are particularly potent protein folding chaperones, magnitudes of orders of magnitude more potent on a mass basis than GroEL. Like GroEL, they can accelerate protein folding and like the periplasmic chaperone Spy they can facilitate folding while the substrate is chaperone bound. SERF related proteins specifically bind to G4 quadruplexes distorting their structure and in doing so inhibit quadruplex chaperone activity.

Liquid-liquid phase condensates govern a wide range of protein-protein and protein-RNA interactions *in vivo*. However, the large and complex nature of key proteins and RNA components involved in the formation of condensates such as stress granules has inhibited a detailed understanding of both how condensates form and the structural interactions that take place within them. We show that the tiny human protein SERF2, initially isolated for its ability to speed disease-associated amyloid formation, is also vital for *in vivo* stress granule establishment. We find SERF2 specifically interacts with non-canonical RNA structures called G-quadruplexes, structures which have previously been linked to stress granule formation. The excellent biophysical amenability of both SERF2 and G-quadruplexes have allowed us to understand the structural transitions leading to liquid-liquid phase transitions and have allowed us to obtain an atomic level structural model for the early stages of ribonucleoprotein condensate formation.

Given SERF's action in speeding protein aggregation and our observations that SERF2 is important for stress granule formation and can engage in liquid-liquid phase separation, it is tempting to consider possible links between these processes. Stress introduced by overexpressing amyloidogenic proteins can lead to persistent stress granules leading to protein aggregation. Within the high-concentration environment of the stress granule, fibrillization of amyloid may be accelerated by SERF2. We wonder if SERF2 family members interacting with G4 quadruplexes may affect the protein folding activities of both G4 quadruplexes and SERF family members. An appropriate balance of the pro-folding activity of G4 quadruplexes and the pro-aggregation properties of SERF2 may be necessary for maintaining liquid-like droplets in a dynamic and reversible state and preventing solidification reactions that can have an irreversible effect on protein structure and function.

SESSION VI

Identification of yeast Hsp90 mutants with differing effects on client proteins (talk title)
Characterization of yeast Hsp90 mutants with distinct in vivo effects

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Hsp90 is an abundant and essential molecular chaperone that mediates the folding and activation of client proteins in a nucleotide-dependent cycle. Hsp90 inhibition directly or indirectly impacts the function of 10-15% of all proteins due to degradation of client proteins or indirect downstream effects. We identified groups of temperature-sensitive Hsp90 mutants that vary depending on how they impact transition through the Hsp90 folding cycle, then conducted a quantitative proteomic analysis to determine whether the mutants impact different sets of clients. Out of 2,482 proteins in our sample set (approximately 38% of yeast proteins), we observed statistically significant changes in abundance of 350 of those proteins. Of these, ~73% with the strongest differences in abundance were previously connected to Hsp90 function. Notably, mutants that affect steps prior to forming the closed, ATP bound state, had distinct impacts from those that affect later steps. We examined the requirement for Hsp90 and cochaperones more closely and found that Hsp90 and cochaperones have two distinct roles in regulating the function of eukaryotic elongation factor 2 (eEF2), which promotes GTP-dependent translocation of the ribosome during translation. Overall, our data suggests that the ability of Hsp90 to sample a wide range of conformations allows Hsp90 and cochaperones to mediate folding of a broad array of clients and that disruption of conformational flexibility results in client defects dependent on those states.

Organismal proteostasis

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I will discuss how proteostasis at the organismal level coordinates and communicates external environmental stresses with internal physiological stress signals to regulate the properties and function of the proteostasis network (PN) across scales of cells and tissues to control proteostasis health and resilience at the organismal level. We will also discuss the evidence for proteostasis collapse in aging and the unexpected complexity to maintain a stable proteome among individuals in a population.

POSTER ABSTRACTS

Delineating mechanisms of HSF1 activation in cancer

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Heat Shock Factor 1 (HSF1) is a key regulator of the heat-shock response (HSR) and crucial for protein homeostasis. In cancer, HSF1 drives a transcriptional program that includes not only canonical heat shock proteins (HSPs) involved in proteostasis, but also non-canonical genes linked to processes such as proliferation, metabolism, and cellular adhesion. We recently identified one such non-canoncial target, JMJD6, as an essential mediator of HSF1 activity through a genome-wide RNAi screen. JMJD6 enhances HSF1 activation by disrupting repressive HSP70-HSF1 complexes. In a positive feedback loop, HSF1 binds to and promotes JMJD6 expression, which reduces HSP70 R469 monomethylation, further disrupting HSP70-HSF1 complexes and enhancing HSF1 activation. This underscores the importance of exploring the broader network of HSF1 interactions. We are now investigating the protein interactions of HSF1 bound at canonical and non-canonical targets with the goal of uncovering key regulators of HSF1 activation in cancer and relevant proteotoxic stresses.

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 peptides into amyloid fibrils. Familial, or genetically inherited, forms of disease arise from mutations in the precursor peptides (including amyloid-β, tau, and TDP-43). However, most cases are sporadic, and amyloid deposition is identified only after symptom onset. While genetic testing can 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 exploit this templating activity as a means to detect the amyloids themselves. We co-transfect seeds with plasmids that express amyloidogenic monomers tagged with the photoconvertible fluorophore mEos3.2. The monomers use the seeds as templates for assembly, and the mEos3.2 tags on those monomers achieve closer interactions that result in high FRET signals. We detect these positive signals using 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 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 factors that differentiate neurodegenerative diseases at the molecular level, improving the understanding of disease mechanisms and allowing for development of specialized therapeutics.

Role of nuclear CCT in regulating RNA pol II transcription

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Chaperonins were initially identified as family of heat shock proteins, grouped by their molecular mass of 60 kDa. The eukaryotic chaperonin CCT (chaperonin-containing complex TCP-1), is composed of two stacked rings of subunits CCT1-8. The functions of the CCT complex have been primarily understood in a cytosolic context, where it works to fold nascent peptides and assemble multi-subunit complexes. However, chaperones have become increasingly relevant in a nuclear context, where they regulate transcription and chromatin organization. Notably, the CCT complex also resides in the nucleus, with nuclear factors composing 48% of its known interactome. However, the specific nuclear functions of the CCT complex remain unclear. We find that the global inactivation of the CCT complex using a temperature sensitive mutant, cct1-2, results in accumulation of high levels of aberrant transcripts. Levels of nascent transcripts produced by RNA Pol II are also dramatically elevated across the genome, indicating that the CCT complex is capable of modulating RNA Pol II activity and fidelity. Our results suggest that the CCT complex is instrumental in maintaining error-free transcription by limiting RNA Pol II activity and enhancing site specificity. We investigate the nature of the CCT-RNA Pol II interaction and the impact upon the transcriptional landscape, under conditions of global CCT inactivation and nuclear CCT depletion.

Synonymous codon substitutions enhance expression of an upstream, divergent gene in E. coli via intragenic transcription

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Synonymous codon substitutions can affect mRNA transcription and translation without changing the protein sequence. For gene expression, understanding how these mutations maintain a functional proteome is important. Using an E. coli plasmid, our lab found that synonymous mutations within cat upregulate the expression of divergent, upstream tetR. We hypothesized that an intragenic mRNA originating from a transcription start site (TSS) within cat facilitates this expression. This mRNA includes a non-coding region from cat and the tetR coding sequence. We then found that synonymous mutations in cat upregulate tetR expression by enhancing intragenic transcription. Unlike canonical tetR transcription, intragenic transcription bypasses tetR auto-repression. For proof, the addition of an antisense terminator downstream of the intragenic TSS suppresses intragenic transcription. We found that the level of intragenic mRNA- not total tetR mRNA, is predictive of TetR protein level. To streamline translational studies, we replaced tetR with mCherry. Our mCherry results show that the translational impact of synonymous mutations is detectable in vivo and in vitro. Intragenic transcription could rescue gene expression when canonical transcription is insufficient, highlighting how synonymous mutations help maintain a functional proteome.

Characterizing direct TDP-43 interactions using crosslinking mass spectrometry

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that affects nerve cells in the brain and spinal cord, leading to progressive muscle weakness and paralysis. More than 30 genes are strongly associated with ALS including the TAR DNA-binding protein 43 (TDP-43). Mutations in TDP-43 lead to its mislocalization from the nucleus to the cytoplasm and formation of insoluble hyper-phosphorylated and ubiquitinated aggregates. These aggregates attract molecular chaperones, ubiquitin, and proteasomal machinery, overwhelming the proteostasis system. Such stress on these pathways leads to further dysregulation of protein turnover and contributes to cellular toxicity. Although interactors of TDP-43 have been identified, the nature of these complexes remains poorly defined. To characterize the direct interactions of TDP-43, we purified TDP-43 complexes from HEK293 cells that had been treated with the MS-cleavable crosslinker DSSO. We identified internal TDP-43/TDP-43 peptides representing both internal TDP-43 crosslinks as well as those from TDP-43 oligomers. Studying TDP-43/interactor peptides uncovered both known and novel TDP-43 protein interactions. We are currently analyzing PTMs present on TDP-43/interactor peptides to understand how PTMs may regulate TDP-43 interactions.

Intestinal Hsp90 promotes neuroprotection via “gut-to-brain” signaling and lipid metabolism

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Heat shock protein 90 (Hsp90) is an essential molecular chaperone involved in maintaining protein homeostasis and regulating organismal stress responses. Previous research has demonstrated that Hsp90 coordinates transcellular chaperone signaling (TCS), a protective inter-tissue communication pathway. Both the intestine and nervous system serve as key tissue hubs for Hsp90-mediated TCS. In this study, we investigated the impact of intestinal Hsp90 overexpression on neuronal health using *C. elegans* models of Alzheimer’s disease that express amyloid beta 1-42 in the nervous system. Using chemotaxis assays, we find that overexpression of Hsp90 in the intestine conferred greater neuroprotection than overexpression within the nervous system itself, suggesting a gut-to-brain signaling mechanism that is initiated by intestinal Hsp90. To elucidate the molecular basis of this Hsp90-mediated gut-to-brain signaling, we performed transcriptomic and metabolomic analyses using RNA-Seq and free fatty acid profiling on our worm strain constitutively overexpressing Hsp90 in the intestine. Differentially expressed genes were significantly enriched in pathways related to lipid metabolism and proteasomal degradation. Utilizing RNA interference-mediated knockdown, we screened these genes in the Alzheimer's disease model using chemotaxis assays. Our preliminary findings highlight the involvement of specific lipid metabolism genes in improving neuronal function and reducing age-associated neuronal toxicity. Using metabolomic profiling we identified pentadecanoic acid, an odd-chain saturated fatty acid commonly found in dairy products, as a potential mediator of the observed neuroprotection. Ongoing chemotaxis and paralysis assays aim to delineate the molecular mechanisms by which pentadecanoic acid and other odd-chain fatty acids confer neuronal protection. These findings suggest that modulation of lipid metabolism by intestinal Hsp90 overexpression plays an important role in reducing amyloid beta-and age-induced neurotoxicity, which could offer future insights into gut-brain communication and potential therapeutic avenues for neurodegenerative diseases.

Understanding an interplay between HSF1 and ERRα in breast cancer

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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. ERRα activity has been shown to be associated with poor outcomes in breast cancer patients. Moreover, in endometrial and esophageal cancer, ERRα confers resistant phenotypes by modulating mitochondrial homeostasis. In oncogenesis, HSF1, a key regulator of stress response, is implicated in promoting cell proliferation and invasion. Despite these insights, any interplay between ERRα 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 ERRα. 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. Because we observed overlapping binding peaks, we further tested and confirmed that co-Immunoprecipitation of either HSF1 or ERRα results in detection of the other, suggesting a transcriptional complex with HSF1 and ERRα. Inhibition of ERRα (XCT790) led to reduction of ERRα as well as HSF1 protein levels while inhibition of HSF1 (DTHIB) caused a reduction in HSF1 as well as ERRα protein levels. Furthermore, inhibition of Hsp90 led to loss of ERRα protein and transcriptional activity, suggesting that transcriptional activity and stability of ERRα may be influenced by heat shock proteins and chaperone systems. Taken together, our results shed light on a novel interplay between HSF1 and ERRα, two critical regulators of cellular stress response and metabolic processes.

Stress or signal? Heat shock's modulation of sporulation efficiency and fitness

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Heat shock, a sudden increase in temperature, is normally considered a cellular stress or a maladaptive change. Across the tree of life, cells encountering heat shock mount a conserved molecular response, including the induction of molecular chaperones and the formation of stress granules and other biomolecular condensates. However, under certain physiological conditions, heat shock may play a role as an adaptive signal rather than a stress. Saccharomyces cerevisiae, or budding yeast, experience a variety of changes in environmental input, including temperature and pH, when undergoing ecological dispersal by birds. In their environments, if there is not sufficient nutrient availability, S. cerevisiae will sporulate into four haploid spores and remain in this state of dormancy until the necessary nutrients are present or they undergo cell death. I hypothesize that by mimicking the physiological signals of ecological dispersal with a concerted 1-hour 42°C heat shock and pH drop to 3, we will observe increased sporulation efficiency and fitness compared to a milder heat shock and acidification such as 1-hour at 37ºC at pH 5.5. Although both temperatures, 37°C and 42°C, induce a measurable heat shock response, sporulation genes are upregulated at 42°C compared to 37°C. This is an indication that there is distinct information encoded in specific temperatures. In addition to revealing the extent to which heat shock of sporulating lab strains alters their sporulation efficiency and viability, I am investigating this question in wild S. cerevisiae strains that are ecologically likely to experience avian-mediated dispersal.

Structural basis for gain-of-activity (GOA) in disease-causing mutation of human mitochondrial chaperonin

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Chaperonins Hsp60s are required for cellular vitality by assisting protein folding in an ATP-dependent mechanism. Human mitochondrial chaperonin hmHsp60 is shown associated with human health conditions including Alzheimer's disease, diabetes, and cancers. Mutations in hmHsp60 are associated with neurodegenerative diseases. Given its central role in biological and disease processes, hmHsp60 has been proposed as a potent diagnostic/prognostic biomarker as well as drug target.

Despite its importance, the mechanistic understanding of how hmHsp60 assists in the folding of mitochondrial proteins remains limited. To address this knowledge gap, we have applied an interdisciplinary approach involving cell-free biochemical assays, cryo-EM, and molecular dynamics (MD) simulations. Our studies have uncovered the basis for subunit dynamics of hmHsp60, the gain-of-activity (GOA) of the pathological mutant, and the underlying mechanism for this GOA. Our ongoing research includes mechanistic studies of binding of ATP and substrate protein to hmHsp60. Together, our research will provide a much needed, comprehensive understanding of hmHsp60 mechanism.

Neuron-specific roles of Hsp90 in regulating proteostasis and aging in Caenorhabditis elegans

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Hsp90 is a molecular chaperone protein that plays an integral role in protein folding within cells and the maintenance of cellular proteostasis, especially under stress conditions. In the nervous system, Hsp90 and other chaperones prevent neuronal protein aggregation implicated in neurodegenerative diseases such as Alzheimer's or Parkinson's Disease. Our lab previously demonstrated that pan-neuronal overexpression of Hsp90 in *C. elegans* induces Transcellular Chaperone Signaling (TCS), which suppresses aggregation of human amyloid beta (Aβ) protein expressed in *C. elegans* muscle cells. Hsp90 is known to influence neuronal chemosensory functions either directly or through its client proteins. Specifically, gustatory and olfactory neurons are essential for regulating developmental decisions (e.g. dauer), lifespan and organismal proteostasis. We hypothesize that Hsp90 modulates these cell nonautonomous processes by interacting with client proteins in these specific neuronal subsets. To investigate the endogenous neuronal expression of Hsp90 and its neuron-specific interactome, we are employing a dual approach. First, using CRISPR-Cas9, we generated a strain with endogenously tagged Hsp90 fused to split GFP. This allows us to visualize Hsp90 expression patterns in neurons and provides us with insights into which neurons require Hsp90 for proper function. Second, we created a strain expressing a GFP nanobody fused to TurboID specifically in neurons. This enables us to identify the neuron-specific Hsp90 interactome via proximity labeling. By analyzing the neuronal expression and interactome of Hsp90 we aim to advance our understanding of how Hsp90 regulates organismal proteostasis through TCS and the nervous system.

Investigating Pab1 condensate dispersal differences among J-domain proteins using HDX-MS

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Across the tree of life, sudden maladaptive changes—stresses—cause cells to form biomolecular condensates and induce molecular chaperones. After the stress subsides, chaperones disassemble these condensates and return the cell to its functional state. However, the molecular mechanisms by which chaperones disperse condensates remains unclear. Here, we explore how poly(A)-binding protein (Pab1, in yeast)—which condenses in response to temperature stress—differentially interacts with the Class A and B J-domain proteins (JDPs), a family implicated in the disassembly of luciferase aggregates. In the case of luciferase aggregates, both classes of JDPs are required, while only Class B JDPs are required for the dispersal of Pab1 condensates. Preliminary work shows that JDPs modulate the temperature at which Pab1 condenses. Specifically, we observe that in the presence of Sis1, a class B JDP, Pab1 condenses at a higher temperature whereas Ydj1, a class A JDP, shifts Pab1 condensation to a slightly lower temperature. This could suggest that Pab1 interacts with Sis1 more than Ydj1 due to the greater shift in condensation temperature. To get a better sense of how Pab1 interacts with JDPs, I used hydrogen-deuterium exchange mass spectroscopy (HDX-MS) to inform on where proteins may be interacting based on how fast they uptake deuterium. So far, HDX-MS has alluded to slightly faster deuterium uptake in Ydj1 compared to Sis1. Future work will explore changes in the deuterium uptake of each JDP in the presence of Pab1 monomers and Pab1 condensates to gain a sense of where these proteins may be interacting.

Intestinal colonization by Prevotella corporis provides protection against protein misfolding and aggregation

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There is no cure or effective treatment for neurodegenerative protein conformational diseases (PCDs), such as Alzheimer's, Parkinson's, and Huntington's. All these ailments are characterized by toxic protein aggregation, resulting in progressive neuronal damage and cell death. Emerging evidence reveals that bacteria play a significant role in the pathogenesis of neurodegenerative diseases; however, the microbial identity and the underlying mechanisms remain elusive. In a screen of over 220 unique bacterial species from the Human Microbiome Project, we found a single bacterial strain, Prevotella corporis, that provides protection against misfolding and aggregation. We employed the Caenorhabditis elegans and Drosophila melanogaster models expressing polyglutamine and Aβ42 to demonstrate that colonization of the gut suppresses aggregation in both models. Further analysis revealed that P. corporis activates the expression of heat shock genes, including hsp70, daf-21/hsp90, and a small heat shock protein, hsp16.2. Our preliminary data indicate that heat shock factor-1 (HSF-1) is not required to induce an hsp70 fluorescent reporter. The observed increase in thermotolerance, lifespan, and disaggregation activity suggests that P. corporis enhances general host proteostasis upon intestinal colonization, and its decreased abundance associated with patients could play a role in the pathogenesis of PCDs.

Evolutionary origins of cellular adaptation and stress response prioritization revealed by single-cell transcriptomics

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Cells activate transcriptional stress response programs to adapt to environmental fluctuations. While cellular responses to individual stressors like heat or hyperosmotic shock are well characterized, responses to complex multi-stress environments remain unpredictable. In the model eukaryote S. cerevisiae, a prevailing model of adaptation is that cells reduce the complexity of the environment into a generic representation of stress via the Environmental Stress Response (ESR), leading to repressed ribosome biogenesis and reduced growth rates. However, this model fails to account for genetic evidence showing that specific factors are essential for responding to distinct stressors. Here, we reconcile transcriptomics with genetics by demonstrating that environmental information is hierarchically represented in transcriptional variation. We exposed 10^5 cells to a structured set of 20 complex environments encompassing changes in carbon source, temperature, osmotic stress, and oxidative stress, and conducted single-cell RNA sequencing. Using spectral decomposition and information theory, we found that information about each environmental perturbation appears in semi-discrete channels of the principal component spectrum, suggesting a functional hierarchy and prioritization scheme by which cells integrate environmental information. Finally, we conducted a directed evolution experiment where cells were grown in constant, osmotic stress for ~3000 generations before exposure to complex environments. We found that cells evolved under a constant environment exhibited a collapse in hierarchical information processing. Rather than compressing the environment into a single ESR parameter or maintaining modular stress responses, cells adapt to complex environments according to a prioritization hierarchy evolved to reflect the statistics of natural environmental fluctuations. Our results establish a direct link between the adaptability of extant natural systems and their cumulative history of selection.

Redox regulation of spatial dynamics and assembly of the sequestrase Hsp42 in yeast

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Molecular chaperones undergo post-translational modifications at specific amino acids under various conditions that determine their relevant functions. These modifications have been interpreted to constitute a type of chaperone code. Under oxidative stress, the chaperone code involves the oxidation of cysteine or methionine residues, leading to changes in chaperone function, such as converting a foldase into a holdase. While the redox chaperone code of the Hsp70, Hsp90, and nucleotide exchange factor families have been extensively studied, the small heat shock proteins have not received the same attention. Therefore, we focus on the oxidation of the small heat shock protein in the model organism budding yeast. Previously, we found that redox-challenged cells lacking thioredoxin reductase (trr1Δ) activate the heat shock response and induce the hyperaccumulation of the small heat shock protein/sequestrase Hsp42 with misfolded proteins. Building on that finding, this study reveals that cysteine 127 (C127) of Hsp42 is redox-active and becomes oxidized in trr1Δ cells, forming a disulfide bond that contributes to Hsp42 oligomerization. This disulfide bond formation at C127 was confirmed by a cys-to-ser mutation and SDS-PAGE with and without reducing agents. Additionally, we detected Hsp42 C127 oxidation in wild-type (WT) cells using divinyl sulfone (DVSF) crosslinking and in chronically aging/stationary-phase cells. The apparent molecular weight of the oxidized form of Hsp42 observed under non-reducing SDS-PAGE is three times the size of the monomer form. Mass spectrometry analysis of this high molecular-weight band indicates that it consists solely of Hsp42, suggesting it is a novel homotrimer. Interestingly, when the prion-like domain (PrLD) at the N-terminus is deleted, the trimer form is not detected; instead, a dimer form is observed. This result suggests that the PrLD domain contributes to the formation of the oxidized Hsp42 trimer. Taken together, our data demonstrate that the small heat shock protein Hsp42 can sense redox signaling through the oxidation of cysteine 127, which subsequently influences Hsp42 oligomerization and may affect its sequestrase ability. Further analyses are needed to examine in detail how C127 oxidation triggers trimer formation, as well as how it affects Hsp42-protein client interactions and foci stability.

DnaJB6 mitigates tau pathology and cognitive deficits

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Tauopathies, including Alzheimer's disease, are neurodegenerative disorders characterized by the pathological accumulation and prion-like spread of tau across synaptically connected brain regions. While molecular chaperones—critical regulators of protein homeostasis—are implicated in tau biology, their specific roles in tau seeding and aggregation remain underexplored.

To address this gap, we conducted a medium-throughput screen of 50 chaperones spanning five distinct families, using Tau RD P301S FRET biosensor cells to identify key modulators of tau seeding. Among these, DnaJB6 emerged as one of the most potent inhibitors, demonstrating robust anti-seeding activity across the screen and in multiple cellular follow up assays. Mechanistic studies revealed that DnaJB6 interacts with tau in cellular aggregation assays, suggesting direct or indirect modulation of tau's pathological behavior. Additional experiments with proteasome and lysosome inhibitors indicated that DnaJB6 may mediate its effects, at least partially, through proteasomal degradation pathways.

To investigate these findings in vivo, we overexpressed DnaJB6 in the bilateral hippocampi of PS19 tauopathy model mice using AAV. Behavioral assays revealed significant improvements in cognitive performance, particularly memory. Ongoing histopathological analyses of brain tissues aim to correlate these behavioral outcomes with tau pathology and chaperone expression levels.

These findings highlight DnaJB6 as a promising modulator of tau pathology, offering new avenues for therapeutic intervention in tauopathies.

Exploring how an active site mutant of the yeast thioredoxin reductase Trr1 causes growth arrest

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Thioredoxin reductase (TrxR) partners with the disulfide reductase thioredoxin (Trx) to protect against ROS-mediated cell death and promote dNTP biosynthesis. To keep thioredoxin in its active state, two catalytic residues (C142 and C145 in yeast Trr1) carry out a thiol-disulfide exchange with thioredoxin, with C145 being the principal catalytic cysteine and C142 serving as the resolving cysteine to liberate the reduced Trx. Here, we report that yeast lacking Trr1 arrest growth when they overexpress a C145A mutant form of Trr1. However, the mechanism through which growth arrest occurs is yet to be determined. To investigate which genetic backgrounds are sensitive to the expression of the C145A Trr1 variant, we further tested whether C145A Trr1 overexpression arrests growth in a wild-type background (BY4742), a Trr1 background (trr1Δ) a thioredoxin-deficient background (trx1Δ trx2Δ), and a background lacking Trr1 and its corresponding thioredoxins (trr1Δ trx1Δ trx2Δ) using a galactose-inducible system. Of these background strains, only yeast lacking Trr1 showed sensitivity to C145A Trr1 overexpression. We are currently investigated whether Trr1 forms disulfide-linked complexes with other proteins (which are potentially essential), using standard disulfide trapping approaches and non-reducing SDS-PAGE. Since Trr1 principally interacts with Trx via C145, we propose that other conserved cysteine residues in Trr1 (notably either C142 or a semi-conserved C-terminal cysteine) may be acting in a primary redox capacity and forming non-productive complexes with previously undescribed proteins. As such, our study may uncover Trx-independent functions of Trr1 and related low-molecular weight TrxRs.

Ribosome association excludes stress-induced gene mRNAs from stress granules

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Cells respond to stress via the integrated stress response (ISR), which causes translation suppression, stress-induced gene expression, and assembly of biomolecular condensates called stress granules. Despite the temporal connection between stress-induced gene expression and stress granules, little is known about how these two ISR branches interface. No direct, quantitative, and mechanistic analysis of the relationship between stress-induced gene mRNAs and stress granules has been performed. Stress granules sequester non-translating mRNAs away from the translation machinery and stress-induced genes evade translational suppression via mechanisms including upstream open reading frames (uORFs). These genes include the transcription factor ATF4 and the translation depressor GADD34, which are crucial for remodeling gene expression in response to stress. Therefore, we hypothesized that stress-induced gene mRNAs would be excluded from stress granules to enable their expression. The key stress-induced gene mRNAs ATF4 and GADD34 shifted from light to heavy polysomes during arsenite stress, suggesting they are translationally upregulated. Through single-molecule fluorescence in situ hybridization, we observed only ~25% of endogenous ATF4 and GADD34 mRNA molecules co-localize with stress granules. Using a panel of small-molecule translation inhibitors, our data reveal association with one or more ribosomes limits stress-induced gene mRNA localization to stress granules. These results suggest translation complexes prevent mRNA assembly into stress granules independently of ribosomal translocation or occupancy across the open reading frame and suggest a role for uORFs in preventing mRNA condensation during stress. We further tested reporter constructs with intact and disrupted uORFs of ATF4 and GADD34. We observed that the presence of uORFs was sufficient to inhibit mRNA condensation into stress granules. Together, we provide evidence that uORFs inhibit mRNA condensation by increasing ribosome association on major stress-induced gene mRNAs.

Death perception in *C. elegans*: Behavioral and physiological responses to the death of neighboring worms

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In *Caenorhabditis elegans*, the death of neighboring worms triggers a range of behavioral and physiological responses that influence the survival and behavior of the living organisms. Here we explore how *C. elegans* perceives the death of other worms and how this information is processed within the organism. We focus on the sensory mechanisms that enable *C. elegans* to detect death cues and examine how these cues alter the behavior of surviving worms, including motility, and reproduction. Understanding how *C. elegans* perceives and responds to the death of conspecifics can provide valuable insights into the broader biological processes of communication, survival, and adaptation in multicellular organisms.

Systematic chemical-genetic profiling as a path to expand precision medicine in cancer

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The advent of cancer genome sequencing technology has promised to revolutionize cancer therapy and drug development by generating extensive catalogs of cancer-specific gene alterations. However, functional and clinical implications of most of these alterations remain poorly understood. To address this gap, we used QMAP-Seq, our high-throughput chemical-genetic profiling assay that leverages next-generation sequencing (NGS) to evaluate the effects of common somatic genome alterations on cell viability in the presence of a collection of compounds. We constructed a CRISPR-screening library targeting the 62 most commonly altered genes contributing to genomic integrity in patient-collected invasive breast carcinoma samples. We then profiled this library of variants against a panel of 760 compounds targeting DNA damage, cell cycle regulation, protein homeostasis, and epigenetic pathways. We identified many expected chemical-genetic interactions, best demonstrated by the synthetic lethal effect of PARP inhibition in BRCA1/2-mutated breast cancer cells. In addition, we identified off-target compound effects, revealing common targets between compounds that share a common chemical scaffold. Finally, we identified many novel clinically-relevant chemical-genetic interactions, comprising numerous events in which engineered cancer variants either increase the sensitivity or resistance of tumor cells treated with a given chemotherapeutic. These findings collectively underscore the transformative potential of QMAP-Seq in bridging the gap between genomic data and clinically-relevant therapeutic strategies.

Effect of vectorial appearance on folding of multimeric proteins in vitro

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A major issue with the study of protein folding in vitro is that many proteins, particularly large proteins, refold inefficiently or not at all. The conventional method for studying protein folding in vitro involves observing protein refolding after a protein has been diluted out of a chemical denaturant (e.g., guanidinium hydrochloride). This method has been successful in studying the folding of small proteins (<150 amino acids) but often does not work with proteins that are large, multimeric, or have otherwise complex native structures, as they often misfold and aggregate upon dilution from denaturant. This limitation has created a bias towards small proteins that do not represent the diversity of proteins in a cell. Within cells, many proteins including large proteins commence folding co-translationally, meaning, vectorially as the protein appears from the ribosome from the N-terminus even before the entire protein is synthesized. An in vitro protein folding study approach that can recapitulate vectorial appearance is required in order to determine the impact of this universal component of the cellular environment on folding success. Here, ClpX, an E. coli enzyme that unfolds and translocate proteins in cells was used to recapitulate vectorial appearance of proteins in vitro. ClpX can recognize substrate proteins tagged at either the N- or C-terminus. Recently, our lab has adapted ClpX to initiate vectorial appearance of fluorescent proteins and shown that translocation through ClpX increases refolding yield. We are now using this approach to investigate the refolding of large proteins including multimeric proteins. Chloramphenicol acetyltransferase (CAT) and Tailspike are homotrimeric proteins and do not refold after dilution from chemical denaturant, but rather misfold with significant aggregation. Preliminary results indicated that ClpX-unfolded and translocated homotrimer e.g., CATssrA is less prone to aggregation when folding is initiated after translocation through ClpX.

Chaperone-dependent aging of single heat shock-induced ribosomal protein condensates

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Protein aggregates formed during heat shock and other cell stresses do not necessarily result from unregulated protein misfolding and are not necessarily detrimental to cells. In fact, recent work has shown that protein aggregation during heat shock, and later recovery through the heat shock response (HSR) pathway, are adaptive mechanisms that are important for cellular fitness. Although the rheological properties of heat shock-induced condensates appear to influence recovery during HSR, it remains unclear how these properties are maintained. In yeast, heat shock causes a pause in ribosomal biogenesis and condensation of newly synthesized “orphan” ribosomal proteins in the nucleolus. Here, we have probed the material properties of single condensates by a variety of in vitro microrheological methods that reveal diffusion and mixing across different length- and timescales. Results from (1) fluorescence recovery after photobleaching (FRAP), (2) fluorescence fluctuations, and (3) bead tracking within single condensates all implicate the chaperone proteins Hsp70 and Sis1, as well as ATP, in the biochemical maintenance of the dynamic liquid-like state of the condensates. In the absence of these factors, we observe reductions in mixing and slowing diffusion over the course of minutes within single condensates, indicating that they may become more viscous over time. Understanding the morphology and material properties of stress-induced protein condensates is critical for discovering the mechanisms by which cells maintain protein homeostasis in changing environments, with broader implications for disease, aging, and adaptation.

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 goals for the successful large-scale production of protein-based pharmaceuticals including interferon and insulin. Generation of protein-based pharmaceuticals by recombinant-DNA technologies is more convenient and affordable in prokaryotic host organisms (e.g., E. coli) than eukaryotes (e.g., baculovirus, Chinese hamster ovary cells). Yet, prior research shows that prokaryotic in-vivo protein overexpression often suffers from the formation of insoluble 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. I hypothesize that fast ejection from the ribosomal exit tunnel in E. coli hampers the ability of newly synthesized proteins to attain their native fold before aggregation takes over. In this work, I took advantage of the selective pressure imposed by the macrolide antibiotic erythromycin to enable the generation of bacterial strains bearing mutated ribosomal proteins (r-proteins) by directed evolution. I found that many of the resulting mutations face the interior of the ribosomal exit tunnel, given that they are typically located across the L4 and L22 r-proteins (substitution mutations) and the 23S rRNA (deletion mutations). These results were confirmed by whole-genome sequencing. The ability of the mutant strains to prevent inclusion-body formation upon protein overexpression was tested. Interestingly, most of the mutant strains significantly increase expressed-protein solubility. Genomic recombineering was carried out to confirm that the mutations close to the ribosome exit tunnel are necessary and sufficient to generate enhanced production of soluble protein. Overall, this study shows that protein-folding quality upon release from the ribosome can be improved by altering the geometry of the E. coli ribosomal exit tunnel. These results have relevant implications for basic-science, biotechnology and the production of protein-based pharmaceuticals. In the future, we plan to generate additional mutations to further enhance protein solubility upon overexpression. In addition, we will probe the ribosome-release time course of the most promising strains to elucidate the mechanism of aggregation-free protein folding at birth.

Putative redox partners and properties of the yeast mitochondrial Thioredoxin Trx3

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Mitochondria represent a major site of reactive oxygen species (ROS) production in eukaryotic cells. To mitigate the adverse impacts of oxidative protein damage caused by ROS, eukaryotes rely on compartmentalized thioredoxin systems that consist of the protein disulfide reductase thioredoxin and its partner enzyme thioredoxin reductase. Although cytoplasmic thioredoxins have been studied extensively, less is known about the mitochondrial thioredoxins, particularly with regard to their substrate proteins/redox partners. In a baker's yeast model, we identified potential redox partners of the mitochondrial thioredoxin Trx3 using the small, thiol-reactive cross-linker divinyl sulfone (DVSF). This analysis revealed a number of proteins involved in redox homeostasis, amino acid metabolism (including members of the branched chain amino acid synthesis pathway), aerobic metabolism and respiration, sulfur metabolism, and protein folding. Experiments are currently underway to investigate the impact of the mitochondrial thioredoxin system on these processes. Separately, Trx3 is targeted by DVSF on multiple cysteine residues, suggesting that non-active site residues may have a redox role. Further work is underway to investigate this possibility. Long-term, our work has the potential to identify novel substrate proteins of the mitochondrial thioredoxin, thereby uncovering how this conserved protein reduction system influences mitochondrial metabolism, protein folding, and other processes during oxidative stress.

Conformational Hsp90 mutants reveal opposing roles in germline development, stress resilience, and longevity

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Hsp90 is an essential and highly conserved molecular chaperone that is required for the folding of hundreds of client proteins. It is crucial for the maintenance of cellular proteostasis and is involved in the regulation of stress responses and aging. While mutational analyses of Hsp90 in unicellular organisms like yeast have elucidated key conformational dynamics that govern its interactions with co-chaperones and clients, the effects of such mutations in multicellular organisms remain poorly understood. Here, we employ the metazoan *C. elegans* to investigate two key Hsp90 point mutations, G304S and E372K (corresponding to yeast Hsc82 G309S and Hsp82-E381K, respectively), which perturb distinct stages of the Hsp90-conformational cycle. Using CRISPR-Cas9 technology, we introduced these point mutations in the sole cytosolic Hsp90 isoform of *C. elegans*. The Hsp90 G304S variant affects an early stage of the Hsp90 conformational cycle that reduces its ability to interact with Hsp70 and client proteins. Conversely, the E372K variant affects a late stage of the Hsp90 conformational cycle with a reduced capacity to release client proteins. Our characterization of these Hsp90 mutants revealed that both mutations significantly impact reproduction and germline development but elicit opposing effects on organismal stress resilience and aging. Hsp90-G304S homozygous mutants are sterile, exhibit germline aplasia, and demonstrate enhanced thermotolerance and increased lifespan relative to wild-type. In contrast, E372K homozygous mutants possess a developed germline and display normal lifespan and thermotolerance. However, they exhibit markedly reduced egg-laying rates despite producing viable offspring. These divergent phenotypes suggest that Hsp90 interactions with germline-specific client proteins may influence germline-to-soma signaling pathways that have implications for reproduction, stress resilience, and aging. Our ongoing research aims to identify germline-specific Hsp90 interactors that modulate these processes, potentially providing novel insights into the mechanisms of germline-to-soma signaling influencing healthspan and stress resistance.

Characterizing the distinctive role of ATP binding in Hsp110 "holdase" chaperone activity

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Heat shock proteins (HSPs) are universally expressed molecular chaperones that are upregulated as a response to cellular stressors such as elevated temperatures. Hsp110s are a unique class of HSPs that act not only as co-chaperones for Hsp70s, but also as independent chaperones capable of preventing the aggregation of proteins exposed to cellular stresses. Our mechanistic understanding of this so-called "holdase" activity is limited, and the importance of ATP binding for holdase activity has been disputed. In this study, we discovered an undocumented divalent cation inhibitory effect that is reversed by ATP. This finding led us to reveal a novel function of the enigmatic C-terminal segment that differentiates in importance between a pair of human/fungal Hsp110 homologs. Furthermore, we have shed light on the role of ATP binding in regulating Hsp110's holdase activity as it pertains to preserving downstream protein refolding competency. Specifically, Hsp110s display a concentration-dependent reduction in the rate of downstream luciferase refolding in exchange for enhanced maximal recovery. However, when using concentrations of holdase beyond a threshold, the reduction of refolding kinetics translates to submaximal recovery. ATP alleviates this negative effect, a finding which was further validated by testing an ATP-binding knockout mutant of Hsp110. Moreover, using an Hsp110 mutant designed to be functionally deficient in Hsp110-Hsp70 complex formation, our results suggest that direct, functional Hsp110-Hsp70 interaction does not alleviate the inhibitory effect of excess Hsp110 on downstream refolding, nor is it essential for transitioning substrates from holdase to folding chaperone machineries. Overall, our biochemical analyses have revealed unique properties of ATP binding as it relates to holdase function. Our current investigations are aimed to characterize the overall effect of Hsp110s on luciferase to dissect the mechanisms of holdase activity.

Interplay between ribonucleotide reductase, peroxiredoxins and disulfide reduction pathways in regulating genomic instability in baker's yeast

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Genetic factors influencing genomic instability, a hallmark of cancer, have been identified as protecting agents against oxidative stress and controlling agents of the rate of dNTP synthesis in Saccharomyces cerevisiae. Disulfide reductases, such as thioredoxins (Trx) and glutaredoxins (Grx), partner with both oxidant defense enzymes such as peroxiredoxins (e.g., Tsa1) and dNTP biosynthesis enzymes (e.g., ribonucleotide reductase (RNR)), but the interplay between these enzymes remains unclear. In this study, we are employing both computational and cellular approaches to understand the relationship between RNR and Tsa1 in maintaining genomic instability. An ordinary differential equation model was constructed with apparent rates to examine if alterations in disulfide reduction pathways cause an imbalance in dNTPs. Without any allosteric regulation of RNR, removal of the disulfide reduction pathways (Trx1, Trx2, Grx1, and Grx2) decreased overall dNDP production but maintained the same ratio of dNDPs as wild type. When removing other oxidant defense enzymes (Glr1, Tsa1, Tsa2, and Trr1), the equilibrating time increased and overall dNDP production was lowered, but the ratio of dNDPs was unaffected. On a cellular level, we are investigating how glutathione reductase (Glr1) influences mutation rates in tsa1-deficient yeast and whether Tsa1 glutathionylation is correlated with mutation rates in certain instances. We anticipate that these approaches will yield new insights into how these interconnected proteins influence genomic stability in cells.

A bipartite tool for systematic manipulation of subcellular protein phase behavior

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Diverse cellular factors modulate the assembly of proteins into biomolecular condensates and aggregates that broadly impact their functions and dysfunctions. Mechanistic details of this modulation are often difficult to elucidate however, because simple genetic and pharmacological perturbations typically do not distinguish direct from indirect effects, while in vitro reconstitutions lack relevant features of the cell. Seeking to bridge this gap, we developed a tool to localize specific factors to self-assembling proteins of interest in a quantitative high-throughput platform. We utilized a bipartite expression system in Saccharomyces cerevisiae in which two proteins of interest are co-expressed as artificial binding partners through genetic fusion to either mEos3.1 – a fluorescent protein uniquely compatible with the flow cytometry-based assay for protein self-assembly, DAmFRET – or a newly developed nanobody against mEos3.1 that is itself fused to an orthogonal fluorescent protein. Thus, the mEos3.1 nanobody functions as a tool to tether specific factors to the mEos3.1 tagged “target” allowing the impact of the interaction on self-assembly to be quantified by DAmFRET. An initial screen of 667 factor and target combinations, including natural and synthetic co-condensates with functional and pathological self-assembling targets, revealed a diversity of assembly-promoting or -inhibiting activities, even for the same factor against different targets. For example, a CAHS (cytosolic abundant heat soluble) IDP involved in desiccation tolerance in tardigrade dramatically increased nucleation frequency of human ASC, a functional non-amyloid polymer, but had no effect on assembly of human tau, a disease-causing amyloid. Additionally, our bipartite approach captures complex concentration-dependencies of both components, such as re-entrant phase transitions. This mEos3.1/nanobody-enabled platform thus allows for the systematic exploration of mechanisms modulating protein self-assembly.

Understanding the role of endocytic coat proteins in de novo [PSI+] prion formation

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Prion diseases are infectious neurodegenerative disorders associated with Creutzfeldt-Jakob disease, Bovine spongiform encephalopathy and Kuru. In prion disease, a normal protein can misfold and further convert normal protein to misfolded forms. These misfolded proteins can form aggregates, which are associated with these diseases. While much work has been dedicated to understanding how prion aggregates impact cellular health, less is known about the cellular mechanisms that contribute to the formation of the prion. To dissect these mechanisms, we use the budding yeast, Saccharomyces cerevisiae, which provides a powerful tool for studying prion formation. The yeast translation termination factor, Sup35, is similar to the human prion protein in that it can misfold and aggregate to form the [PSI+] prion. Sup35 contains an N-terminal prion forming domain (PrD), which is rich in glutamine (Q) and asparagine (N) residues, followed by a C-terminus that is important for translational termination. This PrD domain is required for the formation of the prion. Sup35, as well as the PrD alone, can misfold to form aggregates that are transmitted from mother to daughter cell in a process called propagation. The cellular mechanisms that underlie the formation of [PSI+] is poorly understood. Genetic screens have shown that loss of some endocytic coat proteins (ECP), SLA1 and LAS17, lead to lower prion formation. There are over 60 proteins involved in endosome formation. The goal of this project is to identify other endocytic coat proteins and understand their role in prion formation. Systematic deletion of EDE1, ENT1, ENT2, SLA2, SLA1 and LAS17 ECP genes and testing for prion formation showed that prion formation was only reduced in sla1Δ and las17Δ strains. Since las17Δ strains are associated with growth defects, we turned our efforts towards Sla1. Here, we will present our recent results focused on understanding the role of Sla1 on [PSI+] formation.

Chemical-genetic mapping Identifies TMED2 as a critical factor in cellular adaptation to cancer-associated ER stress

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Cancers must adapt to a diverse set of stressors in order to proliferate and survive. These include but are not limited to oxidative stress, heat shock, and endoplasmic reticulum (ER) stress. The proteostasis network (PN) comprises molecular chaperones, co-chaperones, and a variety of additional support proteins which enable cells to cope with myriad stresses. As such, targeting components of the PN is a strategy for the development of novel treatments for cancer. 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. Recently, I have used an unbiased screen of proteostasis factors to identify genes most critical for adapting to chronic, growth-associated stress. Notably, this screen used a diverse collection of compounds that broadly modulate the proteostasis milieu (e.g. proteasome inhibitors, ERAD inhibitors, heat shock protein inhibitors) at doses far lower than most acute stress studies, thus identifying factors most important for proliferating through stress. Using this approach, I found that TMED2, a transmembrane protein involved in vesicular transport between the Golgi and ER, was among the most critical factors for surviving growth-associated ER stress in all conditions. Remarkably, TMED2 is upregulated and essential in a subset of particularly aggressive triple-negative breast cancers (TNBCs), even in the absence of exogenous stressors. Mechanistically, TMED2 and another TMED family member TMED10 interact and are strongly coessential across cancer cell lines. From these data, I hypothesize that a subset of TNBCs have high levels of cell-autonomous ER stress and are therefore dependent on TMED2, and that loss of TMED2 will suppress TNBC proliferation, and render TNBC tumors more susceptible to existing proteotoxic stress-inducing therapeutics. This new understanding of TMED2 will allow for further insight into the mechanisms behind breast cancer and reveal a potential new target for future treatment.

Sequence-encoded nucleation barriers govern adaptor protein self-assembly

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Adaptor proteins like Bcl10 and ASC drive the assembly of macromolecular complexes in innate immune signaling. These proteins encode nucleation barriers in their sequences, preventing premature aggregation by maintaining a metastable, supersaturated state. Dynamic conformational changes further stabilize this state by forming an ensemble of interchanging structures that delay spontaneous assembly.

Using fluorescence imaging and flow cytometry, we show that the nucleation barrier size depends on the degree of protein supersaturation: higher supersaturation enables sensitivity to weaker stimuli, while lower supersaturation requires stronger signals to initiate assembly. In the CBM complex, we confirm that nucleation barriers regulate signal amplification, while AIM2 inflammasome studies highlight the role of conformational dynamics in controlling aggregation, measured through pyroptosis.

Our findings reveal how sequence-encoded nucleation barriers and protein dynamics precisely control self-assembly, enabling robust immune signaling while preventing aberrant activation.

The effect of aggregate size, thermodynamic stability and protein folding/unfolding on saturation concentration

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The aberrant aggregation of folded proteins is linked to the pathology of several lethal neurodegenerative maladies, including amyotrophic lateral sclerosis and Huntington's disease. Aggregation also poses significant setbacks for the large-scale production and storage of protein-based pharmaceuticals. Previous studies showed that proteins only start aggregating when the total monomer concentration is greater than a parameter known as saturation concentration (SC). The latter is conceptually analogous to the critical micelle concentration of lipids (CMC). However, there is a lack of understanding on how aggregate size and thermodynamic stability affect SC values and aggregation behavior. Our study attempts to address this gap of knowledge via kinetic simulations. The computational results show that, at equilibrium, proteins with small and thermodynamically unstable aggregates undergo some aggregation, in dilute solution. Moreover, "clearcut" SCs are only observed for large aggregate sizes. In contrast, proteins with small and thermodynamically stable aggregates undergo massive aggregation even in dilute solution. In this thermodynamic regime, SCs are "clearcut" at all aggregate sizes, and larger aggregates have larger SC values. Taken together, our study reveals that the best way to escape massive aggregation is to render aggregates as thermodynamically unstable as possible and, surprisingly, to promote large aggregate size. In addition, as expected, total monomer concentration should also be kept as low as possible. These results have implications for understanding the fundamental nature of aggregation and for the design of anti-aggregation agents in the context of neurodegenerative diseases and long-term shelf life of pharmaceuticals.

Investigating thioredoxin-independent roles of Trr1 in saccharomyces cerevisiae: Phenotypic and biochemical characterization of interaction partners

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Thioredoxin reductase partners with the disulfide reductase thioredoxin to maintain redox homeostasis. However, baker's yeast lacking the thioredoxin reductase Trr1 exhibit a more severe slow-growth phenotype than yeast lacking the corresponding thioredoxins, suggesting that Trr1 has thioredoxin-independent roles. Using a thiol-dependent cross-linking approach, we found that Trr1 interacts with additional proteins besides thioredoxin. Using standard proteomic methods, we identified six novel interaction partners of Trr1 and confirmed these interactions via co-immunoprecipitation. We are currently investigating how each of these proteins influences the slow growth phenotype, oxidative stress sensitivity, and ER stress response elevation that is observed in Trr1-deficient cells. Additionally, we are exploring how oxidants influence the activity of a subset of these redox partners, with hopes of determining whether Trr1 can reverse the impacts of oxidation on their activity. Our work has the potential to shed new light on thioredoxin-independent roles of Trr1 and comparable microbial thioredoxin reductases.

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

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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 (Get1/2). We have recently confirmed that Hsp90 directly interacts with Get1 via proximity ligation assay in yeast, indicating that Hsp90 docks to Get1/2 to deliver nascent TAs to the translocase. Additionally, using fluorescently tagged TAs and Hsp104, 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. These results suggest that Hsp90 has a role in TA membrane insertion by driving an alternative route to the conventional 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.

Hsp70s modulate stress protein aggregation and heat shock recovery

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Molecular chaperones play a pivotal role in maintaining proteostasis under normal conditions. Under transient stress, such as heat shock, certain proteins and RNAs assemble into membrane-less, protective organelles called stress granules (SGs). However, SGs are dynamic and reversible. Once stress subsides, the upregulated chaperone network efficiently disassembles SGs. Hsp70s, specifically cytosolic isoforms Ssa1-4 in yeast, are central players in this disassembly process. In addition to disassembly, the Hsp70 family also regulates the heat shock response (HSR) by repressing the transcriptional regulator, Hsf1, under basal conditions. While Hsp70s have many roles, it is unclear how Hsp70s limit protein aggregation of endogenous, stress responsive proteins. Using a ssa1Δssa2Δ double knockout, we explore the involvement of Hsp70 in limiting aggregation of the SG protein, Pab1. In ssa1Δssa2Δ cells, Pab1-GFP forms large cytoplasmic inclusions under basal conditions. While introduction of Ssa1 prevented the formation of these inclusions, as expected, so did the overexpression of Hsp104, or the J-domain protein, Sis1. Following heat shock, normal SG puncta form in all ssa1Δssa2Δ cells, regardless of whether a large inclusion is present. Cells with only the small SG puncta resolved quickly, and those containing a large inclusion resolved slower. However, it is important to note that heat shock leads to the complete recovery of these inclusions, which does not happen in non-heat shocked cells. We suspect that the recovery of these inclusions is likely due to the increased HSR, and therefore upregulated chaperone levels observed in this mutant. Taken together, our data supports the role of Hsp70s in limiting protein aggregation, specifically of endogenous stress-responsive proteins. Furthermore, the increased HSR of ssa1Δssa2Δ cells primes them for fast SG disassembly.

Understanding the structure and dynamics of nascent proteins in the presence of the yrigger factor chaperone

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Correctly folded proteins, bearing a proper three–dimensional structure, are essential for proper biological activity. Many proteins, however, are prone to undesirable misfolding and aggregation, and the mechanism by which this occurs is poorly understood. In the cellular environment, the immediately post–translational events accompanying the release of newly synthesized chains from the ribosome lead to both soluble native states and soluble and/or insoluble aggregates. Given that all three classes of species are typically kinetically trapped from each other, after being initially generated, the early stages of a protein's life are of seminal importance. Here, we focus on the interactions between the ribosome and ribosome–bound fully synthesized nascent chains in the presence of molecular chaperones. Specifically, the cotranslationally active chaperone, trigger factor (TF), is ribosome–associated, and extensively interacts with nascent chains during biosynthesis. The three-dimensional structure and dynamics ribosome nascent–protein complexes have been explored in the absence and presence of TF via a combination of biochemical and structural–biology approaches. Cell–free transcription–translation, unnatural tRNA technologies, chemical crosslinking, single–particle cryo–electron microscopy and fluorescence anisotropy decays contributed to provide insights on nascent–chain interactions and dynamics. In all, this work paves the way to understand the origin of kinetic partitioning between protein folding and aggregation at birth.

Predicting the nucleating structure of amyloids through artificial evolution

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Amyloids can have roles both in normal physiology and in disease states. For example, some amyloids can drive signal propagation in the innate immune system, while others cause aggregation-associated diseases like Alzheimer's. The nucleus structure is the rate-limiting structure on path to amyloid formation, therefore it is the ideal target for blocking aggregation of disease-causing amyloids. However, the nucleus structure cannot be determined experimentally due to its short lifetime on pathway to a more stable amyloid structure. We hypothesize that computational structural prediction techniques such as AlphaFold2 can be used to elucidate the nucleus structure. AlphaFold2 utilizes an evolution-based approach that requires large numbers of sequences presumed to form the same structure to create multiple sequence alignments (MSA). By generating a synthetic mutant pool and functionally selecting for mutations that increase the aggregation rate, a multiple sequence alignment specific to an amyloid's nucleus structure can be created. This alignment could then be used by AlphaFold2 to predict the amyloid nucleus. As proof of principle, we have generated a random mutant pool of human TICAM1. TICAM1 is an innate immune signaling protein containing a conserved amyloid-forming RHIM domain for functional signal propagation. The mutant pool was sorted based on the expression level and extent of self-assembly in yeast cells with the use of Distributed Amphifluoric FRET (DAmFRET) as the functional selection. Next-generation sequencing was used to determine the frequencies of each mutation within the sorted bins and the fold change relative to wild-type aggregation. Mutations with an increased rate of aggregation were used as the MSA for AlphaFold2 structural prediction. This artificial evolution-based approach will be extended for robust determination of the rate-limiting structure to amyloid formation for any amyloid-forming protein in living cells.

Multisite phosphorylation of Hsp70 regulates the response to replication stress via the fine-tuning of ribonucleotide reductase (RNR) complex subunits

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Organisms have developed well-conserved and robust systems to detect and repair damage to their genetic material. A key part of this response involves the multi-subunit complex ribonucleotide reductase (RNR) key for DNA base synthesis. The (RNR) complex comprises four subunits, Rnr1, 2, 3, and 4. Previous studies from our lab have demonstrated that RNR is a client of the molecular chaperone Hsp70 in yeast and mammalian cells. In this study, we queried the impact of Hsp70 phosphorylation on RNR activity in yeast. We screened 146 yeast strains expressing mutations in Hsp70 phosphorylation sites (73 phospho-mutants and 73 phospho-mimics) for sensitivity to the well-characterized RNR inhibitor Hydroxyurea (HU). We 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. 3 of the 13 HU-sensitive Hsp70 phosphorylation mutants displayed reduced Rnr2 or Rnr4 abundance. Interestingly, we also observed an HU-dependent decrease in Rnr1 abundance in 9 of the 13 HU-sensitive mutants. To determine whether Rnr1 abundance changes were due to transcriptional or stability effects, we performed a series of RNR1 promoter-lacz assays. Several Hsp70 mutants in which Rnr1 levels were decreased also showed significant attenuation of RNR1 transcription. We are currently studying whether these mutants display defects in Ixr1, the transcription factor responsible for RNR1 expression. Taken together our results reveal the first example of the Hsp70 chaperone controlling a multisubunit enzyme complex. Fascinatingly, the diverse nature of the Hsp70 phosphorylation sites and motifs involved, it is likely that multiple kinases (and thus signaling pathways) regulate the response to regulation stress through Hsp70 phosphorylation.

Understanding the ubiquitin code: From cellular homeostasis to pathology

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Protein degradation is an essential element of proteostasis and its misregulation is implicated in numerous pathological conditions. Eukaryotic cells clear the damaged proteins or organelles via proteasomes or lysosomes. While specificity of these pathways is dependent on the nature of substrates and their associated proteins like ubiquitin ligases, there is increasing evidence of crosstalk between the pathways under cellular stress conditions initiated by altered ubiquitin linkages on substrates to be degraded. The diverse ubiquitin code (modification) is generated by the huge conformational flexibility of ubiquitin protein itself, which in turn regulates the proteome health by modulating a myriad of signaling pathways. Association of ubiquitin with proteins involves the code writers (E1, E2s and E3s), and the code erasers (DUBs) and their altered levels can be beneficial or detrimental for cellular health. An understanding of specific and global alterations in the ubiquitin modifications on proteins under stress conditions is imperative to restore the normal ubiquitin code in a disease relevant and substrate specific manner. To understand the ubiquitin network, we have employed a deep mutational scanning approach, which has uncovered a spectrum of dominant-negative mutations in ubiquitin protein. The widespread nature of these mutations in ubiquitin implicates the significance of each residue in modulating its complex interactions with the code writers, erasers and the substrates. Many of the mutations which occur outside of the critical lysine residues alter polyubiquitination patterns, leading to defects in ubiquitin conjugation and deconjugation. Our work has identified variants that alter the proteasomal/ autophagic degradation pathways to selectively clear the aggregating proteins. Collectively, our studies not only enhance our understanding of the proteostasis network but also opens new avenues for therapeutic intervention in protein aggregation related diseases

Spatiotemporal analysis of the Mpk1 stress-activated protein kinase reveals a potential role In stress granule dynamics

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The stress-activated protein kinase Mpk1 is a core component of the yeast cell integrity pathway required for multiple stress responses, including heat, caffeine, and hydroxyurea. Previous studies have focused on the activation of Mpk1 and its control over key transcriptional regulators, including Rlm1 and Sw4/Swi6. In this study, we set out to understand the spatiotemporal regulation of Mpk1. As a first step, we assessed the subcellular localization of GFP-tagged Mpk1 under critical activatory stresses. Treatment of cells with caffeine and hydroxyurea promoted relocalization from the cytoplasm to the nucleus in a time-dependent manner. In contrast, heat shock at 39℃ triggered the rapid formation of Mpk1 puncta. Co-localization studies with the PolyA-binding protein Pab1 determined these Mpk1 puncta were present in yeast stress granules. Interestingly, this heat-dependent localization was independent of Mpk1 phosphorylation and kinase activity. Truncation studies confirmed that the long C-terminal domain of Mpk1 was dispensable for localization to stress granules. Follow-up co-immunoprecipitation studies revealed a novel heat-induced interaction between Mpk1 and Pab1. We are currently performing experiments to determine whether Mpk1 can directly phosphorylate Pab1 and whether Mpk1 impacts heat-induced Pab1 phase separation.

Mechanism of protein processing by bacterial ClpB

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Protein disaggregase ClpB belongs to the AAA+ family of ATPases associated with various activities and forms cylindrical hexameric complexes with an open central pore. Since ClpB is not produced in metazoans but is essential for survival and infectivity of many bacterial pathogens, it is considered a promising target for antimicrobial therapies, which generates interest in dissecting its mechanism. During ATP-dependent reactivation of protein aggregates, substrate polypeptides are translocated through the ClpB pore and released in an unfolded conformation. However, the sequence and/or structural motifs recognized by ClpB in its substrates are currently unknown. To investigate the substrate processing by E. coli ClpB, we employed its engineered variant BAP (ClpB, which, like ClpA, interacts with ClpP). Instead of reactivating substrates, BAP translocates them to the associated peptidase ClpP which leads to their degradation. ClpB/BAP activity was considered strictly ATP-dependent but a recent study (1) showed enhanced degradation of □-casein by BAP-ClpP in the absence of nucleotides. Our group has previously found that ClpB forms heptamers in the absence of nucleotides, rather than the canonical hexamers. The central pore in ClpB heptamers is larger in diameter than the hexamer pore. We hypothesized that BAP, like ClpB, forms heptamers in the absence of nucleotide, leading to the observed increased substrate degradation rates supported by diffusion across an enlarged central pore. Indeed, sedimentation velocity experiments showed an increased sedimentation coefficient for BAP-ClpP complexes in the absence of nucleotides, as compared to the ATP-bound complexes. Mass spectrometry analysis of □-casein degradation fragments generated with BAP-ClpP confirmed a higher rate of degradation in the absence of ATP than with ATP. Identification of the peptides generated by BAP-ClpP from aggregated substrates during the early stages of disaggregation suggests that ClpB preferentially recognizes internal sequence segments in its substrates and does not show a preference towards the N- or C-terminal regions. Thus, the mechanism of substrate processing by ClpB differs from that of other Clp ATPases (ClpA, ClpX) that recognize the N- or C-terminal sequences in substrates and initiate translocation from the termini.

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. Additionally, we will use arrest-peptide force profiling analysis to probe CAT folding intermediates. Together, these assays should prove useful for defining the in vivo folding pathway of CAT.

Effects of hydrophobic sequence patterns on the disordered conformational ensembles of foldable proteins

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We have previously shown that the N-terminus of pertactin (PNt) is disordered and highly expanded in isolation, despite having the sequence composition of a well-folded protein. In contrast, the pertactin C-terminus (PCt) is stably folded in isolation. Interestingly, PNt has unusually low clustering of its hydrophobic residues. Increasing the clustering significantly contracts its conformational ensemble. To further test the impact of hydrophobic clustering on folding and disorder, we constructed a series of PCt sequence swaps, preserving the sequence composition (including the charge distribution) of WT PCt but lowering hydrophobic clustering by reordering a small fraction of its residues. Using a combination of CD spectroscopy, SAXS, and SEC, we show that altering PCt hydrophobic patterning leads to unfolded conformational ensembles. However, while these disordered sequences are remarkably soluble, they remain in the poor solvent regime with v < 0.5. Further analyses showed that the position of a pair of Trp residues distant in sequence but forming a pi-pi contact in WT native PCt is unchanged in the PCt sequence swaps, potentially driving the observed collapse. Surprisingly, increasing the sequence distance between this Trp pair led to a further collapse of the ensemble, suggesting an important role for these Trp residues in overall ensemble dimensions. More experiments are underway to test the potential roles of Trp residues in the disordered conformational ensembles of foldable proteins.

Interaction of J-domain proteins with Hsp90 chaperones in yeast and human

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Molecular Chaperones are a critical component of the cellular proteosome, involved in the folding of newly synthesized proteins, protection and recovery of proteins from environmental conditions such as heat or chemical stress, and targeting damaged and unfolded proteins to degradation pathways. The Hsp70 chaperone along with co-chaperones of the J-domain protein (JDP) family, are involved in folding and maintenance of cellular proteins. Some proteins require the cooperation of the Hsp70 and Hsp90 chaperones for their folding, remodeling, and activation. Previous work with E. coli proteins demonstrated that Hsp90 and Hsp70, DnaK in E. coli, directly interact and the interaction is facilitated by a J-domain protein, DnaJ or CbpA in E. coli. Further work showed E. coli Hsp90 forms binary complexes with DnaJ and CbpA, and S. cerevisiae Hsp90, Hsp82, forms binary complexes with yeast JDP, Ydj1. Additionally, ternary interactions between E. coli Hsp90, DnaK and CbpA were observed. In the present study we tested if the interaction between Hsp90 and JDP is conserved in higher eukaryotes. Using human proteins, we observed by both pull-down experiments and biolayer interferometry (BLI) that Hsp90AA1, a human Hsp90, directly interacts with DnaJB1, a human JDP. We also observed that yeast Hsp82 interacts with Sis1, a yeast type II JDP, as well as with Ydj1. Crosslinking followed by mass spectrometry was performed to identify the sites of interaction between Hsp82 and Sis1, Hsp82 and Ydj1, and Hsp90AA1 and DnaJB1. Our results showed that there are multiple sites on JDPs that interact with Hsp90 and multiple sites on Hsp90 that interact with JDPs. A series of cysteine mutants in Hsp82 and Sis1, which lack cysteines in the wild type proteins, were constructed and used in crosslinking experiments with a sulfhydryl reactive crosslinker to confirm sites of Hsp90-Sis1 interaction. Altogether our observations show that complex formation between Hsp90s and JDPs is conserved in higher organisms, although the physiological importance of the interaction remains to be understood.

HSF1 remodels mitochondrial biogenesis and function in cancer cells via TIMM17A

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Mitochondria play critical roles in energy production and cellular metabolism. Despite the Warburg effect, it is evident that mitochondria are crucial for cancer cell survival and proliferation. Heat Shock Factor 1 (HSF1), a key transcription factor in cellular heat shock response, promotes malignancy and metastasis when aberrantly activated. To understand the multifaceted roles of HSF1 in cancer, we performed a genome-wide CRISPR screen to identify epistatic interactors of HSF1 in cancer cell proliferation. Individually verified interactors of HSF1 include those functioning in DNA replication and repair, transcriptional and post-transcriptional gene expression, and mitochondrial functions. Specifically, we found that HSF1 promotes cell proliferation, mitochondrial biogenesis, respiration, and ATP production in a manner dependent on TIMM17A, a subunit of the inner membrane translocase. Our results suggest that HSF1 upregulates the steady-state level of TIMM17A protein via its target genes, HSP60 and HSP10, which are responsible for protein refolding once imported into the matrix. Our proteomics and metabolomics data support a model that the HSF1-HSP60/HSP10-TIMM17A axis remodels mitochondrial biogenesis, protein composition, and energy metabolism to support malignancy and metastasis.

HSF1-amplified ovarian cancer is sensitive to HDAC inhibition

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Heat Shock Factor 1 (HSF1) is a gene that is often amplified in cancers and is known to lead to worse prognosis in multiple cancer types through mechanisms such as increased survival, metastasis, and immune evasion. The cancer with the highest rate of HSF1 amplification is high-grade serous ovarian cancer, a disease with limited treatment options and a 5-year relative survival rate of only 49%. Therefore, targeting HSF1 in the context of ovarian cancer could be a promising new treatment strategy for ovarian cancer patients. Many inhibitors have been developed to directly target HSF1 through mechanisms such as inhibiting its transcriptional activity or targeting it for degradation. These inhibitors, however, have not successfully made it through clinical trials to be translated into available treatment options. Since HSF1 is a transcription factor and relies on the correct epigenetic landscape in order to promote gene expression at its target genes, we hypothesized that there may be epigenetic inhibitors that will be able to decrease HSF1 transcriptional activity and lead to death in ovarian cancer cells that are dependent on the high rate of HSF1 activity. We screened for these drugs in a cell viability assay with a library of epigenetic inhibitors and found that ovarian cancer cells that carry amplification of HSF1 are sensitive to Class I histone deacetylase inhibitors (HDACi). Experiments with HDACi from this screen have revealed that HSF1 is decreased at the mRNA and protein level when treated with Entinostat. Consistent with this, the transcriptional activity for HSF1 is decreased as determined through decreased expression of its canonical downstream targets of heat shock proteins and non-canonical targets as determined from Cut-n-Run performed in the amplified ovarian cacner cell line OVCAR8. Further studies will be aimed to determine the mechanism of HSF1 loss by decreasing class I HDAC activity using class I HDAC inhibitors, and whether HSF1 amplification can be used as a biomarker for sensitivity for class I HDAC inhibitors.

Validating Aha1 as a therapeutic target to reduce tau accumulation

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ATPase homolog 1 (Aha1) is a co-chaperone protein that stimulates the 90-kDa heat shock protein (Hsp90). ATPase activity is essential for Hsp90's regulatory function on client proteins, like tau. Tauopathies, like Alzheimer's disease (AD) are hallmarked by the abnormal accumulation and aggregation of the tau protein. Prior work from our lab using postmortem human AD brain tissues identified Aha1 colocalizes with tau. As part of this prior study, we also demonstrated that Aha1 overexpression in tau transgenic mice increased oligomeric and insoluble tau and led to neuron loss and cognitive impairments. Based on this work, we hypothesize that the reduction of Aha1 will reduce abnormal tau accumulation and related behavioral deficits in PS19 tau transgenic mice. We are examining this in 5.5-month-old PS19 and non-transgenic littermates using viral expression of shRNA to reduce Aha1. At this age tau has already started to accumulate in the brains of PS19 mice. To deliver the shRNA, a retro-orbital injection of AAV-PHP.eB is being used, which allows for broad brain distribution of the Aha1 and control shRNA. The virus also expresses the fluorescent control protein mKATE, which will allow for distribution confirmation. A total of 12 mice per AAV per genotype per sex will be injected. At 8-months old, these mice will be assessed for cognitive and motor behavioral phenotypes. Following behavior tissues will be collected and analyzed for effects on tau as well as to confirm AAV distribution. While this study is still in progress, our pilot studies demonstrate excellent distribution of virus using retro-orbital injection of AAV-PHP.eB. Through this work, we expect to demonstrate whether the reduction of Aha1 is beneficial for tau. We expect that Aha1 ablation will lead to reduced oligomeric and insoluble tau, while improving cognitive behavior. This study is significant, if our hypothesis is confirmed, it will strongly support the further development of Aha1-targeted therapeutics for tau.

Investigating the role of the extracellular scl gene family in TDP-43-mediated neurotoxicity in *C. elegans*

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of neurons controlling voluntary muscles, leading to muscle weakness, atrophy, and paralysis. In ALS, TAR DNA-binding protein 43 (TDP-43) forms abnormal aggregates in the cytoplasm of neurons, which may spread in a prion-like manner. Manipulating extracellular matrix components may lead to inhibition of this transmission. To investigate TDP-43-related neurotoxicity, we use the nematode *C. elegans*, which is amenable to genetic manipulation and has a simple nervous system. Ribosome profiling of young adult C. elegans expressing human TDP-43 pan-neuronally and wild-type animals identified differentially translated genes in the scl gene family, with some members upregulated over 50 fold in TDP-43 transgenic animals. These genes are associated with longevity, stress resistance, and are expressed in the extracellular matrix. Genetic crosses were performed to introduce specific scl gene deletions into the TDP-43 transgenic line. To evaluate the effects of these deletions on TDP-43 neurotoxicity, behavioral assays were conducted to monitor movement. In the thrashing assay, worms are placed in a buffer, and their body bends (thrashes) are counted over 30 seconds. We hypothesize that deleting scl gene members will alleviate motor neuron deficits, increasing the thrashing rate of TDP-43 animals. Wild-type *C. elegans* exhibit approximately 50 thrashes, while TDP-43 transgenic animals average 10 thrashes. Preliminary data indicates that deletion of the scl-9 gene significantly improves the thrashing rate of TDP-43 animals, doubling the average number of body bends. Given that scl genes share homology with human PI16, an inhibitor of matrix metalloproteinase-2 (MMP-2), which is critical for extracellular matrix (ECM) remodeling, our study suggests that scl gene deletions could impact TDP-43 transmission by altering the ECM environment. MMP-2 facilitates the breakdown of ECM components and the deletion of its inhibitor, PI16 orthologs, likely enhances MMP-2 activity. This increased activity alters the ECM potentially reducing transmission of TDP-43 and its associated toxicity. Thus, remodeling ECM components, such as those altered by scl gene expression, may offer a therapeutic approach to attenuate TDP-43 neurotoxicity.

A high-throughput assay to measure autotransporter protein secretion

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Autotransporter (AT) virulence proteins represent the largest family of secreted virulence proteins in Gram-negative bacterial pathogens. All AT proteins have the same basic components for translocation and virulence encoded on a single polypeptide chain, including a β-helical passenger domain that comprises the mature, functional virulence protein. Despite having the same secretion mechanism and basic structure, ATs have a variety of diverse functions such as cell adhesion, invading host cells, and disrupting the host's immune system. Approaches to measure AT secretion are not well studied, and currently, there are no reports of any high-throughput methodologies to directly measure and/or inhibit AT secretion. However, due to their abundance and relevance to infection, it is imperative to develop a high-throughput assay compatible with drug screening and deep-mutational analyses required for identifying factors that will further our understanding of the AT secretion mechanism and how to inhibit it. Here, we present an immunofluorescent high-throughput screening (HTS) assay that measures AT secretion across the outer membrane. The HTS assay will be used to screen AT mutants to elucidate more details about the AT secretion mechanism. Additionally, the HTS assay will be used to screen small molecules to identify those that inhibit AT secretion, enabling new avenues for the development of antimicrobials that target a wide range of Gram-negative pathogens.

Type I myosins actively mediate chromosome organization and nuclear structure

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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. Surprisingly, we did not see a correlation between global genome organization and gene expression. Instead, there is a striking increase in interaction across the long arm of Chromosome XII, where the rDNA is, and is further accompanied by nucleolar spreading. Overall, we have discovered novel roles for Type I Myosins in the maintenance of nuclear organization and processes.

HSP90 mutator alleles: A new mechanism of chemotherapy resistance in head and neck squamous cell carcinoma

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Drug resistance remains one of biggest challenges in Head and Neck Squamous Cell Carcinoma (HNSCC) treatment and contributes to a low 5-year survival rate in HNSCC patients. This issue has remained unchanged over the past decade, while the incidence rates of HNSCC have drastically increased world-wide. There is an urgent need to understand the mechanisms driving drug resistance in HNSCC. Much of drug resistance involves the acquisition of new mutations in cancer cells. The heat shock protein 90 KDa (HSP90) has been previously shown to foster the evolution of acquired drug resistance in yeast and tumors by acting as a potentiator for genetic variation. As a potentiator, HSP90 amplifies the effects of mutations, including oncogenic mutations that confer resistance to cancer drugs. However, efforts over the past decade to target this HSP90 function led to disappointing results in the clinic. Here we present evidence for a new role for HSP90 in cancer drug resistance, involving the less understood mechanism of mutational buffering (that is mitigation). This buffering function enables HSP90 to act as a “capacitor” for genetic variation, fostering the accumulation of conditional mutations within populations, similar to how an electrical capacitor conditionally stores and releases electrical energy. While HSP90 has been shown to act as a capacitor in various model organisms, experimental evidence for the HSP90 capacitor model in tumors is lacking. Work from our lab revealed that HSP90 can buffer disease mutations in humans. We now show that HSP90 buffers mutations in HNSCC cancer cells and tumors. Specifically, we show that HSP90 can buffer mutator alleles in DNA repair genes. Mutator alleles are mutations that increase the mutation rate and have been shown to accelerate the evolution of acquired drug resistance in microorganisms and tumors. Looking closely at the tumor cells with scDNAseq and scDNAseq, HSP90 seems to accelerate the selection of oncogenic variations in the tumor by promoting a low degree of genome instability within tumor lineages with HSP90-buffered mutator alleles. Furthermore, low-level inhibition of HSP90 had a significant synergistic effect with chemotherapy in suppressing drug-resisted linages with HSP90-buffered mutations in tumors. In conclusion, HSP90 acts as a capacitor for therapy resistance in HNSCC, and low-level HSP90 inhibition suppressed this acquired drug resistance. This work reignites enthusiasm for the use of HSP90 inhibitors in cancer therapy.

Exposure to microplastics triggers proteostasis decline in *C. elegans*

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Proteostasis, the ability to maintain a healthy proteome, is essential to cellular function and naturally declines with age. This progressive collapse can be exacerbated by risk factors such as genetic background and the presence of disease-associated proteins and contribute to conformational disorders such as Alzheimer's (AD) and Huntington's Disease (HD). Environmental risk factors, especially air pollution, have also been shown to exacerbate symptoms of AD. Our laboratory previously showed that this disease risk may be partly due to an accelerated collapse of proteostasis. Here, we use *C. elegans* as a model system to determine whether exposure to other pervasive environmental contaminants, especially microplastics, similarly contribute to proteostasis decline. To address this, we tested the effects of nano-polystrene, micro-rubber, and nano-gold on polyQ and Aβ misfolding in *C. elegans*. We found that these disease-associated metastable proteins experienced accelerated misfolding in body wall muscle cells in response to all three materials. We then asked whether exposure to microplastics triggers transcriptional responses to stress, including the heat shock response (HSR), oxidative stress response, the UPRER and the UPRmt, but found no statistically significant differences. However, we found that exposure to some microparticles influences the ability of the animals to launch an HSR. Specifically, we addressed this with a reporter strain that expresses GFP under the control of the C12C8 (inducible cytosolic Hsp70) promoter. While microplastics and micro-rubber did not trigger HS gene expression in the absence of HS, micro-rubber suppressed the transcriptional response following an acute HS. Altogether, our findings suggest that environmental pollutants exacerbate protein misfolding and may interfere with cellular stress responses, potentially exacerbating the progression of neurodegenerative disease.

Hsp70 and Hsp110 chaperones influence human transthyretin protein aggregation

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Molecular chaperones play a crucial role in maintaining proper protein folding and limiting protein aggregation. However, as we age, the decline of the proteostasis network can lead to protein aggregation, which is commonly associated with protein aggregation diseases. Despite significant progress in understanding molecular chaperones, the mechanisms by which chaperones influence intracellular protein aggregation in vivo is not fully clear. This gap in knowledge is particularly evident in diseases like transthyretin amyloidosis (ATTR), which remains significantly understudied. ATTR is characterized by aggregation of the transthyretin (TTR) protein, which forms oligomers and larger aggregates that can infiltrate organs, accumulate intracellularly, and are associated with cell death. To investigate the in vivo relationship between molecular chaperones and intracellular protein aggregation, we express human TTR in a tractable yeast-based system. Our focus is on the highly conserved Hsp70/Hsp110 chaperone network, which has been shown to use ATP hydrolysis and nucleotide exchange factor (NEF) activity for protein disaggregation. We find that the loss of the yeast Hsp110 (Sse1) results in a decrease in monomeric TTR and an increase in SDS-resistant oligomers and high molecular weight (HMW) aggregates. Similarly, the loss of yeast Hsp70s (Ssa1 and Ssa2) results in a modest increase in HMW aggregates. Taken together, our data suggest that Hsp70 and Hsp110 act on TTR aggregates, possibly through an ATPase/NEF dependent mechanism.

Computationally designed binders inhibit spontaneous nucleation of Aβ and tau in cellulo

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Amyloid β (Aβ) and tau are amyloid-forming proteins associated with incurable neurodegenerative diseases. Amyloidogenic sequences are often intrinsically disordered, which leads to challenges in the design of amyloid inhibitors. Sahtoe et al. (2024) leveraged computational tools for the design of monomer binders, overcoming the complications that arise from the disordered nature of these segments. Given the association of amyloid formation with various diseases, these binders hold promise as diagnostic or therapeutic tools. In our study, we validate these binders using DAmFRET, a high-throughput assay that quantifies protein self-assembly in vivo. Our findings demonstrate that the binders selectively inhibit the spontaneous nucleation of Aβ and tau in yeast and a mammalian cell line. However, even when expressed at a 1:1 ratio with the protein of interest, the binders fail to reduce amyloid growth in the presence of pre-existing fibers. These results suggest that computational binder design combined with high-throughput testing via DAmFRET offers a unique pipeline for accelerating therapeutic discovery. For therapeutic applications, targeting amyloid fibers directly may prove more effective, but the "fuzzy coat" surrounding the fibers hinders target accessibility. To address this challenge, we propose tagging the binder to the monomer protein to bypass the fuzzy coat and cap amyloid growth.

Surveying the proteostatic effects of arm-selective UPR activators on the GPCRome

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G-protein coupled receptors (GPCRs) are the largest family of human genes and are a major class of therapeutic targets. Many of the drugs that target these receptors are used to treat diseases that involve various cellular stress responses. However, it is unclear how changes in cellular stress responses may impact the cellular proteostasis of various target receptors. To identify receptors that are sensitive to the unfolded protein response (UPR), we developed a deep receptor scanning platform to measure the expression of 946 GPCRs and their response to small molecules that selectively activate the ATF6 pathway (AA147) or the IRE1 pathway (IXA4). Our preliminary results suggest that most GPCRs are relatively insensitive to the activation of either of these pathways. However, our measurements identify a set of 43 receptors that are activated by one of or both of the small molecules. We are currently engaged in various computational approaches to identify structural features that give rise to the selective proteostatic response of these particular receptors. Together, our findings highlight specific GPCRs that appear to have divergent modes of proteostatic regulation. Our findings also highlight the utility of deep receptor scanning technology as a promising approach to survey the mechanisms of GPCR proteostasis.

Computational prediction of prion proteins in E. coli

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Prions are proteinaceous infectious particles that are unique in their ability to convert folded conformations of the same protein into inactive aggregated counterparts upon seeding. In mammals, prion aggregates cause deadly diseases including Creutzfeldt-Jakob and Chronic Wasting Disease. Prions have been found in many vertebrates and several fungi. However, there are virtually no known prions in bacteria. Both vertebrate and yeast prions comprise an intrinsically disordered and a folded region. In this study, we developed a Python script denoted as CIDR (Cumulative Index of Disorder per Residue) to accurately predict intrinsically disordered and folded regions within proteins. The entirety of the E. coli proteome was analyzed to generate CIDR plots. Based on the plot outcomes in comparison with those of known prions, and on the peculiar distribution of tryptophan in prions, we predicted the presence of six distinct soluble prion proteins in E. coli. Interestingly, some of these putative prions have presently unknown functions in E. coli, while others are known to be involved in various aspects of gene expression. Yet, the latter have not been associated with prion-like behavior, to date. We are currently experimentally testing the above candidates for prion-like behavior in the context of protein misfolding cyclic amplification (PMCA) assays. This work is biomedically significant because it may propel the discovery of new antimicrobial agents based on controlling bacterial populations by ad-hoc triggering prion conversion.

Thermal regulation of innate immune signalosome nucleation – implications for a new function of fever

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Innate immunity serves as the body's primary defense system against infections and maintains immune homeostasis. Dysregulation of this system facilitates autoimmune diseases, cytokine storms, and impaired pathogens defense, all of which contribute to substantial morbidity and mortality. Inflammasomes are the multi-protein complexes that act as key sentinels of innate immunity, triggering inflammatory responses to pathogens and cellular damage. These complexes assemble through a tightly regulated process, but the regulation of inflammasomes with physical conditions remains poorly understood. A critical gap is the role of fever in modulating inflammasome assembly, in part due to the challenges in studying the assembly process in a qualitative way in living cells. To address this, we developed Distributed Amphifluoric FRET (DAmFRET) to quantify protein nucleation with protein concentration in living cells. We have found many inflammasomes component proteins containing death fold domains (DFDs) are supersaturated in cells and require overcoming a nucleation barrier for assembly. We hypothesize that the heat during fever lowers the barrier. Using DAmFRET in Saccharomyces cerevisiae and 293T cell lines, we observed that heat can specifically promote the nucleation of ASC, an adapter protein critical for inflammasome formation. Microscopy revealed that heat does not alter the morphology of aggregates. Cross-species comparisons showed that ASC proteins from different species exhibited enhanced nucleation at higher temperatures in the yeast system, suggesting an evolutionary convergence in ASC behavior. Further investigation with drug treatments indicated that heat-related stressors, such as mistranslation and reactive oxygen species (ROS), can modulate the ASC nucleation barrier. In the future, we aim to define the role of fever-induced cellular stress on ASC nucleation and identify the residues involved by mutation pool. We also plan to determine whether heat directly enhances ASC nucleation with an in vitro system and examines conformational changes in ASC that favor nucleation with NMR. By completing these aims, we will advance our understanding of how fever regulates innate immune protein nucleation. This work will provide a novel platform for studying the regulation of DFDs assembly and offer new insights into patient care for fever.

Regulation of Hsp90 dynamics during stress recovery and aging.

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Multicellular organisms such as Caenorhabditis elegans encounter a multitude of external and internal stressors throughout life, stemming from environmental factors and the aging process. Molecular chaperones, specifically heat shock proteins, are vital for maintaining the organismal stress response and proteostasis machinery. Previous research has highlighted a crucial role for the molecular chaperone Hsp90 in coordinating organismal stress responses via transcellular chaperone signalling, with lysosomal degradation of Hsp90 in the *C. elegans* intestine post-heat shock as a mechanism that promotes stress protection. Here, we are investigating the dynamics and regulation of Hsp90 during the recovery phase following heat shock to understand its contribution to stress resilience amid external stressors and aging-related internal stressors. Utilizing CRISPR-Cas9 technology we have generated *C.elegans* strains expressing endogenously tagged Hsp90 (hsp-90) on the genome with a green fluorescent (GFP) tag and the photoconvertible mEOS2 tag. This allowed us to closely monitor Hsp90 expression and turnover during stress and aging. Our findings reveal that Hsp90 transiently increases after heat shock and subsequently declines in a sinusoidal pattern across all tissues. Photoactivation studies using HSP-90::mEOS2 showed a reduction in fluorescence after heat shock, indicating active degradation of the protein. Interestingly, we find that heat-shocking worms at the pre-fertilization stage led to a more pronounced decrease in Hsp90 fluorescence throughout the organism, whereas post-fertilized mature adults showed a less marked effect. This suggests that the germline may significantly influence Hsp90 dynamics. Moreover, in worms with a developed germline, HSP-90 was secreted from embryos and accumulated extracellularly. RNAi mediated knockdown of the lysosomal components indicated reduced HSP-90 secretion during the recovery phase, further corroborating the involvement of lysosomal degradation in post-HS recovery mechanisms. Based on our data, we hypothesize that Hsp90 expression and degradation are controlled by the germline, which could unveil a novel mechanism by which the germline modulates stress resilience and aging. Our ongoing studies aim to elucidate molecular pathways underpinning this regulation.

On a broader scale, we believe that, understanding this dynamic process will provide us with important insights into how the system can potentially modulate the chaperone expression in specific tissues under the effect of stressors induced by neurodegenerative disorders and other age-associated diseases.

Repurposing an anti-tumor drug candidate for targeting bacterial ATP-dependent chaperones and proteases

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Protein turnover is a critical process for maintaining proteostasis. The bacterial AAA+ chaperones ClpX and ClpA, coupled with the peptidase ClpP form complexes (ClpXP and ClpAP, respectively) which mediate the degradation of proteins in many bacterial species by threading a substrate through the central pore of the chaperone into the proteolytic site of ClpP. Due to the essential function of these proteins in cell survival, they make for promising targets of novel antimicrobial drugs. Another AAA+ chaperone, ClpB, does not bind to ClpP but bears a similar function of threading substrates through a central channel for subsequent reactivation by other chaperones. We have previously discovered that N2,N4-dibenzylquinazoline-2,4-diamine (DBeQ), an inhibitor of the mammalian AAA+ protein p97, an anti-tumor target, inhibits ClpB and suppresses the growth of E. coli. In this study, we investigated the effects of DBeQ on the engineered ClpB variant, BAP that, like ClpX and ClpA, binds to ClpP and mediates degradation of substrates, instead of their reactivation. We found that DBeQ inhibits the BAP/ClpP-mediated degradation of casein with an apparent IC50 ~ 7μM, but does not affect the intrinsic peptidase activity of ClpP. The loss of BAP/ClpP activity is linked to DBeQ-induced dissociation of the BAP-ClpP complex, as determined by sedimentation velocity, size exclusion chromatography, and dynamic light scattering experiments. Despite the similarities between members of the Clp ATPase family, DBeQ inhibits ClpA and ClpX with a significantly lower potency than BAP/ClpB. Analysis of photo-crosslinking of DBeQ derivatives with BAP suggests that DBeQ binds in the vicinity of the coiled-coil middle domain of ClpB. Our results demonstrate that DBeQ shows a significant degree of selectivity towards BAP/ClpB, possibly due to a lack of the coiled-coil domain in ClpA and ClpX.

Polyglutamine aggregation accelerates proteome conformational metastability in aging

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Loss of proteostasis and the resultant accumulation of misfolded and aggregated proteins is a hallmark of aging and age-related neurodegenerative diseases, including Huntington's disease. However, the underlying mechanisms are still elusive. Here, we measured the proteome conformational stability and solubility in the isogenic organism Caenorhabditis elegans expressing different polyglutamine (polyQ) expansions in the context of biological aging. Using limited proteolysis of native extracts together with tandem mass tag-based quantitative proteomics, we found a Q-length-dependent increase in proteome conformational metastability and insolubility in young animals. Moreover, expression of a short polyQ expansion accelerates the age-dependent accumulation of metastable proteins. Our results suggest polyQ exaggerates the age-associated decrease in the conformational dynamics of the substrate unfolding and translocation channel in the proteasome, indicating misfolded proteins are stuck in the proteasome. We are currently using state-of-the-art cryoEM to test the hypothesis.

Neurodegenerative VCP mutant and wild type co-assembly and stoichiometry assessment by single-molecule pull-down assays

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The ATPase valosin-containing protein (VCP), with its hexametric structure, is crucial for unfolding ubiquitinated protein substrates to facilitate proteasomal degradation. A single amino acid mutation within VCP can cause amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD). The mechanism of pathogenesis remains undefined. Studies have revealed persistent ubiquitin-positive TDP-43 aggregates in patient tissues with the VCP mutation, indicating that the ubiquitinated misfolded proteins may not be properly unfolded and degraded. In vitro studies have demonstrated enhanced ATPase activity and unfoldase rate in mutated VCP homomers when compared to the wild type. Notably, while most studies focus on homomeric mutant VCPs patients usually have heterozygous VCP mutations. Therefore, we ask whether mutant VCP monomers can co-assemble with wildtype, and how the mutations affect VCP's ATPase and unfolding function. To test whether wildtype and mutant VCP co-assemble, we co-expressed SNAP- and Halo-tagged, distinctly fluorophore-labeled wildtype and mutant VCP, respectively, in HeLa cell lysate and visualized the assembly stoichiometry using single-molecule pull-down (SiMPull) combined with total internal reflection fluorescence (TIRF) microscopy-based single-molecule photobleaching (SMPB). Our results show ~50% of the wildtype VCP co-assemble with the mutated ones in various. We also found the VCP monomers exchange after maturation. Next, we study the ATPase hydrolysis mechanism of wildtype and mutant co-assembled VCPs using fluorescently labeled ATP in single-molecule TIRF. Unfolding assays will also elucidate the unfolding mechanism of co-assembled VCPs.

Loss of FIC-1-mediated AMPylation activates the UPRER and upregulates cytosolic HSP70 chaperones to suppress polyglutamine toxicity

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Targeted regulation of cellular proteostasis machinery represents a promising strategy for the attenuation of pathological protein aggregation. Recent work suggests that the unfolded protein response in the endoplasmic reticulum (UPRER) directly regulates the aggregation and toxicity of expanded polyglutamine (polyQ) proteins. However, the mechanisms underlying this phenomenon remain poorly understood. In this study, we report that perturbing ER homeostasis in Caenorhabditis elegans through the depletion of either BiP ortholog, hsp-3 or hsp-4, causes developmental arrest in worms expressing aggregation-prone polyQ proteins. This phenotype is rescued by the genetic deletion of the conserved UPRER regulator, FIC-1. We demonstrate that the beneficial effects of fic-1 knock-out (KO) extend into adulthood, where the loss of FIC-1-mediated protein AMPylation in polyQ-expressing animals is sufficient to prevent declines in fitness and lifespan. We further show that loss of hsp-3 and hsp-4 leads to distinct, but complementary transcriptomic responses to ER stress involving all three UPRER stress sensors (IRE-1, PEK-1, and ATF-6). We identify the cytosolic HSP70 family chaperone F44E5.4, whose expression is increased in fic-1-deficient animals upon ER dysregulation, as a key effector suppressing polyQ toxicity. Over-expression of F44E5.4, but not other HSP70 family chaperones, is sufficient to rescue developmental arrest in polyQ-expressing embryos upon hsp-3 knock-down. We further show that knock-down of ire-1, pek-1, or atf-6 blocks the upregulation of F44E5.4 in fic-1-deficient worms. Taken together, our findings support a model in which the loss of FIC-1-mediated AMPylation engages UPRER signaling to upregulate cytosolic chaperone activity in response to polyQ toxicity.

Polysome-sized, translation-inhibited mRNA condensates lurk in polysome profiles of unstressed cells

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In virtually all eukaryotic cells facing sudden maladaptive changes or stresses, such as heat shock or oxidative damage, RNA and protein molecules form cytosolic condensates, or clusters of biomolecules. When these condensates collect into microscopically visible foci, they are referred to as stress granules. During stress, cells can preferentially translate transcripts that avoid stress granule recruitment amidst global translational shutdown. While investigating the link between selective translation and stress-induced condensation, we discovered mRNA condensates whose abundance correlates with the degree of translation initiation blockage in unstressed cells. To establish causality, we engineered yeast strains with synthetic genes encoding GFP with varying hairpin strengths in their 5' untranslated region to block translation initiation. Stronger blocks in translation initiation resulted in stronger condensation as monitored by sedimentation, confirming that initiation blockade drives mRNA-specific condensate recruitment, even in unstressed cells. Unlike stress granules, these condensates are not microscopically visible and are referred to as translation-initiation inhibited condensates (TIICs). When performing polysome profiling, a widely used method for assessing the translational status of mRNAs by separating them into lighter (free) and heavier (ribosome/polysome-associated) fractions via sedimentation, we discovered that translation initiation blocked transcripts can be isolated in fractions associated with polysomes despite not undergoing active translation. We hypothesized that TIICs could be large enough to co-sediment with polysomes, while being translationally inactive—and subsequent experiments confirm this interpretation. Our results raise important questions: How do these findings alter the interpretation of polysome profiling data? Has information gathered from polysome profiling been contaminated with the presence of mRNA condensates correlated with translation initiation inhibition? TIICs may represent a new level of molecular organization that occurs basally in unstressed cells, emphasizing the need to consider what possible regulatory roles condensates may play during normal cellular behavior in addition to their role during stress.

TDP-43 C-terminal domain sequence writes the story of its complex phase behavior

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One in five people beyond 80 years, and most patients with an Alzheimer's Disease Related Dementia (ADRD), have intraneuronal inclusions of TDP-43. This ubiquitous nucleocytoplasmic protein transitions with age and pathology from initially dynamic oligomers and condensates to insoluble aggregates involving its prion-like C-terminal domain (CTD). TDP-43 CTD is known to condense as well as form the core of disease-associated amyloid, as documented by cryoEM of patient samples. Using Distributed Amphifluoric FRET, we recapitulated its phase boundary for condensation, as well as capturing its kinetic barrier to amyloid formation and reveal a requirement of CTD's condensation, as well as buffering of amyloid by the CTD's condensed phase. Here, we used high throughput DAmFRET and microscopy, and atomistic molecular simulations, along with biochemical and microscopy techniques to study the effects of over 300 rational mutations on TDP-43 C-terminal domain phase behavior in cells. We deployed ESM2, a protein language model, and UMAP clustering to decipher the sequence grammar of TDP-43's complex phase behavior from our rich dataset. While condensation of TDP-43 CTD can be reconciled by rational mutations, the sequence grammar to amyloid formation proved more intricate. In this ongoing work, we discuss several elements that contribute to the amyloid kinetics of TDP-43, the most striking of which is the conformation of a single proline at residue 363 (P363) that profoundly influences amyloid nucleation. Any mutation to this residue, regardless of its properties, strongly promoted amyloid formation. This suggests that proline's ability to exist in a cis conformation is crucial for preventing aggregation. Furthermore, this cis conformation appears to be dominant, as most molecules (~90%) exist in the amyloidogenic trans conformation. By conducting the largest atomistic simulations yet undertaken for a disordered protein, we confirmed that the cis isoform of P363 but not other prolines, profoundly changes the global conformational ensemble of TDP-43 CTD. This finding is exciting because it provides a promising mechanistic link between TDP-43 aggregation and the greatest genetic risk factor for ALS: a hexanucleotide repeat expansion at C9ORF72. The latter causes an aberrant accumulation of proline-arginine dipeptide repeat polypeptides, which have been shown to inactivate the major prolyl isomerase, PP1A, which functions to accelerate trans-to-cis isomerization of proline residues. Genetic ablation of PP1A was independently shown to induce TDP-43 pathology in mice. Together with our new findings, these observations allow us to propose that TDP-43 pathology is intimately linked to the cis/trans equilibrium of P363.

Reexamining the role of proteasomal inhibition in amyloid beta 42 nucleation

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Alzheimer's disease (AD) is an uncured, fatal, neurodegenerative disease that impacts ~10% of persons over the age of 65. Both the development and progression of AD are associated with the de novo assembly, or nucleation, of amyloid beta 42 (Aβ42) fibrils. This conformational ordering of Aβ42 monomers into highly ordered amyloid fibrils is both kinetically and thermodynamically limited, making it an extremely rare event. As the occurrence of AD cannot be explained via randomness, it is plausible that the kinetic/thermodynamic nucleation barriers to Aβ42 can be overcome by disease-specific factors. One extrinsic factor thought to facilitate the formation of Aβ42 fibrils is proteostasis decline. It is hypothesized that age-related declines in proteasomal function may facilitate the conformational transition of Aβ42 by increasing the local concentration of both monomers and oligomers. However, Aβ42 nucleation is also kinetically limited. Thus, increased concentration alone is not sufficient to initiate a "seeding" event. In fact, using distributed amphifluoric FRET (DAmFRET) in HEK293T cells, we show that Aβ42 self-assembly is reduced in the presence of the proteasomal inhibitor Bortezomib (0.5μM; 20h). We also show, in vitro, that Aβ42 fibrillization is not directly related to 20S proteasome concentration, nor inhibition. Thus, our central hypothesis is that indirect effects of proteasomal inhibition increase Aβ42's kinetic barrier to nucleation – counter to the longstanding hypothesis that reduced proteasomal function results in favorable conditions for assembly. To this end, we will use DAmFRET to determine whether compensatory increases in alternate proteastasis networks, such as autophagy, result in selective degradation, or sequestering, of on-pathway Aβ42 species required for self-assembly. Using a CRISPRi screen, we will also determine what candidate gene(s) are involved with this phenomenon. These data will determine key regulatory networks that directly impact Aβ42's kinetic barriers – a critical nuance not well explored in the field of AD.

The DnaJB1 chaperone recognizes the N-terminus of tau to enable discrimination of different tau species

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The microtubule-associated protein tau accumulates as beta-sheet-rich amyloids in a number of neurodegenerative diseases, including Alzheimer's disease. To recognize and neutralize misfolded proteins, organisms have evolved a network of proteins called chaperones that maintain cellular homeostasis, and molecular chaperones play an important role in regulating tau misfolding to limit disease. The Hsp70/Hsp40 system is a key component of this network, responsible for folding newly translated, misfolded, or aggregated proteins but it is unclear how chaperones can discriminate between different conformational states of tau. We characterized the interactions between tau protein and DnaJB1, a member of J-domain protein (JDP/Hsp40) family to understand how this chaperone can recognize fibrils. Using a combination of cell biological, biochemical, and biophysical approaches, we explored how DnaJB1 selectively recognizes tau fibrils while omitting soluble monomeric tau species. We first use a cellular model of tau aggregation to discover that DnaJB1 interacts with the acidic N-terminal region of tau, modulating the efficiency of tau assembly. We next confirm in vitro that DnaJB1 and the N-terminal region of tau bind with nanomolar affinity. To understand the details of the interaction, we use a combination of HSQC NMR and cross-linking mass spectrometry to identify potential interaction sites of the DnaJB1:tau complex. Our findings enhance our understanding of how the protein homeostasis network distinguishes between different forms of misfolded tau protein and provide new insight into the mechanism by which DnaJB1 is recruited to tau amyloid fibrils, but not the soluble tau monomers.

A mitochondrial unfolded protein response-independent role of DVE-1 in longevity regulation

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The Special AT-rich sequence-binding (SATB) protein DVE-1 is widely recognized for its pivotal involvement in orchestrating the retrograde mitochondrial unfolded protein response (mitoUPR) in *C. elegans.* In our targeted exploration of downstream factors contributing to the prolonged lifespan observed in sensory ciliary mutants, we discovered that DVE-1 plays a crucial role in facilitating the longevity conferred by these mutants. Intriguingly, this effect is independent of mitoUPR, the canonical function of DVE-1. Furthermore, our investigations unveiled that DVE-1 also participates in extending lifespan under the influence of dietary restriction and germline signaling, again distinct from its role in mitoUPR. Thus, our findings shed light on a novel mitoUPR-independent function of DVE-1 in regulating lifespan. Mechanistically, while mitochondrial stress typically prompts nuclear accumulation of DVE-1 to initiate the transcriptional mitoUPR program, the long-lived daf-10 sensory ciliary mutant facilitates the cytosolic translocation of DVE-1. This observation suggests a cytosolic role for DVE-1 in lifespan extension. Overall, our study implies that, in contrast to the more narrowly defined role of the mitoUPR-related transcription factor ATFS-1, the transcriptional regulator DVE-1 may possess broader functions than previously recognized in modulating longevity and defending against stress.

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 neurodegenerative disease characterized primarily by degeneration of motor neurons leading to 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 shuttling mRNA between the nucleus and cytosol. In ALS, TDP-43 accumulates in the cytosol of affected neurons as 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 examine the connection between proteostasis imbalances and TDP-43 neurotoxicity, we are taking advantage of the simple nervous system and well characterized behavioral assays of the nematode *C. elegans*. Using a ribosome profiling dataset of wildtype and a transgenic line expressing TDP-43 pan-neuronally, three upregulated genes, usp-33, asp-17, and lgmn-1 involved in protein clearance were identified. Genetic crosses and molecular genotyping were used to select TDP-43 expressing worms homozygous for the deletions. To investigate the impact of protein clearance genes on TDP-43 toxicity, organismal and neuronal health were assessed through fecundity and thrashing assays. Fecundity assays were performed by removing hatched larva and counting the progeny. Preliminary data indicates that wildtype animals average 350 progeny while TDP-43 transgenic animals average 250 progeny. Introducing gene deletions further reduced progeny production. Thrashing assays were performed by measuring the body bends of individual worms placed in a buffer over 30 seconds. Preliminary data shows an average of 50 and 10 body bends for wildtype and TDP-43 transgenic animals, respectively. Consistent with the fecundity data, a reduction in thrashing was observed in strains carrying the deletions. To determine the steady state level of TDP-43, western analyses are being conducted. Based on preliminary data, we expect mutants to have increased levels of TDP-43, indicating the genes' involvement in protein clearance. Elevated TDP-43 levels and decline in organismal and neuronal health demonstrate the genes' role in removing toxic TDP-43 in affected neurons. Understanding mechanisms neurons use to clear proteins may reveal therapeutic targets to alleviate ALS pathology.

ERR and HSF cooperatively regulate cellular metabolism

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Metabolism must be precisely regulated to provide appropriate energy and building blocks for cellular homeostasis, growth, and survival. Inappropriate alterations in metabolic flux underlie a wide range of human diseases and even represent a hallmark feature of cancer cells. In this regard, one of the most notable metabolic alterations observed in cancer cells is known as the Warburg effect (aerobic glycolysis), which is characterized by elevated levels of glycolytic flux and aerobic lactate production. The resulting glycolytic program is suited to rapidly metabolize carbohydrates for biomass production in tumors, thus supporting cell growth and proliferation. While aerobic glycolysis is commonly associated with cancer cells, this form of glycolytic metabolism can also promote normal growth and development. In Drosophila melanogaster, aerobic glycolysis is activated prior to the onset of larval development, thus promoting a glycolytic state that supports rapid larval growth. This observation establishes the fly as an ideal model for studying the endogenous molecular mechanisms that regulate the Warburg effect. Previous studies demonstrated that the Drosophila Estrogen-Related Receptor (dERR), an orphan nuclear receptor, is a key activator of aerobic glycolysis. In dERR mutants, late-stage embryos fail to activate aerobic glycolysis, rendering larvae unable to use dietary carbohydrates to support biomass accumulation. While the role of dERR in regulating the larval glycolytic program is well-documented, the mechanisms that control dERR activity during this time remain poorly understood. During our ongoing studies of dERR regulation, we found that heat shock causes dERR to translocate from the cytoplasm into the nucleus in the somatic follicle cells of the ovary. This result raises the possibility that ERR activity is coordinated with the cellular heat response, and implicates heat shock transcription factor (HSF), which is the master regulator of cellular heat response, in mediating environmental regulation of dERR activity and cellular metabolism. Consistent with this idea, our multi-omics data suggested that mutations in the Drosophila ortholog of the HSFs (dHsf) result in larval lethality and induce defects in glycolytic processes and gene expression, similar to those observed in dERR mutants. Moving forward, our goal is to identify the molecular mechanisms by which dHsf regulates dERR activity in the context of glycolytic metabolism. By leveraging the special insights offered by Drosophila, our long-term aim is to not only identify the conserved mechanisms linking cellular stress response, metabolic regulation, and developmental growth but also reveal new metabolic vulnerabilities within cancer cells.

Identification of interneurons involved in stress response

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Organisms experience a variety of environmental stress throughout their life. Most stressors, including heat, hypoxia, radiation, oxidative, osmotic, and pathogenic stress, cause cellular dysfunction and homeostasis imbalances that trigger intracellular stress responses. The ability of an organism to respond to external stress often impacts short-term survival and long-term health. Discoveries in the past 15 years have found a clear role of neural signaling in improving stress resistance across multiple species. However, our understanding of the different types of neurons involved is still limited. Although most sensory neurons are reported to regulate stress resistance, very few interneurons have been identified and studied for stress resistance. We aimed to identify interneurons to outline the neuronal regulatory mechanisms of stress response. Stress assays such as heat stress, oxidative stress, ER (endoplasmic reticulum) stress and mitochondrial stress were performed with neuron specific ablation *C. elegans* strains to screen the interneurons involved in stress response. We found that PVQ neuron ablation worm showed more stress resistance compared with control strain N2, which indicated that PVQ neurons may play a negative role in stress response. We will test whether identified interneurons neurons are involved in integrating multiple stress response pathways.