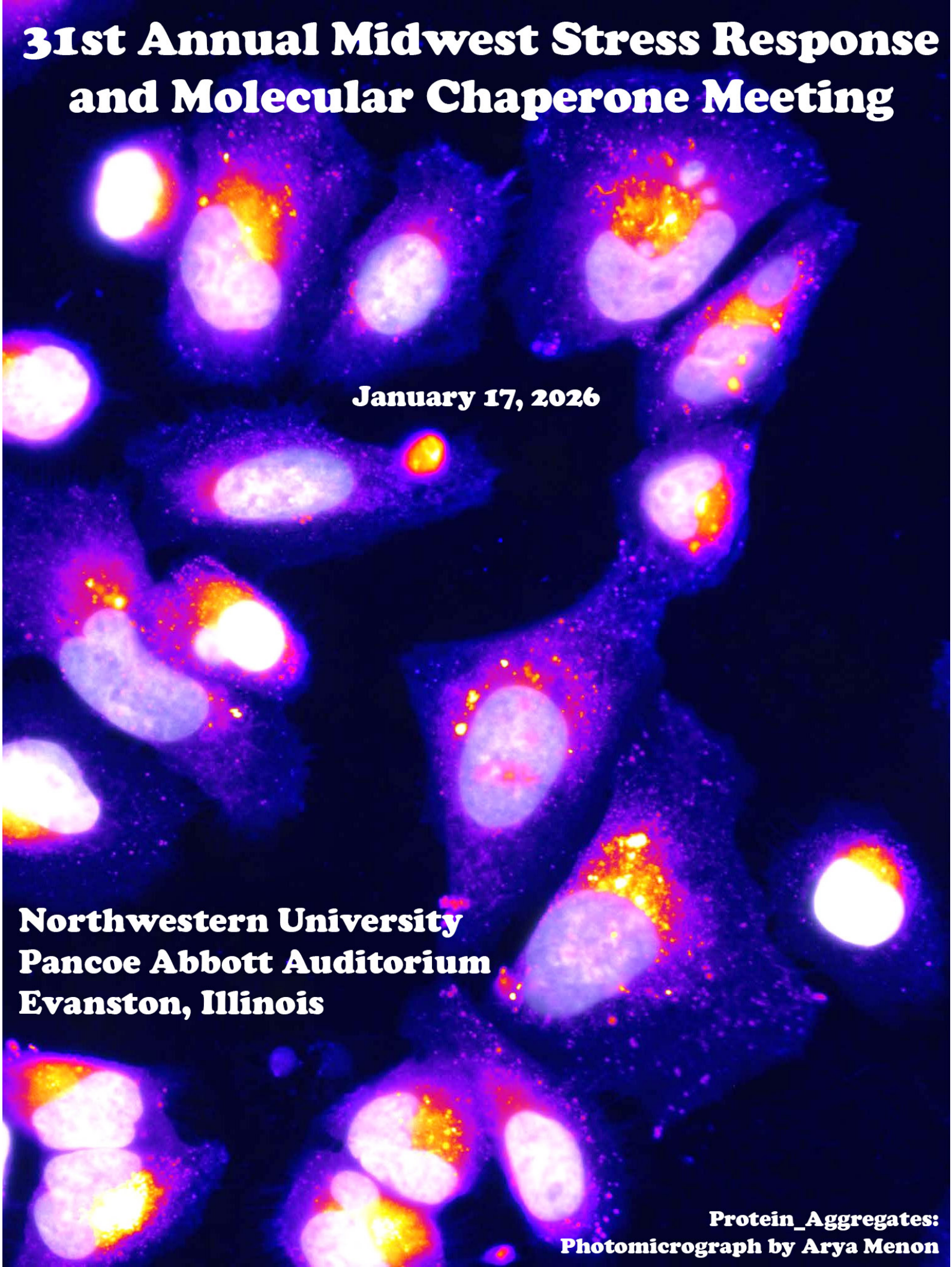
31st Annual Midwest Stress Response and Molecular Chaperone Meeting



January 17, 2026

Northwestern University
Pancoe Abbott Auditorium
Evanston, Illinois

Protein_Aggregates:
Photomicrograph by Arya Menon

31st Annual Midwest Stress Response and Molecular Chaperone Conference

Saturday, January 17, 2026
Hosted by Northwestern University
Northwestern University
Pancoe Abbott Auditorium & Cafe
2200 Campus Drive
Evanston, Illinois 60208

Program Chairs
Randal Halfmann, *Stowers Institute for Medical Research*

Emily Sontag, *Department of Biological Sciences, Marquette University*

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

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

PROGRAM

All times in CST

8:00 - 9:00 AM	**Continental Breakfast**
9:00 - 9:05 AM	**Opening remarks- Rick Morimoto, Northwestern University**
Session I-	**Chaperone Networks**
Session Chair:	**Danish Khan, Virginia Tech and Thomas Stoeger, Northwestern University**
9:05 - 9:20 AM	J-domain proteins' distinct interactions with Hsp70 impact condensate dispersal **Estefania Cuevas-Zepeda** *University of Chicago*
9:20 - 9:35 AM	Elucidating Hsp90's Role in Buffering Mutations in Client Proteins Which Function in rRNA Synthesis and Processing **Isabel Hunsberger** *University of Idaho*
9:35 - 9:50 AM	Chaperone regulation of orphan ribosomal protein condensates **Maya Igarashi** *University of Chicago*

9:50 - 10:05 AM Mechanosensor-mediated Hsp70 phosphorylation orchestrates the landscape of the heat shock response

Siddhi Omkar
The University of North Carolina at Charlotte

Session II- **Regulation of Proteostasis**

Session Chair: **Stephanie Moon, University of Michigan and Tony Pedley, University of Iowa**

10:05 - 10:20 AM Epigenetic control of proteostasis dynamics by RBBP5–mediated H3K4 trimethylation

Bokai Zhu
The University of Pittsburg

10:20 - 10:35 AM Predictive gene expression connects environmental sensing to cell-fate determination

Leah Chaney Winner
University of Chicago

10:35 - 10:50 AM Defective TRAP1 chaperone-mediated Complex II assembly underlies the pathogenesis of SDHAF2-mutant disease

Gianna Mochi
SUNY Upstate Medical University

10:50 - 11:05 AM Heat shock factor 1 maintains mitochondrial proteostasis during copper-induced stress in pancreatic cancer cells

Rejina Shrestha
University of Toledo

11:05 - 11:20 AM **Coffee Break**

Session III- **When Proteostasis Fails**

Session Chair: **Caitlin Wong Hickernell, North Park University and Jian Li, New York Medical College**

11:20 - 11:35 AM Interindividual variation in proteostasis shapes phenotypic traits and stress resilience

Laura Bott
Northwestern University

11:35 - 11:50 PM TPR domain chaperone Sgt2 alters spatial sequestration of mutant huntingtin

Chloe Langridge
Marquette University

11:50 - 12:05 PM A multi-omic approach to HSF1 in the mammalian central nervous system reveals non-canonical roles in synaptic development, maintenance, and integrity

Nicholas Rozema
University of Minnesota

12:05 - 12:20 PM Flash talks: Lexie Berkowicz, Martin Duennwald, Stephanie Gates, Deepika Gaur, Rocio Gomez-Pastor, Rachael Halby, Joshua Mitchell, Stephanie Moon, Akshata Moorthy, Audrey Peng, Tulika Sharan, Shriram Venkatesan

12:20 – 12:30 PM **Group picture**

12:30 – 2:00 PM **Lunch**

1:00 – 2:45 PM **Poster Session**

Session IV- **Protein Degradation**

Session Chair: **Stephanie Gates, University of Missouri and Kuo-Hui Su, University of Toledo**

2:45 - 3:00 PM Deletion of the STI1 domain of yeast Dsk2 causes accumulation of proteasome substrates and enhances assembly of proteasome condensates

Emily Daniel
University of Kansas Medical Center

3:00 - 3:15 PM Proteasomal decline activates HRI kinase and triggers ISR through oxidative stress

Arya Menon
University of Michigan

3:15 - 3:30 PM E3 ligase recruitment by UBQLN2 protects substrates from proteasomal degradation

Sachini Thanthirige
University of Pittsburgh

3:30 - 3:45 PM Reexamining Translation as a Convergent Regulator of Amyloid Nucleation

Alex Von Schulze
Stowers Institute for Medical Research

Plenary session

3:45 - 3:50 PM **Introduction of Plenary Speaker by Randal Halfmann**

3:50 - 4:35 PM **Endoplasmic Reticulum Quality Control and Protein Conformational Diseases**

Jeffrey L. Brodsky
Director, Center for Protein Conformational Diseases
University of Pittsburgh

4:35 - 4:50 PM Q&A

4:50 - 5:00 PM **Closing Remarks by Emily Sontag**

5:00 - 6:30 PM **Reception w/food & beverages in the Pancoe Cafe**

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

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

SPEAKER ABSTRACTS

SESSION I

Chaperone Networks

J-domain proteins' distinct interactions with Hsp70 impact condensate dispersal

Cuevas-Zepeda, Estefania(1), Lin, Kyle(1,2), Sosnick, Tobin R.(1,3), Drummond, D. Allan(1,3,4)

(1) Biophysical Sciences, The University of Chicago, Chicago, IL
(2) Medical Scientist Training Program, The University of Chicago, Chicago, IL
(3) Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL
(4) Department of Medicine, Section of Genetic Medicine, The University of Chicago, Chicago, IL

Molecular chaperones—Hsp104, Hsp70, and Sis1—disperse condensates after stress subsides. The molecular mechanisms for how chaperones engage condensates remain elusive but much is known about how they behave with model substrate, luciferase. Interestingly, to take apart luciferase aggregates requires Hsp104, Hsp70, Sis1, and Ydj1. However, Ydj1 is not required for condensate dispersal and, in fact, inhibits the dispersal of condensates. What makes this inhibitory behavior so puzzling is that Ydj1 and Sis1 are both members of the same J-domain family and are co-chaperones for Hsp70. To investigate the behavioral differences of Ydj1 and Sis1, we noted that Sis1's J-domain is autoinhibited while Ydj1's J-domain remains uninhibited suggesting that the accessibility of the J domain relates to the efficiency of condensate dispersal. To test the impact of the J domain on dispersal, we created two domain-swap mutants where we observed that if the mutant had an uninhibited J-domain, it strongly inhibits condensate dispersal while an autoinhibited J-domain led to dispersal as long as Sis1 was present. With a strong case that the accessibility state of the J-domain could be responsible for inhibition of condensate dispersal, we looked toward its interaction with Hsp70. The J-domain stimulates the ATPase activity in Hsp70. If Ydj1 is prematurely interacting with Hsp70 via its J-domain, then increasing the concentration of Hsp70 should allow for recovery of dispersal. Indeed, we observe that increasing Hsp70 concentration is sufficient to rescue condensate dispersal which supports our model that Ydj1 inhibition likely stems from an uninhibited J-domain. These results suggest that J-domain interactions are key for dispersal and that inhibition is likely to occur off-substrate.

Elucidating Hsp90's Role in Buffering Mutations in Client Proteins Which Function in rRNA Synthesis and Processing

Hunsberger, Isabel, Johnson, Jill

University of Idaho, Microbiology, Molecular Biology, and Biochemistry, Moscow, ID

Heat-shock protein 90 (Hsp90), is a highly conserved and abundant molecular chaperone estimated to interact with approximately 20% of the proteome. In yeast, 14 cochaperones help to fold client proteins and it is known that not every cochaperone is required for every client. Previous high-throughput studies have sought to identify client proteins dependent on a specific cochaperone. Our focus is whether the requirement for Hsp90 or its cochaperones is altered when the client is mutated. After finding a link between yeast Hsp90 and two proteins that function in the synthesis and maturation of ribosomal RNA (rRNA), we sought to understand the role Hsp90 is playing. Rpc19, a subunit of RNA Polymerase I and III, is involved in synthesizing rRNA and Utp21, part of the small subunit processome, is required for the maturation of 18S rRNA. Mutations in both of these essential genes have been linked to human disease; mutations in rpc19 (human: POLR1D) have been linked to Treacher Collins Syndrome 2 and mutations in utp21 (human: WDR36) have been linked to primary open-angle glaucoma. We took advantage of the yeast system to determine Hsp90 and its cochaperones' impact on disease-associated mutations of rpc19 or utp21. We found that deletion or mutation of Hsp90 or cochaperone exacerbated or suppressed growth defects of the mutated clients, suggesting that there are differences in how Hsp90 and its cochaperones mask deleterious effects. We also found that mutants are differentially affected by a specific change, highlighting that a single point mutation in the client protein changes the requirements for the Hsp90 folding cycle. Further analysis of these differences will help elucidate Hsp90 and cochaperone function.

Chaperone regulation of orphan ribosomal protein condensates

Igarashi, Maya, Schaffer, Olivia, Ali, Asif, Lin, Kyle, Lin, Diangen, Cuevas-Zepeda, Estefania, Pincus, David, Squires, Allison

University of Chicago, Chicago, IL

Protein aggregates formed in response to heat shock and other cell stresses are not necessarily caused by protein misfolding nor are they necessarily detrimental to cells. Recent work has shown that these aggregates play an adaptive role in cell fitness during both stress and recovery, and that biomolecular chaperones including Hsp70 facilitate this role. In yeast, newly synthesized “orphan” ribosomal proteins (oRP) aggregate in the nucleolus when heat shock halts ribosome biogenesis. Critically, the rheological properties of these nucleolar oRP condensates are correlated to cell recovery from this stress. However, the location and small size of oRP condensates make them particularly challenging to probe in vivo, so it is unclear how chaperones influence oRP condensate morphology and recovery. We have developed an in vitro cell-free lysate system and an in vitro fully reconstituted system to characterize fluorescently labeled oRP condensates and their interactions with biomolecular chaperones including Hsp70. Using the lysate system, we have shown that Hsp70 activity is necessary for maintaining dispersible oRP condensates. Using a minimal system reconstituted from purified ribosomes to mimic oRP condensates, here we probe whether Hsp70 is also sufficient to ensure condensate dispersibility. By reintroducing components needed for Hsp70 activity (ATP, Hsp70, Sis1), we systematically evaluate their effects and interactions to establish a set of baseline requirements for oRP dispersal. The results indicate that the dynamic properties and dispersibility of oRP condensates depend on concentration of ATP and the Hsp70 chaperone system. The in vitro system we establish here affords us the opportunity to precisely measure the chaperone-dependent biophysical properties of oRP condensates and connect these to the adaptive function of oRP preservation during stress in vivo.

Mechanosensor-mediated Hsp70 phosphorylation orchestrates the landscape of the heat shock response

Omkar, Siddhi, Kline, Jake T. , Grissom, James H., Sun, Diyun, Chi, Richard J., Bard, Jared A. M., Fornelli, Luca, Truman, Andrew W.

Department of Biological Sciences, The University of North Carolina at Charlotte, Charlotte, NC
Department of Biology, University of Oklahoma, Norman, OK
Department of Biology, Texas A&M University, College Station, TX

Cells must respond rapidly to heat stress by activating multiple signaling pathways that preserve proteostasis. In budding yeast, this includes the induction of Hsf1 and Msn2/4-mediated transcription, cell integrity signaling, stress-triggered phase separation of proteins, and inhibition of translation. How these pathways are so rapidly activated and coordinated remains unclear. We show that the mechanosensor Mid2 senses heat-induced membrane stretch and leads to rapid phosphorylation of the cytosolic Hsp70 Ssa1 at a well-conserved threonine (T492). Phosphorylation of T492 leads to epichaperome rearrangement, promoting fine-tuning of multiple cellular processes, including translational pausing, HSF activity, MAPK signaling, and stress granule resolution. Taken together, these results provide a comprehensive, unified theory of the global yeast heat shock response mediated by the Hsp70 chaperone code.

SESSION II

Regulation of Proteostasis

Epigenetic control of proteostasis dynamics by RBBP5–mediated H3K4 trimethylation

Zhu, Bokai, Kubra, Syeda, Sun, Michelle, Dion, William, Catak, Ahmet, Luong, Hannah, Wang, Haokun, Pan, Yinghong, Liu, Jia-Jun, Ponna, Aishwarya, Liu, Lijun, Sipula, Ian, Luo, Jian-Hua, Jurczak, Michael J., Liu, Silvia

University of Pittsburgh, Pittsburgh, PA

Proteostasis is essential for cellular health, with its disruption contributing to aging, neurodegeneration, and metabolic disorders. While the upstream proteostatic stress-sensing and protein-folding mechanisms in the ER and cytosol are well studied, the transcriptional regulation of proteostasis remains incompletely understood, particularly concerning the temporal epigenome dynamics, chromatin landscapes, and co-regulatory networks underlying dynamic proteostasis control. Traditionally, proteostatic stress responses were viewed as acute reactions to noxious stimuli, but recent evidence shows that many proteostasis genes exhibit ~12-hour ultradian rhythms under physiological conditions, driven by a XBP1s-dependent oscillator independent of the circadian clock and cell cycle. By mapping the chromatin landscape of the murine 12-hour oscillator, we identified RBBP5—an essential subunit of the COMPASS complex responsible for H3K4 trimethylation—as a pivotal epigenetic regulator of proteostasis dynamics. In contrast, histone acetyltransferases and H3K9 acetylation were dispensable for dynamic proteostasis gene expression. RBBP5 is not only indispensable for the 12-hour oscillator but also essential for the transcriptional regulation of diverse proteotoxic stresses response, by coactivating XBP1s and promoting H3K4me3 deposition at the promoters of proteostasis genes. As a result, loss of RBBP5 sensitizes cells to proteostatic stress due to impaired autophagy. Proximity labeling of H3K4me3 further uncovered a dynamic chromatin-associated proteomic architecture, including components of COMPASS, the Integrator complex and SWI/SNF remodelers, that constitutes the transcriptional response to proteostatic stress. Together, these findings reveal a previously unappreciated epigenetic timing mechanism distinct from circadian control and establish RBBP5 as a central regulator of proteostasis dynamics, essential for maintaining cellular resilience.

Predictive gene expression connects environmental sensing to cell-fate determination

Chaney Winner, Leah (1), Pincus, David (1,2,3), Drummond, D. Allan (2,4,5)

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(2) Institute for Biophysical Dynamics, University of Chicago, Chicago, IL
(3) Center for Physics of Evolving Systems, University of Chicago, Chicago, IL
(4) Department of Biochemistry & Molecular Biology, The University of Chicago, Chicago, IL
(5) Department of Medicine, Section of Genetic Medicine, The University of Chicago, Chicago, IL

Saccharomyces cerevisiae, or budding yeast, experiences heat shock (ambient temperature to 42ºC) when ingested by birds, which are known ecological dispersal vectors for yeast. After dispersal, yeast cells are expelled into a new environment where they are likely to experience starvation. Under starvation conditions, budding yeast can undergo sporulation, a meiotic process that increases cell survival in unfavorable conditions. I am investigating the predictive capacity of heat shock on cell-fate determining processes such as sporulation. It is well understood that budding yeast's ability to sporulate diminishes at higher temperatures. Yet, I observe a large overlap between the transcripts induced 6 hours into sporulation and transcripts induced after a 42ºC heat shock for 90 minutes. These data raise the possibility that heat shock induces a transcriptional program that prepares cells to undergo sporulation. Additionally, this shared transcriptional response is not solely dependent on regulation by Hsf1 (heat-shock-induced transcription factor) and Msn2/4 (general and nutrient stress-induced transcription factors). I discovered that a subset of these upregulated transcripts is under the control of Sum1, a middle-sporulation transcriptional repressor known for its role in regulating the meiotic recombination checkpoint. My working hypothesis is that heat shock can act as a signal that prepares cells for sporulation by upregulating sporulation transcripts normally induced at the meiotic recombination checkpoint. Using a high-throughput quantitative sporulation assay and transcriptomics, my work aims to define the transcriptional regulation underlying this uncharacterized long-term heat shock response and reveal its downstream functional effects on cell-fate determination.

Defective TRAP1 chaperone-mediated Complex II assembly underlies the pathogenesis of SDHAF2-mutant disease

Mochi, Gianna(1,2,3), Nahhas, Nathan(1), Nguyen, Mylinh H.(1), Khan, Ariba(1), Burkacki, Julia K.(1), Woodford, Mark R.(1,2,3)

(1) Department of Urology
(2) Department of Biochemistry & Molecular Biology
(3) Upstate Cancer Center, SUNY Upstate Medical University, Syracuse, NY

Succinate dehydrogenase (SDH) couples the Krebs cycle to electron transport and is integral to oxidative phosphorylation. Dysregulation of SDH activity is known to contribute to tumorigenesis, while loss of function mutations in SDH or the assembly factor SDHAF2 are associated with pheochromocytomas, paragangliomas and related familial tumor syndromes. Mortality in affected patients is high due to symptomatic dysregulation of adrenal function. Despite this, no targeted therapies are available. The mitochondrial chaperone TNF-receptor associated protein 1 (TRAP1) controls mitochondrial respiration in part through regulation of the SDH subunits SDHA and SDHB, but impact of TRAP1 in SDH assembly is poorly characterized. Our data showed that the pathogenic SDHAF2-G78R mutant exhibited reduced SDHA interaction and diminished SDH activity. We also found SDHAF2-G78R mutation precluded TRAP1-SDHA interaction, while TRAP1 knock-out disrupted SDHA-SDHAF2 complex formation. Taken together, our data suggest a previously unrecognized interplay between SDHAF2 and TRAP1 in regulating SDH complex assembly.

Heat shock factor 1 maintains mitochondrial proteostasis during copper-induced stress in pancreatic cancer cells

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Department of Cell and Cancer Biology, College of Medicine and Life Sciences, The University of Toledo, Toledo, OH

Pancreatic ductal adenocarcinoma (PDAC) cells exhibit elevated copper levels (an essential cofactor that is tightly regulated under normal conditions) to support tumor growth. However, excessive copper can disrupt mitochondrial function by downregulating essential mitochondrial metabolic proteins, including iron–sulfur (Fe–S) cluster proteins, triggering a proteotoxic stress response and copper-mediated programmed cell death. This vulnerability suggests that PDAC cells require an adaptive mechanism to preserve mitochondrial proteostasis when copper levels rise. Heat shock factor 1 (HSF1), a key regulator of the proteotoxic stress response, is highly expressed in PDAC and supports protein stability, mitochondrial function, and tumor progression. Whether HSF1 plays a role in the stability of Fe-S cluster proteins in PDAC cells under copper-induced stress remains unclear. We hypothesize that HSF1 sustains PDAC cell viability during copper overload by preventing the copper-mediated loss of mitochondrial Fe–S cluster proteins. In PDAC cell lines MIA PaCa-2 and PANC-1, treatment with the copper ionophore elesclomol-copper (ES-Cu) reduced protein levels of Fe–S cluster proteins, including ferredoxin 1 (FDX1), aconitase 2 (ACO2), and lipoic acid synthase (LIAS), without altering their mRNA levels. Notably, HSF1 overexpression rescued the copper-induced decrease in expression levels of FDX1, ACO2, and LIAS. We identified the mitochondrial protease Lon protease 1 (LONP1) as a key mediator of ES-Cu-induced decreases in ACO2 and LIAS. Pharmacological inhibition of LONP1 or genetic knockdown of LONP1 reversed ES-Cu-reduced expression of Fe-S cluster proteins. Interestingly, manipulating HSF1 did not affect LONP1 protein levels, and HSF1 translocated to the mitochondria upon copper stress and increased its binding to LONP1. The ES-Cu-mediated decrease in cell viability could be rescued by copper chelator tetrathiomolybdate, suggesting copper-specific cytotoxicity. A combination of HSF1 inhibitor with ES-Cu significantly decreased PDAC cell viability compared to the individual treatments. Our findings suggest that HSF1 protects PDAC cells from copper-induced mitochondrial dysfunction via maintaining the stability of Fe–S proteins. Targeting HSF1 in combination with copper-based therapies may enhance treatment efficacy by disrupting mitochondrial stress adaptation in PDAC.

SESSION III

When Proteostasis Fails

Interindividual variation in proteostasis shapes phenotypic traits and stress resilience

Bott, Laura, Kim, Jeonghyun, Brielmann, Renee, Imran, Ghania, Sala, Ambre, Morimoto, Richard I.

Northwestern University, Department of Molecular Biology, Evanston, IL

Protein homeostasis (proteostasis) is essential for cellular function, promoting health and resilience. Genetic mutations or proteotoxic stress can disrupt this process, destabilizing the proteome, driving disease onset, and influencing evolutionary trajectories. Here we show that even among isogenic individuals, variations in proteostasis contribute to phenotypic heterogeneity and differential stress resilience. To investigate this, we engineered a conformational switch to monitor protein folding stability in C. elegans in real time. This system assesses proteostasis function and the role of triage mechanisms in balancing folding and aggregation. Our study reveals a bimodal response to proteotoxic stress, with individuals either managing the stress or reaching a threshold of proteostasis insufficiency. Beyond this point, quality control mechanisms fail, leading to misfolded protein accumulation. This threshold acts as a switch within physiological conditions, where variations in proteostasis determine whether an individual maintains homeostatic balance or succumbs to proteotoxic burden. These findings demonstrate that intrinsic differences in proteostasis function drive variability in health and stress resilience, leaving some individuals more vulnerable to proteome damage. Our results underscore the importance of population-level heterogeneity in linking gene variants, proteotoxic stress, and phenotypic consequences, with implications for disease susceptibility and treatment strategies.

TPR domain chaperone Sgt2 alters spatial sequestration of mutant huntingtin

Langridge Chloe, Sontag, Emily

Marquette University, Milwaukee, WI

Huntington's disease (HD) is an inherited fatal neurodegenerative disease that degenerates the basal ganglia impairing voluntary movement and cognitive function. A mutation in the huntingtin gene causes the formation of protein deposits of mutant huntingtin (mHTT) in the striatum and cortex of the brain. There is evidence of a breakdown in protein quality control (PQC) mechanisms, but how HD impairs proteostasis remains unclear. One mechanism of PQC called spatial sequestration sequesters misfolded proteins into spatially distinct membrane-less compartments. Chaperones are well known for sorting misfolded proteins, soluble oligomers, and insoluble aggregates into these compartments. However, the role of co-chaperones in spatial sequestration remains unclear. Using a yeast model, we are investigating the role of the Hsp90 co-chaperone Sgt2 in the spatial sequestration of mHTT. Deletion of Sgt2 increases mHTT toxicity by 6% and increases mHTT inclusion formation by 25%. We find that the dosage of Sgt2 alters the dynamics of mHTT. Mild overexpression of Sgt2 increases the number of mHTT inclusions and the inclusions more frequently locate near the endoplasmic reticulum, however severe overexpression of Sgt2 greatly reduces the number of mHTT inclusions. Because Sgt2 is well known for inhibiting the ATPase activity of Ssa1, we are investigating the interaction between Sgt2 and Ssa1 in altering mHTT solubility and inclusion formation, and we propose Sgt2 inhibits Ssa1 refolding to promote stabilization of misfolded proteins by Ssa1. Understanding the role of Sgt2 in protein quality control will uncover its potential as a target for protein misfolding diseases.

A multi-omic approach to HSF1 in the mammalian central nervous system reveals non-canonical roles in synaptic development, maintenance, and integrity

Rozema, Nicholas(1), Zarate, Nicole(1), Gerlach, Kinsey(1), Pennell, Harrison(1), Zhang, Ying(2), Gomez-Pastor(1)

(1) Department of Neuroscience, Medical School, University of Minnesota, Minneapolis, MN
(2) Minnesota Supercomputing Institute, University of Minnesota, Minneapolis, MN

Heat Shock transcription Factor 1 (HSF1) is a stress protective transcription factor canonically known for its role in cellular stress responses and the regulation of protein quality control systems. However, recent evidence suggests HSF1 might play distinct roles in the central nervous system. Through a combination of ChIP-seq, RNA-seq, and proteomic analyses in the mouse striatum, a brain region that controls movement and distinct forms of cognition, we have demonstrated HSF1 primarily controls the expression of key synaptic genes related to cytoskeletal stability and synaptic receptor anchoring. HSF1 displays distinct DNA binding profiles depending on developmental stage, promoting cell proliferation and axon/dendrite elongation early in life and transitioning to synaptic specialization later. Further, we demonstrate that these binding patterns are disrupted in Huntington's disease (HD), a dominantly inherited neurodegenerative disease affecting the striatum, in preference for genes related to immune response. Synaptic deficits are a hallmark of early HD and we hypothesize that the disruption of HSF1 binding activity during these critical windows may contribute to the decrease in synaptic density observed in HD. Striatal synaptic connections also decline during normative aging and contribute to alterations in synaptic dysfunction and behavioral deficits. HSF1 levels also decline during aging, but whether HSF1 is responsible for age-dependent loss of striatal synapses is unknown. We have utilized both chronic and acute reduction of HSF1 levels to demonstrate that HSF1 depletion results in a specific reduction in excitatory striatal synapses and cognition. We are currently making strides towards understanding the role of specific HSF1-regulated genes at the synapse in the context of individual pathways within the striatum. These results demonstrate an emerging role for HSF1 in synaptic gene regulation that has important implications in synapse maintenance and memory during aging and in neurodegeneration that can result in effective therapeutic interventions to ameliorate cognitive deficits.

SESSION III

Flash Talks

Disease Subtyping via a Novel DAmFRET Biosensing Approach

Berkowicz, Lexie(1, 2), Sancho Salazar, Laura(1), Sansaria, Shrutika(1), Wu, Jianzheng(1), Von Schulze, Alex(1), Mehojah, Justin(1), Song, Xiaoqing(1), Halfmann, Randal(1, 2)

(1) Stowers Institute, Kansas City, MO
(2) University of Kansas Medical Center, Department of Biochemistry, Kansas City, KS

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. Cryo-EM studies suggest that amyloids in these diseases are structurally unique, propagating through self-templating "seeds" that maintain the conformation of the original amyloid. Using our innovative biosensing system, we exploit this templating activity as a means to detect the amyloids themselves. We co-transfect HEK293T cells with seeds and plasmids that express amyloidogenic monomers, and these monomers use the seeds as templates for assembly. This assembly is then measured using Distributed Amphifluoric FRET (DAmFRET), a flow cytometry assay that quantifies in vivo protein self-assembly. This method has higher throughput than current biosensor systems that require either transient or stable lentiviral transduction of the reporting construct prior to a transfection step with the seeds. The improved throughput allows for larger screens without sacrificing sensitivity. Additionally, this technique can use both in vitro and patient derived amyloids as seeds for assembly. In an effort to differentiate amyloid structures between disease subtypes, we screened brain lysates from patients with Frontotemporal Lobar Degeneration with TDP-43 Deposits (FTLD-TDP) Types A, B, and C. Our preliminary results show potential differences in assembly profiles, and further refinement and screening may uncover mutations with specific recognition of one subtype over another. Further expansion of these biosensing constructs to include other amyloids and diseases 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.

Hop/Sti1, a condensing holdase

Duennwald, Martin, Rutledge, Benjamin, Choy, Wing-Yiu, Prado, Marco

University of Western Ontario, London, Ontario, Canada

Hop/Sti1 is well established as a key player in proteostasis for its regulation of the ATPase and chaperoning activities of Hsp90. We find that under stress, Hop/Sti1 adopts previously unappreciated functions to prevent detrimental effects associated with protein misfolding. Specifically, we show that upon exposure to stress in yeast and neuronal cells, Hop/Sti1 forms cytoplasmic inclusions, which do not overlap with previously stress induced structures, such as stress granules, IPOD and JUNQ. Hop/Sti1 inclusions rapidly dissolve once the stress subsides. Furthermore, our in-vitro studies document that Hop/Sti1 forms condensates driven by intermolecular interactions of its TPR domains together with Hsp70 and that Hsp90 dissolves these condensates. We propose a mechanism by which Hop/Sti1 holds misfolded proteins under acute stress conditions, thereby preventing their toxic accumulation and enabling efficient refolding or degradation once the stress subsides. In neurodegenerative diseases, however, this holding by Hop/Sti1 can stabilize toxic conformers of misfolded proteins, such as alpha-synuclein and TDP-43, thus possibly contributing to their cellular toxicity.

Characterizing the BAG-1 co-chaperone interaction with p97 and the 26S proteasome

Bansah, Lois, Hsieh, Yu-Shao, Koomson, Victoria, Gates, Stephanie

Department of Biochemistry, University of Missouri, Columbia, MO

BAG-1 is a nucleotide exchange factor and Hsp70 co-chaperone. BAG-1 regulates cell survival and protein stability through its interactions with heat shock proteins, anti-apoptotic factors, and the 26S proteasome. It is a promising cancer therapeutic target due to its roles in promoting proliferation and preventing drug-induced apoptosis. Recent proteomics studies identified a novel interaction between BAG-1 and p97, a highly conserved AAA+ ATPase that extracts ubiquitinated substrates from the ER lumen for proteasomal degradation during ER-Associated Degradation (ERAD). However, the mechanistic basis of the p97-BAG-1 interaction and its impact on ERAD remain unclear. BAG-1 contains a Ubiquitin-like domain and may serve as a shuttle factor for delivering ubiquitinated proteins to the proteasome. We have used many biophysical techniques to characterize the interaction between p97 and BAG-1, including enzymatic assays, isothermal titration calorimetry, and single-molecule cryo-EM. We are studying how BAG-1 impacts the unfoldase and ATPase activity of p97 to determine how BAG-1 may facilitate substrate handoff to the 26S proteasome. We have also developed fluorescence polarization assays to study how BAG-1 influences ubiquitination of chaperone clients and its effect on substrate degradation by the 26S proteasome. These studies will reveal how BAG-1 interacts with chaperones and co-chaperones and the downstream impact on proteasomal degradation, and their implications in ERAD.

Multiple extractases ensure fidelity in organelle targeting and orientation of small membrane proteins

Gaur, Deepika, Acquaviva, Brian, Wohlever, Matthew

Department of Cell Biology, University of Pittsburgh, Pittsburgh, PA

The correct targeting and integration of small membrane proteins (<70 residues) is essential for organelle function. However, small membrane proteins depend on error-prone post-translational targeting and insertion pathways. These small proteins often lack structural features for accurate targeting, resulting in mislocalization and mixed membrane topologies. To dissect the trafficking behavior of small membrane proteins we developed a split-luciferase based in-vivo assay that reports on membrane protein insertion and orientation in real time. Using Ost4, an ER-anchored small membrane protein, our assay showed that it inserts into both the ER and the outer mitochondrial membrane (OMM) with mixed topologies. Deletion of the OMM extractase Msp1 leads to the accumulation of Ost4 on the OMM. Similarly, deletion of the ER extractase Spf1 leads to the accumulation of Ost4 inserted in the ER with the wrong topology. Thus, both extractases act post insertion to maintain targeting and orientation fidelity. To test whether this is a general phenomenon, we analyzed Tom5 and Tom6, two small mitochondrial membrane proteins chosen for their minimal cytosolic domains. Both proteins exhibited mixed topologies and Spf1 dependent clearance from the ER, supporting the model that orientation errors are a common feature of small membrane proteins. Our findings establish in-vivo luminescence assays as powerful tools for monitoring membrane protein trafficking and highlight the critical role of extractases in correcting topological errors during biogenesis.

Silmitasertib, an FDA-designated orphan CK2 Inhibitor, ameliorates neuropathology and motor dysfunction in a Huntington's disease mouse model

Pelzel, Ross, Herbst, Miaya, Rozema, Nicholas, Solem, Melissa, Gomez-Pastor, Rocio

University of Minnesota, Minneapolis, MN

Huntington's disease (HD) is a devastating autosomal dominant neurodegenerative disease that manifests with progressive motor, cognitive, and psychological impairments. HD is caused by a polyQ (CAG) repeat expansion in the huntingtin (HTT) gene, leading to the misfolding and aggregation of mutant HTT protein (mHTT) and the preferential degeneration of the striatum. Previously in our lab, we identified Protein Kinase CK2 as an important kinase involved in the pathophysiology of HD. Specifically, the alpha prime catalytic subunit of CK2 (CK2α') is upregulated in HD, and genetic depletion of CK2α' in HD mice results in improved motor behavior, decreased mutant Htt aggregation, and improved neuronal function. Silmitasertib (CX-4945) is an FDA designated orphan drug that inhibits CK2. This study aims to investigate whether CX-4945 treatment ameliorates HD pathology. We treated prodromal and late symptomatic HD mice, and used a variety of immunohistochemical, biochemical, physiological and behavioral approaches. We found that CX-4945 presented benefits in the amelioration of HD pathophysiology in both treated groups. Importantly, we found CX-4945 decreased mHtt aggregation, increased DARPP-32 expression and excitatory synapse density, restored homeostatic astrocyte phenotypes and ameliorated neuroinflammation and microgliosis, altogether resulting in improved motor behavior. These results support CX-4945 as a strong candidate for a targeted therapy to treat HD.

Cellular Stress to Collective Action: How Heat and Social Context Influence Honeybee Fanning Behavior

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Social insects depend on collective behaviors to mitigate various environmental stressors; however, the physiological mechanisms linking individual stress responses to group-level outcomes remain unclear. In honeybees (Apis mellifera), thermoregulatory fanning is essential for maintaining brood development and colony homeostasis. Our preliminary results suggest that prior heat exposure impacts fanning behavior and that the social environment during heat stress further shapes these responses. Here, we investigate the role of heat shock proteins (HSPs), conserved markers of cellular stress, in modulating fanning behavior after different thermal and social experiences. Bees previously exposed to heat exhibited elevated HSP expression, altered fanning dynamics, and responded at higher temperatures, indicating a physiological cost of thermal stress. Prior social context modulated this relationship, suggesting an interaction between temperature and social experience on an individual's physiology and collective behavior. These findings highlight a mechanism by which social context and past stress shape individual behavior, revealing implications for understanding group-level resilience during stress.

Not just your grandpa's FAIM: eukaryotic FAIM proteins can broadly inhibit protein aggregation

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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. Aggregate 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. 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 FAIM protein from other species was active in a manner like human FAIM, and whether the substrate profile for this protein shared similarity to FAIM. Eukaryotic FAIM proteins have variable sequence identity, with mouse FAIM sharing high identity (~90%) and fruit fly FAIM having much lower identity (~37%). However, the AlphaFold predicted structure of these proteins reveals near identical structures, with the same topology and fold found in human FAIM. After expressing and purifying FAIM proteins from several eukaryotes, we found that all the FAIM proteins were active, with similar activity to human FAIM. These proteins were able to prevent aggregation of aggregation-prone proteins not found in their native environment (human IAPP, AB42, etc.), demonstrating that FAIM substrate recognition is not species-specific. This work suggests that FAIM is either a broadly acting protein with high promiscuity, or that FAIM substrates can all be defined by a common motif that FAIM identifies. Further investigation of this protein and its function across multiple species will reveal greater 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.

tRNA synthetase activity is required for stress granule and P-body assembly

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Translation elongation defects trigger the integrated stress response (ISR), however, if and how ribosome stalls are resolved to permit mRNA release for ribonucleoprotein (RNP) granule formation is unclear. We find that preventing tRNA aminoacylation leads to the accumulation of persistent uncollided ribosome stalls, which block stress granule and P-body formation despite ISR activation. Collided ribosomes exhibit rapid clearance via ZNF598-dependent ribosome-associated quality control within 4 hours, whereas uncollided stalls resist clearance and persist for at least 16 hours. Puromycin treatment releases these persistently stalled ribosomes and restores RNP granule assembly. The block in stress granule formation is generalizable across tRNA synthetase inhibitors and amino acid deprivation. Therefore, stress granules function as signal integrators reporting translation elongation status when translation initiation is inhibited. These results demonstrate translation quality control pathways selectively clear collided ribosome stalls and that translation elongation stress inhibits RNP granule formation despite ISR activation. The results of this study suggest uncollided ribosome stalls may be tolerated and serve an adaptive role in co-translational processes essential for cell function.

Exploring Protein Birth in Real Time and Directed Evolution as Complementary Approaches to Probe and Control Protein Folding and Aggregation in the Cell

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Optimization of protein folding and elimination of protein aggregation are important goals to achieve the large-scale production of a variety of protein-based pharmaceuticals including monoclonal antibodies, interferon and insulin. Towards this end, recombinant-DNA technologies in prokaryotic hosts (e.g., E. coli) rather than eukaryotes (e.g., baculovirus, Chinese hamster ovary cells) are in principle more convenient and affordable. Yet, prior research shows that prokaryotic in-vivo protein overexpression often suffers from the formation of aggregates known as inclusion bodies. While it is presently unknown why bacterial cells are more likely to yield insoluble overexpressed proteins, we hypothesize that the extremely fast release of fully synthesized nascent chains from the ribosome and suboptimal molecular-chaperone concentrations contribute to protein misfolding and aggregation at birth. Fast ejection from the ribosomal tunnel in E. coli may hamper the ability of newly synthesized proteins to attain their native fold before aggregation takes over. In this research, we take advantage of the selective pressure imposed by the macrolide antibiotic erythromycin to generate bacterial strains bearing mutated ribosomal proteins via directed evolution. Many of the resulting genomic mutations face the interior of the ribosomal exit tunnel and are located across the uL4 ribosomal protein and the 23S rRNA. Interestingly, some of these mutations are computationally predicted to reshape the geometry of the tunnel by slightly narrowing its width. Importantly, the uL4 and 23S-rRNA mutant strains were found to increase newly-synthesized protein solubility. In order to understand the origin of this effect, we also explored the kinetics of newly synthesized protein folding and aggregation in real time by fluorescence anisotropy. These studies, which were performed in the absence and presence of molecular chaperones, provide a conceptual foundation to understand and reshape the mechanism of protein birth. In all, this research will promote novel approaches targeting the large-scale and efficient production of a variety of practically useful proteins, including pharmaceutically active nanobodies, antibodies and bioinspired materials.

Type I Myosins Actively Mediate Chromosome Organization and Nuclear Structure

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Genome organization correlates with cell fate including cell-type and physiological states. The disruption of sequence-specific organization correlates with deleterious phenotypes including cancer, limb deformations, and neurological defects. However, it is not known if such a relationship is causative as the mechanism(s) responsible for faithfully re-arranging chromosomes into a cell-type dependent organization through each round of cell division is still lacking. Based on our previous work that Type I Myosins are required for efficient transcription-dependent long-range gene movement, we investigated if myosins have a more global role in the nucleus. To do this, we used an Anchor-Away strategy to deplete Type I Myosins from the nucleus. Significantly, we found that nuclear Type I Myosins are required for growth. Upon subjecting nuclear Type I Myosin depleted cells to Hi-C, we found an overall increase in global contact frequency across the genome accompanied by changes in nuclear morphology. Surprisingly, the collapse in nuclear morphology was non-correlative to major changes in gene expression. Overall, our data suggest that nuclear Type I Myosins play a role in nuclear shape maintenance and we plan on further elucidating its roles in genome organization.

Molecular Determinants of Generalist Function in Hsp70

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Generalist molecular chaperones like Hsp70 occupy a unique position in biology, using a single, deeply conserved molecular architecture to recognize and triage an enormous variety of diverse misfolded proteins. How this simple scaffold encodes such expansive, but not unlimited, client specificity remains a central open question in proteostasis biology. Here, we decode these molecular rules by systematically mapping the sequence-function landscape of DnaK (E.coli Hsp70) through deep mutational scanning and screening across a panel of rewired chaperone networks and defined client contexts. We have developed a high-throughput in vivo screen for CHAperone-Mediated Protein Folding (CHAMPFold) that couples DnaK activity to a sequencing-based readout. Using CHAMPFold, we quantify the effects of thousands of DnaK mutations during heat stress and in strains lacking key partner chaperones and co-chaperones (ΔdnaJ, Δtig, ΔhtpG, ΔclpB), revealing how generalist specificity emerges from both intrinsic determinants and inter-chaperone cooperation. To capture client-specific rules, we further challenge each DnaK variant with a library of folding-defective mutants of the model client chloramphenicol acetyltransferase (CAT-I). This design exposes how perturbations in DnaK remodel its ability to rescue distinct misfolding client topologies, providing an orthogonal, client-centered view of specificity that complements the proteome-wide assays. Together, these datasets uncover previously unrecognized functional hotspots and identify condition- and client-specific specificity determinants, defining how Hsp70 architecture encodes generalist activity across diverse proteostatic contexts. CHAMPFold thus establishes a scalable platform for mapping the molecular logic of chaperone-client interactions with both proteome-wide and client-specific resolution.

Unraveling TDP-43 sequence contributions to its complex amyloid phase behavior

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Intraneural amyloid deposits involving the C-terminal domain of TDP-43, a ubiquitous nucleocytoplasmic protein, is implicated in most patients with Alzheimer's Disease Related Dementia (ADRD) and in 20% of cognitively normal people above 80 years. TDP-43 oligomerizes via its N-terminus, localizes to the nucleus via an NLS, binds RNA via two RRMs, and phase separates via its disordered C-terminus (CTD). Recent cryoEM findings reveal polymorphism in amyloid core involving the CTD, in different clinical presentations of dementia. Understanding the underlying sequence contributions to pathologic TDP-43 species remains a key problem. Using yeast as a model, high-throughput Distributed Amphifluoric FRET (DAmFRET), semi-denaturing agarose gel electrophoresis and fluorescence microscopy, we queried inactivating point mutants and truncations of TDP-43. We find that CTD is sufficient on its own and is necessary for amyloid formation, while multiple regions of the protein inhibit it. Amyloid propensity anticorrelates with visible condensation of the various TDP-43 mutants. CTD exhibits sub-diffraction sized emulsions with a sharp phase boundary, and amyloid formation exclusively happens beyond that point. Mounting evidence points to amyloid nucleation in living cells happening predominantly at the surface of oligomers in vivo. We also show that the yeast model of amyloid formation holds true in human cell lines as well as when seeded in yeast by human functional amyloids expressed in trans.

Overall, we present a rich resource on the complex phase behavior of TDP-43 CTD that would serve to understand kinetic and thermodynamic drives at play en route pathological aggregation.

SESSION IV

Protein Degradation

Deletion of the STI1 domain of yeast Dsk2 causes accumulation of proteasome substrates and enhances assembly of proteasome condensates

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Dsk2/Ubiquilin is a partially disordered protein with multiple domains that are conserved across eukaryotic kingdoms. The N-terminal ubiquitin-like (UBL) domain and C-terminal ubiquitin-associated (UBA) domain enable it to function as an adapter of the ubiquitin-proteasome system by binding proteasomes and ubiquitin chains, respectively. Between these domains, Dsk2/Ubiquilin commonly has one or two STI1 domains that have been associated with (co)chaperone-like activity for specific clients and display an ability to bind helical peptides in vitro and in cells. This suggests that Dsk2/Ubiquilin functions in protein stability and degradation. We found that S. cerevisiae Dsk2 has three transiently helical regions that interact with its STI1 domain and contribute to Dsk2 phase separation in vitro. In yeast, Dsk2 contributes to the phase separation of proteasomes after gradual carbon starvation. These proteasome condensates contain Dsk2, Rad23, proteasomes, and polyubiquitinated substrates. To determine whether the internal domains of Dsk2 contribute to the formation of proteasome condensate assembly, we deleted the STI1 domain or the three transient helices from Dsk2. Our data show that the interaction between these domains is not essential for proteasome condensate formation. Furthermore, the STI1 domain and these transient helixes appear to function beyond their binary interaction, as we observed pleotropic effects when comparing the mutants. Nevertheless, in general, proteasome condensate assembly positively correlated with Dsk2 protein levels and the accumulation of K48-linked polyubiquitinated substrates. Our data suggest that the STI1 domain of Dsk2 is involved in limiting the size of proteasome condensates under at least two carbon stress conditions and processing of polyubiquitinated proteins in yeast, supporting expansion of the definition of Dsk2/Ubiquilin beyond that of a simple ubiquitin-proteasome system adaptor.

Proteasomal decline activates HRI kinase and triggers ISR through oxidative stress

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Proteasome function declines with age and aging-associated neurodegeneration. The Ubiquitin Proteasome System (UPS) maintains protein homeostasis by degrading nascent, regulatory, and misfolded proteins. During stress, the Integrated Stress Response (ISR) preserves proteostasis by phosphorylating the translation initiation factor eIF2α by one of four kinases, GCN2, PERK, PKR, or HRI, leading to global translational inhibition. ISR dysregulation is associated with aging and neurodegeneration, yet the mechanisms by which the ISR and proteasome suppression are interconnected remain unclear. Through genetic, molecular, and microscopy assays, we examined ISR activation under chronic proteasome inhibition by treating human cells with low doses of the chemotherapeutic bortezomib (BTZ) for 24 hours. We show this system recapitulates many aspects of proteasome decline associated with aging and disease. We find that chronic BTZ exposure induces eIF2α phosphorylation and stress-induced gene expression, indicating persistent ISR activation. CRISPR/Cas9-mediated HRI knockout eliminates eIF2α phosphorylation under chronic proteasome suppression, identifying HRI as the primary ISR kinase in this context. We demonstrate that HRI is activated by oxidative stress upon chronic BTZ treatment and rule out heme deprivation and the OMA1/DELE1 pathway. We find that reactive oxygen species are detectable early and remain elevated during BTZ treatment, and the addition of antioxidants inhibits ISR activation. Therefore, chronic proteasome dysfunction triggers ROS production, leading to persistent HRI-dependent ISR activation. These results may have therapeutic implications for aging, cancer, and neurodegenerative diseases.

E3 ligase recruitment by UBQLN2 protects substrates from proteasomal degradation

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Ubiquilins are a family of cytosolic proteins essential for cellular proteostasis that are traditionally recognized for their role as shuttle factors within the ubiquitin-proteasome pathway. Paradoxically, Ubiquilins have also been observed to stabilize substrates. The basis of this triage decision is unknown. Ubiquilins are also associated with neurodegenerative diseases, as specific mutations in UBQLN2 are causative for X-linked forms of ALS. In addition, Ubiquilins undergo LLPS (Liquid-Liquid Phase Separation) in a concentration-dependent manner. Several mechanistic aspects of Ubiquilin function remain unresolved, including the physiological role of phase separation, the distinct contributions of each paralog, and the effects of ALS-associated mutations on Ubiquilin function. To further elucidate these mechanisms, we adopted a system of isogenic triple knockout (TKO) rescue cell lines engineered to express single Ubiquilin paralogs or ALS disease mutant forms at physiological expression levels under a doxycycline-inducible promoter. Our investigations demonstrate that UBQLN2 is unique among Ubiquilin paralogs in that it can actively stabilize a model substrate. This stabilization correlates with the unique enhanced recruitment of multiple E3 ubiquitin ligases by UBQLN2, including SCFbxo7. We propose that E3 ligase recruitment facilitates UBQLN2-driven phase separation, thereby shielding substrates from proteasomal degradation. Notably, this mechanism also extends to the amyloid precursor protein (APP), which is protected from degradation by the formation of biomolecular condensates. Collectively, our findings introduce a novel conceptual framework for the roles of Ubiquilins in neurodegenerative disease, providing fresh insights into how UBQLN2 modulates proteostasis and substrate fate in the context of neurodegenerative diseases.

Reexamining Translation as a Convergent Regulator of Amyloid Nucleation

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Amyloid nucleation is an extremely rare, rate-limiting event in neurodegeneration, yet the cellular factors that tune its probability remain unclear. Using Distributed Amphifluoric FRET (DAmFRET) in HEK293T cells, we find that acute proteasome inhibition with bortezomib (BTZ) robustly suppresses nucleation of mEos-tagged Aβ42, poly-TA, and mHTT. In contrast, Aβ42 fibrillization in vitro is unchanged across 20S proteasome concentrations and insensitive to inhibition, arguing against a direct proteasome–substrate interaction. Inhibition of the AAA-ATPase VCP likewise reduces nucleation. Although BTZ and VCP act at distinct proteostasis nodes, both perturbations lower global translation, suggesting a shared axis. Consistently, proteomics of mEos immunoprecipitates reveal BTZ-dependent enrichment of ribosome/translation Gene Ontology pathways. To test causality, we engineered Kozak and 5′-UTR variants to modulate initiation efficiency: dampening initiation consistently decreased nucleation, whereas enhancing translation restored or increased it. Differences in nucleation were not attributable to expression level, as total construct expression was comparable across experimental paradigms. Alternative mechanisms—including autophagy, HSP70 induction, cytoplasmic crowding, and aggresome formation—did not account for the effects observed. Together, these findings indicate that diverse cellular stresses converge on translational output to calibrate the kinetic barrier for amyloid nucleation. Rather than proteasome impairment promoting assembly via simple concentration effects, our data support a model in which translational initiation/flux actively tunes nucleation barriers. This establishes a direct functional link between protein synthesis and the emergence of proteopathic assemblies, reframing how proteostasis pathways may influence disease-relevant amyloid formation.

POSTER ABSTRACTS

Disease Subtyping via a Novel DAmFRET Biosensing Approach

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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. Cryo-EM studies suggest that amyloids in these diseases are structurally unique, propagating through self-templating "seeds" that maintain the conformation of the original amyloid. Using our innovative biosensing system, we exploit this templating activity as a means to detect the amyloids themselves. We co-transfect HEK293T cells with seeds and plasmids that express amyloidogenic monomers, and these monomers use the seeds as templates for assembly. This assembly is then measured using Distributed Amphifluoric FRET (DAmFRET), a flow cytometry assay that quantifies in vivo protein self-assembly. This method has higher throughput than current biosensor systems that require either transient or stable lentiviral transduction of the reporting construct prior to a transfection step with the seeds. The improved throughput allows for larger screens without sacrificing sensitivity. Additionally, this technique can use both in vitro and patient derived amyloids as seeds for assembly. In an effort to differentiate amyloid structures between disease subtypes, we screened brain lysates from patients with Frontotemporal Lobar Degeneration with TDP-43 Deposits (FTLD-TDP) Types A, B, and C. Our preliminary results show potential differences in assembly profiles, and further refinement and screening may uncover mutations with specific recognition of one subtype over another. Further expansion of these biosensing constructs to include other amyloids and diseases 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.

Poster number: 1

TRiC regulates RNA Polymerase II in the Nucleus

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The eukaryotic chaperonin CCT (chaperonin-containing complex TCP-1), remains one of the most structurally complex members of the proteostasis network, built by two stacked rings of paralogous 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, as well as the crosstalk between the chaperonin's role n proteostasis vs its role in regulating transcription.

Poster number: 2

Untangling the Granule: systematically parsing out the impact of stress granules on gene expression

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Stress granules (SG) are ribonucleoprotein condensates formed when cells encounter a stress that triggers the inhibition of translational initiation, but allows elongation to proceed for ribosomes to run off the message and free mRNAs from ribosomes. While SGs form during this global repression of translation, there is simultaneous selective translation of stress-induced genes. This process is termed the Integrated Stress Response (ISR). Lack of experimental systems to perturb SGs without interfering with essential SG proteins like G3BP1/2 that can have other, non-SG functions has obscured the role that SGs may have in regulating gene expression. G3BP1/2 are core components of SGs, as knocking them out blocks SG assembly, but they have also been implicated in a number of cellular processes beyond scaffolding SGs. The goal of this project is to deconvolve the functional impacts of stress granules from the roles of their constituent proteins. We established a set of cell lines that allow for the separation of stress granules from G3BP1/2 without genetically depleting them. Validation of our cell models has shown that SG formation is blocked and that bona fide SGs are rescued under arsenite stress. Using this validated approach, and in combination with the traditional G3BP1/2 depleted, stress granule-deficient model, we have begun to collect measurements on the impact SGs versus their constituent G3BP1/2 proteins have on gene expression. Through this research, we will have established a new model system to evaluate the potential emergent impact of SGs versus those of their constituent proteins. Further, we will have benchmarked this system against the widely used G3BP1/2 knockout approach, and established the role of SGs, if any, in regulating gene expression in response to stress.

Poster number: 3

Selective Detection of Neurotransmitters, Pharmaceuticals and Metabolites at Physiological Concentration in Biological Media via Optically Enhanced NMR

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Detection of neurotransmitters, pharmaceuticals and metabolites in biological media is important for disease diagnosis and other clinical investigations. However, traditionally employed techniques, including nuclear magnetic resonance (NMR) spectroscopy, high-performance liquid chromatography (HPLC) and mass spectrometry (MS), often involve cumbersome equipment, long experiment times, expensive setup and maintenance, as well as complex handling. Here, we show that a recently developed magnetic-resonance technology known as low-concentration photochemically induced dynamic nuclear polarization (LC-photo-CIDNP) combined with benchtop NMR enables fast, inexpensive, and easy detection of compounds carrying an aromatic moiety at physiological concentrations in biological media. LC-Photo-CIDNP is a nuclear spin hyperpolarization technique that involves photoexcitation of a photosensitizer dye by a LED or LASER, followed by collisions (that create a radical pair) and a nuclear spin-sorting process that generates non-equilibrium nuclear-spin populations, greatly increasing sensitivity. By using a 1.88 T low-field benchtop NMR spectrometer, a variety of pharmaceutically relevant compounds were readily observed at physiological concentrations (low µM) in biological media via LC-Photo-CIDNP. This advance highlights the promise of benchtop NMR spectrometers combined with LC-photo-CIDNP for clinical diagnosis. Upon employing the 13C-RASPRINT (a 1H-detected-13C Photo-CIDNP pulse sequence), 1H-PASS-WET (a tailored 1H-WET LC-Photo-CIDNP pulse sequence), and 1H-PASSWORD (a CPMG and WET-solvent-suppression scheme integrated with LC-Photo-CIDNP), we achieved efficient detection of neurotransmitters (e.g., L-DOPA, serotonin, melatonin and epinephrine), pharmaceuticals (e.g., rizatriptan, reserpine and harmaline) and metabolites (e.g., tryptophan, tyrosine and indoxyl sulfate) in protonated solvents, complex cell-like media and unaltered biofluids. In all, the above advances bring optically enhanced benchtop NMR spectroscopy within the realm of standard laboratory settings, enabling it to effectively compete with more expensive and cumbersome approaches, like high-field NMR and HPLC-MS.

Poster number: 4

Understanding Nucleolar Heat Shock Protein 90 Proteome Dynamics at the Organismal Level

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Cells have developed complex, highly conserved stress response mechanisms to protect themselves against environmental and physiological challenges. Cellular protein quality control networks are part of these stress response mechanisms with molecular chaperones such as heat shock protein 90 (Hsp90) playing an essential role in this process7,12 to ensure cellular health and proteome integrity throughout environmental challenges. These stress response pathways have been largely uncovered in the cytosolic heat shock response, unfolded protein response of the mitochondria, and unfolded protein response of the endoplasmic reticulum, but remain largely unexplored in the nucleus7. It has been demonstrated that when cells are recovering from stress, the nucleolus - a dynamic nuclear condensate – can play a crucial role for the storage of damaged proteins for refolding and degradation2. Using a CRISPR-Cas-9 engineered C. elegans strains that express the essential Hsp90 endogenously tagged with red or green fluorescent protein (RFP or GFP) at its C-terminus, our lab identified a specific Hsp90 nuclear localization pattern during heat shock and recovery. While Hsp90 is known to be present in the nucleus by continuously shuttling between the cytosol and the nucleus8, its function in the nucleus and particularly the nucleolus under stress conditions is largely unknown. I will determine the physiological relevance and molecular function of Hsp90 in the nucleolus during different environmental challenges and throughout different life stages, using the multicellular organism C. elegans as a model system. These studies will contribute to a broader understanding of the complex proteostasis networks in the nucleolus and expand our understanding of Hsp90s role in these compartments during different stress conditions.

Poster number: 5

Interactions between the Yeast Mitochondrial Thioredoxin Trx3 and Enzymes Involved in Amino Acid Biosynthesis Pathways

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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). Our analysis revealed many proteins involved in amino acid biosynthesis, including members of the branched chain amino acid (BCAA) synthesis pathway and the lysine synthesis pathway. Our lab has validated Trx3's interactions with most of the proteins identified to be involved with BCAA and lysine synthesis. In preliminary phenotypic studies, yeast lacking mitochondrial disulfide reduction pathways are sensitive to oxidative stress in media lacking BCAAs or lysine. Future work will focus on quantifying these amino acids in cells lacking mitochondrial disulfide reduction pathways. In addition, we are developing an in-silico approach using a constraint-based model to serve as a predictive tool and holistically map Trx3's functions in mitochondria. By identifying novel substrate proteins of mitochondrial thioredoxins, we hope to uncover how this conserved protein reduction system regulates key metabolic processes during homeostasis and oxidative stress.

Poster number: 6

Understanding an interplay between HSF1 and ERRα in triple negative breast cancer

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Breast cancer is the most common cancer in women, with 1 in 8 at risk during their lifetime. Despite decades of research, incidence and mortality continue to rise. Oncogenesis often stems from genetic alterations that drive transcriptional dysregulation, enabling cancer cells to withstand oxidative, metabolic, and other stresses, ultimately promoting survival. ERRα (Estrogen Related Receptor α), an orphan nuclear receptor regulating metabolic gene expression, has been linked to ovarian and breast cancers, among others. While inhibiting ERRα shows therapeutic promise, its mechanisms remain unclear. HSF1 (Heat Shock Factor 1), a master transcriptional regulator of heat shock response and protein homeostasis, is critical for oncogenesis and has diverse tumor-promoting roles, including proteostasis, EMT, and immune evasion. However, any cooperation between ERRα and HSF1 has not been explored. ChIP-seq analyses revealed extensive overlap in binding peaks and shared target genes between ERRα and HSF1, enriched for cancer-relevant pathways such as proliferation, cell junction assembly, and Wnt signaling. Using a novel ERRα transcriptional activity signature, ERRα activity strongly correlated with HSF1 activity in breast cancer patients, with high co-activity predicting poorer prognosis. Functionally, ERRα enhanced HSF1 activity, and HSF1 expression induced ERRα activity. Treatment of HEK293FT and HCC1937 cells with an ERRα inhibitor (XCT790) reduced HSF1 protein, while an HSF1 inhibitor (SISU102) reduced ERRα protein. These results suggested a possible protein interaction between HSF1 and ERRα, which was confirmed with co-immunoprecipitation. Taken together, we propose a model whereby HSF1 and ERRα form a protein complex stabilizing both and co-regulating genes that promote the growth and progression of tumors. Future studies will investigate the role of this complex in oncogenesis, progression, and therapy resistance in breast cancer, with the goal of exploiting this interaction for improved chemotherapeutic response.

Poster number: 7

HSP90 Capacitance for Chemotherapy Resistance in Head and Neck Squamous Cell Carcinoma

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Drug resistance remains one of the biggest challenges in cancer treatment and contributes to a low 5-year survival rate in Head and Neck Squamous Cell Carcinoma (HNSCC) patients. This issue has become more prominent over the past decade, as the global burden of HNSCC has increased. There is an urgent need to understand the mechanisms driving drug resistance in HNSCC. Drug resistance often involves the acquisition of new mutations in cancer cells. The heat shock protein 90 kDa (HSP90) has previously been shown to foster the evolution of acquired drug resistance in yeast and tumors by acting as a potentiator of genetic variation. As a potentiator, HSP90 amplifies the effects of mutations, including oncogenic mutations that confer resistance to cancer drugs. However, previous efforts to target HSP90's potentiator function led to disappointing clinical results primarily due to dose-limiting toxicities. Here we present evidence for a new role for HSP90 in cancer drug resistance, involving the less-understood mechanism of mutational buffering (mitigation). This buffering function enables HSP90 to act as a "capacitor" for genetic variation, fostering the accumulation of conditional mutations within populations, much as 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 the effects of disease mutations in humans. We now demonstrate 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 development of drug resistance in microorganisms and tumors. Examining tumor cells with scDNA-seq (single-cell DNA sequencing) and scRNA-seq (single-cell RNA sequencing), HSP90 appears to speed up the selection of oncogenic variants by promoting low levels of genome instability within tumor lineages with HSP90-buffered mutator alleles. Furthermore, low-level HSP90 inhibition worked synergistically with chemotherapy to suppress drug-resistant tumor lines containing HSP90-buffered mutations. In conclusion, HSP90 functions as a capacitor for therapy resistance in HNSCC, and low-level HSP90 inhibition reduced this acquired drug resistance. This work suggests a potential new strategy for using HSP90 inhibitors in cancer treatment.

Poster number: 8

A Neurodegenerative Disease–Linked hmHsp60 Mutation Enhances ATPase Activity via an Optimized ATP Pathway and Binding Stereochemistry

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The human mitochondrial chaperonin hmHsp60 plays an essential role in mitochondrial proteome homeostasis by facilitating folding of mitochondrial proteins in an ATP-dependent mechanism. The V72I mutation in hmHsp60, linked to a form of hereditary spastic paraplegia characterized by progressive gait impairment, results in increased ATPase activity. To investigate the structural basis for this gain of activity, we determined the cryo-EM structures of ATP-bound wild-type hmHsp60 (hmHsp60wt) and hmHsp60V72I, complemented by comprehensive molecular dynamic (MD) simulations analyses. We show that this mutation enhances ATPase activity by optimizing ATP binding and hydrolysis. Cryo-EM structures reveal a more favorable ATP-binding geometry in the mutant, while molecular dynamics simulations demonstrate more efficient ATP engagement, lower energetic barriers, and stronger binding. These findings provide a quantitative biophysical basis for how a single substation perturbs chaperonin dynamics and contributes to disease.

Poster number: 9

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), suppressing aggregation of human amyloid beta (Aβ) protein expressed in C. elegans muscle cells. Hsp90 is also 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 processes by interacting with client proteins in 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 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. Preliminary data indicates that whole-worm Hsp90 levels remain relatively constant with age, but we hypothesize that neuron-specific expression patterns may vary and influence organismal decisions affecting healthspan and aging. By analyzing the neuronal expression and interactome of Hsp90 we aim to advance our understanding how Hsp90 regulates organismal proteostasis through TCS and the nervous system.

Poster number: 10

Microbial Modulation of Host Proteostasis: Prevotella corporis as a Protective Member of the Gut Microbiota

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Emerging evidence supports a microbial role in neurodegenerative protein conformational diseases (PCDs), including the most prevalent: Alzheimer's and Parkinson's disease. While the exact mechanisms that affect pathogenesis at the host-microbe interface remain elusive, metagenomic studies consistently reveal that gut dysbiosis is strongly associated with these disorders. Although considerable attention has been given to proteotoxic bacteria, the mechanisms by which commensal microbes confer proteoprotection are not well understood. We have previously employed Caenorhabditis elegans models to characterize the role of over 220 bacterial isolates on host proteostasis. Strikingly, members of the Prevotella genus exhibited proteoprotective effects. Most notably, P. corporis uniquely induced Hsp70, a critical molecular chaperone that maintains proteostasis, and significantly reduced aggregation of polyglutamine (polyQ), Aβ1-42, and α-synuclein upon intestinal colonization. In the present study, we expand on these findings, demonstrating that among 13 Prevotella species tested, P. corporis robustly activates the heat shock response (HSR) and confers conserved protection in Drosophila melanogaster. We further demonstrate that transient exposure to P. corporis results in the disaggregation of existing intestinal polyQ aggregates in C. elegans, leading to a general enhancement of global proteostasis. This is supported by significantly improved survival and enhanced thermotolerance, as well as a buffered age-dependent decline in fecundity. Interestingly, we found that the protective role of P. corporis is only observed when the bacteria are cultured under defined media conditions, suggesting that metabolic changes of this species drive its protective role. Together, our findings reveal a beneficial niche for P. corporis in activating the HSR to enhance organismal proteostasis. This work highlights the therapeutic potential of targeting the gut microbiota for the management of PCDs, the importance of species-level resolution in microbiome studies, and the emerging view of the intestine as a proteostasis-modulating organ.

Poster number: 11

Evolutionary Plasticity of Environmental Stress Response Prioritization

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The capacity to adapt is a defining property of living systems but is difficult to resolve through reductionist approaches. Statistical analysis of yeast populations exposed to 22 diverse environments and profiled by single-cell transcriptomics revealed a hierarchical organization of adaptation in which responses to some environmental features take precedence over others. We experimentally validated this adaptive prioritization and found that it mechanistically arises from differential regulation of translation initiation. Long-term laboratory evolution under constant stress for >5,000 generations collapsed this hierarchy, reduced adaptive capacity, and rewired stress-regulated translation. These results reveal a hierarchical logic to cellular adaptation that is contingent on evolutionary history, suggesting that adaptability may naturally emerge from the structure of iterative selection and variation through time.

Poster number: 12

Activation of the GCN2-eIF2α-ATF4 signaling axis downregulates HSF1 and inhibits cancer phenotypes

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Under states of proteotoxic stress, Heat Shock Factor 1 (HSF1) serves as the primary transcription factor responsible for sensing stress and transcribing proteins essential for restoring protein homeostasis. Also tasked with alleviating cellular stress is General Control Nonderepressible 2 (GCN2), a member of the Integrated Stress Response (ISR). Activated by a shortage in amino acids, GCN2 triggers the phosphorylation of eIF2α and the subsequent downregulation of global protein synthesis while selectively translating proteins, such as Activating Transcription Factor 4 (ATF4), to promote cell survival. Due to rapid proliferation, uncontrolled growth, and increased metabolic demands, cancer cells often exploit the HSF1 and GCN2 pathways to mitigate cellular damage. In Glioblastoma (GBM), HSF1 promotes tumor growth, migration, invasion, and apoptosis evasion, while GCN2 is critical in maintaining cancer cell homeostasis, proliferation, and survival. Recently, NXP800 (CCT361814) has emerged as both an HSF1 pathway inhibitor and a GCN2 pathway agonist, leading to antitumor activity both in vitro and in vivo. However, it remains unclear whether there is a mechanism by which HSF1 and GCN2 biologically integrate to modulate glioblastoma fate. Using GBM patient data, we first demonstrated a negative correlation between the GCN2 and HSF1 pathways. Further studies in GBM cell lines reveal pharmacological stimulation (via NXP800 and Halofuginone) and GCN2 overexpression resulted in decreased HSF1 protein levels, without altering HSF1 mRNA expression. While the decrease in HSF1 protein could partially be attributed to an overall decrease in translation, altering ATF4 expression alone also led to changes in HSF1 protein levels and pathway activity. Further elucidation of the antagonistic mechanism of GCN2/ATF4 on HSF1 will enhance our therapeutic approach to targeting the pathways and improving cancer therapy.

Poster number: 13

Hop/Sti1, a condensing holdase

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Hop/Sti1 is well established as a key player in proteostasis for its regulation of the ATPase and chaperoning activities of Hsp90. We find that under stress, Hop/Sti1 adopts previously unappreciated functions to prevent detrimental effects associated with protein misfolding. Specifically, we show that upon exposure to stress in yeast and neuronal cells, Hop/Sti1 forms cytoplasmic inclusions, which do not overlap with previously stress induced structures, such as stress granules, IPOD and JUNQ. Hop/Sti1 inclusions rapidly dissolve once the stress subsides. Furthermore, our in-vitro studies document that Hop/Sti1 forms condensates driven by intermolecular interactions of its TPR domains together with Hsp70 and that Hsp90 dissolves these condensates. We propose a mechanism by which Hop/Sti1 holds misfolded proteins under acute stress conditions, thereby preventing their toxic accumulation and enabling efficient refolding or degradation once the stress subsides. In neurodegenerative diseases, however, this holding by Hop/Sti1 can stabilize toxic conformers of misfolded proteins, such as alpha-synuclein and TDP-43, thus possibly contributing to their cellular toxicity.

Poster number: 14

Mitochondrial Thioredoxin as a Potential Redox Regulator of Citric Acid Cycle Activity in S. cerevisiae

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Eukaryotes express compartment-specific thioredoxins to reduce disulfide bonds in substrate proteins, thereby protecting against oxidative stress. In the baker's yeast, Saccharomyces cerevisiae, we have used a cross-linking approach with the bifunctional electrophile divinyl sulfone (DVSF) to obtain a list of potential redox partners for Trx3. Many of the proteins we identified are involved in core metabolic pathways like the citric acid cycle. Given the key role of the citric acid cycle (TCA) in cellular energy metabolism, we hypothesize Trx3 regulates the activity of numerous TCA enzymes. To confirm whether specific TCA enzymes interact with Trx3, we co-expressed epitope-tagged forms of these enzymes in yeast, treated with DVSF, and tested whether TCA enzymes co-immunoprecipitate with Trx3. We observed complexes between the TCA enzymes Cit1, Fum1, Aco2, and Idh2 and Trx3. Going forward, we will measure cellular ATP, NADH, and isocitrate concentrations with ROS treatment in yeast lacking mitochondrial disulfide reduction mechanisms to further establish a regulatory link between Trx3 and this pathway. Ultimately, identifying the individual enzymatic interactions of Trx3 and the pathways it regulates will provide key insight into Trx3's involvement in mitochondrial metabolism.

Poster number: 15

Investigating spatial dynamics of small heat shock proteins in chronologically aging yeast

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A hallmark of aging is the decrease in protein homeostasis (proteostasis) which can lead to accumulation of misfolded and damaged proteins that contribute to age-related diseases. As part of the first line of defense, small heat shock proteins (sHSPs) hold a crucial role in mitigating aggregation by acting as chaperone holdases, sequestrases, and aggregases. They interact with misfolded or damaged proteins, direct them to specific cellular locations, and form larger substrate-chaperone complexes presumably to minimize deleterious effects of uncontrolled protein aggregation. However, the precise roles of sHSPs in maintaining proteostasis during aging remain incompletely understood. In this study, we investigated the dynamics of the three S. cerevisiae sHSPs, Hsp26, Hsp42, and Btn2, all of which share the conserved □-crystallin domain, in chronologically aging/stationary yeast cells using previously generated GFP fusions. In line with earlier studies, we confirmed that both Hsp42 and Hsp26 form protein foci in stationary-phase cells. In contrast, we found that Btn2 expression is very low and that Btn2 does not accumulate in large foci. Interestingly, Hsp42 and Hsp26 foci show partial colocalization, and a larger fraction of Hsp26 foci overlap with Hsp42 than vice versa. Deletion of HSP42 reduces both the proportion of cells containing Hsp26 foci and the size of those foci, whereas the absence of HSP26 does not affect Hsp42 foci formation, suggesting that Hsp26 foci formation partly depends on Hsp42 in chronologically aging yeast. We also found that the Prion-Like Domain (PrLD) at the N-terminus of Hsp42 is critical for foci formation in stationary phase. Notably, replacing the N-terminal domain (NTD) of Btn2 with the Hsp42 PrLD enables Btn2 to form foci. However, the Hsp42 PrLD alone is unable to direct GFP into foci and requires the □-crystallin domain to do so. Similarly, the NTD of Hsp26 is essential for Hsp26 foci formation; however, in contrast to the Hsp42 PrLD, the Hsp26 NTD can form foci when fused to GFP in the absence of the □-crystallin domain, suggesting distinct interactions mediate foci formation of small heat shock proteins. Taken together, our findings indicate that both Hsp26 and Hsp42, by virtue of their N-terminal domains, likely play a major role in the sequestration of proteins in chronologically aging yeast cells. Identifying the protein complement of these different structures will be key to understanding how protein sequestration occurs in aging yeast cells, as well as potential impacts on cell fitness and adaptation to environmental stress.

Poster number: 16

Hsp-12.6 can selectively protect myosin-containing thick filaments under proteotoxic stress

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Muscles experience stress throughout an organism's life. This stress can come in different forms, for example, everyday mechanical stress like exercise, or intense muscle remodeling under starvation, which can alter the protein folding environment of the muscle and therefore disrupt protein homeostasis. As a result, maintenance of muscle function is heavily reliant on protein regulation by molecular chaperones. Specifically, small heat shock proteins (sHsp) are important in muscle maintenance and, interestingly, mutations in sHsp have been implicated in myopathies. However, the protective roles of sHsp in the muscle are not well understood at molecular level. In C. elegans, one sHsp, Hsp-12.6, is induced in the body wall muscles (BWM) under stress conditions, such as during starvation and in an arrested dauer state, and is specifically localized to myosin-containing thick filaments. We asked whether expression of Hsp-12.6 can protect (muscle function?) from proteotoxic stress caused by misfolding or mis-assembly of myofilament proteins. Our data show that muscle-specific expression of mCherry-tagged Hsp-12.6 improves motility and development in animals with mutations that disrupt folding or assembly of structural or organizing thick filament proteins, such as paramyosin, myosin, or the titin homologue, UNC-22. However, expression of Hsp-12.6 does not improve phenotypes caused by assembly mutations in actin-containing thin filament proteins. Interestingly, Hsp12.6 also does not improve phenotypes of animals with mutations causing a loss of sarcomere proteins, including those in thick filament proteins. These data suggest that Hsp-12.6 can play a role as a highly selective chaperone protein to protect and maintain myosin-containing thick filaments. Indeed, we find that Hsp12.6 preferentially localizes to the aggregates of thick but not thin filament proteins, and not to the aggregates of unrelated toxic proteins like polyglutamine expansions. The mechanism of protection Hsp-12.6 provides to the myofilaments is currently unknown. Understanding its role in chaperoning myosin-containing filaments may provide insight into how sarcomeres are preserved in healthy muscles, as well as in diseases such as protein aggregate myopathies (PAMs).

Poster number: 17

Elucidating the chaperone interactors of FGAMS

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When proteins are synthesized, they need to be folded into the correct structure to function as intended. This process is done by proteins known as chaperones. A large question plaguing the chaperone community is how selectivity is conferred in recruiting immature proteins to these chaperones. The protein FGAMS rely on Hsp70 and Hsp90 for its catalytic activities. This protein is a glutamine amidotransferase that catalyzes the fourth step in the de novo synthesis of purines, which are essential molecules that make up DNA backbones and cellular energy (ATP). Before the immature FGAMS client binds Hsp70, we hypothesize that a subset of co-chaperones known as DNAJs (Hsp40s) must first bind the client protein. To decipher the co-chaperone(s) that facilitate this process, we have been developing a yeast two-hybrid assay to test for protein-protein interactions between the bait (FGAMS) and preys (DNAJ proteins). The positive interactions will be validated via a co-immunoprecipitation analysis. Identifying those DNAJ proteins that bind to this enzyme can provide a therapeutic strategy to prevent its folding and inhibit nucleotide metabolism which may slow the rate of diseases that require them. Since the proliferation of cancer cells require de novo purine biosynthesis to compensate for the large number of resources that they use, the proliferation of Huh7 hepatocarcinoma cells was measured upon FGAMS knock down to determine the possibility of cancer therapeutics. Further, the DNAJ proteins that interact with FGAMS might extend to other glutamine amidotransferases since there are structural similarities that define this metabolic enzyme class.

Poster number: 18

Characterizing the BAG-1 co-chaperone interaction with p97 and the 26S proteasome

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BAG-1 is a nucleotide exchange factor and Hsp70 co-chaperone. BAG-1 regulates cell survival and protein stability through its interactions with heat shock proteins, anti-apoptotic factors, and the 26S proteasome. It is a promising cancer therapeutic target due to its roles in promoting proliferation and preventing drug-induced apoptosis. Recent proteomics studies identified a novel interaction between BAG-1 and p97, a highly conserved AAA+ ATPase that extracts ubiquitinated substrates from the ER lumen for proteasomal degradation during ER-Associated Degradation (ERAD). However, the mechanistic basis of the p97-BAG-1 interaction and its impact on ERAD remain unclear. BAG-1 contains a Ubiquitin-like domain and may serve as a shuttle factor for delivering ubiquitinated proteins to the proteasome. We have used many biophysical techniques to characterize the interaction between p97 and BAG-1, including enzymatic assays, isothermal titration calorimetry, and single-molecule cryo-EM. We are studying how BAG-1 impacts the unfoldase and ATPase activity of p97 to determine how BAG-1 may facilitate substrate handoff to the 26S proteasome. We have also developed fluorescence polarization assays to study how BAG-1 influences ubiquitination of chaperone clients and its effect on substrate degradation by the 26S proteasome. These studies will reveal how BAG-1 interacts with chaperones and co-chaperones and the downstream impact on proteasomal degradation, and their implications in ERAD.

Poster number: 19

Multiple extractases ensure fidelity in organelle targeting and orientation of small membrane proteins

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The correct targeting and integration of small membrane proteins (<70 residues) is essential for organelle function. However, small membrane proteins depend on error-prone post-translational targeting and insertion pathways. These small proteins often lack structural features for accurate targeting, resulting in mislocalization and mixed membrane topologies. To dissect the trafficking behavior of small membrane proteins we developed a split-luciferase based in-vivo assay that reports on membrane protein insertion and orientation in real time. Using Ost4, an ER-anchored small membrane protein, our assay showed that it inserts into both the ER and the outer mitochondrial membrane (OMM) with mixed topologies. Deletion of the OMM extractase Msp1 leads to the accumulation of Ost4 on the OMM. Similarly, deletion of the ER extractase Spf1 leads to the accumulation of Ost4 inserted in the ER with the wrong topology. Thus, both extractases act post insertion to maintain targeting and orientation fidelity. To test whether this is a general phenomenon, we analyzed Tom5 and Tom6, two small mitochondrial membrane proteins chosen for their minimal cytosolic domains. Both proteins exhibited mixed topologies and Spf1 dependent clearance from the ER, supporting the model that orientation errors are a common feature of small membrane proteins. Our findings establish in-vivo luminescence assays as powerful tools for monitoring membrane protein trafficking and highlight the critical role of extractases in correcting topological errors during biogenesis.

Poster number: 20

Silmitasertib, an FDA-designated orphan CK2 Inhibitor, ameliorates neuropathology and motor dysfunction in a Huntington's disease mouse model

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Huntington's disease (HD) is a devastating autosomal dominant neurodegenerative disease that manifests with progressive motor, cognitive, and psychological impairments. HD is caused by a polyQ (CAG) repeat expansion in the huntingtin (HTT) gene, leading to the misfolding and aggregation of mutant HTT protein (mHTT) and the preferential degeneration of the striatum. Previously in our lab, we identified Protein Kinase CK2 as an important kinase involved in the pathophysiology of HD. Specifically, the alpha prime catalytic subunit of CK2 (CK2α') is upregulated in HD, and genetic depletion of CK2α' in HD mice results in improved motor behavior, decreased mutant Htt aggregation, and improved neuronal function. Silmitasertib (CX-4945) is an FDA designated orphan drug that inhibits CK2. This study aims to investigate whether CX-4945 treatment ameliorates HD pathology. We treated prodromal and late symptomatic HD mice, and used a variety of immunohistochemical, biochemical, physiological and behavioral approaches. We found that CX-4945 presented benefits in the amelioration of HD pathophysiology in both treated groups. Importantly, we found CX-4945 decreased mHtt aggregation, increased DARPP-32 expression and excitatory synapse density, restored homeostatic astrocyte phenotypes and ameliorated neuroinflammation and microgliosis, altogether resulting in improved motor behavior. These results support CX-4945 as a strong candidate for a targeted therapy to treat HD.

Poster number: 21

Interplay of nucleoid protein paralogs modulates dynamics and stability of bacterial heterochromatin condensates

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Intragenomic paralogs are prevalent among bacteria and have important physiological and clinical implications. However, functional delineation of specific members within a paralogous family is often challenging due to compensatory effects among paralogs. H-NS is an archetypal nucleoid-associated protein widely present in Gram-negative bacteria that acts primarily as a transcriptional suppressor. H-NS is often intra-genomically present with one or more paralogs, yet the function of these paralogs is largely unknown due to the lack of phenotype upon deletion. Combining bacterial culture, in vitro reconstitution and in silico simulation, we show that interplay between H-NS and its paralog StpA in the model bacterium Escherichia coli modulates the biophysical properties of heterochromatin condensates, thereby improving bacterial fitness under stress. We demonstrate that H-NS forms dynamic yet metastable biomolecular condensates, whereas StpA forms stable but poorly soluble fibrillar oligomers. Co-presence of both proteins gives rise to a heterotypic condensate whose fluidity and stability can be dynamically tuned by varying the relative stoichiometry of H-NS and StpA. This corroborates the fact that E. coli increases StpA to H-NS ratio upon stress such as heat, which results in condensates that are stable but less dynamic. We further show that deletion of stpA has no effect upon optimal growth conditions but significantly disrupted H-NS condensation upon stress. This further leads to widespread gene de-repression, reduction in growth rate and decrease in bacterial competitive fitness. Finally, we identified physicochemical differences at dimerization site 2 as the reason underlying the paralogs' different phase behaviors and stabilities. Taken together, our data reveal a novel paradigm in which organisms utilize interacting paralogs, which lie at the two opposite ends of the phase spectrum, to fine-tune the properties of biomolecular condensates under fluctuating environments, thereby allowing them to survive among a wider range of ecological niches.

Poster number: 22

Transcriptome Condensation as a Conserved Strategy for Preferential Translation During Heat Shock

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Cell survival is contingent on the cell's ability to coordinate an effective gene regulatory response during changing environments, such as a rapid temperature change. In addition to the induction of stress response transcripts, a reprioritization of translating transcripts occurs during stress. Biomolecular condensation, the assembly of proteins and RNAs, is hypothesized to contribute to this reorganization during stress as a survival response. A recent Drummond lab publication showed stress-induced condensation occurs transcriptome-wide while stress-related transcripts escape condensation. These escaped transcripts tend to be translated more often during stress indicating stress-induced biomolecular condensates aid in the sequestration of non-stress related transcripts, allowing increased ribosome accessibility for stress-induced transcripts. Whether transcriptome condensation is a conserved strategy for preferential translation during stress is unknown. I hypothesize the timing of a transcript's production and their condensation behavior are conserved phenomena used for preferential translation. Using two additional species of yeast from distinct thermal niches, Saccharomyces kudriavzevii (cryophile) and Kluyveromyces marxianus (thermotolerant), I am investigating transcriptome condensation and preferential translation during their respective heat shock temperatures. Preliminary data suggests the conservation of transcriptome condensation that is specific to each organism's ecological thermal niche. These data support a conserved function of condensation in regulating translation during stress conditions.

Poster number: 23

Cellular Stress to Collective Action: How Heat and Social Context Influence Honeybee Fanning Behavior

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Social insects depend on collective behaviors to mitigate various environmental stressors; however, the physiological mechanisms linking individual stress responses to group-level outcomes remain unclear. In honeybees (Apis mellifera), thermoregulatory fanning is essential for maintaining brood development and colony homeostasis. Our preliminary results suggest that prior heat exposure impacts fanning behavior and that the social environment during heat stress further shapes these responses. Here, we investigate the role of heat shock proteins (HSPs), conserved markers of cellular stress, in modulating fanning behavior after different thermal and social experiences. Bees previously exposed to heat exhibited elevated HSP expression, altered fanning dynamics, and responded at higher temperatures, indicating a physiological cost of thermal stress. Prior social context modulated this relationship, suggesting an interaction between temperature and social experience on an individual's physiology and collective behavior. These findings highlight a mechanism by which social context and past stress shape individual behavior, revealing implications for understanding group-level resilience during stress.

Poster number: 24

uORFs inhibit mRNA localization to stress granules

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The integrated stress response (ISR) is critical for cellular adaptation to stress and is implicated in neurodegeneration, neurodevelopmental disorders, and cancers. During the ISR, translation is repressed, stress-induced genes are translated, and translationally repressed mRNAs condense into stress granules. The relationship between stress-induced gene expression and stress granules is poorly understood. To begin to address this gap, we asked if and how stress-induced gene mRNAs localize to stress granules. We measured endogenous stress-induced gene mRNA localization to stress granules using single-molecule fluorescence in situ hybridization in the presence or absence of small-molecule translation inhibitors that modulate ribosome occupancy. We observed that reducing ribosome occupancy increases the localization of stress-induced gene mRNAs to stress granules, while increasing ribosome association inhibits localization to stress granules. The presence of an upstream open reading frame (uORF) in mRNA reporters reduces mRNA localization to stress granules in a ribosome-dependent manner. We also observed that stabilizing a single ribosome globally in the cell inhibits the formation of stress granules. Together, our results suggest that uORF-mediated ribosome association inhibits stress-induced gene mRNA localization to stress granules. Our results imply a novel role of uORFs beyond their known translational regulation, as regulators of mRNA localization, and highlight that ribosome-bound mRNAs are incompatible with condensation.

Poster number: 25

Elucidating the function of Cdc48 and alternative ubiquitin linkages in cellular stress adaptation

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Protein homeostasis (proteostasis) is a dynamic cellular state describing the essential balance of protein synthesis, folding, organization, and degradation. When disrupted, proteostasis dysregulation contributes to various diseases, ranging from cancer to neurodegeneration. To maintain proteostasis, cells have evolved a diverse array of proteins collectively referred to as the proteostasis network (PN). The PN encompasses molecular chaperones, degradation machinery, and signaling pathways that contribute to proper protein folding, degradation, and maintenance. The PN's ability to rapidly adapt to environmental perturbations through the activation of stress response pathways is fundamental to cell survival. One underappreciated aspect of the PN is the roles of the ubiquitin-proteasome system (UPS) and Cdc48 in conferring PN reorganization and dynamics in cell stress adaptation.

In Saccharomyces cerevisiae, a hallmark of many stress responses is the reorganization of the PN, resulting in the relocalization of important molecular chaperones and other machinery. One such example of this occurs during the heat shock response (HSR), a highly conserved eukaryotic stress response that is activated upon an abrupt increase in temperature. Similar to the HSR, reactive oxygen species (ROS) are another environmental stressor that results in this reorganization of the PN. A critical component in restoring proteostasis upon heat shock and ROS exposure is Cdc48, a conserved AAA+ ATPase with established roles in protein degradation and stress response across eukaryotes. While biochemical studies have elucidated many mechanistic aspects of Cdc48, its functional role in stress response remains poorly understood, particularly regarding its coordination with other proteostasis factors and the UPS. Cdc48's ability to interact with ubiquitin chains in concert with cofactors suggests it is a central hub for managing the reorganization of the proteome during heat shock and ROS exposure. In parallel, emerging evidence shows alternative ubiquitin chains, such as K29 linkages, are formed during cell stress. These noncanonical ubiquitin chains may provide novel regulatory mechanisms for PN reorganization during stress. However, their function in restoring proteostasis remains enigmatic. Together, these emerging findings raise intriguing questions about the intersection of Cdc48 function, alternative ubiquitin chains, and their collective role in proteostasis during cellular stress response.

Poster number: 26

Systematic Chemical-Genetic Profiling as a Path to Expand Precision Medicine in Breast Cancer

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Large-scale tumor sequencing has revealed extensive catalogs of cancer-specific genetic alterations, yet the functional consequences of most remain unknown. We applied QMAP-Seq, a high-throughput chemical–genetic profiling platform, to systematically define how common alterations in genome maintenance pathways influence drug response. A CRISPR library targeting 62 genes frequently altered in invasive breast carcinoma was profiled against 760 compounds spanning DNA damage, cell cycle, protein homeostasis, and epigenetic pathways. The resulting >45,000 chemical–genetic interactions uncovered 84 strong therapeutic and 39 resistance interactions. These included clinically validated or clinically tested strategies across multiple categories: targeted therapies such as PARP inhibition for BRCA1 mutations, resistance mechanisms such as RB1 loss conferring CDK4/6 inhibitor resistance, and combination approaches such as cisplatin with ATR inhibition. We also identified novel therapeutic candidates, off-target effects, and divergent sensitivity profiles among compounds with shared mechanisms, underscoring the influence of chemical structure on biological activity. Together, these findings demonstrate the power of QMAP-Seq to connect genomic alterations with drug response, enabling discovery of clinically actionable vulnerabilities and resistance mechanisms.

Poster number: 27

Coordination of Metabolons with Organelle Morphologies in Response to Cell Stress

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Metabolism is the network of biochemical reactions that supplies cells with the energy and biomolecules required for survival. Regulation of this process enables cells to dynamically respond to internal and environmental cues and meet fluctuating nutrient demands. Cell stress brought on by nutrient depletion is a common environmental cue that reshapes metabolism. Cells respond to this new stress in two unique ways: the assembly of multi-enzyme complexes such as purinosomes to help alleviate intracellular purine demands and the induction of morphological changes in key organelles, most notably mitochondria and the endoplasmic reticulum. While previous studies have examined how enzyme assemblies and organelle morphologies individually influence metabolic activity, the coordination between these two processes remains largely unexplored. Here, we investigate how purinosomes associate with adjacent organelles to coordinate purine production under stress. Key to this investigation is a potential role of the mitochondrial chaperone mortalin in modulating these interactions and its impact on purine biosynthesis.

Poster number: 28

Exploring Protein Birth in Real Time and Directed Evolution as Complementary Approaches to Probe and Control Protein Folding and Aggregation in the Cell

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Optimization of protein folding and elimination of protein aggregation are important goals to achieve the large-scale production of a variety of protein-based pharmaceuticals including monoclonal antibodies, interferon and insulin. Towards this end, recombinant-DNA technologies in prokaryotic hosts (e.g., E. coli) rather than eukaryotes (e.g., baculovirus, Chinese hamster ovary cells) are in principle more convenient and affordable. Yet, prior research shows that prokaryotic in-vivo protein overexpression often suffers from the formation of aggregates known as inclusion bodies. While it is presently unknown why bacterial cells are more likely to yield insoluble overexpressed proteins, we hypothesize that the extremely fast release of fully synthesized nascent chains from the ribosome and suboptimal molecular-chaperone concentrations contribute to protein misfolding and aggregation at birth. Fast ejection from the ribosomal tunnel in E. coli may hamper the ability of newly synthesized proteins to attain their native fold before aggregation takes over. In this research, we take advantage of the selective pressure imposed by the macrolide antibiotic erythromycin to generate bacterial strains bearing mutated ribosomal proteins via directed evolution. Many of the resulting genomic mutations face the interior of the ribosomal exit tunnel and are located across the uL4 ribosomal protein and the 23S rRNA. Interestingly, some of these mutations are computationally predicted to reshape the geometry of the tunnel by slightly narrowing its width. Importantly, the uL4 and 23S-rRNA mutant strains were found to increase newly-synthesized protein solubility. In order to understand the origin of this effect, we also explored the kinetics of newly synthesized protein folding and aggregation in real time by fluorescence anisotropy. These studies, which were performed in the absence and presence of molecular chaperones, provide a conceptual foundation to understand and reshape the mechanism of protein birth. In all, this research will promote novel approaches targeting the large-scale and efficient production of a variety of practically useful proteins, including pharmaceutically active nanobodies, antibodies and bioinspired materials.

Poster number: 29

Cellular and molecular mechanisms of PERK-dependent olfactory defects in neurodegeneration

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In most familial neurodegenerative diseases (NDs), specific subsets of neurons are affected despite broad expression of disease-causing mutant proteins. The cause of this selective susceptibility is unknown. Interestingly, olfactory dysfunction long precedes clinical symptoms in many of these diseases. The commonality and early onset of olfactory dysfunction across NDs suggest that olfactory neurons are especially susceptible to neurodegeneration. The molecular mechanisms underlying this susceptibility may indicate shared initiating mechanisms. I found that PERK, a known risk factor for progressive supranuclear palsy and Alzheimer's disease, is necessary for olfactory function in Drosophila. The disease risk alleles of PERK were shown to be hypomorphic in patient-derived neuron culture, therefore I hypothesize that decreased PERK activity enhances the olfactory system's susceptibility to neurodegeneration. PERK's function in the olfactory system may therefore provide new targets for earlier and more effective treatment of neurodegenerative diseases.

Because PERK may contribute to olfaction through multiple cellular and molecular functions, this project will determine where and when PERK is needed in the olfactory system, and through which molecular pathways. PERK's canonical function is to respond to ER stress by phosphorylating eIF2α, thereby attenuating translation. However, PERK has additional, novel roles in axon guidance, Ca2+ dynamics, and growth factor sorting. These functions may affect different populations of neurons and operate within different timeframes. To develop therapies that target PERK-dependent mechanisms driving neurodegeneration without undesirable side effects, it is critical to resolve which of these functions is necessary for olfaction. I am utilizing the extensive genetic tools available in Drosophila to probe for selective PERK dependence among olfactory system neurons of different functional types (e.g., sensory vs. interneuron) and belonging to circuits mapped to different odors. Likewise, to determine whether PERK is necessary in the development, function, or aging of olfactory neurons, I will temporally control PERK expression and activity and assess olfactory function at multiple life stages. Finally, I will determine whether ER stress, eIF2α phosphorylation, or another pathway downstream of PERK is necessary or sufficient to explain the olfactory system's dependence on PERK. This work will elucidate how PERK modulates early ND symptoms, providing new possible routes towards effective treatment of neurodegeneration and deepening our knowledge of PERK's broader neuronal functions.

Poster number: 30

Resolving Stalled Protein Synthesis: From Mechanism to Physiology

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The faithful translation of mRNAs into proteins is crucial for cellular function and life. However, defective mRNAs or amino acid deficiencies can cause ribosomes to stall and collide, threatening proteome integrity, disrupting homeostasis, and generating incompletely synthesized polypeptides that impose proteotoxic stress. To address these challenges, all domains of life have evolved the ribosome-associated quality control (RQC) pathway, which detects collided ribosomes and disassembles them into subunits. Remarkably, the large ribosomal subunit can then resume protein synthesis without an mRNA template or the small subunit, adding amino acids to the C-terminus of stalled polypeptides. These untemplated extensions, known as “CAT tails,” can be up to ~60 amino acids long, and represent a central dogma-defying form of protein synthesis. Our research in yeast revealed that in the absence of mRNA, mechanical pulling forces on stalled nascent chain regulates the selective incorporation of alanine and threonine into stalled polypeptides, resolving a longstanding question about how CAT tail sequence is determined by the ribosome independent of mRNA. We have discovered new functions for CAT tails and established a framework for understanding the paradoxical role of CAT tailing in both targeting stalled polypeptides for clearance and predisposing them to aggregation. Dysfunctional CAT tailing contributes to motor neuron disorders and neurodegenerative diseases in humans, flies and mouse, highlighting the fundamental role of RQC and CAT tailing in maintaining organismal health.

Poster number: 31

A Conformational Biosensor Reveals Inter-Individual Variation in Proteostasis

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Maintaining proteostasis—the balance between protein folding, degradation, and aggregation—is essential for organismal health and is perturbed by environmental stress. To examine protein fate under stress, we expressed a temperature-sensitive DHFR-based fluorescent biosensor in somatic tissues of C. elegans and monitored its behavior under heat stress. High-throughput imaging enabled quantitative analysis of biosensor fluorescence across large populations of genetically identical animals, providing a readout of protein conformational state. DHFR(P67L) fluorescence decreases with increasing temperature up to 30 °C, consistent with protein destabilization and proteasome-mediated clearance, but increases at higher temperatures, reflecting aggregation and increased inter-individual heterogeneity. In contrast, DHFR(WT) remains stable across all temperatures. Native PAGE and western blot analyses confirm the presence of distinct folding and solubility states of the protein under different stress conditions. To assess how disease-associated proteotoxic stress influences organism-wide proteostasis, the biosensor was crossed into established neuronal polyglutamine models. Animals expressing a non-aggregating repeat (Q19) retain temperature-dependent folding responses similar to those observed in the absence of polyglutamine repeats, whereas animals expressing an aggregation-prone repeat (Q40) lose this response and exhibit increased inter-individual variability. Together, these results demonstrate that protein fate under stress is not uniform across individuals, but instead bifurcates between degradation and aggregation, and that neuronal proteotoxic stress shifts both the distribution and variability of proteostasis responses under identical conditions. Ongoing work extends this framework to aging to examine how protein fate decisions and variability change with age. Preliminary analyses reveal age-dependent shifts in DHFR folding dynamics, consistent with a progressive decline in proteostasis across the lifespan.

Poster number: 32

Investigating the mechanisms of TDP-43 nuclear-to-cytoplasmic mislocalization and aggregation

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The mislocalization and aggregation of TDP-43, a nuclear RNA-binding protein, are hallmarks of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Understanding the subcellular localization of TDP-43 and its assembly into aggregates is of importance given the body of evidence linking it to neurodegenerative diseases. While 90-95% of ALS cases are sporadic, 3-5% of familial ALS and <1% of sporadic ALS is associated with mutations in the TDP-43 gene. However, models of TDP-43 mislocalization have almost exclusively relied on the overexpression of engineered transgenes that remove the nuclear localization signal and/or introduce pathogenic mutations. Thus, we are establishing CRISPR-engineered human cell lines that have been modified to include fluorescent tags on the N- or C-terminus of TDP-43 in order to track endogenous, wild-type TDP-43 using quantitative microscopy approaches. We are characterizing TDP-43 behavior in response to stress and perturbation of different nodes of the proteostatic pathway, such as proteasome inhibition, to mimic chronic disease states. Future studies will expand into more disease-relevant cell model systems, such as iPSC-derived cortical and lower motor neurons. This research will suggest mechanisms of TDP-43 mislocalization and condensate-mediated aggregation and contribute to furthering our understanding of ALS/FTD pathogenesis.

Poster number: 33

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. One of the central nodes within the PN is the membrane trafficking system which involves transport of membrane bound proteins between the ER, Golgi, and plasma membrane. As such, targeting components of membrane trafficking is a strategy for the development of novel treatments for cancer. Thus, understanding the regulation and function of membrane trafficking in the context 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 membrane trafficking milieu (e.g. autophagy inhibitors, ER stress inducers) at doses ~1000x lower than most acute stress studies which highlighted factors most important for surviving chronic membrane trafficking stress. In my preliminary data, I found: (1) TMED2, a transmembrane protein involved in vesicular transport between the Golgi and ER, was among the most critical factors for surviving growth-associated ER and autophagic stress in all conditions; and (2) TMED2 is upregulated in many cancer types, including triple negative breast cancer (TNBC) (3) TMED2 and TMED10 are strongly coessential across cancer cell lines; and (4) AGN192403, a compound capable of targeting TMED2, reduces proliferation and growth of TNBC cell lines. From these data, I hypothesize that a subset of TNBCs have high levels of cell-autonomous ER stress and are thus dependent on TMED2, and that loss of TMED2, genetically or pharmacologically, will suppress TNBC proliferation. 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.

Poster number: 34

Unlocking High-Affinity Binders: A Novel DAmFRET-Based High-Throughput Screening Platform.

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Protein interactions are fundamental to life, and the capability to engineer such interactions has immense potential for therapeutics and synthetic biology. Established methods for screening protein binders, such as yeast and phage display are limited by the need to first purify the target protein, and by the need to express binders extracellularly. These requirements limit throughput, preclude screening against complex or insoluble targets, and require hit binders to be subsequently validated for intracellular activity before they can be used in synthetic biology applications. To overcome these limitations, we are developing the Intracellular Binder–Antigen Screening System (iBASS), a high-throughput screening platform that operates intracellularly and can screen for high affinity binders to an antigen of interest in a pooled fashion. iBASS exploits a prion-like domain to amplify a fluorescent signal triggered by a stable interaction of antigen with binder. For this purpose we have designed a simple diblock amyloid forming sequence whose saturating concentration and nucleation barrier can be independently tuned, allowing for its intracellular supersaturation and nucleation upon recruitment to a multivalent seed. This sequence is fused to the binder pool and mEos3, a photoconvertible fluorescent protein that reports on the protein's self-association using a flow cytometry technique, DAmFRET (Distributed Amphipofluoric FRET). The antigen is expressed in trans as the multivalent seed by fusing it to a homo-oligomeric domain. Interaction of binder to antigen increases its local concentration and triggers amyloid nucleation, which then drives essentially the entire cellular pool of binder fusion into a high density aggregate, resulting in a massive digital gain in ratiometric FRET (AmFRET). We use fluorescence-activated cell sorting (FACS) to isolate FRET-positive cells followed by targeted sequencing to identify successful binders. Using the well-characterized Alfa/AlfaNb pair, we validated and optimized iBASS, demonstrating its ability to perform pooled, intracellular binder screening that is faster, easier and cheaper to use than other binder screening platforms.

Poster number: 35

TPR domain chaperone Sgt2 alters spatial sequestration of mutant huntingtin

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Huntington's disease (HD) is an inherited fatal neurodegenerative disease that degenerates the basal ganglia impairing voluntary movement and cognitive function. A mutation in the huntingtin gene causes the formation of protein deposits of mutant huntingtin (mHTT) in the striatum and cortex of the brain. There is evidence of a breakdown in protein quality control (PQC) mechanisms, but how HD impairs proteostasis remains unclear. One mechanism of PQC called spatial sequestration sequesters misfolded proteins into spatially distinct membrane-less compartments. Chaperones are well known for sorting misfolded proteins, soluble oligomers, and insoluble aggregates into these compartments. However, the role of co-chaperones in spatial sequestration remains unclear. Using a yeast model, we are investigating the role of the Hsp90 co-chaperone Sgt2 in the spatial sequestration of mHTT. Deletion of Sgt2 increases mHTT toxicity by 6% and increases mHTT inclusion formation by 25%. We find that the dosage of Sgt2 alters the dynamics of mHTT. Mild overexpression of Sgt2 increases the number of mHTT inclusions and the inclusions more frequently locate near the endoplasmic reticulum, however severe overexpression of Sgt2 greatly reduces the number of mHTT inclusions. Because Sgt2 is well known for inhibiting the ATPase activity of Ssa1, we are investigating the interaction between Sgt2 and Ssa1 in altering mHTT solubility and inclusion formation, and we propose Sgt2 inhibits Ssa1 refolding to promote stabilization of misfolded proteins by Ssa1. Understanding the role of Sgt2 in protein quality control will uncover its potential as a target for protein misfolding diseases.

Poster number: 36

Using C. elegans to uncover the link between the cellular unfolded protein response and TDP-43 proteotoxicity

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Neurodegenerative diseases are categorized by deterioration of nerve cells and chronic progression of fatal symptoms. A hallmark of these diseases is the aggregation of specific proteins in affected neurons. TAR DNA Binding Protein 43 (TDP-43) is found in aggregates in several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Cells possess an evolutionarily conserved system that normally prevents protein aggregation called proteostasis. However, in these diseases, this system no longer functions properly. To study the link between proteostasis and TDP-43 related toxicity, we are using the small, transparent nematode, Caenorhabditis elegans. In the Voisine lab, a transgenic line expressing human TDP-43 expressed in the nervous system has been generated. Using this transgenic line, I will perform a genetic cross to introduce a deletion of catp-3 (Δcatp-3). This gene encodes for a protein product that is predicted to enable the unfolded protein response (UPR), a proteostatic system that is responsible for maintaining proteostasis within the endoplasmic reticulum (ER) by responding to accumulation of unfolded or misfolded proteins. Once this new strain is generated, I will examine the physiological effects of altering proteostasis by deleting catp-3 on TDP-43 toxicity. Using a well-established behavioral assay called a thrashing assay, I will measure the movement of the worms in liquid to assess motor neuron function. I hypothesize that removing the catp-3 gene will improve motor neuron function in worms expressing TDP-43. Observing the behavioral effects of Δcatp-3 would provide insight into the role of proteostasis in neurodegenerative pathology.

Poster number: 37

E. coli J-domain proteins, DnaJ and CbpA, interact with Hsp90 through multiple distinct and overlapping regions

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Molecular chaperones play a crucial role in maintaining proteostasis by assisting polypeptide folding, reactivating misfolded proteins, preventing aggregation, and facilitating degradation. Hsp90 is a highly conserved ATP-dependent molecular chaperone that is upregulated under stress and implicated in cancers and neurodegenerative disorders. It frequently collaborates in proteostasis with Hsp70 and Hsp70 cochaperones, including J-domain proteins (JDPs) and a nucleotide exchange factor. JDPs bind and transfer the client protein to Hsp70. Hsp70 interacts directly with Hsp90, enabling the transfer of the client protein from Hsp70 to Hsp90, where the client undergoes final maturation and reactivation. Recent work has shown that E. coli Hsp90 (Hsp90Ec) can form binary complexes with E. coli JDPs, DnaJ and CbpA. The aim of our current work is to identify the specific domain or domains of DnaJ that interact with Hsp90Ec and to determine whether these interaction(s) are conserved in CbpA. Both DnaJ and CbpA consist of a highly conserved J-domain, a G/F rich linker, two C-terminal domains (CTD I and CTD II), and a dimerization domain. In addition, DnaJ contains an additional zinc-finger-like region that is embedded in the CTD I domain. We generated truncation mutants of both DnaJ and CbpA, each consisting of one or more domains. The DnaJ and CbpA wild type and mutant proteins were purified, characterized in functional assays, and tested for interaction with Hsp90Ec using a pulldown assay, where the association of Hsp90Ec with biotinylated DnaJ or CbpA wild type or mutants was monitored. Chemical cross-linking between DnaJ and Hsp90Ec using the lysine-reactive crosslinker DSSO, followed by mass spectrometry, was used to identify specific interaction sites between DnaJ and Hsp90Ec. The results were then compared with previously identified DSSO cross-linked sites between CbpA and Hsp90Ec. Altogether the preliminary results suggest that JDPs utilize multiple sites in various domains for binding Hsp90Ec. It remains to be shown whether these differences in interacting regions reflect functional differences between the DnaJ-Hsp90Ec and CbpA-Hsp90Ec complexes.

Poster number: 38

Exploring the role of Hsp90 in tail-anchored protein biogenesis

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Tail-anchored proteins (TAs) require the collaboration between molecular chaperones, TA transporters, and TA translocases to be properly inserted into their membranes. Previous studies have shown a network of multiple pathways including the guided entry of tail-anchored protein (GET) pathway, the ER membrane protein complex (EMC) pathway, and the signal recognition particle independent (SND) pathway work in parallel to insert TAs post-translationally. 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 key components of each of these parallel pathways as well as 34 different TAs in yeast. 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 indicating that Hsp90 may have a role in aiding TA insertion. While the protein-protein interactions between Hsp90 and TA insertion machinery from the GET, EMC, and SND pathways have been confirmed in vivo via proximity ligation, intriguingly Hsp90 cannot compensate for the loss of components from all 3 pathways. This indicates that although Hsp90 does not work in parallel to GET, EMC, and SND to insert TAs it may help prevent TA aggregation by maintaining TAs in an insertable form. 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.

Poster number: 39

Modulating the Chaperone-like Behavior of the Bacterial Ribosome

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Proper protein folding and protection from aggregation are critical for the function and viability of all organisms. Molecular chaperones and other cellular machineries, e.g., the ribosome, have been evolutionarily optimized for these tasks. Yet the extent of the interactions between nascent proteins and “folding helpers” remains unclear. Recent studies revealed that nascent proteins interact with the uL23 ribosomal protein within the outer surface of the ribosome. This interaction involves nonpolar residues and is displaced in the presence of molecular chaperones, including trigger factor. Therefore, we hypothesize that the nascent-chain-uL23 interaction is chaperone-like in nature and promotes proper native state formation. To test this hypothesis, we introduced various hydrophobicity-altering mutations within the uL23 nonpolar pocket and probed their effects on protein solubility. Lambda-Red recombineering was employed to introduce these mutations, and protein overexpression and solubility assays were conducted to assess soluble vs insoluble protein production for both mutant and wild-type strains. If successful, modulation of the ribosome’s intrinsic chaperone activity could be employed in the future to enhance de novo protein-folding quality. This strategy is appealing because it could reduce the production cost of protein-based therapeutics and render them more accessible to the broader community.

Poster number: 40

Structural and Dynamic Insights into Nascent-Protein Interactions with the Trigger Factor Chaperone

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Molecular chaperones are central to protein homeostasis, guiding nascent polypeptides towards their native conformations and preventing misfolding and aggregation within the crowded cellular environment. Immediately post-translational events accompanying the release of newly synthesized chains from the ribosome lead to 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 protein life are crucial. Understanding how chaperones behave at this stage is essential to define the origin of folding versus aggregation pathways. Here, we focus on the ribosome-associated trigger factor (TF) chaperone, whose cotranslational interaction with nascent proteins at the ribosomal exit tunnel is poorly understood. Using a monomeric globin as a model system, we probe how TF interacts with the globin chain and how it influences its structure and dynamics during biosynthesis. This work synergistically employs cell-free transcription-translation, unnatural tRNA technologies, single-particle cryo-electron microscopy and fluorescence lifetime/anisotropy-decay spectroscopy to probe three-dimensional structure and dynamics of ribosome nascent-protein complexes. Taken together, our results reveal how TF prevents cotranslational aggregation while preserving nascent-chain conformational sampling. This research paves the way to a deeper molecular-level understanding of kinetic partitioning between protein folding and aggregation at birth.

Poster number: 41

Multisite Hsp70 phosphorylation acts as a rheostat for the DNA damage response

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Organisms have evolved highly conserved and efficient mechanisms to detect and repair genomic damage. A central feature of this response is the activation of the upstream kinases Mec1 and Rad53, which promote the expression of ribonucleotide reductase (RNR), the essential enzyme responsible for deoxyribonucleotide synthesis. The RNR complex comprises three subunits: Rnr1, Rnr2, and Rnr4. Previous work from our laboratory established that RNR is a client of the molecular chaperone Hsp70 in both yeast and mammalian systems. In this study, we investigated how Hsp70 phosphorylation influences the DNA damage response in yeast. We screened 146 yeast strains expressing mutations at Hsp70 phosphorylation sites (73 phospho-deficient and 73 phospho-mimetic mutants) for sensitivity to the RNR inhibitor hydroxyurea (HU). This screen identified 13 phosphorylation sites critical for HU resistance. To examine the effects of these sites on the RNR complex and upstream signaling pathways, we assessed subunit abundance by Western blotting. Three mutant strains exhibited reduced levels of the small subunits Rnr2 and Rnr4, while nine strains displayed an HU-dependent decrease in Rnr1 abundance. We also analyzed Rad53 protein levels and found three phosphorylation sites that affect its stability. Notably, one previously studied site, T36, was linked to reduced levels of all three RNR subunits and Rad53. Since T36 is also activated by nutrient starvation stress, our results suggest T36 phosphorylation site connects nutrient stress with RNR regulation, indicating that Hsp70 may act as a hub integrating multiple stress signals.

Poster number: 42

Investigating the conservation and molecular basis of acquired thermotolerance in budding yeast

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Cells across the tree of life have had evolved resilience to environmental stresses to survive through evolutionary time. One such stress is the ability to withstand high temperatures. While budding yeast cells have a canonical heat shock response, they can also acquire thermotolerance, meaning if they are first exposed to a mild heat stress, they are then protected against a subsequent lethal heat stress. While this phenomenon has been observed for many years, there are open questions that remain, regarding both its molecular mechanism and the extent to which it is conserved in cells adapted to different thermal niches.

S. cerevisiae and S. kudriavzevii are budding yeast strains that have only diverged 15-20 MYA (reviewed in Replanksy et al., 2008). However, S. kudriavzevii is considered a cryophile, as its maximum growth rate occurs as at 36.8°C and its optimum growth rate occurs at 23.6 °C (Salvadó 2011). While it has been established that the heat shock response is conserved for these two species (Keyport Kik et al., 2023), it is not clear whether the acquired thermotolerance response is also conserved.

Here, we explore both the molecular mechanism and the evolutionary conservation of acquired thermotolerance in S. cerevisiae and S. kudriavzevii. We identify the minimum amount of time S. cerevisiae cells must experience a mild stress to survive a lethal stress, and we also map this against an increase in HSP104 mRNA, a known chaperone that has been established as necessary for cells to acquire thermotolerance (Sanchez 1990). Ultimately, this work seeks to better characterize the bounds of acquired thermotolerance, and seeks to reveal how molecular changes lead to cell survival and resilience.

Poster number: 43

Not just your grandpa's FAIM: eukaryotic FAIM proteins can broadly inhibit protein aggregation

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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. Aggregate 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. 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 FAIM protein from other species was active in a manner like human FAIM, and whether the substrate profile for this protein shared similarity to FAIM. Eukaryotic FAIM proteins have variable sequence identity, with mouse FAIM sharing high identity (~90%) and fruit fly FAIM having much lower identity (~37%). However, the AlphaFold predicted structure of these proteins reveals near identical structures, with the same topology and fold found in human FAIM. After expressing and purifying FAIM proteins from several eukaryotes, we found that all the FAIM proteins were active, with similar activity to human FAIM. These proteins were able to prevent aggregation of aggregation-prone proteins not found in their native environment (human IAPP, AB42, etc.), demonstrating that FAIM substrate recognition is not species-specific. This work suggests that FAIM is either a broadly acting protein with high promiscuity, or that FAIM substrates can all be defined by a common motif that FAIM identifies. Further investigation of this protein and its function across multiple species will reveal greater 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.

Poster number: 44

Defective TRAP1 chaperone-mediated Complex II assembly underlies the pathogenesis of SDHAF2-mutant disease

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Succinate dehydrogenase (SDH) couples the Krebs cycle to electron transport and is integral to oxidative phosphorylation. Dysregulation of SDH activity is known to contribute to tumorigenesis, while loss of function mutations in SDH or the assembly factor SDHAF2 are associated with pheochromocytomas, paragangliomas and related familial tumor syndromes. Mortality in affected patients is high due to symptomatic dysregulation of adrenal function. Despite this, no targeted therapies are available. The mitochondrial chaperone TNF-receptor associated protein 1 (TRAP1) controls mitochondrial respiration in part through regulation of the SDH subunits SDHA and SDHB, but impact of TRAP1 in SDH assembly is poorly characterized. Our data showed that the pathogenic SDHAF2-G78R mutant exhibited reduced SDHA interaction and diminished SDH activity. We also found SDHAF2-G78R mutation precluded TRAP1-SDHA interaction, while TRAP1 knock-out disrupted SDHA-SDHAF2 complex formation. Taken together, our data suggest a previously unrecognized interplay between SDHAF2 and TRAP1 in regulating SDH complex assembly.

Poster number: 45

Exploring Structural and Functional Effects of Mutating a Semi-Conserved C-Terminal Cysteine in the Yeast Thioredoxin Reductase Trr1

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Thioredoxin reductase plays a crucial role in maintaining redox homeostasis by reducing thioredoxin, a key component in the cellular defense against oxidative stress. While the active site cysteines of Trr1 from Saccharomyces cerevisiae are well-characterized, whether a semi-conserved cysteine residue near its C-terminus has a redox or functional role remains underexplored. This study investigates the role of the cysteine residue at position 305 in Trr1 by substituting it using a semi-saturated mutagenesis approach. Growth assays revealed that yeast strains expressing C305 variants largely rescued the slow growth phenotype associated with Trr1 deletion. However, some C305 variants exhibited increased sensitivity to hydrogen peroxide when compared to wild-type, with the most pronounced variants being proline, tryptophan, lysine, and histidine, indicating compromised oxidative stress tolerance with charged or bulky residues. Growth curve analyses further support these phenotypic characterizations. These results implicate C305 in maintaining redox balance, potentially by stabilizing enzyme structure or facilitating interactions with redox partners. Future work will involve purification of mutant proteins to assess biochemical defects, including whether flavin binding, dimerization, and enzymatic activity are affected. Our studies will help elucidate whether the C-terminal cysteine modulates Trr1's redox interactions, potentially providing new insights into microbial oxidative stress defenses and potential avenues for antimicrobial strategies.

Poster number: 46

MYC and HSF1 co-amplification as a biomarker for treatment in high-grade serous ovarian cancer

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Ovarian cancer is a disease that affects approximately 1 in 91 women. The 5-year relative survival percentage for those with the disease is 51%. The most lethal and common form of ovarian cancer is high-grade serous ovarian cancer (HGSOC). Current precision-based medicine approaches to treating HGSOC are limited to PARP inhibitors for BRCA mutant and homologous combination deficient patients. There is a desperate need for more options for ovarian cancer patients. Preferably these new treatments would both work for a large population of those with high-grade serous ovarian cancer and be precise to a specific biomarker of the disease. We have shown that one possible biomarker for disease treatment is the MYC and HSF1 (Heat Shock Factor 1) co-amplification which occurs in approximately 31% of ovarian cancer patients. We have shown in cell viability assays, colony formation, and spheroid assays that ovarian cancers with this co-amplification are more sensitive to the PLK1i volasertib compared to ovarian cancers that do not carry this co-amplification. Since volasertib is not being pursued clinically as a monotherapy due to its own adverse effect concerns, we aimed to find if there are other drugs that HGSOC with the MYC and HSF1 co-amplification are sensitive to. Considering that HSF1 and MYC are both transcription factors, we hypothesized that an epigenetic inhibitor could be an effective disruptor of MYC and HSF1 activity in HGSOC. We found in a drug screen of 479 drugs that ovarian cancers with this co-amplification are sensitive to class I HDAC inhibitors. We have shown using one of these top-performing HDAC class I inhibitors, entinostat, that MYC and HSF1 protein levels are decreased after 24 hours of treatment with MYC protein stability being able to be rescued by blocking the proteasome with MG132. Further studies will aim to establish the mechanism between how HDAC class I inhibition leads to a decrease in MYC and HSF1 protein and if this mechanism is responsible for cell death seen after 48 hours of treatment is unique to MYC and HSF1 coamplified ovarian cancer.

Poster number: 47

Mechanosensor-mediated Hsp70 phosphorylation orchestrates the landscape of the heat shock response

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Cells must respond rapidly to heat stress by activating multiple signaling pathways that preserve proteostasis. In budding yeast, this includes the induction of Hsf1 and Msn2/4-mediated transcription, cell integrity signaling, stress-triggered phase separation of proteins, and inhibition of translation. How these pathways are so rapidly activated and coordinated remains unclear. We show that the mechanosensor Mid2 senses heat-induced membrane stretch and leads to rapid phosphorylation of the cytosolic Hsp70 Ssa1 at a well-conserved threonine (T492). Phosphorylation of T492 leads to epichaperome rearrangement, promoting fine-tuning of multiple cellular processes, including translational pausing, HSF activity, MAPK signaling, and stress granule resolution. Taken together, these results provide a comprehensive, unified theory of the global yeast heat shock response mediated by the Hsp70 chaperone code.

Poster number: 48

A non-canonical function of DNAJA1 in R2-R2B heterodimer formation: Dimer Exchange and Remodeling Factor (DERF)

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Ribonucleotide reductase (RNR) is the rate-limiting enzyme in deoxyribonucleotide biosynthesis and a well-established therapeutic target in cancer. Human RNR consists of the catalytic subunit R1 and the small regulatory subunits R2 and R2B. We previously identified the Hsp40 co-chaperone DNAJA1 as a key stabilizer of R2 and R2B, yet the molecular basis of this regulation remained unclear. Here, we define the structural and functional mechanisms underlying DNAJA1-mediated control of RNR assembly. Using pull-down assays and cross-linking mass spectrometry (XL-MS) with purified proteins, we demonstrate direct DNAJA1–R2/R2B interactions and map the specific contact interfaces. Modified pull-down assays show that R2 recruits Hsp70 only in the presence of DNAJA1, indicating that DNAJA1 is essential for initiating chaperone engagement. Mass photometry reveals that DNAJA1 promotes formation of R2–R2B heterodimer complexes. Förster resonance energy transfer (FRET) analysis further shows that DNAJA1 destabilizes R2 homodimers and drives their conversion into functional R2–R2B complexes. Ongoing work focuses on understanding how post-translational modifications (PTMs) within the DNAJA1-binding region of R2 affect R2 stability and DNAJA1 recognition, and on developing small-molecule inhibitors to disrupt DNAJA1–R2 interactions. Together, these results uncover a previously unrecognized mechanism of chaperone-mediated RNR regulation and highlight the DNAJA1–R2 interface as a potential therapeutic target in cancer.

Poster number: 49

Micro- and Nano-Sized Plastic Particles Disrupt Proteostasis in C. elegans

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Progressive decline in the ability to preserve proteome integrity is an established hallmark of aging. Metastable proteins, including those implicated in neurodegenerative disease, such as Alzheimer's disease (AD) and Huntington's disease (HD), seem to be especially vulnerable to misfolding. While genetic risk factors for these diseases are increasingly well defined, the extent to which environmental pollutants impact neurodegenerative disease-related protein misfolding is largely unknown. Micro- and nanoplastics are an emerging health concern and have been detected in water, air and soil. Importantly, they are able to penetrate physiological barriers and develop a protein corona that modifies their bioactivity, facilitates their coalescence, and may disrupt proteostasis. Previously, our laboratory found that nano-size particulate matter (nPM) air pollution disrupts proteostasis in Caenorhabditis elegans. Here, using transgenic proteostasis sensor strains expressing aggregation-prone polyglutamine as a model system, we asked whether two plastic-derived pollutants – commercially available nanopolystyrene (nPS) and micro-rubber particles from artificial sports turf – similarly disrupt proteostasis. We found that exposure to both nPS and micro-rubber significantly exacerbates protein aggregation and associated toxicity of the aggregates. Together, our findings suggest that these plastic-derived pollutants represent environmental threats that may contribute to proteostasis collapse and progression of neurodegenerative disorders.

Poster number: 50

Do all phase separating proteins really need intrinsically disordered regions?

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Liquid-liquid phase separation (LLPS) has emerged as mechanism contributing to the protein aggregation observed in diseases like amyotrophic lateral sclerosis (ALS). Aggregating proteins associated with these diseases can undergo LLPS to form dynamic, liquid-like biomolecular condensates. However, persistence of these condensates can lead to their subsequent solidification and transition into stable aggregates. The ability for proteins to undergo LLPS is largely attributed to intrinsically disordered regions (IDRs) and low complexity domains (LCDs). IDRs generally lack defined tertiary structures, and LCDs can promote multivalent interactions. However, the ability of proteins without IDRs or LCDs to form biomolecular condensates has been poorly explored. Transthyretin (TTR) is the amyloid forming protein associated with the fatal systemic disease, transthyretin amyloidosis (ATTR). TTR is a β-strand rich protein with no prominent IDRs or LCDs. The generally accepted model is that TTR uses a nucleation mechanism to form amyloid fibrils. However by expressing human TTR fused to eGFP in yeast, we find that TTR may have abilities to undergo LLPS in vivo. Biochemical data suggests that TTR-eGFP does not form insoluble aggregates commonly associated with β-strand rich amyloid forming proteins. Under normal conditions, TTR-eGFP is cytoplasmically diffuse and occasionally forms a visible focus in a small portion of cells. However after heat shock, about 75% of cells exhibit numerous, small TTR-eGFP puncta. This data suggests that during heat shock TTR-eGFP rapidly de-mixes from a dilute, diffuse state into a punctate phase, a feature of phase separation. These puncta mostly co-localize to the stress granule marker, Pab1-mCherry, and disassemble after release from heat shock. The dynamic and reversible nature suggests that TTR, a non-IDR nor LCD protein, is capable of forming biomolecular condensate-like bodies.

Poster number: 51

Understanding the role of chaperones in the regulation of purine biosynthesis enzyme FGAMS

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Purines are some of the most abundant metabolites in the cell, making up the building blocks for DNA and RNA as well as energy containing molecules such as ATP. The molecular chaperone heat shock protein 90 (Hsp90) interacts directly with phosphoribosylformylglycinamidine synthetase (FGAMS), the enzyme that catalyzes the 4th step in de novo purine biosynthesis. In this study, we found that inhibition of Hsp90 led to FGAMS aggregation and reduction in fluorescence recovery after photobleaching as well as decreased isotope incorporation into newly synthesized purines. Truncated FGAMS constructs interacting with Hsp90 all contained the synthetase domain while expression of the glutaminase domain was reduced compared to other domains. This indicates that the glutaminase domain of FGAMS may be unstable and provides insight into the interaction interface between FGAMS and Hsp90. Gaining a better understanding of the interaction between these proteins may lead to the development of new therapeutic strategies for targeting chaperone-client interactions in the treatment of disease.

Poster number: 52

Linking stresses: molecular transducers after heat shock response enable resilience to pathogens

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Metazoans have evolved conserved stress responses to restore homeostasis against biotic or abiotic conditions. On one hand, a heat shock response activates an expression profile mediated by the Heat Shock Factor 1 to maintain protein homeostasis. On the other hand, innate immunity is an equally ancient property of metazoans to protect against pathogens and other forms of stress conditions. Here we examine how these two mechanisms interact to induce resilience at the organismal level. We performed transcriptomic analysis immediately after stress and during a 24-hour recovery to profile the effect of hormetic heat shock stress. Applying a clustering approach reveals distinct groups of genes, which include the classical heat shock-responsive genes that peak immediately after heat shock and decrease within a 6-hour recovery period. A significant portion of the transcriptional changes followed a pattern that consistently increased expression within the 24-hour recovery period. Among the genes with delayed expression kinetics, there are several protective gene programs, such as innate immunity. Consistent with this finding and prior observations, we found that exposing C. elegans to heat shock induces survival against the opportunistic pathogen Pseudomonas aeruginosa. Genetic analysis shows that the self-oligomerization capacity of TIR-1, a key component of innate immunity, is critical for mounting pathogen resilience after heat shock. Further examination of TIR-1 revealed that it can form discrete foci with low mobility when assessed by FRAP. We are currently exploring the potential positive feed-forward loop connecting the heat shock response to TIR-1 in C. elegans by exploring its biophysical properties and transcript regulation upon heat shock. Altogether, this work could offer insight into how biomolecular condensates can function as transducers of distinct environmental cues to elicit robust stress responses.

Poster number: 53

A multi-omic approach to HSF1 in the mammalian central nervous system reveals non-canonical roles in synaptic development, maintenance, and integrity

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Heat Shock transcription Factor 1 (HSF1) is a stress protective transcription factor canonically known for its role in cellular stress responses and the regulation of protein quality control systems. However, recent evidence suggests HSF1 might play distinct roles in the central nervous system. Through a combination of ChIP-seq, RNA-seq, and proteomic analyses in the mouse striatum, a brain region that controls movement and distinct forms of cognition, we have demonstrated HSF1 primarily controls the expression of key synaptic genes related to cytoskeletal stability and synaptic receptor anchoring. HSF1 displays distinct DNA binding profiles depending on developmental stage, promoting cell proliferation and axon/dendrite elongation early in life and transitioning to synaptic specialization later. Further, we demonstrate that these binding patterns are disrupted in Huntington's disease (HD), a dominantly inherited neurodegenerative disease affecting the striatum, in preference for genes related to immune response. Synaptic deficits are a hallmark of early HD and we hypothesize that the disruption of HSF1 binding activity during these critical windows may contribute to the decrease in synaptic density observed in HD. Striatal synaptic connections also decline during normative aging and contribute to alterations in synaptic dysfunction and behavioral deficits. HSF1 levels also decline during aging, but whether HSF1 is responsible for age-dependent loss of striatal synapses is unknown. We have utilized both chronic and acute reduction of HSF1 levels to demonstrate that HSF1 depletion results in a specific reduction in excitatory striatal synapses and cognition. We are currently making strides towards understanding the role of specific HSF1-regulated genes at the synapse in the context of individual pathways within the striatum. These results demonstrate an emerging role for HSF1 in synaptic gene regulation that has important implications in synapse maintenance and memory during aging and in neurodegeneration that can result in effective therapeutic interventions to ameliorate cognitive deficits.

Poster number: 54

Cell-Type Specific Amyloid Nucleation Kinetics Underlying Selective Vulnerability in Neurodegenerative Disease

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Neurodegenerative diseases (NDs) encompass a range of disorders characterized by the progressive degeneration of the nervous system, affecting millions of individuals globally. Although the pathological proteins associated with each disease, such as TDP-43, huntingtin, α-synuclein, or tau, are broadly expressed, their aggregation and toxicity are strikingly restricted to particular brain regions and cell types. The mechanisms underlying this selective vulnerability remain poorly understood. A critical early event in disease progression is amyloid nucleation, which is the rate-limiting step governing protein aggregation kinetics. A fundamental gap in knowledge is the absence of a systematic, quantitative comparison of amyloid nucleation kinetics in the most vulnerable cells relative to other cell types residing in the brain. The purpose of this study is to understand how nucleation kinetics differ among distinct neuronal and glial cells to unravel the mechanisms governing this selective vulnerability. To address this, we will quantify cell-type specific nucleation behavior using Distributed Amphifluoric FRET (DAmFRET) in neurons and glial cells differentiated from isogenic hiPSCs. DAmFRET exploits a photoconvertible fluorophore, heterogeneous expression, and large cell numbers to quantify the extent of a protein's self-assembly as a function of cellular concentration via flow cytometry. Building on our prior establishment of DAmFRET in yeast, HEK293T, and SH-SY5Y cells, we are now extending this platform to human brain-lineage cells. We will also compare aggregate morphology, spatial distribution, and early nucleation sites using high-resolution microscopy. This work will generate the first mechanistic, cell-type map of amyloid nucleation kinetics in human neural lineages, providing insight into the molecular basis of selective vulnerability and guiding future strategies to modulate nucleation propensity or enhance resilience in vulnerable neuronal populations.

Poster number: 55

Molecular Determinants of Generalist Function in Hsp70

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Generalist molecular chaperones like Hsp70 occupy a unique position in biology, using a single, deeply conserved molecular architecture to recognize and triage an enormous variety of diverse misfolded proteins. How this simple scaffold encodes such expansive, but not unlimited, client specificity remains a central open question in proteostasis biology. Here, we decode these molecular rules by systematically mapping the sequence-function landscape of DnaK (E.coli Hsp70) through deep mutational scanning and screening across a panel of rewired chaperone networks and defined client contexts. We have developed a high-throughput in vivo screen for CHAperone-Mediated Protein Folding (CHAMPFold) that couples DnaK activity to a sequencing-based readout. Using CHAMPFold, we quantify the effects of thousands of DnaK mutations during heat stress and in strains lacking key partner chaperones and co-chaperones (ΔdnaJ, Δtig, ΔhtpG, ΔclpB), revealing how generalist specificity emerges from both intrinsic determinants and inter-chaperone cooperation. To capture client-specific rules, we further challenge each DnaK variant with a library of folding-defective mutants of the model client chloramphenicol acetyltransferase (CAT-I). This design exposes how perturbations in DnaK remodel its ability to rescue distinct misfolding client topologies, providing an orthogonal, client-centered view of specificity that complements the proteome-wide assays. Together, these datasets uncover previously unrecognized functional hotspots and identify condition- and client-specific specificity determinants, defining how Hsp70 architecture encodes generalist activity across diverse proteostatic contexts. CHAMPFold thus establishes a scalable platform for mapping the molecular logic of chaperone-client interactions with both proteome-wide and client-specific resolution.

Poster number: 56

Heat shock factor 1 maintains mitochondrial proteostasis during copper-induced stress in pancreatic cancer cells

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Pancreatic ductal adenocarcinoma (PDAC) cells exhibit elevated copper levels (an essential cofactor that is tightly regulated under normal conditions) to support tumor growth. However, excessive copper can disrupt mitochondrial function by downregulating essential mitochondrial metabolic proteins, including iron–sulfur (Fe–S) cluster proteins, triggering a proteotoxic stress response and copper-mediated programmed cell death. This vulnerability suggests that PDAC cells require an adaptive mechanism to preserve mitochondrial proteostasis when copper levels rise. Heat shock factor 1 (HSF1), a key regulator of the proteotoxic stress response, is highly expressed in PDAC and supports protein stability, mitochondrial function, and tumor progression. Whether HSF1 plays a role in the stability of Fe-S cluster proteins in PDAC cells under copper-induced stress remains unclear. We hypothesize that HSF1 sustains PDAC cell viability during copper overload by preventing the copper-mediated loss of mitochondrial Fe–S cluster proteins. In PDAC cell lines MIA PaCa-2 and PANC-1, treatment with the copper ionophore elesclomol-copper (ES-Cu) reduced protein levels of Fe–S cluster proteins, including ferredoxin 1 (FDX1), aconitase 2 (ACO2), and lipoic acid synthase (LIAS), without altering their mRNA levels. Notably, HSF1 overexpression rescued the copper-induced decrease in expression levels of FDX1, ACO2, and LIAS. We identified the mitochondrial protease Lon protease 1 (LONP1) as a key mediator of ES-Cu-induced decreases in ACO2 and LIAS. Pharmacological inhibition of LONP1 or genetic knockdown of LONP1 reversed ES-Cu-reduced expression of Fe-S cluster proteins. Interestingly, manipulating HSF1 did not affect LONP1 protein levels, and HSF1 translocated to the mitochondria upon copper stress and increased its binding to LONP1. The ES-Cu-mediated decrease in cell viability could be rescued by copper chelator tetrathiomolybdate, suggesting copper-specific cytotoxicity. A combination of HSF1 inhibitor with ES-Cu significantly decreased PDAC cell viability compared to the individual treatments. Our findings suggest that HSF1 protects PDAC cells from copper-induced mitochondrial dysfunction via maintaining the stability of Fe–S proteins. Targeting HSF1 in combination with copper-based therapies may enhance treatment efficacy by disrupting mitochondrial stress adaptation in PDAC.

Poster number: 57

BFSP1-C: A Novel Vertebrate Functional Amyloid in the Lens

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Amyloids are widely associated with neurodegenerative disease; however, growing evidence shows that cells can also employ amyloid assembly for essential physiological functions. The mechanisms that enable functional amyloids to assemble safely—while avoiding pathogenic aggregation—remain poorly understood. Here, we propose to characterize BFSP1-C—the C-terminal fragment of the lens-specific protein filensin—as a novel vertebrate functional amyloid. Using AlphaFold2 predictions and experimental validation, we show that the predicted BFSP1-C amyloid core forms amyloid-like fibrils in vitro and that similar fibrillar assemblies are present in the native ocular lens. Genetic disruption of the BFSP1-C amyloid core in mice causes severe defects in lens fiber cell organization and depth vision, despite the lenses remaining transparent, suggesting a specific role in supporting lens biomechanical properties and visual acuity. This project aims to systematically establish BFSP1-C as a functional amyloid and uncover the principles governing its physiological assembly. Aim 1 will determine the atomic structure of BFSP1-C fibrils by cryo-EM and characterize their assembly properties using biochemical approaches. Aim 2 will determine the functional relevance of the BFSP1-C amyloid by assessing fiber-cell organization, biomechanical properties, and visual performance; dissect the underlying mechanism by examining how the amyloid core directs BFSP1-C membrane targeting and modulates AQP0's dual functions in water transport and cell adhesion; and distinguish the functional roles of amyloid structure and condensation by replacing the native core with synthetic self-assembling sequences. Aim 3 will define the regulatory mechanisms that ensure precise spatiotemporal assembly, including potential inhibition by the BFSP1 N-terminal domain before cleavage and phosphorylation after cleavage, membrane targeting via the upstream amphipathic helix, and spatial restriction through AQP0 membrane arrays seeding. This work will establish a vertebrate functional amyloid model, providing fundamental insights into how cells harness self-templated protein assembly for lens function. The findings will also have broad implications for amyloid-associated diseases.

Poster number: 58

Role of heat shock factor 1 in copper-induced PDAC ferroptosis through regulation of iron-sulfur cluster protein stability

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Pancreatic ductal adenocarcinoma (PDAC) exhibits a high metabolic dependency, supported by metal homeostasis, including copper and iron. Although copper is known to support cellular function and tumor progression, its accumulation can lead to toxicity that causes iron dysregulation and lipid peroxidation–driven programmed cell death, known as ferroptosis. Biochemical studies show that copper can displace iron from iron–sulfur (Fe–S) clusters, leading to labile iron release, but whether this mechanism operates in mammalian cancer cells has not been established. Copper stress also activates cytoprotective heat shock factor 1 (HSF1), a transcription factor in the regulation of tumoral proteostasis, to combat stress-induced protein instability. However, the specific sources of labile iron during copper stress and the role of HSF1 in copper stress-mediated iron homeostasis that drives ferroptosis in PDAC remain unclear. We hypothesize that HSF1 serves as a molecular switch that preserves Fe–S cluster protein integrity and iron homeostasis under copper stress. This regulation thereby protects PDAC cells from lipid peroxidation and ferroptosis. In human PDAC cell lines, copper treatment downregulated mitochondrial Fe–S cluster proteins, increased cellular labile iron, and induced lipid peroxidation. The copper-induced accumulation of free iron was rescued by an iron chelator and a ferroptosis inhibitor in human PDAC cells. Overexpression of HSF1 prevented the decrease in Fe–S cluster proteins and reduced lipid peroxidation induction in PDAC under copper stress. Notably, we found that inhibition of mitochondrial protease preserved the decrease of Fe–S cluster proteins and prevented copper-induced labile iron accumulation. Pharmacological or genetic inhibition of HSF1 enhances copper-induced decrease in human PDAC cell viability. In summary, our results suggest that HSF1 functions as a redox switch coordinating cellular defense against oxidative damage in PDAC cells.

Poster number: 59

A conformationally metastable subproteome drives aging in C. elegans

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Aging and neurodegeneration are associated with the accumulation of metastable proteins, yet their role in systemic proteome failure remains unclear. Using quantitative structural proteomics in C. elegans, we demonstrate that early aging alone alters the structure of approximately one-third of the proteome, associated with broad proteostasis decline. Specifically, many conformationally altered proteins are ubiquitinated and accumulate on proteasomes, indicating stalled degradation as a driver of age-associated failure. To decode the mechanisms of this vulnerability, we challenged the proteome of young animals with diverse single metastable proteins. We found that individual metastable proteins are sufficient to trigger extensive proteome-wide conformational changes that mirror the instability of aging. Crucially, these diverse proteotoxic stresses converge with normal aging on a structurally vulnerable subproteome, enriched for highly abundant proteins and those containing beta-sheets or intrinsically disordered regions. Consistent with this structural vulnerability driving organismal decline, we show that polyglutamine overexpression directly accelerates aging. Together, these data support an “edge of conformational stability” model, wherein the presence of metastable proteins triggers a positive feedback loop of proteostasis decline and accumulation of conformationally unstable proteins, driving widespread proteome failure in aging.

Poster number: 60

Exploring functional redundancy between glutaredoxins and thioredoxins in Saccharomyces cerevisiae

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The thioredoxin (Trx) and glutaredoxin (Grx) systems maintain redox balance in cells by reducing disulfide bonds in substrate proteins in an NADPH-dependent manner. Functional redundancy has been proposed between the two systems, since a single cytosolic Trx or Grx is required for viability in Saccharomyces cerevisiae. To explore this idea of Trx-Grx functional redundancy further, we overexpressed each Grx from S. cerevisiae in cells lacking the cytosolic Trx proteins Trx1 and Trx2. Unexpectedly, we found that no Grx could rescue the slow growth or peroxide sensitivity phenotypes in yeast lacking cytosolic Trx. These results suggest that Grx proteins may have distinct redox partners and functions. To identify potential redox partners of Grxs, we are using crosslinking approach with the bifunctional electrophile divinyl sulfone (DVSF). All eight Grx proteins from yeast are targets of DVSF. We are currently determining which cysteines in the cytosolic Grx proteins Grx1 and Grx2 are targeted by DVSF, as well as using a candidate approach to determine whether there is truly overlap between the interaction partners of Trx and Grx. To perform the latter, we are co-expressing epitope-tagged forms of Grx1 and Grx2 with known Trx and Grx substrates and using DVSF and co-immunoprecipitation to irreversibly capture interactions between potential binding partners. These results will pave the way for future proteomic studies aimed at fully elucidating the interactomes of the cytosolic Grx proteins.

Poster number: 61

E3 ligase recruitment by UBQLN2 protects substrates from proteasomal degradation

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Ubiquilins are a family of cytosolic proteins essential for cellular proteostasis that are traditionally recognized for their role as shuttle factors within the ubiquitin-proteasome pathway. Paradoxically, Ubiquilins have also been observed to stabilize substrates. The basis of this triage decision is unknown. Ubiquilins are also associated with neurodegenerative diseases, as specific mutations in UBQLN2 are causative for X-linked forms of ALS. In addition, Ubiquilins undergo LLPS (Liquid-Liquid Phase Separation) in a concentration-dependent manner. Several mechanistic aspects of Ubiquilin function remain unresolved, including the physiological role of phase separation, the distinct contributions of each paralog, and the effects of ALS-associated mutations on Ubiquilin function. To further elucidate these mechanisms, we adopted a system of isogenic triple knockout (TKO) rescue cell lines engineered to express single Ubiquilin paralogs or ALS disease mutant forms at physiological expression levels under a doxycycline-inducible promoter. Our investigations demonstrate that UBQLN2 is unique among Ubiquilin paralogs in that it can actively stabilize a model substrate. This stabilization correlates with the unique enhanced recruitment of multiple E3 ubiquitin ligases by UBQLN2, including SCFbxo7. We propose that E3 ligase recruitment facilitates UBQLN2-driven phase separation, thereby shielding substrates from proteasomal degradation. Notably, this mechanism also extends to the amyloid precursor protein (APP), which is protected from degradation by the formation of biomolecular condensates. Collectively, our findings introduce a novel conceptual framework for the roles of Ubiquilins in neurodegenerative disease, providing fresh insights into how UBQLN2 modulates proteostasis and substrate fate in the context of neurodegenerative diseases.

Poster number: 62

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

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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.

Poster number: 63

mRNAs blocked in translation initiation form condensates that lurk in polysome fractions

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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. We discovered a new class of mRNP condensates whose abundance correlates with the degree of translation initiation blockage in the absence of stress. 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 measured 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 being translationally inactive. We hypothesized that TIICs could be large enough to co-sediment with polysomes—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 mRNP condensates correlated with translation initiation inhibition? Using EDTA, a chelating agent that disassembles polysomes into free ribosomal subunits and free mRNAs, we discovered EDTA had no effect on the sedimentation behavior of TIICs. More importantly, transcripts that were ribosome-associated sediment less following EDTA treatment, providing a strategy to distinguish TIICs from actively translating mRNAs. Incorporating EDTA treatment alongside polysome profiling will be essential for resolving TIIC-derived sedimentation from translational activity. Taken together, TIICs may represent a hidden layer of mRNA organization that has been co-sedimenting with polysomes and has remained invisible until now.

Poster number: 64

Unraveling TDP-43 sequence contributions to its complex amyloid phase behavior

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Intraneural amyloid deposits involving the C-terminal domain of TDP-43, a ubiquitous nucleocytoplasmic protein, is implicated in most patients with Alzheimer's Disease Related Dementia (ADRD) and in 20% of cognitively normal people above 80 years. TDP-43 oligomerizes via its N-terminus, localizes to the nucleus via an NLS, binds RNA via two RRMs, and phase separates via its disordered C-terminus (CTD). Recent cryoEM findings reveal polymorphism in amyloid core involving the CTD, in different clinical presentations of dementia. Understanding the underlying sequence contributions to pathologic TDP-43 species remains a key problem. Using yeast as a model, high-throughput Distributed Amphifluoric FRET (DAmFRET), semi-denaturing agarose gel electrophoresis and fluorescence microscopy, we queried inactivating point mutants and truncations of TDP-43. We find that CTD is sufficient on its own and is necessary for amyloid formation, while multiple regions of the protein inhibit it. Amyloid propensity anticorrelates with visible condensation of the various TDP-43 mutants. CTD exhibits sub-diffraction sized emulsions with a sharp phase boundary, and amyloid formation exclusively happens beyond that point. Mounting evidence points to amyloid nucleation in living cells happening predominantly at the surface of oligomers in vivo. We also show that the yeast model of amyloid formation holds true in human cell lines as well as when seeded in yeast by human functional amyloids expressed in trans.

Overall, we present a rich resource on the complex phase behavior of TDP-43 CTD that would serve to understand kinetic and thermodynamic drives at play en route pathological aggregation.

Poster number: 65

Reexamining Translation as a Convergent Regulator of Amyloid Nucleation

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Amyloid nucleation is an extremely rare, rate-limiting event in neurodegeneration, yet the cellular factors that tune its probability remain unclear. Using Distributed Amphifluoric FRET (DAmFRET) in HEK293T cells, we find that acute proteasome inhibition with bortezomib (BTZ) robustly suppresses nucleation of mEos-tagged Aβ42, poly-TA, and mHTT. In contrast, Aβ42 fibrillization in vitro is unchanged across 20S proteasome concentrations and insensitive to inhibition, arguing against a direct proteasome–substrate interaction. Inhibition of the AAA-ATPase VCP likewise reduces nucleation. Although BTZ and VCP act at distinct proteostasis nodes, both perturbations lower global translation, suggesting a shared axis. Consistently, proteomics of mEos immunoprecipitates reveal BTZ-dependent enrichment of ribosome/translation Gene Ontology pathways. To test causality, we engineered Kozak and 5'-UTR variants to modulate initiation efficiency: dampening initiation consistently decreased nucleation, whereas enhancing translation restored or increased it. Differences in nucleation were not attributable to expression level, as total construct expression was comparable across experimental paradigms. Alternative mechanisms—including autophagy, HSP70 induction, cytoplasmic crowding, and aggresome formation—did not account for the effects observed. Together, these findings indicate that diverse cellular stresses converge on translational output to calibrate the kinetic barrier for amyloid nucleation. Rather than proteasome impairment promoting assembly via simple concentration effects, our data support a model in which translational initiation/flux actively tunes nucleation barriers. This establishes a direct functional link between protein synthesis and the emergence of proteopathic assemblies, reframing how proteostasis pathways may influence disease-relevant amyloid formation.

Poster number: 66

Protein kinase CK2α' as a dual modulator of immune signaling and synaptic dysfunction in Tauopathy

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Tauopathies are a group of neurodegenerative diseases characterized by tau accumulation, neuroinflammation, and synaptic dysfunction, yet effective treatments remain elusive. Protein Kinase CK2 has been previously associated with different aspects of tau pathology but genetic evidence for the contribution of CK2 to tauopathy remained lacking. Here, we show CK2α', one of the two catalytic subunits of CK2, as a novel regulator of tau-mediated neurodegeneration. We found that CK2α' expression is elevated in postmortem brains of dementia patients and in the hippocampus of PS19 tauopathy mice, especially in neurons and microglia. Using genetic haploinsufficiency in PS19 mice, we demonstrated that reduced CK2α' levels significantly decrease phosphorylated tau and total tau burden in the hippocampus and cortex. CK2α' depletion also attenuated microglial activation, pro-inflammatory cytokine production, and microglia synaptic engulfment, enhanced synaptic gene expression, synaptic density, and LTP. Importantly, CK2α' depletion rescued cognitive deficits assessed in the Barnes maze. These effects appear to be mediated through both neuronal and glial functions and may involve CK2α'-dependent modulation of tau-associated phosphorylation and neuroinflammatory and immune signaling pathways.

Poster number: 67

Delineating a regulatory mechanism of Hsp90 buffering

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Genome instability arising from genetic alterations often drives pathological disorders, including cancer. Therefore, mitigating the detrimental effects of these genetic alterations is crucial to disease prevention. The protein-folding chaperone heat shock protein 90 (Hsp90) has been described as a “mutational buffer” that can mitigate the effects of mutations and influence the course of diseases. Hsp90 buffering capacity is known to fluctuate in response to intrinsic and extrinsic factors, such as proteotoxic stress and fever, respectively. Notably, our lab previously demonstrated that disrupting Hsp90 buffering unmasks the deleterious phenotypes of FANCA and BRCA1 mutations, resulting in genome instability and the development of cancerous lesions. However, the genetic basis of Hsp90 buffering is poorly understood. Here, I propose that Hsp90’s buffering capacity can be genetically determined. Higher Hsp90 buffering capacity presumably increases mutational tolerance, which in turn confers protection against complex diseases. To identify genetic factors influencing Hsp90’s buffering capacity in cells, we conducted quantitative trait locus (QTL) mapping of eight complex traits in yeast. This revealed a genetic locus, IRA2, as a highly pleiotropic hit underlying the trait sensitivity to Hsp90 inhibition. We hypothesize that IRA2 modulates the buffering activity of Hsp90. My preliminary data showed that IRA2 knockout compromised Hsp90 chaperoning of its two well-known clients, Mal63 and glucocorticoid receptor (GR). IRA2 depletion increased the turnover rate of Mal63 in a cycloheximide chase assay and reduced the activity of GR in a β-galactosidase-based reporter assay, implicating a role for IRA2 in Hsp90 regulation. Interestingly, the human ortholog of IRA2, neurofibromatosis type 1 (NF1), is a tumor suppressor frequently mutated in various cancers, resulting in aberrant RAS activation. One aspect of the ongoing work is to investigate the functional conservation between yeast IRA2 and human NF1 in regulating Hsp90 buffering. Importantly, delineating the mechanism of IRA2/NF1-mediated Hsp90 buffering regulation will provide valuable insights into the molecular underpinnings of NF1-related disorders. We present data from our ongoing efforts based on quantitative multi-omics and cancer genetics to understand how IRA2/NF1 influences Hsp90’ buffering capacity and alters adaptive traits in yeast and cancer cells.

Poster number: 68

HSP90 Buffers Deleterious Mutations in BRCA1

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Protein-folding chaperone heat shock protein 90 (HSP90) buffers mutations in diverse organisms, but the significance of HSP90 buffering in human disease remains unclear. Here, we report that HSP90 buffers deleterious mutations in the breast cancer type 1 susceptibility gene BRCA1, the poster child of precision medicine. Inactivating mutations in BRCA1 predispose carriers to breast and ovarian cancer by compromising genome maintenance pathways critical for cellular fitness. Using cell fitness assays, we found that HSP90 can support the fitness of BRCA1 mutant cells, and this can be disrupted by inhibiting HSP90 with small molecules. Importantly, the seemingly benign proteotoxic stressor fever also reduced the fitness of BRCA1 mutant cells, showing that HSP90 renders the deleterious effect of mutations conditional on clinically relevant proteotoxic stressors found in the environment. We also determined the mechanism by which HSP90 buffers BRCA1 mutations. Using cycloheximide chase assays, we found that inhibiting HSP90 using small molecules or fever increased the turnover rate of BRCA1 mutants relative to wild-type BRCA1, suggesting that HSP90 stabilizes mutant proteins. Finally, we show that HSP90 buffers about 18% of known BRCA1 mutations in the BRCT domain, promotes mutation accumulation in the general population, and delays breast cancer onset in patients. Altogether, our study extends the clinical significance of HSP90 buffering to a major cancer susceptibility gene, highlighting its importance in cancer predisposition.

Poster number: 69

Constructing a cellular stress response atlas using integrated chemical-genetic and transcriptomic approaches

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A wide variety of environmental stressors, from heat shock to chemical therapeutics, induce proteotoxic stress. To cope with these challenges, organisms have developed cellular stress response mechanisms to maintain cellular homeostasis and functionality. Dysregulation of cellular stress response is associated with numerous human diseases, ranging from cancer to neurodegenerative disorders. Therefore, understanding which members of these cytoprotective programs are the most critical to resilience and the mechanisms by which cells adapt to stressful environments is increasingly important in understanding disease susceptibility and developing targeted therapeutics.

To systematically study cellular stress responses, we employed an integrated approach consisting of high-throughput functional genomics and transcriptomic profiling. An sgRNA library targeting more than 1,500 chaperones, co-chaperones, regulators, and effectors was profiled across 80 distinct compounds that broadly modulate the cellular stress milieu, generating over 124,000 high-confidence gene–compound interactions. In parallel, we characterized acute transcriptional responses to 60 of these compounds using RNA-Seq and categorized stressors by their transcriptomic profiles. From these data, we revealed compounds that induce canonical stress responses, identified novel marker genes for various stress response pathways, and identified chemicals that potently and specifically modulate distinct stress response pathways. Finally, we integrate our genetic data with transcriptome studies to reveal fundamental principles for how cells cope with constitutive stresses and provide the research community with a comprehensive atlas to understand cellular stress response programs and discover novel drug targets to modulate cellular stress response in diseases.

Poster number: 70

Epigenetic control of proteostasis dynamics by RBBP5–mediated H3K4 trimethylation

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Proteostasis is essential for cellular health, with its disruption contributing to aging, neurodegeneration, and metabolic disorders. While the upstream proteostatic stress-sensing and protein-folding mechanisms in the ER and cytosol are well studied, the transcriptional regulation of proteostasis remains incompletely understood, particularly concerning the temporal epigenome dynamics, chromatin landscapes, and co-regulatory networks underlying dynamic proteostasis control. Traditionally, proteostatic stress responses were viewed as acute reactions to noxious stimuli, but recent evidence shows that many proteostasis genes exhibit ~12-hour ultradian rhythms under physiological conditions, driven by a XBP1s-dependent oscillator independent of the circadian clock and cell cycle. By mapping the chromatin landscape of the murine 12-hour oscillator, we identified RBBP5—an essential subunit of the COMPASS complex responsible for H3K4 trimethylation—as a pivotal epigenetic regulator of proteostasis dynamics. In contrast, histone acetyltransferases and H3K9 acetylation were dispensable for dynamic proteostasis gene expression. RBBP5 is not only indispensable for the 12-hour oscillator but also essential for the transcriptional regulation of diverse proteotoxic stresses response, by coactivating XBP1s and promoting H3K4me3 deposition at the promoters of proteostasis genes. As a result, loss of RBBP5 sensitizes cells to proteostatic stress due to impaired autophagy. Proximity labeling of H3K4me3 further uncovered a dynamic chromatin-associated proteomic architecture, including components of COMPASS, the Integrator complex and SWI/SNF remodelers, that constitutes the transcriptional response to proteostatic stress. Together, these findings reveal a previously unappreciated epigenetic timing mechanism distinct from circadian control and establish RBBP5 as a central regulator of proteostasis dynamics, essential for maintaining cellular resilience.

Poster number: 71