Proteostasis Researchers in Canada, Eh (PRinCE)

Inaugural meeting

Donnelly Centre, University of Toronto

June 10-11, 2019

Financial support

Organizers

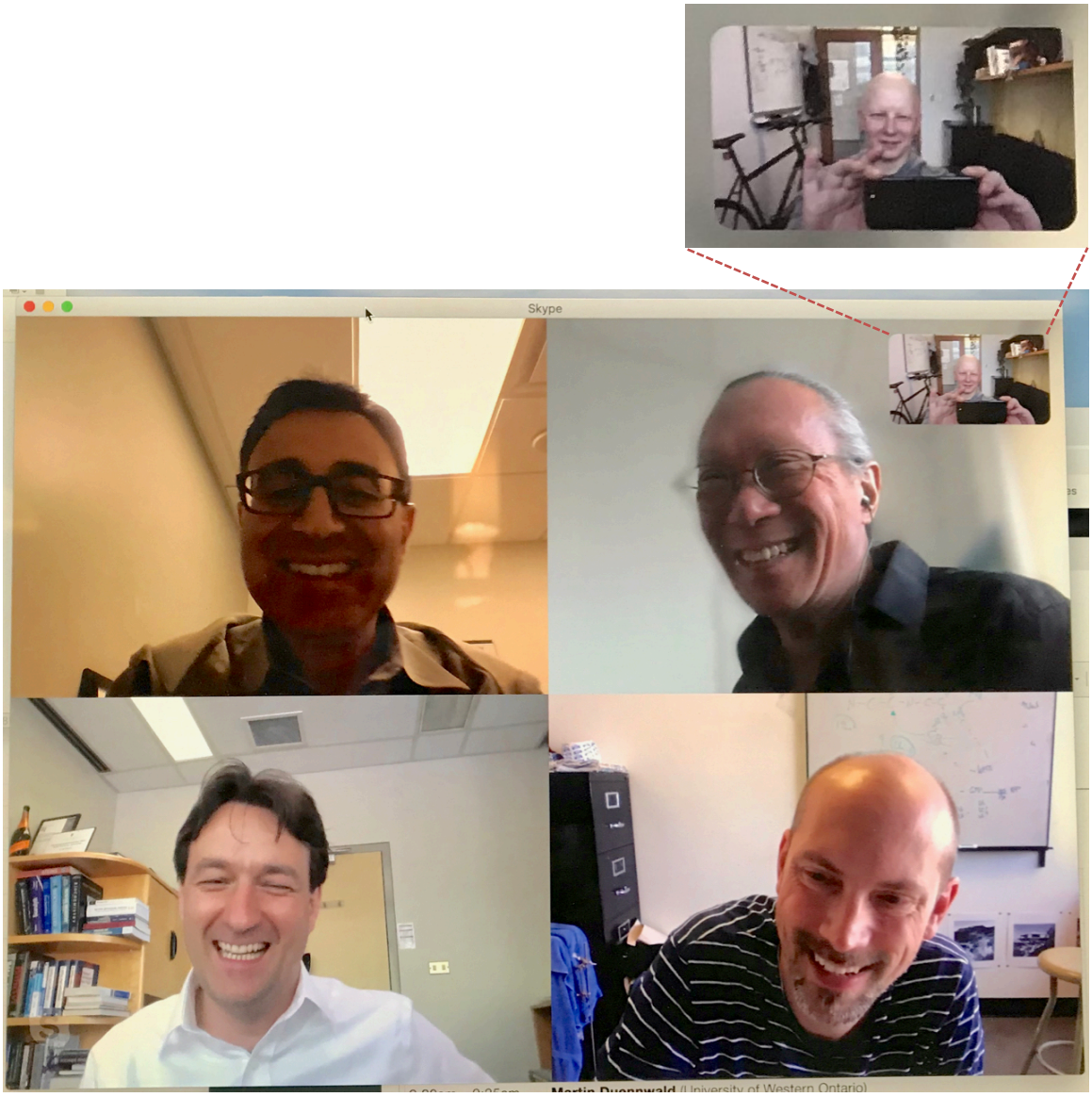

Figure 1. PRinCE organizers hard at work.

Jörg Gsponer, University of British Columbia

Walid Houry, University of Toronto

Thibault Mayor, University of British Columbia

Mikko Taipale, University of Toronto

Jason Young, McGill University

Monday, June 10

8:15am – 8:45am	Registration

Session 1: Hsp90 and systems biology – Chair: Walid A. Houry

8:45am – 9:00am	Opening remarks by organizers
9:00am – 9:20am	Leah Cowen (University of Toronto)
9:20am – 9:35am	Martin Duennwald (University of Western Ontario)
9:35am – 9:50am	Rongmin Zhao (University of Toronto Scarborough)
9:50am – 10:05am	Leo Spyracopoulos (University of Alberta)
10:05am – 10:20am	Georgios Karras (MD Anderson Cancer Center)
10:20am – 10:35am	Andrew Woolley (University of Toronto)

10:35am – 11:00am	Coffee break

Session 2: Keynote speaker – Chair: Jason Young

11:00am – 12:00pm	Jim Bardwell (University of Michigan and HHMI)

12:00pm – 1:30pm	Group picture and lunch break

Session 3: Ubiquitin and proteostasis – Chair: Thibault Mayor

1:30pm – 1:50pm	Kalle Gehring (McGill University)
1:50pm – 2:05pm	Cordula Enenkel (University of Toronto)
2:05pm – 2:20pm	Wei Zhang (University of Guelph)
2:20pm – 2:35pm	El Bachir Affar (Maisonneuve-Rosemont Hospital Res Centre)
2:35pm – 2:50pm	Gil G. Prive (Princess Margaret Cancer Centre)

2:50pm – 3:10pm	Coffee break

Session 4: Protein misfolding diseases 1 – Chair: Mikko Taipale	
3:10pm – 3:30pm	Joel Watts (University of Toronto)
3:30pm – 3:45pm	Neil Cashman (University of British Columbia)
3:45pm – 4:00pm	Sue-Ann Mok (University of Alberta)
4:00pm – 4:15pm	Timothy Audas (Simon Fraser University)
4:15pm – 4:30pm	Patrick Lajoie (University of Western Ontario)

4:40pm – 5:30pm	Panel discussion – future steps for PRinCE
6:00pm – 8:30pm	Cocktail reception and poster session (Stone Lobby, Medical Sciences Building)
8:30pm – late	Dinner and drinks at REDS, 384 Yonge St. (Yonge & Gerrard)

Tuesday, June 11

Session 5: Chemical approaches – Chair: Jörg Gsponer	
9:00am – 9:20am	David Vocadlo (Simon Fraser University)
9:20am – 9:35am	Sharon Gorski (Michael Smith Genome Sciences Centre)
9:35am – 9:50am	Ellen Vieux (C4 Therapeutics)
9:50am – 10:05am	Gerold Schmitt-Ulms (University of Toronto)

10:05am – 10:40am	Coffee break

Session 6: Protein misfolding diseases 2 – Chair: Jason Young	
10:40am – 11:00am	Paul LaPointe (University of Alberta)
11:00am – 11:15am	Gergely Lukacs (McGill University)
11:15am – 11:30am	Sebastian Pechmann (Université de Montréal)
11:30am – 11:45pm	Mohan Babu (University of Regina)
11:45pm – 12:00pm	Avi Chakrabartty (Princess Margaret Cancer Centre)
12:00pm - 12:20pm	Christine Vande Velde (Universite de Montreal)

12:2s0pm – 2:00pm	Lunch

Session 7: Stress response & Compartmentalization – Chair: Thibault Mayor	
2:00pm – 2:20pm	Julie Forman-Kay (Hospital for Sick Children)
2:20pm – 2:35pm	P. H. St George-Hyslop (Univ of Cambridge & Univ of Toronto)
2:35pm – 2:50pm	Hyun Kate Lee (University of Toronto)
2:50am – 3:05pm	Peter Stirling (Terry Fox Laboratory, BC Cancer Agency)
3:05pm – 3:20pm	Janice Braun (University of Calgary)
3:20pm – 3:35pm	Marianne Koritzinsky (Princess Margaret Cancer Centre)

3:35pm – 4:00pm	Poster prizes and final comments

Global Proteomic Analyses Define an Environmentally Contingent Hsp90 Interactome and Reveal Chaperone-Dependent Regulation of Stress Granule Proteins and the R2TP Complex in a Fungal Pathogen

Teresa R. O'Meara[1], Matthew J. O'Meara[2], Elizabeth J. Polvi[1], M. Reza Pourhaghighi[1], Sean Liston[1], Zhen-Yuan Lin[3], Andrew Emili[1,4], Anne-Claude Gingras[3], Leah E. Cowen[1]

[1]University of Toronto, [2]University of California, San Francisco, [3]Lunenfeld-Tanenbaum Research Institute, [4]Boston University

Hsp90 is a conserved molecular chaperone that assists in the folding and function of diverse cellular regulators, with a profound impact on biology, disease and evolution. As a central hub of protein interaction networks, Hsp90 engages with hundreds of protein-protein interactions within eukaryotic cells. These interactions include client proteins, which physically interact with Hsp90 and depend on the chaperone for stability or function, as well as co-chaperones and partner proteins that modulate chaperone function. Currently, there are no methods to accurately predict Hsp90 interactors and there has been considerable network rewiring over evolutionary time, necessitating experimental approaches to define the Hsp90 network in the species of interest. This is a pressing challenge for fungal pathogens, for which Hsp90 is key regulator of stress tolerance, drug resistance, and virulence traits. To address this challenge, we applied a novel biochemical fractionation approach to examine the entire proteome upon Hsp90 perturbation, identified proteins that co-elute with Hsp90, and performed affinity purification coupled to mass spectrometry to define interacting partners for Hsp90 and the Hsp90 co-chaperones in a leading human fungal pathogen, *Candida albicans*. Through this, we defined 37 putative protein clusters in *C. albicans* and identified 195 Hsp90 interacting proteins, including 137 that are specific to the pathogen. We also performed the first analysis of the Hsp90 interactome upon antifungal drug stress and demonstrated that Hsp90 stabilizes P-body and stress granule proteins that contribute to drug tolerance. We also describe novel roles for Hsp90 in regulating post-translational modification of the Rvb1-Rvb2-Tah1-Pih1 (R2TP) complex and the formation of protein aggregates in response to thermal stress. This study provides a global view of the Hsp90 interactome in a fungal pathogen, demonstrates the dynamic role of Hsp90 in response to environmental perturbations, and highlights a novel connection between Hsp90 and the regulation of mRNA-associated protein granules.

Sti1 chaperones protein misfolding in ALS

Martin Duennwald[1]

[1]University of Western Ontario

Protein misfolding is a hallmark of many neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease (PD), and ALS (amyotrophic lateral sclerosis). Cellular protein quality control, i.e. all mechanisms involved in protein synthesis, maintenance, and degradation, typically protects cells from the detrimental consequences of protein misfolding. Clearly, in neurodegenerative diseases, protein quality control fails thus allowing neuronal dysfunction and death to ensue. The central molecular chaperone Hsp90 and its co-chaperones control specific cellular functions and determine specific client protein interactions. Employing a complementary approach in yeast models, mammalian cells, and in vitro biochemistry, we elucidate the molecular and cellular mechanisms by which Hsp90 and its co-chaperones modulate the misfolding of ALS proteins, such as TDP-43, FUS1, and Sod1. We find the co-chaperone St1p uniquely recognizes misfolded proteins and reduces their toxicity.

Role of molecular chaperone HSP90C in regulating PsbO1 homeostasis in the chloroplast stroma

Rongmin Zhao[1,2], Tim Jiang[1,2], Edward Oh[1,2], Christine Yeung[1]

[1]Department of Biological Sciences, University of Toronto Scarborough, [2]Department of Cell & Systems Biology, University of Toronto

HSP90C is an HSP90 family chaperone and localized in chloroplast stroma. The essential role of HSP90C in regulating chloroplast protein import and chloroplast maturation has been well documented, however the exact client spectrum and the detailed mechanism of action of HSP90C is still lacking. In an attempt to modify the expression level of HSP90C in planta, we observed that the expression level of endogenous HSP90C is tightly regulated by plant development, and altered HSP90C expression results in leaf variegation and abnormal chloroplast maturation. We also identified by proteomics analyses that HSP90C interacts with the thylakoid lumen protein PsbO1, which is encoded by a nuclear gene and imported into the thylakoid lumen via the stroma. PsbO1 is a protein that is required for efficient O_2 evolving by the photosystem II, and a native substrate of the thylakoid SEC translocon. To understand the mechanism of HSP90C in regulating PsbO1 homeostasis in chloroplast, we studied the retention and active transport of a PsbO1-GFP fusion protein *in planta* and *in organello*. Our results indicated that HSP90C binds to and facilitates the thylakoid targeting of PsbO1. HSP90C not only binds PsbO1 in the stroma but also associates with the SEC translocon machinery. Our data also suggested that active HSP90C ATPase activity is required to keep the PsbO1 in soluble state which might be critical for its proper thylakoid transport. With analysis of the PsbO1 protein accumulation *in vivo*, our study revealed a mechanism by which HSP90C regulates the chloroplast protein homeostasis.

Hsp90 - A perfect enzyme.

Brian L. Lee[1], Suad Rashid[1], Benjamin Wajda[1], Annemarie Wolmarans[2], Paul LaPointe[2], Leo Spyracopoulos[1]

[1]Department of Biochemistry, University of Alberta, [2]Department of Cell Biology, University of Alberta

Hsp90 is a crucial chaperone whose ATPase activity is fundamental for stabilizing and activating a diverse array of client proteins. Binding and hydrolysis of ATP by dimeric Hsp90 drives an enigmatic conformational cycle characterized by fluctuations between a compact, N- and C-terminally dimerized catalytically competent closed state, and less compact open state which is largely C-terminally dimerized. We used ^{19}F and ^{1}H dynamic nuclear magnetic resonance (NMR) spectroscopy to study the opening and closing kinetics of Hsp90, and to determine the k_{cat} for ATP hydrolysis. We derived a set of coupled ordinary differential equations describing the rate laws for the Hsp90 kinetic cycle, and used these to analyze the NMR data. We find that the kinetics of closing and opening for the chaperone are slow, and that the lower limit for k_{cat} of ATP hydrolysis is ~1 s^{-1}. Our results show that the chemical step is optimized, and that Hsp90 is indeed, a "perfect" enzyme. ^{19}F NMR spectroscopy is a powerful tool for developing an understanding of the mechanistic principles underlying the enzymatic activity of Hsp90. Furthermore, the rate laws we developed for the mechanism of Hsp90 can be applied to a variety of biophysical techniques used to study the conformational cycle, in order to facilitate development of a unified view for the catalytic mechanism.

Fantastic Protein-Folding Mutants and Where to Find Them

Mélody Mazon[1], Brant Gracia[1], Georgios Karras[1]

[1]Department of Genetics, MD Anderson Cancer Center, Houston, TX 77030, USA

Genetic mutations underlie the inheritance of phenotypic traits and the etiology of many diseases. Yet, identical mutations in the same gene, and often, identical genomes, typically lead to qualitatively different phenotypes. The variable penetrance and expressivity of mutations is a consequence of interactions between genes and environment and their effects on the function of gene products in cells, proteins. The challenge for precision medicine is that there are probably several millions of relevant genetic mutations that interact with arguably an infinite number of environmental factors. I propose a new approach to tackle this confounding problem, based on a seemingly distant field, protein folding. To achieve their purpose in life, proteins must properly fold into intricate three-dimensional shapes. To this end, they rely on a specialized suite of proteins, called protein folding chaperones. My work demonstrated that the central protein folding chaperone heat shock protein 90 (HSP90) can "buffer" (that is – mitigate) the deleterious effects of disease mutations in human cells. Furthermore, in doing so, HSP90 renders the effects of mutations conditional upon seemingly benign environmental factors that perturb HSP90 function in the cell, such as fever. A particularly compelling example is provided by a serendipitous "experiment of nature" involving a somatic mutation in monozygotic twins with Fanconi Anemia (FA), a congenital cancer predisposition disorder. The twins had inherited an HSP90-buffered mutation in FANCA from their mother, which led them develop several but distinct congenital manifestations of the disease. However, a compensatory mutation only found in their blood cells prevented the development of anemia. We found that the same acquired mutation alleviated the HSP90-dependence and temperature sensitivity of the HSP90-buffered mutant protein the twins had inherited. We find that similar examples of HSP90-buffering are pervasive in nature, and may explain a substantial fraction of gene-environment interactions that dictate the course and expressivity of disease. Utilizing functional proteomics we are revealing principles for harnessing the protein-folding machinery of the cell to render HSP90-buffered mutations actionable for precision cancer medicine.

A yeast system for discovering optogenetic inhibitors of eukaryotic translation initiation

Andrew Woolley[1]

[1]University of Toronto

The precise spatiotemporal regulation of protein synthesis is essential for many complex biological processes such as memory formation, embryonic development and tumor formation. Current methods used to study protein synthesis offer only a limited degree of spatiotemporal control. Optogenetic methods, in contrast, offer the prospect of controlling protein synthesis non-invasively within minutes and with a spatial scale as small as a single synapse. Here, we present a hybrid yeast system where growth depends on the activity of human eukaryotic initiation factor 4E (eIF4E) that is suitable for screening optogenetic designs for the down-regulation of protein synthesis. We used this system to screen a diverse initial panel of 15 constructs designed to couple a light switchable domain (PYP, *Rs*LOV, LOV, Dronpa) to 4EBP2 (eukaryotic initiation factor 4E binding protein 2), a native inhibitor of translation initiation. We identified cLIPS1 (circularly permuted LOV inhibitor of protein synthesis 1), a fusion of a segment of 4EBP2 and a circularly permuted version of the LOV2 domain from *Avena sativa*, as a photo-activated inhibitor of translation. Adapting the screen for higher throughput, we tested small libraries of cLIPS1 variants and found cLIPS2, a construct with an improved degree of optical control. We show that these constructs can both inhibit translation in yeast harboring a human eIF4E *in vivo*, and bind human eIF4E *in vitro* in a light-dependent manner. This hybrid yeast system thus provides a convenient way for discovering optogenetic constructs that can regulate of human eIF4E-dependent translation initiation in a mechanistically defined manner.

Regulation of mitochondrial quality control by parkin and PINK1

Kalle Gehring[1]

[1]McGill University

Protein phosphorylation and ubiquitination are widespread mechanisms for controlling protein activity. By influencing charge and conformation, they induce changes in protein biological activity and interactions with binding partners. The process of mitochondrial quality control is at the intersection of these pathways. PINK1, a protein kinase, and Parkin, an E3 ubiquitin ligase together regulate the disposal of damaged mitochondria by autophagy. When depolarized or damaged, mitochondria accumulate PINK1 which then phosphorylates ubiquitin to recruit cytosolic Parkin. In a second step, PINK1 phosphorylates Parkin leading to the recruitment of the downstream autophagic machinery. Normally quiescent due to autoinhibitory interactions, Parkin is activated by phosphorylation of its Ubl domain. The molecular mechanisms that activate its E2-binding and catalytic sites have been the focus of much speculation. Here, we report the crystal structure of the active conformation of Parkin. Phosphorylation of the Ubl domain by PINK1 leads to a major conformational change to unleash Parkin ligase activity. The work redefines the role of the Ubl domain and opens up new avenues for the identification of small molecules to activate the PINK1-Parkin pathway.

Proteasome Dynamics

Cordula Enenkel[1]

[1]University of Toronto

Proteasomes are key proteases in regulating protein homeostasis. Their holo-enzymes are composed of forty different subunits which are arranged in a proteolytic core (CP) flanked by one to two regulatory particles (RP). Proteasomal proteolysis is essential for protein quality control and time-sensitive processes like cell cycle progression. In dividing yeast cells with high ATP levels, proteasomes are primarily nuclear and assembled as holo-enzymes engaged in the degradation of poly-ubiquitinated proteins. With the transition to quiescence, the reversible absence of proliferation which is induced by glucose depletion and lower ATP levels, proteasomes move from the nucleus into the cytoplasm. In the cytoplasm of quiescent yeast, proteasomes are dissociated into CP and RP and stored with ubiquitin in membraneless organelles, named proteasome storage granules (PSGs). PSGs protect proteasomes from autophagy. With the resumption of growth, PSGs clear and mature proteasomes are transported into the nucleus by Blm10, a conserved 240 kDa protein and proteasome-intrinsic import receptor.

Here, I will present our recent work on nuclear export of proteasomes. Our data based on yeast mutants deficient in nuclear export of proteasomes suggest that proteasomes use intrinsic transport receptors which sense the need for trafficking between the nucleo- and cytoplasm. Mechanistic details will be discussed. The paradigm of a self-compartmentalizing protease may be expanded to its subcellular localization.

Engineering protein-protein interactions to probe and rewire cell signaling

Wei Zhang[1]

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Effective therapeutic strategies rely on our ability to interfere with cellular processes that are deregulated in human diseases. Thanks to the advance of genomic technologies in recent years, components essential for major biological pathways have been identified at the genetic level. Together they constitute signal transduction cascades relying on protein-protein interactions (PPIs) to elicit various biological functions. However, it is still poorly understood about the exact roles of individual PPI in controlling enzyme activity and complex assembly, especially in the context of diverse signaling networks. Traditional mutation-to-function studies have limitations in this regard due to unpredictable changes in protein folding and conformation, and difficulties in the identification of *bona fide* "separation-of-function" alleles. Hence, there is an urgent need for novel approaches that can selectively probe and investigate individual PPIs to dissect their biological roles. To tackle this problem, we have devised a structure-based combinatorial protein design and engineering strategy to develop novel protein-based PPI modulators. In the past three years, we generated inhibitors and/or activators for more than 50 E3 ligases and deubiquitinases, enzymes that determine specificity of ubiquitination and deubiquitination, respectively. With the help of these synthetic molecules, we discovered new biochemical mechanisms and new biological functions of diverse protein families in the ubiquitination system. Importantly, we have established effective delivery methods for these intracellular probes and successfully target therapeutic-relevant genes in cells and organoids.

Multiple layers of quality control regulatory mechanisms of the deubiquitinase BAP1

Haithem Barbour[1], Salima Daou[2], Oumaima Ahmed[1], Louis Masclef[1], Maxime Uriarte[1], Caroline Baril[1], Eric Bonneil[1], Pierre Thibault[1], Frank Sicheri[2], Marc Therrien[1], El bachir Affar[1]

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The tumor suppressor BAP1 regulates chromatin-associated processes and is a major deubiquitinase (DUB) for histone H2AK119, a modification involved in the coordination of transcription and DNA repair. We established that BAP1 is subjected to several quality control mechanisms ensuring proper targeting and deubiquitination of its substrates in the nucleus. First, the ubiquitin-conjugating and -ligase hybrid UBE2O multi-monoubiquitinates the nuclear localization signal of BAP1, thereby inducing its cytoplasmic sequestration. Importantly, intramolecular interactions, involving multiple domains, ensure BAP1 proper conformation and endow this DUB with the ability to autodeubiquitinate, thus counteracting UBE2O action. Significantly, we identified cancer-derived BAP1 mutations that abrogate autodeubiquitination and promote its cytoplasmic retention, indicating that BAP1 autodeubiquitination ensures proper BAP1 nuclear localization and tumor suppression. In the nucleus, BAP1 assembles DUB complexes with the transcription regulators Additional Sex Combs-Like (ASXLs). ASXLs use their DEUBiquitinase ADaptor (DEUBAD) domain to stimulate BAP1 activity. Interestingly, we found that the DEUBAD domain is monoubiquitinated, resulting in an increased stability of ASXLs, which in turn stimulates BAP1 DUB activity. ASXLs monoubiquitination is directly catalyzed by UBE2E family of ubiquitin-conjugating enzymes, which ensure a rapid turnover of free ASXLs while stabilizing ASXLs integrated within the BAP1 complexes, hence tightly regulating the dosage of ASXLs and DUB activity. This constitutes an important quality control mechanism for ASXLs/BAP1 protein levels and DUB activity. Excess of free ASXLs proteins might be quickly primed by UBE2Es for degradation to prevent potential unwanted effects of orphan ASXLs. As we demonstrated that monoubiquitination of DEUBAD is promoted by the proper folding of BAP1, we also concluded that monoubiquitination of DEUBAD could provide a quality control mechanism for the assembly of a DUB activity competent complex.

BTB adaptors in E3 ligase complexes

Gil Prive[1]

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Many E3 ligases assemble as dimeric or higher order structures, and this is often required for the activity of the complexes. E3 self-association allows for the binding of multiple copies of both substrates and E2s. In the Cullin3-Ring-Ligases (CRL3s), BTB domain proteins act as substrate adaptor proteins that bind directly to both Cul3 and substrates. Moreover, BTB domains can self-associate as dimers, pentamers, and oligomers, leading to bivalent, pentavalent, and polyvalent E3 ligase complexes. These complexes can bind multiple copies of Cul3, E2~Ub and substrate, and multivalency is essential for high levels of activity. Crystal structures of various subcomplexes reveal how the architecture of E3 complexes lead to the spatial positioning of substrates for ubiquitination.

Strains of Pathological Protein Aggregates in the Synucleinopathies

Joel Watts[1,2]

[1]Tanz Centre for Research in Neurodegenerative Diseases, [2]Department of Biochemistry, University of Toronto

The defining feature of synucleinopathies such as Parkinson's disease (PD) and multiple system atrophy (MSA) is the presence of pathological **α**-synuclein (**α**-syn) aggregates within the brain. An emerging theory is that the progressive nature of PD and MSA stems from the formation and spread of prion-like, self-propagating protein aggregates. Additionally, evidence is accumulating that **α**-syn can polymerize into distinct "strains" of aggregates. Prion strains, which are encoded by different conformations of protein aggregates, underlie the clinical and pathological heterogeneity observed amongst prion disease subtypes. We have hypothesized that distinct strains of **α**-syn may be responsible for enciphering disease variability in PD, MSA, and related neurological disorders. Using a transgenic mouse model, we have found that distinct clinical and pathological disease phenotypes can be induced by injection with different strains of recombinant or brain-derived **α**-syn aggregates. In particular, variability was observed in the clinical signs of neurological illness, the morphology and neuroanatomical localization of cerebral **α**-syn deposits, and the conformational properties of the induced **α**-syn aggregates. These differences were maintained following multiple passages in transgenic mice, suggesting that **α**-syn strains "breed true" upon transmission. Our results reveal that **α**-syn strains exhibit key biochemical and pathological hallmarks of prion strains, including selective neuronal targeting. This suggests that prion-like conformational templating is the dominant mechanism driving the observed spread of **α**-syn aggregates in PD and related illnesses.

Exploring the chaperone-tau interaction landscape during tau aggregation

Sue-Ann Mok[1,2], Carlo Condello[2], Rebecca Freilich[3], Anne Gillies[3], Taylor Arhar[3], Javier Oroz[7], Harindranath Kadavath[7], Olivier Julien[1,3], Victoria Assimon[3], Jennifer N. Rauch[3], Bryan M. Dunyak[3], Jungsoon Lee[4], Francis T.F. Tsai[4], Mark R. Wilson[5], Markus Zweckstetter[6,7,8], Chad A. Dickey[9], Jason E. Gestwicki[2,3]

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A network of molecular chaperones is known to bind proteins ("clients") and balance their folding, function and turnover. However, it is often not clear which chaperones are critical for selective recognition of individual clients. It is also not clear why these key chaperones might fail in protein aggregation diseases. In this study, we utilized human microtubule-associated protein tau (MAPT or tau) as a model client to survey interactions between ~30 purified chaperones and ~20 disease-associated tau variants (~600 combinations). From this large-scale analysis, we identified human DnaJA2 as an unexpected, but potent, inhibitor of tau aggregation. DnaJA2 levels were correlated with tau pathology in human brains, supporting the idea that it is an important regulator of tau homeostasis. Of significance, we found that some disease-associated tau variants were relatively immune to interactions with chaperones, suggesting a model in which avoiding physical recognition by chaperone networks may contribute to disease. My laboratory's current projects continue to explore biochemical and cellular properties of chaperone-substrate interactions and amyloid aggregation. For example, we are developing high-throughput screens to identify sequence determinants in tau that promote the formation of distinct aggregate conformations. My lab also has a focus in better understanding substrate

recognition and anti-aggregation functions of the chaperone network in cell-type specific contexts.

Physiological Amyloid Aggregation: An Adaptive Response to Cellular Stress

Dane Marijan[1,2], Emma Lacroix[1], Sahil Chandhok[1], Timothy Audas[2]

[1]Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, Canada.,
[2]Centre for Cell Biology, Development, and Disease, Burnaby, Canada.

Exposure to harsh environmental conditions requires the widespread re-programming of molecular networks to maintain homeostasis and ensure cell viability. Our lab studies a novel post-translational program, which responds to common stressors (i.e., extracellular acidosis, heat shock and proteotoxic stress) by inducing the macromolecular formation of RNA-seeded subnuclear aggregates that possess amyloid-like properties, termed Amyloid-bodies (A-bodies)[1,2]. Biophysically, these stress-responsive protein aggregation share many of the hallmark characteristics associated with the toxic amyloid plaques observed in patients suffering from common neurodegenerative disorders (i.e., Alzheimer's, Parkinson's and prion-based diseases). However, contrary to the pathological aggregates, formation of the physiological A-bodies appears to be rapid, protective and fully reversible.

Recently, we found that the A-bodies formed in response to heat shock and acidotic conditions possess vastly different proteomic constituents, suggesting that this amyloid aggregation event has evolved to carefully tailor the cellular response to each stimulus. Using the RNA helicases DDX39A and DDX39B (proteins that share 90% sequence identity, but divergent stress-specificity) as a tool, we have begun mapping the motif(s) and structural elements necessary for A-body targeting under different stimuli. By furthering our understanding of natural amyloid aggregation, we hope to shed light on the pathological events that lead to plaque formation in common neurological diseases.

REFERENCES

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2. 10.1016/j.molcel.2011.12.012

The impact of expanded polyQ proteins on proteostasis: lessons from a yeast model of Huntington's disease

Patrick Lajoie[1]

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Age-dependent accumulation of incorrectly folded proteins is at the root of several neurodegenerative diseases, including Huntington's disease, which affects thousands of people worldwide. Protein misfolding is detrimental to cells and can lead to loss of protein function and cell death. Expansion of polyglutamine (polyQ) repeats within the Huntingtin protein, results in mutant Htt (mHtt) misfolding and aggregation leading to neuronal cell death in Huntington's disease patient. Importantly, mHtt expression is linked to toxic accumulation of misfolded proteins in several models of Huntington's disease, including yeast, mammalian tissue culture and animal models of Huntington's disease, as well as in post-mortem patient samples. However, the underlying mechanisms associated with this phenotype remain poorly understood. Thus, understanding how mHtt promotes protein misfolding stress is essential in designing new approaches to alleviate mHtt toxicity. We employ yeast genetics to understand the basis of polyQ toxicity. We identified new modulators of polyQ toxicity such as Tra1, which are related to the control of histone acetylation and transcriptional regulation. We are currently investigating how these targets impact gene expression to induced age-dependent toxicity associated with human protein misfolding diseases such as Huntington's.

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Combinatorial treatment with heat shock protein inducers and epigenetic drugs to enhance protein quality control and maintain motor neuron integrity in ALS

Heather Durham[1], Rachel Kuta[1], Nancy Larochelle;[1], Mario Fernandez[1], Sandra Minotti[1], Josephine Nalbantoglu[1]

[1]Montreal Neurological Institute, McGill University

Background: Protein misfolding and aggregation are associated with multiple forms of ALS. Mutations, post-translational modifications or increased protein concentration coupled with the biophysical properties of the affected protein(s) increase demands on chaperoning and proteolytic mechanisms. When these systems become overwhelmed, inappropriate molecular interactions, protein mislocalization and formation of aggregates can ensue, promoting multiple pathogenic cascades. A logical therapeutic strategy is to boost the chaperoning capacity of neural cells by inducing heat shock proteins (HSPs) so they can continue to manage the load of aberrant proteins and remain functional; however, certain challenges remain. 1) Motor neurons have a high threshold for inducing expression of HSPs in response to stress. 2) Motor neurons are relatively resistant to drugs that act as co-inducers of HSP expression, such as arimoclomol. 3) Disease worsens response to HSP-inducers. Loss of efficacy in the CNS can occur as a consequence of changes in chromatin architecture.

Histone acetylation and the chromatin landscape influence expression of stress-response pathways including heat shock genes. Our lab reported that major mechanisms of nucleosome remodeling are abrogated in ALS: a) *Disruption of nBAF chromatin remodeling complexes*, which regulate expression of genes for neuronal differentiation and remodeling in response to activity and b) *Post-translational modifications of histones*, including acetylation, which regulate gene expression and activity of chromatin remodeling complexes. These changes were associated with dendritic attrition, a pathological hallmark of ALS.

Objective: To assess whether particular classes of histone deacetylase (HDAC) inhibitors, as epigenetic modifiers, would enhance the efficacy of the HSP co-inducing drug, arimoclomol, to improve protein quality control mechanisms and maintain motor neuron connectivity.

Approach: Dissociated murine spinal cord cultures were heat-shocked or mutant proteins linked to familial ALS (SOD1G93A or FUSR521H) were expressed in motor neurons by plasmid microinjection. Stress-inducible Hsp70 (HSPA1A), SOD1 and FUS were assessed by immunocytochemistry.

Results: Heat shock stress. The pan HDAC inhibitor, SAHA, and the class I/1 and 3 inhibitor, RGFP109, at concentrations that did not constitutively induce Hsp70, did increase expression in heat-shocked motor neurons and enhanced the effect of arimoclomol, itself a weak co-inducer. The HDAC6 inhibitor, tubastatin A, acted as a co-inducer alone and potentiated arimoclomol.

Mutant SOD1 stress. Arimoclomol had a small effect inducing Hsp70 in motor neurons expressing SOD1G93A. HDAC inhibitors, SAHA and RGFP109, were both more potent HSP co-inducers in motor neurons expressing SOD1G93A. Combinatorial treatment, SAHA or RGFP109 + arimoclomol, was more efficient in inducing Hsp70.

Mutant FUS stress. Neither HDAC inhibitors nor arimoclomol nor the combination induced Hsp70 in motor neurons expressing FUSR521H; however, SAHA and RGFP109, but not tubastatin A, maintained mutant FUS in the nucleus, axonal transport and dendritic architecture.

Conclusions: 1) Certain HDAC inhibitors act as HSP-coinducers in neurons and enhance efficacy of other HSP inducing drugs including HSP90 inhibitors (not shown) and arimoclomol. 2) The co-inducing effect of HDAC inhibitors varies with the stress. 3) The heat shock response is suppressed by mutant FUS. HDAC inhibition does not overcome this block, but inhibitors of class I HDACs preserve nuclear FUS and dendritic architecture through epigenetic mechanisms. 4) RGFP109 is a more class-specific inhibitor with a wide spectrum of protective activities. Next generation compounds with improved CNS PK are being tested.

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A non-canonical role for caspases in the regulation of stress-induced autophagy.

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Autophagy is a lysosome-mediated intracellular degradation and recycling process that plays important roles in development, aging, and diseases including neurodegenerative disorders and cancer. Autophagy functions to maintain cell homeostasis and serves as an adaptive survival response to various cellular stresses, like nutrient deprivation, hypoxia and chemotherapy. This survival-promoting or cytoprotective function of autophagy is now well documented and shown to be evolutionarily conserved, but how cells regulate this process in response to various stressors is largely unknown.

Caspases are a family of cysteine-dependent aspartate-directed proteases that are well known for their traditional roles in executing apoptotic cell death. Using a *Drosophila* model, we previously discovered a novel non-apoptotic role for an effector caspase in the regulation of autophagy. We found that the *Drosophila* effector caspase was required for upregulating autophagy in cellular stress conditions, such as nutrient deprivation and proteasome inhibition. Functional loss of Hsp83, the *Drosophila* ortholog of human HSP90 (heat shock protein 90), resulted in reduced proteasomal activity and elevated levels of the effector caspase Dcp-1. Surprisingly, genetic analyses showed that the caspase was not required for cell death in this context, but instead was essential for the ensuing compensatory autophagy, female fertility, and organism viability. The zymogen pro-Dcp-1 was found to interact with Hsp83 and undergo proteasomal degradation in an Hsp83-dependent manner. Our recent studies in mammalian cells identified effector caspases with a similar role in promoting stress-induced autophagy, supporting an evolutionarily conserved mechanism of autophagy regulation to sustain homeostasis.

Towards understanding protein homeostasis at a systems level through constraint-based whole-cell modeling

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Cells maintain protein homeostasis through a complex regulatory network that integrates protein biosynthesis, folding, degradation, and trafficking pathways. Failure of protein homeostasis is directly linked to conditions of aging and aging-associated diseases such as Alzheimer's and Parkinson's. However, our understanding of why protein homeostasis fails as organisms age and how this failure leads to disease onset remains very limited.

A fundamental challenge lies in the shear complexity of the system that is characterized by pervasive redundancies and hitherto unknown feedback mechanisms, as well as competition for limited capacity of individual protein homeostasis pathways. Computational models are proving exceedingly useful for inferring complex systems behavior by bridging the gap between detailed biochemical knowledge and genomic data that provide snapshots of cellular states.

Here, I will present our progress in developing a constraint-based whole-cell model of protein homeostasis in the eukaryotic model organism *S. cerevisiae*. Using constraint optimization and probabilistic sampling of constraint solution spaces, we derive novel insights into the organization of the protein homeostasis network as well as identify some of its most critical components for systems level function. By exploiting the established power of constraint-based modeling as framework for the integration of heterogeneous data across scales, I will further highlight our efforts to derive detailed biochemical hypotheses on protein homeostasis mechanisms that we hope to further explore in collaboration with experimentalists.

Nuclear protein quality control during the DNA damage response in S. cerevisiae

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During the DNA damage response cells must mount a multi-system stress response to promote survival. Canonically, this global response encompasses DNA repair, cell cycle arrest, and transcriptional rewiring. It has recently been recognized that chaperone-dependent protein sequestration and quality control in the nucleus are also likely to be important parts of the DNA damage response. Using the yeast model, we are exploring how post-translational modifications like SUMO, specific nuclear chaperones like Apj1, and disaggregase proteins like Cdc48, together coordinate the transient sequestration and turnover of a set of substrate proteins that undergo quality control during the DNA damage response. This system should reveal native proteins that undergo quality control in the nucleus, and help delineate principles of stress induced nuclear PQC across species.

Targeted Protein Degradation

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The ability to direct proteins for selective degradation by the ubiquitin-proteasome system using heterobifunctional small molecules has created a unique opportunity to treat human diseases. Targeted protein degradation (TPD) offers the potential to reduce systemic drug exposure, counteract increased target protein expression typically observed when proteins are inhibited, and address protein targets that are not currently therapeutically tractable. The BET family protein BRD4 is a well-credentialed target in both hematologic and solid tumor indications. BRD4 inhibition disrupts oncogenic transcription resulting in therapeutic benefit. BRD4 degradation exhibits a deep, sustained, and strong phenotypic response. In this presentation, I will discuss our approach to characterizing targeted protein degraders including the critical assays used and how they inform on BRD4 degrader development.

Deciphering the role of SACSIN in ARSACS

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a complex neurodegenerative disorder caused by mutations in the *SACS* gene encoding the protein SACSIN, a large multidomain protein (4,579-residue) of unknown function localized to the cytoplasm and outer surface of the mitochondria (mt) in neuronal cell line or primary neurons. Based on domain composition, it has been proposed that SACSIN could function as a chaperone to mediate protein folding or assembly, but this may not be physiologically relevant. SACSIN has also been proposed to regulate mt dynamics as fibroblasts from ARSACS patients show hyperfused mt networks, and is physically associated with the dynamin-related protein 1, DNM1L, which is required for mt fission. Detailed investigations have identified many common interacting proteins involved in inherited ataxias yet how SACS functions on a global scale and in neuronal cell contexts, and how its dysfunction contributes to ARSACS, is still unknown. In my talk, I will discuss our findings that are aimed to address some of these questions from our recently completed proteomics and epistatic screens in ARSACS fibroblasts and differentiated neuronal cells. Overall, our multi-pronged strategy have revealed how macromolecular complex assemblies and epsitatic connections in ARSACS contribute to the dynamic changes in mt networks.

Mutations-specific contribution of peripheral protein quality controls to hERG channel loss-of-expression in LQT2 syndrome

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Accumulating evidence suggests that besides the endoplasmic reticulum (ER) quality control (QC), post-ER or peripheral protein QC systems can contribute to the refolding of metastable and/or degradation of terminally unfolded plasma membrane (PM) proteins (1-4). Diseases causing mutations in CFTR, vasopressin and dopamine D4 receptors, as well as MLC1 impose conformational and processing defect, with limited PM accumulation, hampering the interrogation of the peripheral proteostatic mechanism in the pathogenesis of conformational diseases. Here we use the hERG K^+-channel, which impaired functional PM expression is associated with Long-QT syndrome type-2 (LQT2) and increased risk of cardiac arrhythmia. hERG loss-of-function has been largely attributed to the retention and degradation of severely misfolded channel variants by the ER QC. To investigate the cellular processing of hERG variants with limited misfolding, we selected eight disease causing mutations in the Per-Arnt-Sim (PAS) domain, which can constitutively escape the ER QC at 37°C. We show that both ER QC and the PM QC account for the PM expression defect of hERG in mutation-specific manner. In post-ER compartments, the PAS-mutants exhibit accelerated endocytosis, lysosomal delivery and impaired endosomal recycling via a primarily ubiquitination-independent mechanism. At the ER, six of the PAS-mutants undergo ubiquitin-dependent proteasomal degradation, further attenuating the WT conformational maturation efficiency. Folding correction by a hERG pharmacochaperone or low-temperature partially restores hERG-mutants processing at the ER and post-Golgi compartments, implying that conformational defects signal their elimination by the two major QC systems. These results demonstrate that PM QC either alone or jointly with the ER QC can be responsible for the loss-of-expression PM phenotype of a subset of LQT2 mutations.

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Chemical biology methods to investigate the roles of O-GlcNAc in proteostasis

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The modification of nucleocytoplasmic proteins with *N*-acetylglucosamine (GlcNAc) O-linked to serine/threonine residues (O-GlcNAc) is conserved among multicellular eukaryotes. Although thousands of proteins are modified in mammals, only two enzymes regulate the levels of O-GlcNAc within cells. O-GlcNAc transferase (OGT) installs O-GlcNAc and O-GlcNAcase (OGA) removes it, both without clear sequence specificity. Because the substrate of OGT is UDP-GlcNAc, the end product of the hexosamine biosynthetic pathway, levels of O-GlcNAc vary based on nutrient availability. In recent years this modification has started to emerge as playing fundamental roles in modulating diverse cellular processes including, for example, autophagy, mitochondrial stability, transcription, and stress response. Notably, O-GlcNAc levels have been found to increase in response to diverse stressors and to thereby offer cellular protection. Moreover, pharmacological enhancement of O-GlcNAc levels has been found to offer protection in a range of disease models from Alzheimer Disease through to ischemia-reperfusion injury in brain and heart. Emerging data suggest that O-GlcNAc can regulate protein stability by altering proteolysis as well as, in some cases, protein aggregation. Here we provide a brief introduction to O-GlcNAc and summarize some of our selected studies toward illuminating the roles of O-GlcNAc in proteostasis using chemical biology tools in combination with high-throughput proteomics.

Disrupting proteostasis to enhance tumour immunogenicity

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Immunotherapy is one of the most promising cancer treatment strategies of the 21st century. However, poor tumour immunogenicity is a major limitation to its use. Anti-tumour immunity depends on the ability of immune cells to recognize neoantigenic peptides on the surface of cancer cells. Chaperones like Hsp90 have long been known to be critical for the stability and function of mutated gene products in cancer cells. This has spurred the development of ATP-competitive inhibitors of Hsp90 for cancer treatment. Less appreciated is the role that Hsp90 plays in suppressing the degradation these potentially neoantigenic proteins and the subsequent presentation of peptides on the surface of cancer cells. We have found that Hsp90 inhibitors drive a significant increase in the presentation of the Major Histocompatibility Complex I on the surface of cancer cells. This effect appears to be dependent on the expression of immunoproteasome subunits Lmp2 and Lmp7. We are currently exploring Hsp90 inhibition as a means to enhance the efficacy of immunotherapy. Disruption of proteostasis may represent a broadly effective strategy to enhance immunotherapy and anti-tumour immunity in a wide range of tumour types.

Diagnosis and Treatment of AL Amyloidoses

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Immunoglobulin light chain (AL) amyloidosis is caused by the accumulation of toxic AL amyloid. It is a lethal cardiac condition and also a paraneoplastic syndrome which occurs in 15% of multiple myeloma patients. Misfolded immunoglobulin light chains (Ig-**κ/λ**) can aggregate and form extracellular amyloid fibrils that accumulate in organs, especially the heart and kidneys. Pre-fibrillar Ig-**κ/λ** aggregates are cardiotoxic while mature Ig-**κ/λ** fibrils cause cardiac dysfunction. Currently, treatments are aimed at targeting the source of the aberrant light chains: the hyperproliferating, Ig-**κ/λ**-secreting plasma cell clone. To this end, chemotherapy or autologous stem cell transplantation are frontline therapies. However, there are currently no therapies that address misfolded light chain species that have already deposited in blood vessels or organs and adversely affect vasculature or organ function, or to capture soluble misfolded light-chain intermediates in circulation.

To address this problem, we have devised an innovative strategy to detect and clear toxic amyloid using antibodies that bind misfolded Ig-**κ/λ**. Our technology is based on the design of antibodies that specifically target epitopes on a protein that are only exposed when the protein is misfolded, and thus distinguishing the often-scarce pathological form of a protein from its more abundant native/normal counterpart.

These antibodies will facilitate the degradation and clearance of Ig-**κ/λ** amyloid from peripheral circulation by the immune system. This approach is unprecedented as there is currently no treatment for AL amyloidosis that can clear the existing misfolded toxic aggregates. Our pathology-specific antibodies have potential to be therapeutic agents that could slow the progression of disease or clear accumulated amyloid without the side effect profile or mortality risks associated with the current standard treatment.

The Role of J Proteins in Extracellular Vesicles

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Extracellular vesicles (EVs) are a collection of secreted vesicles of diverse size that are implicated in the physiological removal of nonfunctional proteins as well as the cell-to-cell transmission of disease-causing-proteins in several neurodegenerative diseases. EV's come in different sizes and carry complex cargoes of proteins, lipids and RNA that exert profound effects in recipient cells following uptake. We have shown that the molecular chaperone, cysteine string protein (CSP**α**; DnaJC5), is responsible for the export of disease-causing-misfolded proteins from neurons via EVs.

Mutant huntingtin, the disease causing entity in Huntington's disease, has an expanded polyglutamine track at the N terminus that causes the protein to misfold and form toxic intracellular aggregates. The CSP**α**-EV export pathway exports GFP-tagged 72Q huntingtin^{exon1} from the cell. When we analyzed the pool of exported EVs, we found it to be heterogeneous with the misfolded GFP-tagged 72Q huntingtin^{exon1} cargo located in 180-240nm EVs and 18-25μm EVs. Interestingly, cargo-loading of GFP-tagged 72Q huntingtin^{exon1} into EVs was impaired by resveratrol. We further determined that CSP**α**-EVs efficiently deliver GFP-tagged 72Q huntingtin^{exon1} to naive neurons.

Human mutations in CSP**α** cause the neurodegenerative disorder, adult neuronal ceroid lipofuscinosis, we therefore evaluated the effect of CSP$_{\alpha L115R}$ and CSP**α**$_{\Delta 116}$ on GFP-tagged 72Q huntingtin^{exon1} export. CSP$_{\alpha L115R}$ and CSP**α**$_{\Delta 116}$ were both found to export mutant huntingtin. In contrast, although the loss of function CSP**α** mutant, CSP**α**$_{HPD\text{-}AAA}$ was itself exported, however it did not facilitate export of GFP-tagged 72Q huntingtin^{exon1}.

Our data provides new insights into EV export and proteostasis, as the CSP**α**-EV export pathway facilitates delivery of EVs carrying misfolded proteins to recipient cells.

Folding of secreted proteins in hypoxia

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Low oxygen conditions (hypoxia) occurs in many (patho-)physiological conditions, including embryonic development, wounds, infarcts and solid tumors. The biological response to hypoxia relies in part on expression of proteins in the extracellular space. These proteins fold and mature in the endoplasmic reticulum (ER) prior to proceeding through the secretory pathway, where processing steps include glycosylation and disulfide bond formation and isomerization. Introduction of disulfide bonds is an oxidative process carried out by protein disulfide isomerases (PDIs) that in turn are oxidized by ER-associated oxidases (ERO1s). Molecular oxygen is the only known terminal electron acceptor for this process, consistent with rapid and strong activation of the Unfolded Protein Response (UPR) in hypoxia. How hypoxic cells nevertheless secrete proteins remains a conundrum.

Using pulse-chase labelling of candidate proteins such as LDLR, Flu-HA, VEGF and CA9, we have demonstrated the existence of oxygen-independent pathway(s) for disulfide bond formation in living mammalian cells. However, the capacity of individual proteins to utilize the oxygen independent pathway(s) varies widely. LDLR fails to introduce correct disulfide bonds and pass ER quality control in hypoxia, resulting in complete intracellular retention. In contrast, VEGF has no maturation impairment in hypoxia, while Flu-HA and CA9 retain partial ability to fold and traverse the secretory pathway.

This work demonstrates the existence of an alternative electron acceptor to oxygen driving disulfide bond formation in hypoxia. The varying abilities of individual proteins to utilize this pathway and hence properly fold in hypoxia explains the paradoxical observations of strong activation of the UPR accompanied by secretion of selected gene products.

Gel-like Condensates of Annexin 11 on Lipid Membranes Enable RNP Granule Tethering to Lysosomes

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Annexin 11 (ANXA11) is a member of the annexin family of calcium-dependent phospholipid-binding proteins. Like other annexins, ANXA11 contains four conserved annexin Ca^{2+} binding domains at its C-terminus, but unlike most other annexins, it also contains a low complexity (LC) disordered domain at its N-terminus, which likely becomes exposed following lipid binding. Missense mutations in ANXA11 are associated with familial Amyotrophic Lateral Sclerosis and Fronto-Temporal Dementia.

We have recently shown (Liao, Fernandopulle, Wang et al, submitted) that ANXA11 forms reversible tethers that link lysosomes (via the C-terminal Ca^{2+} binding domains) with RNP granules (via the N-terminal LC domain). This tethering allows RNP granules to be co-transported along axons by hitchhiking on lysosomes which shuttle bi-directionally by microtubule-dependent mechanisms. We report here on the biophysical determinants underpinning this specific transport mechanism.

The observation that ANXA11 contains a significant LC disordered domain prompted us to investigate the ability of ANXA11 to form biological condensates. We found that ANXA11 could indeed phase separate into condensates by itself at high concentrations or in the presence of crowding agents, and that the LC domain is necessary and sufficient for this property. In contrast to many other proteins which form biological condensates upon cooling, ANXA11 has a lower critical solution temperature and phase separates upon warming from 4ºC.

ANXA11 forms biological condensates on the surface of lysosome phospholipid (PI3P)-containing liposomes in a time and Ca^{2+}-dependent manner, a process which could be reversed by EDTA. The liposome-associated condensates exhibit reduced mobility by FRAP and stiffer mechanical properties on microfluidic assays, suggesting that they were gel-like, rather than liquid in nature. Cell-free, *in vitro* reconstitution experiments revealed that ANXA11 could tether purified RNP granules to lysosomes in a Ca^{2+} dependent manner.

This ability of ANXA11 to bind liposomes was modulated by several proteins, and by ALS-associated mutations, which increased propensity to form irreversible fibrillary aggregates. These fibrillar gelled condensates had a remarkable ability to assimilate liquid droplet condensates that contacted them, suggesting a mechanism for propagation of aggregates of ANXA11 in neurodegenerative diseases.

These data illustrate a previously poorly recognized mechanism by which regulated condensation of ANXA11 on the surface of lysosomes allows reversible tethering and co-transporting of RNP granules with membrane-bound intracellular organelles.

BTB adaptors in E3 ligase complexes

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Many E3 ligases assemble as dimeric or higher order structures, and this is often required for the activity of the complexes. E3 self-association allows for the binding of multiple copies of both substrates and E2s. In the Cullin3-Ring-Ligases (CRL3s), BTB domain proteins act as substrate adaptor proteins that bind directly to both Cul3 and substrates. Moreover, BTB domains can self-associate as dimers, pentamers, and oligomers, leading to bivalent, pentavalent, and polyvalent E3 ligase complexes. These complexes can bind multiple copies of Cul3, E2~Ub and substrate, and multivalency is essential for high levels of activity. Crystal structures of various subcomplexes reveal how the architecture of E3 complexes lead to the spatial positioning of substrates for ubiquitination.

A mechanism-based small-molecule approach to the treatment of prion diseases that targets PrPC

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A wide range of observations in humans and animals indicate that a reduction in steady-state levels of the cellular prion protein (PrP^C) is both safe and would delay or prevent prion diseases. To derive rational approaches for suppressing PrP^C levels at the plasma membrane, we and others have been studying the molecular environment and function of PrP^C.

This work led us to recognize that prion genes evolved from a ZIP zinc transporter ancestral molecule with roles in a morphogenetic program known as epithelial-to-mesenchymal transition (EMT). The latter guided us to investigate the possible involvement of PrP^C in EMT, revealing PrP^C to control the polysialylation of the neural cell adhesion molecule 1 (NCAM1), a well-studied post-translational modification that serves as a molecular marker of EMT. In several cell models we interrogated, the molecular environment of PrP^C is characterized by its residence within a specialized membrane domain that hosts, in addition to NCAM1, distinct subsets of more than 20 additional proteins. Interestingly, most of these proteins have known roles in the modulation of TGFB1 and integrin sister signaling complexes that are activated during EMT.

The binding of ligands to their cognate receptors often induces the internalization of ligand-receptor complexes, followed by their recycling or degradation. We reasoned that such a turn of events may lead to a passive co-internalization of PrP^C, effectively reducing its levels at the cell surface. Our recent data reveal that PrP^C levels are indeed robustly reduced in the presence of non-toxic nanomolar concentrations of a compound, hereafter referred to as KDC series 100 compound 2 (KDC102), which targets one of the proteins in proximity to PrP^C. As anticipated, this reduction in PrP^C levels depends on the compound-dependent degradation of the targeted receptor and can be observed in co-cultures of human neurons and astrocytes. Although mechanistic insights are not essential for translation into the clinic, their existence should prove an asset going forward. Current efforts are directed toward testing KDC102 in proof-of-concept prion disease treatment studies in rodents.

RNA binding proteins in Amyotrophic Lateral Sclerosis pathogenesis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective loss of motor neurons, a cell type that is vulnerable to exogenous stress since it cannot be replaced. While two pharmaceutical options exist for ALS, neither truly slows disease nor is an outright cure. Our failing to develop viable therapies is due in part to our incomplete understanding of the fundamental cell biology underlying disease initiation and progression. In recent years, RNA binding proteins and their related proteostasis have emerged as exciting new research areas in this disease. Mutations in TDP-43, and other RNA binding proteins such as hnRNP A1, are causative for rare familial ALS cases. In the case of TDP-43, it is found as a major component of neuronal cytoplasmic inclusions in 97% of all ALS patients even in the absence of mutation. TDP-43 participates broadly in several aspects of RNA metabolism, including alternative splicing and the integration of environmental stress via stress granule formation. Disruptions in stress granule dynamics and homeostasis are considered to be at the root of ALS pathogenesis. Current work explores the relevance of TDP-43 loss of function on stress granule dynamics and their role in neuronal vulnerability and degeneration in ALS.

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1 - Discrimination and quantification of α- and β-synucleins in brain tissue from transgenic mouse models of Parkinson's disease using mass spectrometry

Serena Singh[1], Anouar Khayachi[2], Austen J Milnerwood[2], Mari L DeMarco[1,3]

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α-Synuclein deposits are the primary neuropathological finding in Parkinson's disease. A multiple reaction monitoring liquid chromatography tandem mass spectrometry method was developed to quantify soluble **α**- and **β**-synuclein in brain tissue. The method monitored six tryptic peptides from the **α**-synuclein sequence, which included three regions unique to **α**-synuclein and three regions shared between **α**- and **β**-synuclein. The method was applied to the characterization of brain tissue from wild-type and transgenic mouse models of synucleinopathies. With the multiplex mass spectrometry method, total **α**-synuclein and combined **α**- and **β**-synuclein were quantified in a single measurement. The tool also enabled differentiation of species-specific contributions to **α**-synclein burden in a transgenic model expressing both mouse and human **α**-synuclein. Finally, by monitoring multiple tryptic peptides from synuclein, peptide-specific profile differences were observed that may reflect post-translational modifications along the protein sequence. Ongoing applications of this tool include detailed characterization of synuclein pathology in experimental mouse models and human tissues.

2 - Hyperactive TORC1 sensitizes yeast cells to endoplasmic reticulum stress by compromising cell wall integrity

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The disruption of protein folding homeostasis in the ER results in an accumulation of toxic misfolded proteins and activates a network of signaling events collectively known as the unfolded protein response (UPR). While UPR activation upon ER stress has been well characterized, how other signalling pathways integrate into the ER proteostasis network is still unclear. Here, we sought to investigate how the target of rapamycin complex 1 (TORC1) signaling cascade acts in parallel with the UPR to regulate ER stress sensitivity. Using *S. cerevisiae*, we found that TORC1 signalling attenuates during ER stress and constitutive activation of TORC1 confers increased sensitivity to ER stressors such as tunicamycin and inositol deprivation. This phenotype is independent of the UPR. Transcriptome analysis revealed that TORC1 hyperactivation results in cell wall remodeling. Conversely, hyperactive TORC1 sensitizes cells to cell wall stressors, including the antifungal caspofungin. Elucidating the crosstalk between the UPR, cell wall integrity, and TORC1 signalling may uncover new paradigms through which the response to protein misfolding is regulated, and thus have crucial implications for the development of novel therapeutics against pathogenic fungal infections.

3 - Protein misfolding cyclic amplification (PMCA) is an effective method for generating self-propagating α-synuclein fibrils

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Parkinson's disease (PD), multiple system atrophy (MSA), and dementia with Lewy bodies (DLB) are neurodegenerative diseases with very different symptoms and disease progression rates. While they are collectively termed "synucleinopathies" as they all display filamentous aggregates composed of phosphorylated alpha-synuclein in the brain, the afflicted brain regions and cell types are disease-specific. Previous works have shown that, like prions, alpha-synuclein can aggregate as different conformational "strains." We hypothesize that different strains are responsible for producing the variable disease phenotypes in synucleinopathy patients. To investigate this hypothesis, we are polymerizing recombinant alpha-synuclein using a variety of conditions to create a diverse array of fibril strains, then intracerebrally inoculating transgenic mice with these fibril strains to determine the disease they cause. We have found that, under the same protein concentration and buffer conditions, human alpha-synuclein monomers formed different fibril strains depending on whether they were generated through continuous shaking (thermomixing) for 7 days or through periodic sonication (protein misfolding cyclic amplification, PMCA) for 2 days. These conformational differences were visualized by biochemical assays. Upon intracerebral inoculation into hemizygous transgenic M83 mice, PMCA fibrils induced neuropathology and a fatal neurological disease, whereas thermomixed fibrils did not. Our results demonstrate that the PMCA method generates self-propagating alpha-synuclein fibrils in a quick and replicable manner.

4 - Sfp1 links TORC1 and cell growth regulation to the yeast SAGA-complex component Tra1 in response to polyQ proteotoxicity

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Proteotoxic stress triggers transcriptional changes that allow cells to cope with aberrant accumulation of toxic misfolded proteins. Chromatin remodeling controls gene expression in response to the accumulation of misfolded polyQ expansions associated with Huntington's disease (HD). Tra1 is an essential component of both the SAGA/SLIK and NuA4 transcription co-activator complexes and is linked to multiple cellular processes associated with misfolded protein stress. Due to Tra1 incorporation in both SAGA and NuA4, cells with compromised Tra1 activity display phenotypes that are not necessarily recapitulated by cells carrying deletions encoding components of either complex. Thus, Tra1 potentially has unique regulatory roles in the cellular response to protein misfolding. Here, we employed a yeast model of HD to define the relationship between Tra1 expression and functions and misfolded polyQ proteins toxicity. Our data suggested that expression of expanded polyQ proteins mimics deletion of SAGA/NuA4 components and results in growth defects under stress conditions. Moreover, deleting genes encoding SAGA and, to a lesser extent, NuA4 components exacerbates polyQ toxicity. Also, cells carrying a mutant Tra1 allele displayed increased sensitivity to polyQ toxicity. Interestingly, expression of polyQ proteins upregulated the expression of TRA1 and other genes encoding SAGA components, revealing a feedback mechanism aimed at maintaining Tra1 and SAGA functional integrity. Moreover, deleting the TORC1 (Target of Rapamycin) effector SFP1 abolished upregulation of TRA1 upon expression of polyQ proteins. While Sfp1 is known to adjust ribosome biogenesis and cell size in response to stress, we identified a new role for Sfp1 in the control of TRA1 expression, linking TORC1 and cell growth regulation to the SAGA acetyltransferase complex during misfolded protein stress.

5 - Stable propagation of hamster prions in CRISPR/Cas9-engineered CAD5 cells

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Background. Prion research has been hindered by a lack of cellular paradigms for studying the replication of prions from different species. Although hamster prions have been widely used to study prion replication in animals and within *in vitro* amplification systems, they have proven challenging to propagate in cultured cells. Since the murine catecholaminergic cell line CAD5 is uniquely susceptible to a diverse range of mouse prion strains,[1,2] we hypothesized that it might also be capable of propagating non-mouse prions.

Materials and Methods. We generated CAD5 cells lacking endogenous PrP^C (CAD5-$PrP^{-/-}$ cells) using CRISPR/Cas9-mediated genome editing. CAD5-$PrP^{-/-}$ cells were stably transfected with a plasmid encoding hamster PrP and then challenged with brain homogenates containing various strains of hamster prions.

Results. When exposed to the 263K, HY, or 139H strains, hamster PrP-expressing CAD5-$PrP^{-/-}$ cells stably propagated high levels of protease-resistant PrP. Moreover, when these cells were challenged with 10-fold serial dilutions of 263K prions, PK-resistant PrP was obtained with dilutions up to and including 0.000002% brain homogenate. Cellular homogenates from 263K-infected cells exhibited prion seeding activity in the RT-QuIC assay and were infectious to naïve cells expressing hamster PrP. The presence of endogenous mouse PrP in hamster PrP-expressing CAD5 cells completely blocked the replication of hamster prions. Interestingly, murine N2a neuroblastoma cells ablated for endogenous PrP expression were susceptible to mouse prions, but not hamster prions upon expression of cognate PrP.

Conclusions. Our results demonstrate that CAD5 cells, but not N2a cells, can propagate prion strains from non-mouse species following the ablation of endogenous mouse PrP. This suggests that CAD5 cells either possess cellular factors that enhance or lack factors that restrict the diversity of strains that can be propagated. We conclude that transfected CAD5-$PrP^{-/-}$ cells may be a useful tool for assessing the biology of prion strains and dissecting the mechanism of prion replication.

6 - Development of Fungal-Selective Molecules and Strategies to Target Hsp90

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The frequency of invasive fungal infections is on the rise, and new therapeutic strategies are imperative as the three major classes of antifungals are hampered by host toxicity, the emergence of drug resistance, and a narrow activity spectrum. Selective targeting of stress responses in fungal pathogens provides a promising therapeutic strategy to mitigate drug resistance and combat invasive mycoses. The molecular chaperone Hsp90 has been extensively validated as an essential regulator of virulence traits and antifungal resistance in *Candida* species. However, toxicity of the current Hsp90 inhibitors, that inhibit the host chaperone, impedes their use as antifungal treatments. Here, we assess the therapeutic potential of targeting Hsp90 and its regulatory circuit in fungal pathogens. To do so, we are performing structure-guided optimization of diverse analogs of a natural-product Hsp90 inhibitor, radicicol. Inhibitor binding is assessed by a fluorescence polarization-based assay in fungal and human lysates, and confirmed with purified proteins. Through the screening of >160 synthetic compounds, we have identified chemical matter with >25-fold fungal-selectivity for the leading pathogens *Candida albicans* and *Cryptococcus neoformans*. In parallel, we aim to identify fungal-specific Hsp90-interacting proteins that govern stress responses and virulence. Just over 4,000 *C. neoformans* deletion mutants were screened to map the Hsp90 chemical genetic interaction network, revealing twenty robust interactors, including components of the ergosterol biosynthesis and COP9 signalosome pathways.

7 - Aggregation Discrimination: Differential Targeting of Proteins to Natural Amyloid Aggregates

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Amyloids are a highly organized structure most commonly associated with debilitating conditions, such as Alzheimer's, Parkinson's and prion-based diseases. Formed through the polymerization of proteins adopting a beta sheet-rich conformation, mature amyloid fibers are extremely stable and resistant to degradation. This has heavily contributed to their historical reputation as an irreversible and pathological protein state.

Our lab has shown that mammalian cells exploit the natural amyloidogenic propensity of many proteins in order to survive and adapt to environmental stressors. Exposure to harsh stimuli (e.g., heat shock or acidosis) results in the transient expression of a family of non-coding RNAs that trigger the rapid aggregation of a variety of cellular proteins. In contrast to other membraneless structures, these nuclear aggregates exhibit many of the hallmarks of pathological amyloid plaques, including staining with amyloidophilic dyes, proteinase resistance, immobilized constituent proteins, and a dense/fibrous ultrastructure. Therefore, we have termed these physiological foci amyloid bodies (A-bodies). Remarkably, upon stimulus termination a rapid disassembly of the A-bodies can be observed, highlighting the possibility that amyloid aggregation does not always generate intractable and toxic structures.

Recently, we confirmed that many proteins are detained in A-bodies in a stress-specific manner (i.e. only in response to one stressor, but not others), however, a mechanism for stress-specific A-body targeting remains unknown. Our current work has shown that the RNA helicases DDX39A and DDX39B, which share ~90% sequence identity, are differentially sequestered to A-bodies under heat shock conditions, with only DDX39A consistently being targeted. To uncover the mechanism of this divergence in protein aggregation we compared the targeting of a range of constructs made by substituting individual amino acids of DDX39A with their corresponding DDX39B equivalents. Our data strongly suggests that two central amino acids dictate heat shock-specific amyloid aggregation of DDX39A. Current and future work will focus on confirming these findings and examining how these amino acid substitutions affect A-body targeting in these and other cellular proteins.

8 - The role of HspB1 on the solubilization of aggregates by the human disaggregase system

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The aggregation of misfolded proteins can be very toxic to cells. Especially under stress conditions, when the formation of the aggregates is stimulated, it is important that they are efficiently cleared by autophagy, or by solubilization for refolding or degradation by proteasomes in order to avoid potential pathological conditions, such as neuro-degenerative disorders. Information on how metazoans cope with the accumulation of aggregates was limited, since these organisms do not express the AAA disaggregases found in yeast and bacteria. However, studies have shown that the chaperone Hsp70 requires a specific set of co-chaperones to extract polypeptides from aggregates. In addition, polypeptides must be co-aggregated with a small heat shock protein (sHSP) to be effectively solubilized, but the only sHsp reported so far has been the dimeric Hsp26 from yeast. Therefore, our main objective was to investigate the Hsp70 system and the role of the human sHsp (HspB1) on disaggregase activity. HspB1 is the most ubiquitous human sHsp, highly expressed in different tissues and characterized as large oligomers with size that can reach 1 MDa. First, we analyzed the ability of HspB1 wild type, phosphorylation-mimic mutants HspB1-3D (S15D, S78D, S82D) and the oligomerization disrupting mutant HspB1-3D-GxG to prevent luciferase aggregation under thermal stress. Also, we performed *in vitro* disaggregation assays using aggregated luciferase and we show that Hsp70, Hsp110, DNAJA2, DNAJB1 and mutant HspB1 are necessary for disaggregation of luciferase. When a mix of DnaJ class A and B (DNAJA2 and DNAJB1) is present, disaggregation is more efficient. We also observed that HspB1-3D-GxG is more active and more easily dissociable from oligomers to dimers. We propose that disassembly of HspB1 oligomers in aggregates is part of the mechanism for solubilization by Hsp70.

9 - Characterizing the molecular environment of the bank vole prion protein using mass spectrometry

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The bank vole prion protein (BVPrP) can replicate a wide range of prion strains from different species and has been characterized as the universal acceptor of prions.[1,2] The sequence of mature BVPrP differs from that of mouse PrP (MoPrP) at only 8 amino acid positions. We hypothesized that these unique residues may allow BVPrP to tailor its molecular environment through novel protein-protein interactions. To address this hypothesis, we generated knock-in (ki) mice that express BVPrP (M109 isoform) at physiological levels in the brain. BVPrP ki mice, wild-type C57Bl/6 mice, and PrP knockout mice were subjected to time-controlled transcardiac perfusion crosslinking (tcTPC) and then PrP-containing protein complexes were purified from the brain through immunoprecipitation.[3] Triplicate samples were analysed by quantitative mass spectrometry to permit a direct comparison of the interactomes of MoPrP and BVPrP. The interactome analysis was conducted twice, using two different anti-PrP antibodies recognizing distinct epitopes, to ensure that all relevant interactions were captured. In this study we build a map of the molecular environment of BVPrP, and quantitatively compare it to that of MoPrP. Many known MoPrP interactors were found in our experiments, including NCAM, 4F2, basigin, as well as subtle interactors such as ZIP6 and ZIP10. We found that there was significant overlap between the top interactors found in the BVPrP and MoPrP samples. However, we also identified several proteins that were reproducibly enriched in the samples derived from BVPrP ki mice. We conclude that the molecular environments of BVPrP and MoPrP in the brains of mice are very similar. Nonetheless, several proteins appear to preferentially interact with BVPrP. These proteins may help to explain the unique behaviour of BVPrP.

[1] Watts et al. PLoS Pathog 2014; 10(4):e1003990.

[2] Agrimi et al. PLoS Pathog 2008; 4(7):e1000113.

[3] Schmitt-Ulms et al. Nat Biotech 2004;22(6):724-31.

10 - Distinct Conformers Of Alpha-Synuclein In The Human Synucleinopathies

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Objectives: The aggregation and deposition of the alpha-synuclein protein is a hallmark signature of the synucleinopathies, a group of neurodegenerative diseases that includes Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB) and Multiple System Atrophy (MSA). MSA features as two distinct subtypes, the Parkinsonian (MSA-P) and the cerebellar (MSA-C) variants. Mounting evidence suggests that the formation and spread of self-propagating prion-like alpha-synuclein over the course of a synucleinopathy may contribute critically to the progression of disease. Our goal was to assess whether alpha-synuclein aggregates derived from DLB, PD and MSA patient brains displayed distinct disease-related features and whether this may relate to the conformation of the aggregates in question.

Methods: Alpha-synuclein aggregates from DLB, PD and MSA patient brains were assessed using biochemical assays. Intracerebral inoculations using different synucleinopathy-derived alpha-synuclein aggregates were performed in the hemizygous M83 ($M83^{+/-}$) PD mouse model to evaluate their relative abilities for clinical disease induction.

Results: We found that alpha-synuclein aggregates from DLB and PD brains were less stable than those from MSA, which were the only aggregates capable of accelerating clinical disease in $M83^{+/-}$ transmission experiments. Additionally, conformational distinctions were observed between alpha-synuclein aggregates from the different MSA subtypes, but the differences did not exist in all sampled regions of the patient brains.

Conclusions: Our data suggest that alpha-synuclein aggregate stability may relate to its relative efficiency at accelerating clinical disease in the $M83^{+/-}$ mouse. Differences in alpha-synuclein aggregate conformation may exist in different regions of the same afflicted brain.

11 - Chaperone Disaggregation Machinery in Mammalian System

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The protein aggregates are toxic to the cells. A number of neurodegenerative diseases are resulted from the accumulation of mutant, misfolded protein aggregates. The key proteins that manage the misfolded proteins in the cells are the molecular chaperone Hsp70 and its co-chaperones. Hsp70 mediates protein refolding and also direct proteins into degradation, either by ubiquitin-proteasome system (UPS) or autophagy. However, disaggregation of proteins in mammalian cells is not well understood. For a long time, it was thought that animal cells lack disaggregation activity. Recently, disaggregation by a complex of human chaperones, assisted by a yeast small HSP, was observed in pure protein assays. (1, 2) In our study, we aim to demonstrate disaggregation activity in mammalian cells and to elucidate the mechanism of core disaggregation machinery. We have found that Hsp70, Hsp110 and DNAJB1 are involved with disaggregation in cells. To examine the mechanism of disaggregation, Hsp110 and DNAJB1 knockout HEK293 cell lines and certain mutants with abolished interactions have been generated. In addition, we will also address how disaggregation is related to the degradation via UPS and autophagy. Study of disaggregation system in cells will allow us to have a better grasp on the protective mechanism of cells against toxic aggregates.

12 - The role of Cdc48 in nuclear protein quality after DNA damage in S. cerevisiae

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Cdc48 is a regulator of numerous cellular activities, including several related to the maintenance of a stable genome. Of interest to us is its role as a highly conserved ATPase chaperone that is essential in the assembly or disassembly of protein-DNA complexes and the subsequent degradation of misfolded proteins. We have found previously that following genotoxic stress, Cdc48 relocalizes to intranuclear protein quality control (INQ) sites. Here we identified a previously unknown role of Cdc48 in regulating these nuclear protein aggregates. Cdc48 mutants show reduced ability to recover from genotoxic stress, indicating its role in the cellular stress response. Under non-stress conditions, Cdc48 functions to suppress the formation of INQ sites. Our goal is to further understand the role of Cdc48 in the regulation of specific proteins involved with INQ sites. We first seek to evaluate the composition of nuclear protein aggregates in cells following genotoxic stress using mass spectrometry, then validate Cdc48 interactions with candidate proteins. Our second aim is to explore the influence of Cdc48 loss-of-function alleles on candidate protein localization and stability. This will shed new light on an important aspect of the cellular stress response to DNA damage and link Cdc48 to the DNA damage response in a new way.

13 - Identifying chaperone-substrate relationships in DNA damage responsive protein quality control

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Repair of damaged DNA is crucial for cell survival. The cell employs various DNA damage response mechanisms to recognize and rectify the lesions produced as a result of extrinsic or intrinsic stressors. Therefore, it is in the interest of the cell to main protein homeostasis, or proteostasis, to avoid misfolding and mislocalization of important proteins. This is achieved through a complex network of protein quality control (PQC) circuits that aim to recognize, refold or degrade misfolded proteins. Recent studies have found an unappreciated PQC circuit, wherein upon DNA damage, numerous proteins relocalize to a quality control site in the nucleus called the Intranuclear Quality control site (or INQ), including Rpd3, a histone deacetylase. Here, we use *Saccharomyces cerevisiae* as our model system to establish Rpd3 as an INQ marker and to study the relationship between Rpd3 and Apj1, another INQ protein. Interestingly, the only Hsp40 chaperone that relocalizes to this structure, to date, is Apj1. While Apj1 has been implicated in prion curing and SUMO-mediated protein degradation, its role in PQC remains elusive. We aim to elucidate the role of the Hsp40-Hsp70 machinery in the clearance of proteins at INQ and thus help define chaperone-substrate links in the context of replicative stress-induced protein quality control.

14 - Interactions Between Wild Type and Disease-Causing Mutant Subunits of the hERG Channel

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The human *ether-a-go-go-related gene* (hERG1, or KCNH2) encodes the voltage gated Kv 11.1 potassium channel protein hERG in the human cardiomyocyte. Mutations in hERG cause Long QT syndrome type 2 (LQT2), characterized by impaired ventricular repolarization, and predisposition for *torsade de pointes* tachycardia and sudden death. A tetramer of hERG polypeptides form the channel hERG, which functions on the cell membrane. The majority of the LQT2 causing hERG mutations cause a trafficking defect, leading to reduced cell surface channel expression. Typically, misfolded hERG proteins undergo proteasomal degradation, mediated by the endoplasmic reticulum quality control system.

LQT2 is an inherited autosomal dominant disease, where one mutant allele is sufficient to produce the disease phenotype. However, the underlying mechanism for such dominance remains unclear. Since hERG forms a tetrameric complex, it is possible that mutant subunits impair the function of the wild type (WT) subunits via co-assembling with them, in a dominant negative mechanism. Alternatively, mutant subunits may be unable to assemble with WT subunits, resulting in haploinsufficiency, which would have a milder phenotype. Thus, the aim of my project is to detect interactions between hERG WT and trafficking mutants.I hypothesize that depending on the biochemical properties of the mutant, and the structural domain of hERG where the mutation is, mutant subunits will show different levels of association with WT, thus producing different severities of disease phenotypes. Constructs of myc-tagged WT hERG, and HA-tagged hERG trafficking deficient mutants G601S and F805C were co-expressed in HeLa cells.Relative expression levels of WT and mutant were detected with anti-myc and anti-HA antibodies, respectively. Significant changes in both the levels of myc-tagged WT and HA-tagged G601S mutant subunits were detected when co-expressed, indicating a dominant negative effect. In contrast, no changes were detected in either levels of WT or co-expressed F805C mutant, indicating a haploinsufficiency effect. Next, we will survey various other hERG trafficking mutants. Subunit interactions, channel expression/localization and function will be examined by co-immunoprecipitation, fluorescence imaging, and whole cell patch-clamp respectively. Folding will be assessed

by limited proteolysis, and turn-over rates by pulse-chase experiments. These experiments will establish a molecular basis for the severity of the LQT2 disease phenotype.

15 - Uncovering the role of the proteasome in regulating Candida albicans morphogenesis

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Maintenance of protein homeostasis is critical for proliferation and viability of all organisms. For one of the leading human fungal pathogens, *Candida albicans*, protein homeostasis modulates the ability to transition between yeast and filamentous forms, which is critical for virulence. A key regulator of *C. albicans* morphogenesis is the essential molecular chaperone Hsp90, which mediates proteostasis under physiological and stress conditions. Hsp90 regulates filamentation by repressing morphogenetic signalling through the cyclic AMP-protein kinase A (cAMP-PKA) pathway, such that inhibition of Hsp90 causes filamentation in the absence of an inducing cue. Filamentation is also induced under conditions of cellular stress that overwhelm Hsp90's functional capacity, such as elevated temperature. We explored the effect of perturbation of other protein homeostasis pathways and identified that *C. albicans* morphogenesis is also regulated by the proteasome, a large protein complex consisting of a 20S catalytic core and two 19S regulatory particles, which degrade intracellular proteins. We identified a conserved role of the proteasome in regulating fungal morphogenesis, as pharmacological inhibition with bortezomib or MG132 was sufficient to induce filamentation of *C. albicans* and the related species *Candida tropicalis* and *Candida dubliniensis*. For *C. albicans*, the function of the entire proteasome complex is critical for morphogenesis, as genetic depletion of any of 11 subunits of the 19S regulatory particle or two subunits of the 20S catalytic core was identified to induce filamentation. To elucidate the circuitry through which inhibition of the proteasome induces morphogenesis, we performed a functional genomic screen of conditional expression mutants covering ~40% of the *C. albicans* genome, uncovering a substantial overlap in genes required for morphogenesis in response to inhibition of Hsp90 and inhibition of the proteasome, including components of the cAMP-PKA pathway. This work is poised to uncover the mechanisms through which protein homeostasis maintains the yeast form of growth and has the potential to reveal novel approaches to modulate virulence traits of a major fungal pathogen.

16 - Dysregulation of Human Mitochondrial ClpP Protease Activity by Acyldepsipeptides Leads to Apoptotic Cell Death

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Acyldepsipeptides (ADEPs) are compounds that dysregulate the activity of the highly conserved tetradecameric ClpP proteases. In bacteria, ClpP dysregulation by ADEPs has been shown to induce cell death, highlighting their potential as antibiotics. Recently, we have identified ADEP analogs that are potent in dysregulating the activity of human mitochondrial ClpP (HsClpP). These ADEPs interact with HsClpP with high affinity and greatly enhances its degradation of model peptidyl and protein substrates. Importantly, dysregulation of HsClpP by these ADEPs induces cytotoxic effects in human cells via activation of the intrinsic, caspase-dependent apoptosis. Furthermore, ADEP-induced cytotoxicity has far greater impact on cells with high HsClpP expression than cells with low HsClpP levels. Notably, we have solved the ADEP-HsClpP crystal structure for one of the analogs, and it reveals HsClpP in its activated state with a highly complementary binding interface for ADEP that is formed between two neighbouring HsClpP subunits. Given that HsClpP is highly upregulated in multiple human cancers and has important roles in their metastasis, our findings thus highlight the potential of HsClpP-targeting in cancer therapeutics.

17 - Investigating Tauroursodeoxycholic Acid's Role in Endoplasmic Reticulum Stress and the Unfolded Protein Response

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The budding yeast *Saccharomyces cerevisiae* has been used extensively to study basic cellular processes, many of which are conserved from yeast to mammals. Variations in yeast growth following genetic alteration or drug treatment can be used to understand the impact of specific genes and pathways. Many factors contribute to cell viability and growth, including accumulation of toxic misfolded secretory proteins in the endoplasmic reticulum (ER stress), to which the cell responds through activation of ER stress signaling pathways such as the Unfolded Protein Response (UPR). Misfolded proteins and impaired UPR have been identified as factors in several diseases, including neurodegenerative diseases such as Parkinson's, Huntington's, and Alzheimer's diseases. Tauroursodeoxycholic acid (TUDCA), a bear bile acid used for centuries as a traditional Chinese remedy, has been recently shown to improve symptoms and slow progression in these (and many other) conditions. Despite promising results, TUDCA's mechanism of action is unknown. Given the connection between protein misfolding diseases and those improved by TUDCA, we propose that TUDCA may act by decreasing misfolded protein burden, thereby lessening sensitivity to ER stress. Using a yeast model, we aimed to investigate how treatment with TUDCA affects cellular response to ER stress conditions, including UPR induction, cell growth, and expression of UPR-related genes and proteins. We hypothesized that TUDCA would decrease cellular sensitivity to ER stress. We examined this by assessing yeast growth during ER stress with TUDCA treatment, including those with UPR deletions. We also assessed TUDCA's effect on glycosylation and calcium signaling during stressed and non-stressed conditions. We demonstrated that TUDCA improves cell growth in cells with defective UPR, increases baseline UPR expression, and reduces sensitivity to ER stress. The mechanism of action for these effects is still unclear, but could include mechanisms such as increasing misfolded protein turnover/protein folding capacity in the ER or improving cell wall integrity.

18 - Plastid chaperone HSP90C guides precursor proteins to the SEC translocon on the thylakoid membrane

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The role of plastid stroma factors involved in regulating thylakoid protein targeting is poorly understood. Previously, we reported that in *Arabidopsis thaliana*, the stromal localized chaperone HSP90C interacts with the specialized thylakoid targeting peptide as well as the mature protein of PsbO1, a thylakoid lumen protein. This interaction serves to prevent and/or remove misfolded stromal aggregates, a necessary requirement for proper *de novo* biogenesis of the thylakoid. Here, we show that SecY1, the channel protein of the SEC translocon directly interacts with both HSP90C and PsbO1 by using bimolecular fluorescence complementation assay. Disruption of the chaperone ATPase activity using geldanamycin inhibits the transport of PsbO1 into the thylakoid lumen by inhibiting the association of PsbO1 to SecY1 and subsequent membrane docking. Based on these results, we propose a model in which HSP90C forms a guiding complex that interacts with and assists in precursors that target to the SEC translocon.

19 - Comprehensive characterization of the human Hsp70/J protein/client interactome

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Protein homeostasis, or proteostasis, maintains the health of cells and organisms and protects them from diverse extrinsic and intrinsic stresses. Misregulation of proteostasis is associated with a wide range of human diseases from cancer to neurodegeneration to rare Mendelian disorders. The maintenance of proteostasis is orchestrated by a diverse set of chaperones and other protein quality control factors. One of the central mechanisms for maintaining proteostasis involves the Hsp70 heat-shock protein family together with their co-chaperones known as Hsp40 or DNAJ proteins. DNAJ proteins confer specificity to Hsp70s by recruiting them to specific client proteins and cellular compartments. Together, they regulate the function of a large fraction of the proteome. The human genome encodes 12 different Hsp70 proteins and, remarkably, 48 unique DNAJs. However, the client specificity of most DNAJs has remained elusive. Characterizing the client specificity is essential for understanding how the Hsp70 machinery has evolved and expanded to regulate distinct aspects proteostasis.

We have taken a two-pronged proteomic approach to characterize the client specificity of all human Hsp70s and DNAJs. We employed affinity purification coupled to mass spectrometry (AP-MS) to reveal physical interactors. In parallel, we used proximity biotinylation (BioID) to identify transient interactors and putative membrane-associated clients. Together, these approaches uncovered ~2,200 interactors or proximity partners for Hsp70s and DNAJs. AP-MS data showed striking interaction specificity for many DNAJs, connecting them with diverse pathways. For example, one nuclear DNAJ interacts with histones whereas two other nuclear DNAJs interact with the spliceosome, but each associated with a specific splicing complex. In a highly complementary manner, BioID revealed clustering between HSP70s and DNAJs, reflecting their unique and highly specific subcellular localization patterns.

Comprehensive proteomic profiling of DNAJ and Hsp70 interactions will shed light on their unique biological functions and how they are integrated to the larger proteostasis network with other major chaperone and protein quality control factors.

20 - Regulation of cancer drivers by E3 ubiquitin-ligases

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Cancer is a generic term for a complex heterogeneous class of diseases. Cancer is driven by the alteration of molecular interactions, activation of oncogenes and/or inactivation of tumour suppressor genes (collectively known as cancer drivers). Dysregulation of cancer drivers' turnover is a crucial event, mainly regulated by the ubiquitin-proteasome system (UPS).

By regulating protein degradation, the UPS plays a crucial role in maintaining cellular homeostasis. Due to this high versatility, the UPS is commonly deregulated in cancer. E3 ubiquitin ligases typically provide substrate selectivity. The interaction between the substrate and its cognate E3 is therefore a major point of regulation in protein ubiquitination. Although only a limited number of E3-substrate pairs have been identified.

The goal of my research project is to discover and characterize new E3- substrate pairs among cancer drivers targeted by the UPS and to determine the functional consequences of cancer driver ubiquitination in cellular transformation and tumorigenesis.

To date, I have identified some cancer drivers that are subjected to proteasomal degradation among a library of 350 genes causally linked to cancer. Protein stability was evaluated using a novel high-throughput dual luciferase reporter assay.

Currently, the cancer drivers-E3s interaction network is being investigated using CRISPR/Cas9 screens. 293T-Cas9 monoclonal stable cell lines expressing unstable cancer drivers in fusion with a C-terminal GFP protein followed by an IRES and TagRFP are infected with a UPS lentiviral gRNA library. The library, from Mike Tyers (IRIC), contains 12000 sgRNAs against 1273 UPS genes. Cells are sorted based on the GFP/RFP ratio: high (stabilization) and low (further destabilization) ratios. gRNAs from these and the unsorted bulk population are sequenced to assess the relative enrichment in the sorted populations.

Altogether, my project aims to provide novel insights on how protein degradation contributes to tumorigenesis and hence identifies targets for specific therapeutic intervention.

21 - Functional Identification and Characterization of Regulators Involved in Ubiquitin-Mediated Protein Degradation

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The ubiquitin proteasome system (UPS) is comprised of an enzymatic cascade producing polyubiquitin tags and routing proteins for degradation. The highly regulated hierarchal pathway consists of E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes, and E3 ubiquitin-ligating enzymes providing substrate specificity. Over 600 E3 ligases and substrate receptor subunits have been characterized in the human genome, based on the presence of characteristic domains. However, not all E3 ligases or E3 adaptors contain these domains, and vice versa. Thus, there is a need for functional identification of proteins involved in ubiquitin-mediated degradation to better annotate the UPS. The benefits of comprehensive UPS annotation extend to the development of small-molecule therapeutics, targeting E3 ligases, for selective degradation of disease-associated proteins. Here, I will discuss methodology being used to establish a forced-proximity screen in human cells. Following screen selection, large pools of open-reading frames (ORFs) from the human genome will be used in high-throughput for identification and characterization of functional degraders. A comprehensive list will be produced, and functional degraders validated using proteasome inhibitors to ensure ubiquitin-mediated degradation. Analysis of current protein-interactors and pathway-association will be used to annotate these functional degraders within the UPS. Annotation will be validated and furthered using methodology derived from BioID, identifying proximal interactors for degraders of interest. My work will provide comprehensive annotation of ubiquitin-mediated degradation and bolster development of targeted-protein degradation therapeutics.

22 - Investigating cytosolic quality control pathways responsible for the degradation of proteins with missense mutations

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Missense mutations can induce conformational changes in protein structure that lead to misfolding. In order to avoid the accumulation of misfolded proteins and their aggregation, cells target such misfolded conformers for degradation. In fact, many monogenic disorders, such as cystic fibrosis, are driven by loss of function mutations caused by enhanced degradation. While the ER-associated degradation pathway (ERAD), the main degradative pathway in charge of the degradation of CFTR mutants has been well characterized, much less is known about the sentinel system involved in the degradation of cytosolic proteins with missense mutations. We have compiled a panel of 21 human mutant proteins that are less stable than their wildtype counterparts to investigate cytosolic quality control pathways in mammalian cells. We have recently determined that 20 out of these 21 proteins are degraded by the proteasome. Using a combination of proteomic, genetic, and cell biology approaches we aim to identify members of the protein homeostasis network that are responsible for targeting missense mutant proteins for degradation.

23 - Oxidative stress-induced aggregation of Keap1 impairs Nrf2 regulation

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Nrf2 is the master regulator of the oxidative stress response needed to eliminate reactive oxygen species (ROS) in cells. It is negatively regulated by Keap1, a substrate adaptor protein that allows for the polyubiquitination and ensuing degradation of Nrf2. Cancer cells exhibit persistently high levels of ROS because of genetic and metabolic instability that is compensated for by increased antioxidant abilities. This is Nrf2's dark side in cancer: hyperactive Nrf2 protects normal, but also malignant cells, from ROS and chemotherapeutic agents. This has been linked to poor prognosis in numerous cancers, therefore, elucidating the mechanisms of Nrf2 regulation in cancer is of utmost importance. To mimic the environment of a cancer cell, we assess the Keap1-Nrf2 interaction under high ROS conditions. We postulate that Keap1's unusually high content of cysteine residues, which can easily be chemically modified by ROS, makes Keap1 susceptible to oxidative stress-induced protein misfolding by disulfide bond formation. To study Keap1-dependent Nrf2 regulation, we have established *in vitro* cell culture models and furthermore use purified proteins to biochemically assess protein misfolding. Our data show that Keap1 misfolds and forms dense protein aggregates upon exposure to oxidative stress by hydrogen peroxide treatment. Biochemical evidence also suggests that significant disulfide bond formation is present. The oxidative stress-induced aggregation of Keap1 may render the protein inactive, preventing its interaction with Nrf2. This failure to mediate Nrf2 degradation could lead to Nrf2 hyperactivation and chemoresistance, as seen in many human cancers. Our research provides new insight into previously unexplored aspects of Nrf2 regulation by protein aggregation and introduces Keap1 aggregation as a novel therapeutic target in cancer therapy.

24 - Unraveling the cellular functions of deubiquitinases (DUBs) using yeast functional genomics

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Genetic interactions have proven to be valuable in predicting gene function. A genetic interaction refers to an unexpected phenotype not easily explained by combining the effects of individual genetic variants. Negative genetic interactions refer to a more severe fitness defect than expected, (with the extreme case being synthetic lethality) are of particular interest because they often identify genes that impinge on a common, essential biological function. The set of genetic interactions for a particular gene – the genetic interaction profile - provides a rich phenotypic signature that reflects gene function and forms the basis for building the global genetic landscape of a cell that our lab has recently completed (Costanzo *et.al*, 2016). We were able to predict the functions of several uncharacterized genes by this approach. Deubiquitinases (DUBs), however, are poorly characterised enzymes that remove or edit ubiquitin chains on substrates and thereby modulate key cellular signalling processes. Many of the 22 DUBs in yeast do not fall on this genetic landscape owing to their poor genetic interaction profiles. Using Synthetic Genetic Array (SGA) that was developed in our laboratory, we have constructed all possible double mutants of DUBs and using colony fitness as a proxy for fitness, mapped their genetic redundancy under standard and ~20 different physiologically relevant stress conditions. This analysis has functionally profiled the DUBs and shed light on the hidden extensive buffering/condition specificity in their function. As a proof-of-principle of effectiveness of our approach, we have used one such DUB double mutant *ubp2Δubp14Δ* (that is temperature sensitive) to identify their substrates by an integrated strategy that involved genome-wide synthetic dosage lethality, high-content microscopy combined with automated image analysis and mass spectrometry based proteomics. From this analysis, we have identified previously unappreciated role of DUBs in cell cycle, protein quality control and ribosome integrity. Particularly, we have identified about ~200 proteins that accumulate into inclusions including at the Intranuclear Quality Control Compartment (INQ) in the absence of these DUBs- highlighting their role in regulating protein sequestration/degradation. Moreover, with loss of function suppressor screens and dosage lethality screens, we have identified the quality control E3 ligases (Ubr1, San1, Ufd2, Ufd4, Hul5 and Ltn1) whose functions are counter-acted by Ubp2 and Ubp14 and thus highlighting our approach to map the interplay of E3 ligases and DUBs in fine-

tuning the ubiquitin signalling and further increasing the wiring diagram of cellular function.

25 - Construction of fluorescent protein reporters for spatiotemporal analysis of protein quality control networks in budding yeast

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The temperature-sensitive (ts) mutant strain collection contains over 1000 budding yeast strains, each carrying a conditional allele of a yeast essential gene. Mutants in this collection show a severe fitness defect compared to wildtype when grown at higher temperatures. Many ts alleles are thought to express mutant proteins that are prone to misfold, a concept that has not been systematically explored. As proof-of-principle, we tagged a set of 95 ts alleles with GFP and imaged the cells using high-throughput fluorescence microscopy in a time course at the non-permissive temperature. Over half of the fluorescently tagged ts mutants in this set can be classified into one of three molecular phenotypes: degradation, foci formation and mislocalization. The degradation class showed a loss of GFP signal, the foci class showed distinct puncta in the cell and the mislocalized class showed differential subcellular localization through time under heat stress. This survey provides evidence that the essential gene alleles in the ts strains encode proteins that include dynamic and endogenous substrates which are under the influence of protein quality control processes in the cell. Upon completion, the fluorescent substrate library will be a resource to study proteostasis in a large-scale, systematic manner.

26 - Global Characterization and Underlyin Mechanisms of Protein Secretion Rates in Normoxia and Hypoxia

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Background: Hypoxic tumours are resistant to therapy, leading to poor patient survival. Hypoxic changes such as tumour development, growth, angiogenesis and metastasis lead to an aggressive phenotype. Adverse biological changes stimulated by tumor hypoxia are mediated by cell-surface or secreted proteins. Little is known about how hypoxia affects secretion efficiency of different proteins and therefore ultimately influence differential gene expression in the extracellular space in hypoxia.

Hypothesis: ER-localized protein folding can represent a significant modality for regulating differential gene expression in the extracellular space.

Purpose: Definition of the transit kinetics of the proteins through the secretory pathway on a global scale in normoxic and hypoxic environment will create a unique resource to understand differential gene expression in the extracellular space.

Methods: To label all newly synthesized secreted proteins with a short pulse of a non-endogenous amino acid (azido-homoalanine, AHA), precipitation of the proteins and isolation of the AHA-containing proteins from cells and media at various times after the pulse. This allows downstream isolation through incubation with an alkyne-containing biotin that forms a triazole conjugate upon reaction with the azide. This reaction is often referred to as copper catalyzed azide-alkyne cycloaddition (CuAAc). Biotin-conjugated proteins (proteins that underwent CuAAC reaction) can be isolated on a streptavidin resin

and can be identified by mass spectrometry. The detected proteins will then be annotated through the uniprot database.

Results: Here, we established a novel method to determine the transit kinetics of all newly synthesized secreted proteins. We were able to successfully isolate for specific proteins of interest (AHA labeled) and increase the amount of protein of interest (secreted proteins) pulled down in the analysis of the given pilot mass spectrometry data. The pilot mass spectrometry data allowed us to observe over 50 secreted proteins in lysate and media combined. The proteins detected in this dataset were previously proven to have an active role in cancer progression.

Conclusion: We have established a method to determine the secreted proteins that are involved in cancers that are hypoxic in nature. This research aims to provide a fundamental resource of protein kinetics as well as knowledge on protein folding and its transit pathway. This valuable information will establish a novel understanding of protein secretion within the field of proteomics, improving our understanding of translational regulation of extracellular proteins based on differential oxygen conditions.

27 - POTENTIAL ROLE OF THE MITOCHONDRIA-ASSOCIATED MEMBRANE IN PROTEIN FOLDING

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Background:

The tumor microenvironment is characterized by poor oxygen availability, known as hypoxia. Hypoxia-induced biological processes such as angiogenesis, invasion and migration are mediated by proteins that are secreted or expressed on the cell surface. Oxidative disulfide bond formation in the endoplasmic reticulum (ER) plays a critical role in the protein folding and stability of secretory proteins, and is largely thought to depend on oxygen availability. However, our group has found that some of the hypoxia-induced secretory proteins including vascular endothelial growth factor (VEGF) are able to utilize a hitherto unidentified oxygen-independent pathway to complete their disulfide bond formation and traverse the secretory pathway under hypoxic conditions. In mammalian cells, in addition to the ER, mitochondria are also capable of forming disulfide bonds into proteins de novo. Protein oxidation in the ER and mitochondria is mediated by different folding factors but with a similar biochemical mechanism. Mitochondria and the ER maintain close structural and functional contacts via formation of mitochondria-associated membrane (MAM). Several mitochondrial and ER-resident protein folding factors as well as mitochondrial metabolites required for protein folding are enriched at the MAM. Data from our group suggests that VEGF may undergo ER-localized protein folding in proximity to the MAM. Intriguingly, mitochondria have been described to re-localize closer to the ER nuclear domain area in hypoxia.

Aim:

The overall goal of my PhD research project is to understand the underlying mechanisms involved in protein folding in hypoxia.

Hypothesis:

Based on the unique environment at the MAM, rich in folding factors and mitochondrial metabolites required for protein folding, we hypothesize that the MAM has a role in protein folding in the ER.

Methods:

We apply pharmacological and genetic approaches to disturb the integrity of the MAM and assess its effects on protein folding. Additionally, we investigate the effects of knockdown of mitochondrial protein oxidation factors on protein folding in the ER. We also isolate the MAM under normoxia and hypoxia and apply quantitative mass spectrometry-based proteomics technique to identify and assess potential MAM-resident protein folding factors under normoxia and hypoxia.

Results:

Disruption of the MAM integrity and knockdown of mitochondrial oxidoreductases lead to ER stress and unfolded protein response, supporting a possible role for the MAM in ER-localized protein folding. We were able to isolate the MAM and validate its purity by detecting reliable MAM protein markers.

Conclusion:

Our results suggest that the interaction of the ER and mitochondria may be essential for protein folding. Better understanding of protein folding pathways under hypoxic condition may help in the design of novel therapeutics that target protein folding and cell survival in hypoxic cancer cells.

28 - NSAIDs as potential proteostatic therapies for mislocalized proteins in Cystic Fibrosis.

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The cystic fibrosis (CF) transmembrane conductance regulator (CFTR) is an anion channel expressed at the apical surface of secretory epithelia in the pancreas, intestine, exocrine glands and in lung. Loss-of-function mutations in the *cftr* gene cause the lethal autosomal recessive disease cystic fibrosis (CF). There are over 2000 documented mutations in CFTR which can be divided into 7 classes. The most common class (class 2 which includes F508del) causes CFTR misfolding, retention by the ER quality control (ERQC) mechanism, and degradation by the 26s proteasome. Low temperature partially restores F508delCFTR trafficking *in vitro* and the rescued mutant retains channel activity, albeit with lower open probability compared to wild-type CFTR.

There has been an intensive effort to find molecules that correct the folding defect and restore trafficking of the mutant with most effort being concentrated in finding pharmacological chaperones (VX-809). However to date, such correctors even when in combination with channel potentiators (Orkambi) provide only ~4% improvement in lung function measured as FEV1. Also clinical studies show that between 10-30% of cystic fibrosis patients are unable to tolerate Orkambi and that VX809 is ineffective in some class 2 mutations (e.g. G85E and N1303K).

Here we report that glafenine a non-steroidal anti-inflammatory drug (NSAID) that has been used in 71 countries can act as a proteostatic modulator rescuing F508del-CFTR. We show that glafenine can operate synergistically with VX-809 in correcting F508del-CFTR and that it can rescue class 2 CFTR mutants can VX-809 cannot (G85E andN1303K). Further we will show that glafenine works by directly binding with COX2 enzyme and inhibiting its action. Also we note with interest that glafenine can correct the misfolding of another protein SLC4A11 the protein linked to corneal dystrophy suggesting a common MoA for protein trafficking rescue by glafenine. Lastly while glafenine is a modest corrector we have recently developed a panel of glafenine analogues some of which are significantly more potent correctors than glafenine itself.

These results offer not only the possibility of developing future drugs that give therapeutically relevant levels of correction, but also points to a possible future novel therapeutic niche for proteostatic modulators in treating Cystic Fibrosis.

29 - Aging as a major modifier of polyglutamine aggregation and toxicity

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Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by neurodegeneration in the striatum in an age dependent manner. HD is caused by an expansion of the CAG repeat in exon 1 of the huntingtin (htt) protein encoding a polyglutamine (polyQ) region. The expanded polyQ region leads to protein misfolding and aggregate formation yet the role of these aggregates in HD remains unknown. Generally, protein misfolding is greatly exacerbated upon aging, which is the greatest risk factor for the development of neurodegenerative disorders. The relationship between aging, protein misfolding, and aggregation is poorly understood.

Here we aim to use **Saccharomyces cerevisiae** and as model to study protein misfolding in aging. Chronologically aged yeast cells expressing polyQ proteins have been used to investigate the role of aging in protein misfolding and its toxicity. Resultant changes in localization and aggregation have been documented by fluorescent microscopy. PolyQ aggregation has been evaluated biochemically by SDD AGE and filter trap assays. Additionally, a variety of cellular quality control proteins (e.g. molecular chaperones and heat shock proteins) have been co-expressed with htt polyQ in aged yeast cells to gain a greater understanding of protein quality control mechanisms involved in age-dependent polyQ toxicity. Furthermore, htt polyQ expressed in Neuro 2A (N2A) cells serve to validate our findings in mammalian neuron-like cells.

Our results show that aging exacerbates polyQ toxicity in the absence of molecular chaperones. Toxicity is also increased in the presence of yeast prions in the aging paradigm. Remarkably, polyQ aggregates begin to breakdown as the cells age and the loss of such aggregates precedes cell death. Our results suggest polyQ aggregation and toxicity are modulated upon aging in yeast and polyQ toxicity is dependent upon cellular protein quality control mechanisms, which are conserved from yeast to humans. Additionally, loss of aggregation exacerbates the toxicity associated with protein misfolding and may thus play a role in the enhanced cell death in age-dependent neurodegenerative diseases.

30 - A new role for the conserved NxNNWHW motif in Aha-type co-chaperones in regulating ATPase stimulation

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Aha1-type co-chaperones (Aha1 and Hch1) are best known for their ability to stimulate the ATPase activity of Hsp90 in vitro. Despite this conserved function, they share only two sequence motifs. The most strongly conserved of these is the N terminal NxNNWHW motif that up to now was thought to be dispensable for Aha1 activity. However, we have recently found that this motif is essential for the activity of Aha1-type co-chaperones in vivo. Furthermore, we have identified a role for this motif in regulating intra- and inter-subunit dynamics of Hsp90. We have characterized two mutations in Hsp90 that disrupt the inter-subunit interaction between the N domain of one subunit and the middle domain of the opposite subunit that each confer growth defects in yeast. Overexpression of either Aha1 or Hch1 overcome these growth defects suggesting that regulating this inter-subunit interaction is fundamental to the activity of each of these co-chaperones. Enzymatic analysis of these new Hsp90 mutants as well as Aha1-mediated stimulation reveals a critical role for the NxNNWHW motif of Aha1p in regulating ATP binding and hydrolysis by Hsp90. Our provides mechanistic insight into how Aha1p couples conformational changes in Hsp90 to ATP binding and hydrolysis.