Poster #1

Determining Functional Specificity of Yeast Cytosolic Hsp70 Nucleotide Exchange Factors

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Protein homeostasis requires regulation of many processes and events in a protein’s lifetime from biogenesis to degradation, and disruptions in these steps can lead to terminal misfolding and/or aggregation. In higher eukaryotes such as humans, improper protein folding is linked to neurodegenerative disorders such as Alzheimer’s, Parkinson’s, and Huntington’s diseases. The Hsp70 molecular chaperones play critical roles in protein folding, transport, and degradation and are thus central factors in protein homeostasis. Hsp70s interact with hydrophobic regions of proteins through conformational changes that occur in a nucleotide dependent cycle governed by co-chaperones that affect both hydrolysis and nucleotide exchange. The cytosolic Hsp70s in the budding yeast Saccharomyces cerevisiae are encoded by the SSA and SSB genes, and interact with up to three distinct nucleotide exchange factors (NEFs) homologous to human counterparts; Sse1/Sse2/HSP110, Fes1/HspBP1, and Snl1/Bag. The NEFs perform the same biochemical function of stimulating ADP release and have been maintained throughout eukaryotic evolution. It is currently unknown why at least three families of Hsp70 NEF exist in yeast and humans distinct from the bacterial NEF GrpE, and whether these proteins play unique or redundant roles in Hsp70 biology. We have generated a combinatorial collection of NEF deletion mutants that will be used to genetically delineate their contributions to protein folding/biogenesis, translocation, and degradation. Preliminary analysis revealed that loss of FES1 moderately impacts growth while SSE1 disruption dramatically reduces growth rate, and loss of both resulted in additive defects. These effects were exacerbated at cold and heat-shock temperatures. Disruption of either SSE2 or SNL1 alone resulted in no growth defects under normal or heat/cold shock conditions. However, loss of SNL1 enhanced sse1Δ growth defects under normal but not stress conditions. These initial findings will be extended by assessing relative growth defects under additional stress conditions. Experiments are currently underway to monitor Hsp70-mediated nascent protein folding and refolding of a thermally denatured substrate (a firefly luciferase-GFP fusion) using both enzymatic and microscopic assays. We are also examining NEF participation in post-translational translocation of the mating pheromone α-factor, and Hsp70-mediated degradation of model misfolded proteins. Together these lines of investigation will provide the first comprehensive analysis of Hsp70 NEF function in yeast as a prelude to future studies in human cells, where differential NEF function may impact the development of protein folding disorders.
Poster #2

Influence of Proteasome Inhibition on the Baker’s Yeast Antioxidant Response Transcription Factor Yap1 Following Its Activation by Organic Electrophiles

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Cells have evolved a variety of mechanisms to protect against adverse environmental conditions. In baker’s yeast, the transcription factor Yap1 controls the activation of expression of detoxification genes that ultimately protect against cellular stress caused by oxidants and electrophiles. While Yap1 oxidation is reversible by the thioredoxin protein reductase system, the mechanism of Yap1 inactivation following its modification by electrophiles has not been elucidated. Here, we test the hypothesis that electrophile-modified Yap1 is targeted for degradation by the proteasome. To this end, we have conducted assays using a Yap1-regulated reporter gene in the presence of the proteasome inhibitor MG132 and the electrophilic compounds diethyl maleate (DEM) and diethyl acetylenedicarboxylate (DAD). The data from these experiments indicate that proteasome inhibition does not significantly influence Yap1 activation by electrophiles. In a separate line of investigation, we are studying Yap1 degradation by monitoring whether or not it is conjugated to ubiquitin in the presence of organic electrophiles. Collectively, the results from these experiments will be useful in determining how Yap1 activation is terminated following the exposure of cells to protein-damaging electrophiles.

Poster #3

Hsp70 Expression is Induced by an Increase in PKC-alpha Expression Independent of the HSF-1

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Research has shown that PKC isozymes and heat shock proteins contribute to cardioprotection. Recent investigations into the interaction between these protein families revealed that PKC-alpha can confer protection against simulated ischemia by mediating the induction of Hsp70i. Interestingly, Hsp70i expression was found to be independent of the conventional heat shock transcription factor HSF-1. In an effort to elucidate a potentially novel mechanism, luciferase reporter assays were performed on chimeric rat Hsp70i promoter constructs transfected into myogenic H9c2 cells. Adenoviral overexpression of wild-type PKC-alpha demonstrates that inducibility of the rat Hsp70 promoter is preserved within the first 175 base pairs from the start of transcription. Treatment with inhibitors targeting RhoA (C3 exoenzyme), ROCK (Y27693) and MEK (PD98059) suggest that PKC-alpha transduces its signal through the raf-1/MEK/ERK pathway to upregulate c-Fos expression. These observations are supported by electrophoretic mobility shift assays confirming that c-Fos and c-Jun components bind to two AP-1 elements contained within the first 175 base pair segment of the hsp70 promoter. Although both of these AP-1 sites retain modest induction in isolation, a more robust response occurs when present in tandem. In addition, in situ mutagenesis of each individual AP-1 binding element within the hsp70 promoter indicates that the AP-1 element most distal to the start of transcription is the one responsible for most of the PKC-alpha inducibility. Potentially, these investigations may lead to new therapeutic approaches to harness the cardioprotective effects of hsp70.
**Poster #4**

**The Hsp90-Dependent and Independent Roles of Fkbp38 in the Biogenesis of CFTR**

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The cellular chaperone machinery regulates the biogenesis of nascent ion channels such as the human ether-a-go-go-related gene product (hERG), a potassium channel responsible for long QT syndrome; and the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel responsible for cystic fibrosis but also expressed in cardiac tissues. We use CFTR as a model cargo protein to understand how cellular chaperone machinery mediates the maturation and quality control of ion channels. Immunophilins are involved in multiple cellular processes such as protein folding, receptor signaling, protein trafficking and regulation of gene expression. FK506-binding protein 38 (FKBP38), one of the high molecular weight family members, was identified as component of a chaperone network mediating the biogenesis of hERG and CFTR. FKBP38 contains an N-terminal glutamate-rich domain (ERD), an FK506-binding peptidylprolyl cis/trans isomerase (PPIase) domain, a tetratricopeptide repeats (TPR) domain, a calmodulin binding domain (CaM), and a carboxy terminal transmembrane domain. Like FKBP52, FKBP38 binds Hsp90 through its TPR domain. FKBP52 shares similar domain structure as FKBP38 except that the former lacks transmembrane domain. FKBP52 serves as an Hsp90 cochaperone in the activation of steroid hormone receptors. However, the functional relationship between FKBP38 and Hsp90 in the context of CFTR biogenesis remains unclear. We found that FKBP38 knockdown increases the synthesis of CFTR and promotes its ER-associated degradation (ERAD) whereas re-introducing FKBP38 reverses both effects. To understand the regulation of CFTR biogenesis by FKBP38, we introduced a series of shRNA-refractory domain-specific mutants of FKBP38 into an FKBP38-deficient cell line. We found that the PPIase domain is the main contributor of FKBP38 activity in the context of CFTR biogenesis, it reduces CFTR synthesis and inhibits its post-translational ERAD. In contrast, disruption of the FKBP38 interaction with Hsp90 through specific mutations in the TPR domain reduces CFTR synthesis but has little effect on its ERAD. The transmembrane domain of FKBP38 is not necessary for its regulation of CFTR biogenesis. These data support a central role for the PPIase domain in FKBP38-mediated folding of CFTR and a moderate regulatory role for Hsp90 in FKBP38-mediated co-translational folding and/or ERAD of CFTR.

**Poster #5**

**FCS Based Analyses Reveal Age and Length Dependent Changes of the PolyQ Oligomer Distribution in a C. elegans Model**

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Fluorescence correlation spectroscopy (FCS) is a single molecule sensitive technique frequently used to characterize the biophysical properties of proteins in vitro and can also be applied to complex reactions, such as occurs in vivo during protein conformational changes and self-association events that lead to protein aggregation. Protein misfolding and aggregation have been implicated in a number of neurodegenerative diseases including Alzheimer’s, Parkinson’s and at least 8 polyglutamine (polyQ) expansion disorders. A hallmark of these diseases is the
presence of both insoluble aggregates and soluble oligomers. While many techniques have been developed to identify and characterize aggregates, very few are suitable to characterize oligomeric species' properties. We employed FCS techniques to examine oligomers present in a C. elegans polyQ model, where aggregation is both age- and polyQ-length dependent. While the traditionally used autocorrelation curves were able to detect large changes in the distribution of diffuse polyQ protein, we also show that more advanced analyses can distinguish smaller changes as well as provide more detailed information about the distribution of oligomers. The new analysis methodologies show that polyQ is maintained in a heterodisperse state, that the distribution varies with both age and Q-length and that oligomers begin to appear as early as Day 2. Next, we analyzed the oligomeric states present upon suppression of aggregates by genetic modifiers. This analysis revealed that while all modifiers suppressed formation of aggregates, they do not all affect the oligomeric distribution in the same manner. This work demonstrates that FCS based techniques can distinguish populations of oligomeric species formed in vivo, providing a valuable tool to study the role of oligomers in conformational diseases' progression. In addition, this work provides support for the early appearance of oligomers and their heterodisperse distribution as well as evidence of multiple modes of genetic suppression of aggregation.

Poster #6
The N-terminus of the VirG Autotransporter Destabilizes the Entire Passenger In Vitro Implications for In Vivo Secretion
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VirG is a virulence-associated protein of Yersinia pestis, the causative agent of bubonic plague. A homologous protein, IcsA from S. flexneri, is known to bind to the mammalian protein N-WASP to facilitate actin polymerization. VirG belongs to the autotransporter (AT) family of virulence proteins. Like other ATs, it consists of a signal sequence that directs secretion across the inner membrane (IM) via the sec apparatus, a central passenger domain (mature virulence protein), and a C-terminal porin that inserts and forms a β-barrel in the outer membrane (OM), and is essential for the passenger domain transport to the exterior of the cell. The VirG passenger domain is predicted to adopt β-helical structure, which we have proposed is important for AT biogenesis. Previous studies in our lab with two other ATs, pertactin from B. pertussis and Pet from a pathogenic strain of E. coli, have shown that the C-terminal, β-helical portion of the passenger domain adopts a stable structure that is resistant to chemical and thermal denaturation. We have proposed that extracellular folding of this domain could serve as a driving force for OM secretion. This study aims to identify the features of the VirG passenger domain that are important for its biogenesis, including what keeps the passenger domain unfolded during its transit across the periplasm prior to OM secretion. We established a VirG expression system in E. coli. Indirect immunofluorescence revealed that the full length VirG protein is exposed on the outer cell surface and can recruit N-WASP. Two VirG passenger domain constructs have been purified, and their secondary structure and thermal stability have been measured by far-UV CD spectroscopy. Our preliminary results suggest that VirG passenger unfolds and refolds reversibly and has a β-sheet-rich structure. However, unlike pertactin and Pet, VirG does not unfold in two transitions, and does not seem to have a stable core structure, but intriguingly, the shorter C-terminal passenger domain construct is more resistant to chemical and heat denaturation, and is more resistant to protease digestion, than the longer construct with an N-terminal extension. The N-terminal portion of the passenger
domain could therefore be preventing premature folding of the entire passenger domain in the periplasm, rendering it compatible with OM secretion.

**Poster #7**

**Investigating the Environment of an Autotransporter Passenger Domain During Its Transport Across the Bacterial Outer Membrane**

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Autotransporter (AT) proteins are virulence factors in Gram-negative bacteria. They are synthesized with an N-terminal signal sequence, which is cleaved after inner membrane secretion, a central passenger domain (the mature protein), and a C-terminal outer membrane (OM) porin domain. The porin domain is required for OM secretion of the central passenger domain. OM secretion does not require ATP nor a proton gradient, and therefore the driving force for efficient secretion remains unknown. There is an ongoing debate about the role, if any, of the effects of interactions between AT proteins and essential OM proteins (like BamA) on AT secretion. We are using the model AT pertactin from *Bordetella pertussis* to understand the mechanism of AT passenger domain OM transport. Previously, our lab created a pertactin mutant with two Cys residues within the passenger domain that form a disulfide bond in the periplasm and arrest OM secretion. This stalled intermediate is on pathway for OM secretion, as OM transport can resume upon reduction of the disulfide bonds. Currently, we are investigating the environment of this stalled secretion intermediate during OM transport; specifically, whether the passenger domain crosses the OM through its own porin domain, or whether it passes through another OM protein, like BamA. We have incorporated an unnatural, photo-crosslinkable amino acid *in vivo* in the passenger domain. We are currently exposing cells bearing stalled secretion intermediates to UV-light to crosslink the incorporated probe to amino acids in close proximity. If the passenger domain passes through BamA, we expect to see inter-molecular crosslinking to BamA. In contrast, if the passenger domain uses its own porin domain for Om translocation, we expect intra-molecular crosslinking. Results from these experiments will determine the immediate environment of the passenger domain during OM transport, clarifying the mechanism of OM secretion.

**Poster #8**

**Effects of Electrophilic Modification on the Activity of Thioredoxin Peroxidase 1 from *Saccharomyces cerevisiae***

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Organisms have evolved a variety of defense mechanisms to deal with adverse environmental challenges, including exposure to reactive oxidants and organic electrophiles. In *Saccharomyces cerevisiae*, one of the proteins involved in defense against oxidants is the peroxide-detoxifying enzyme, thioredoxin peroxidase 1 (Tsa1p). Tsa1p possesses a highly reactive cysteine residue in its active site that reacts directly with peroxides, undergoing oxidation in the process. Here, we report that Tsa1p provides cellular protection against organic electrophiles as well as oxidants, as tsa1 deletion mutants have reduced survival when treated with toxic doses of the thiol cross-linker diethyl acetylenedicarboxylate (DAD). In addition, we
have determined that Tsa1p is modified by DAD in vitro and in vivo, undergoing cross-linking to itself and other proteins. We found that electrophilic modification of Tsa1p inhibits the ability of recombinant Tsa1p to become oxidized. Collectively, these results suggest that Tsa1p potentially functions in other capacities besides its peroxidase activity to mediate cellular protection against electrophiles.

Poster #9
Folding and Stability of a Polytopic Membrane Protein Involved in a Lysosomal Storage Disease
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Neiman-Pick type C (NPC) disease is a lysosomal storage disorder associated with accumulation of cellular cholesterol, leading to neurological degeneration and death. Most NPC patients have mutations in the integral membrane protein NPC1. While NPC1 is known to localize to late endosomes and participates in lipid transport, its cellular function is not fully understood. This 1278 amino acid protein contains 13 transmembrane segments and three soluble luminal domains. The most C-terminal of these domains (G3) is the location of a disproportionately high number of disease-causing mutations, including the most common NPC1 mutation implicated in NPC disease (I1061T). I1061T is known to affect the folding mechanism of NPC1: most NPC1-I1061T is lost due to ER-associated degradation of misfolded protein, resulting in reduced NPC1 activity; however, the small amount of NPC1-I1061T that reaches the native conformation is active. This mechanism is similar that of the cystic fibrosis-causing ΔF508 mutation in CFTR, also a soluble-domain mutation in a large polytopic transmembrane protein. The effects of these mutations on transmembrane protein folding suggests that understanding the early steps in the biogenesis of these protein, including co-translational folding, will be crucial for understanding these diseases. However, as a large eukaryotic transmembrane protein, NPC1 is not likely to be amenable to bacterial expression or biophysical studies. We reasoned that if the I1061T mutation affects the folding of G3, its effects could be studied in the isolated soluble domain, which could provide a more tractable model system than full length NPC1. For this purpose, a construct was designed that contains the sequence of the human NPC1 G3 domain attached to a C-terminal His tag. The construct was successfully expressed in E. coli, and refolded to high yields in vitro. We are presently investigating the effects of the I1061T mutation on G3 folding and stability.

Poster #10
Proteomic-Scale Analysis of Hsp70 Binding Patterns
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The DnaK molecular chaperone, i.e., E. coli Hsp70, is important for the proper folding of newly synthesized proteins. DnaK is known to have specific affinity for short nonpolar stretches of amino acids flanked by positive charges. A large-scale computational analysis of Hsp70 binding patterns was performed at the proteomic level in E. coli. We found that several membrane proteins, cytosolic proteins and, surprisingly, also intrinsically disordered proteins (IDPs) have local DnaK binding sites. The overall binding site density (number of DnaK binding sites/protein length) increases with mean hydrophobicity except for IDPs, which display little correlation
between these two properties. On the average, IDP are predicted to have a slightly weaker binding affinity for DnaK than all other proteins. Other interesting properties arising from the DnaK binding site distribution across *E. coli* proteins will be discussed.

**Poster #11**

**Reduced Expression or Activity of GRP94 Increases Selectively the Levels of BiP and PDIA6, But Not Other ER Chaperones**

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The Endoplasmic Reticulum (ER) is the major folding and quality control compartment for membrane and secreted proteins, and contains a number of molecular chaperones and resident enzymes. Glucose regulated protein 94 (GPR94) and BiP/GRP78 are two of the most abundant ER chaperones and work sequentially to assist the folding of client proteins. They are also co-regulated by multiple stresses that induce the unfolded protein response (UPR). To explore the coordinated control of expression of BiP, GRP94 and other ER chaperones, we took advantage of loss-of-function approaches and pharmacological inhibition.

When GRP94 level was down-regulated by RNAi, or its activity blocked by geldanamycin, the expression of BiP and PDIA6, a poorly studied member of the protein disulfide isomerases' family, were significantly. This induction was selective and not a global UPR, as calreticulin, PDI, HSP47 and OS9 levels were unchanged or marginally affected. When BiP expression was knocked down, by either RNAi or by cleavage with subtilase AB, reciprocal up-regulation of GRP94 and PDIA6 was observed. Such linked, selective changes in expression lasted up to months, depending on the mammalian cell line used, until the expression of the silenced chaperone recovered. They did not result in cell death, but did inhibit cell proliferation. Importantly, the linked changes in expression were also observed in mouse skeletal muscles, when GRP94 expression was ablated using Cre recombinase. The reciprocal up-regulation is a transcriptional response, as shown by analysis of transcripts and by using transcriptional reporters.

To explore the underlying mechanism, we asked which branch of the UPR was triggered by the depletion of the chaperones. The selective induction of BiP and PDIA6 was still observed when GRP94 expression was silenced in IRE1-/-, XBP1-/-, ATF6a-/-, PERK-/- or ATF4-/- cells, showing that none of these UPR components was needed individually. Furthermore, GRP94-depleted cells failed to show splicing of XBP1 mRNA, the activated form of ATF6 or phosphorylation of eIF2α, the immediate responses downstream of each UPR sensor. Only silencing of GRP94 affected BiP and PDIA6 expression while increased expression of GRP94 did not, suggesting that there is a threshold level of GRP94 below which cells response.

Taken together, our data show that: 1) GRP94 and BiP expressions are finely balanced in reciprocal fashion; 2) The absence or inhibition of either one initiates a specific transcriptional response, involving only a restricted subset of ER chaperones and not a global UPR; 3) This response is not initiated by the common UPR sensing mechanism. We hypothesize that a specific sensor (or combination of sensors) reports on depletion of even the abundant luminal chaperones.
Poster #12

Exploring the Roles of J-Proteins in Cell Wall Maintenance and Hsp70 Substrate Selection

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J-proteins (Hsp40s) are a class of co-chaperones that assist heat shock protein 70 (Hsp70) in carrying out its many cellular roles, which include nascent protein folding, protein disaggregation and protein translocation. J-proteins all share a highly conserved J domain, which stimulates Hsp70 ATPase activity, but they diverge in the structure and function of the C-terminal region. In order to investigate the details of this evolved diversity, which has given rise to 22 J-proteins in Saccharomyces cerevisiae and over 40 in humans, we are pursuing a two-pronged approach. In one set of experiments, we found that deletion of individual J-proteins from S. cerevisiae resulted in a spectrum of cell wall deficiencies, evaluated by sensitivity to the cell wall stressors calcofluor white (CW), congo red (CR), and caffeine. Specifically, deletion of Ydj1 or Zuo1 caused sensitivity to all three stressors, while deletion of Swa2 caused sensitivity to CW and CR but not caffeine. In the second experimental approach, we have developed a microarray platform in which potential substrates are immobilized on a glass slide, and the binding of J-proteins is detected by fluorescence. Using this approach, we uncovered unexpected differences in the substrate-binding preferences for the primary J-proteins of Escherichia coli and S. cerevisiae, DnaJ and Ydj1 respectively. Using these complementary approaches, we plan to explore the physical and functional interactions between J-proteins and putative substrates. These studies are expected to help elucidate specific, cellular roles of J-proteins.

Poster #13

Genetic Regulatory Network that Controls the Heat Shock Response in C. elegans

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The regulation of the Heat Shock Response (HSR) in eukaryotes has been principally studied as an HSF1-dependent stress-inducible transcriptional switch. In metazoans, heat shock genes are expressed in different tissues and HSF1 is essential from early development and throughout aging. A genetic and systems-level approach was taken to identify and analyze the regulators of the HSR in C. elegans. Fifty-nine genes with positive and negative effects on HS gene expression were identified by genome-wide RNAi screens. These enhancers and suppressors of the HSR function in an HSF1-dependent manner and confer differential tissue patterns of HS gene expression. They include known regulators of protein synthesis and gene expression, folding, trafficking, and clearance that collectively form an HSR network. These observations reveal many individual components of the proteostasis machinery that are genetically linked to the stress-sensing HSR, and in concert achieve the necessary balance for the expression of chaperones and other stress-responsive genes under diverse conditions.
**Poster #14**

**[Swi+]**, the Prion Formed by the Chromatin Remodeling Factor Swi1, is Highly Sensitive to Alterations in Hsp70 Chaperone System Activity

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Yeast prions are heritable genetic elements, formed spontaneously by aggregation of a single protein. Prions can thus generate diverse phenotypes in a dominant, non-Mendelian fashion, without a corresponding change in chromosomal gene structure. Since the phenotypes caused by the presence of a prion are thought to affect the ability of cells to survive under different environmental conditions, those that have global affects on cell physiology are of particular interest. One such prion, [SWI+], is formed of heritable amyloid aggregates of the Swi1 protein, results in a partial loss of function of the SWI/SNF chromatin-remodeling complex which widely regulates gene expression. Our analysis revealed that [SWI+] propagation is highly dependent upon the action of members of the Hsp70 molecular chaperone system, specifically the Hsp70 Ssa, two of its J-proteins co-chaperones, Sis1 and Ydj1, and the nucleotide exchange factors of the Hsp110 family (Sse1/2). Notably, while all yeast prions tested thus far require Sis1, [SWI+] is the only one known to require the activity of Ydj1, the most abundant J-protein in yeast. While dependent upon Ydj1 and Sis1 for propagation, [SWI+] is also hypersensitive to overexpression of both J-proteins. Given the plethora of genes affected by the activity of the SWI/SNF chromatin-remodeling complex, it is possible that this hypersensitivity of [SWI+] to the activity of Hsp70 chaperone machinery may serve a regulatory role, keeping this prion in an easily-lost, meta-stable state. Since expression of molecular chaperones, often known as heat shock proteins, are known to vary under diverse environmental conditions, such “chaperone sensitivity” may allow alteration of traits that under particular environmental conditions convey a selective advantage. Indeed, we found that [SWI+] was greatly destabilized when yeast cells are grown at elevated temperatures. Thus, such sensitivity may provide a means to reach an optimal balance of phenotypic diversity within a cell population, to better adapt to stressful environments, and may be a common characteristic of prions formed from proteins involved in global gene regulation. Finally, a comparison of the prion-forming domains of the proteins which form [SWI+] and other prions revealed distinctions which may at least partially account for these observed characteristics.

**Poster #15**

Small Molecules Inhibit the AAA⁺ ATPase Activity and J-Domain-Mediated Hsp70 Stimulation of Polyomavirus Large Tumor Antigen

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Polyomaviruses are common viral agents in the human population, and can become pathogenic in immunosuppressed populations. Because most treatments for polyomavirus-associated diseases non-specifically target DNA replication, treatments for polyomavirus infection without undesirable side effects are currently unavailable. However, all polyomaviruses express a Large Tumor Antigen (T Ag), which is a multi-domain, 708 amino acid protein. The replication of
Simian Virus 40 (SV40), a member of the Polyomaviridae family, requires two domains within the viral-encoded T Ag: The ATPase domain, which serves as a helicase for viral DNA synthesis, and the N-terminal J-domain, which is homologous to Hsp40 and stimulates the ATPase activity of host-encoded Hsp70 molecular chaperones. Previous screening of pyrimidinone-peptoid hybrid compounds in our laboratories identified MAL2-11B as an inhibitor of viral replication, Hsp70 stimulation, and T Ag ATP hydrolysis (IC_{50} of ~50 µM). Therefore, we proposed that the MAL2-11B scaffold might be co-opted to identify more effective inhibitors of T Ag dependent activities. We now report that a MAL2-11B tetrazol derivative inhibits T Ag ATP hydrolysis (IC_{50} ~ 20 µM; Wright et al., *Virus Res.*, 2009). The same derivative also displays significantly more potent inhibition of T Ag-mediated Hsp70 ATP hydrolysis than MAL2-11B. Moreover, the tetrazol is more effective at inhibiting the replication of viral DNA in SV40-infected cells, possibly because the derivative is anticipated to possess a greater ability to permeate the membrane than MAL2-11B. For the additional tetrazol-based MAL2-11B derivatives tested thus far, major structural changes resulted in a loss of inhibition for both T Ag’s endogenous ATPase activity and Hsp70 stimulation. We suggest further refinement of this scaffold will provide a specific, novel therapeutic treatment for polyomavirus infection and related disease.

**Poster #16**

The Hsp90 Kinase Co-Chaperone Cdc37 Regulates Tau Stability

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The microtubule associated protein tau becomes hyper-phosphorylated and pathologically aggregates in a number of diseases collectively termed tauopathies. The tau protein is extremely sensitive to manipulations of chaperone signaling. For example, Hsp90 inhibitors can reduce the levels of tau in transgenic mouse models of tauopathy. Because of this, we hypothesized that a number of Hsp90 accessory proteins, termed co-chaperones, could also affect tau stability. Perhaps by identifying these co-chaperones, new therapeutics could be designed to specifically target these proteins and facilitate tau clearance. Here we report that the co-chaperone, cell division cycle protein 37 (Cdc37), can regulate aspects of tau pathogenesis. We found that suppression of Cdc37 de-stabilized tau, leading to its clearance, while Cdc37 over-expression preserved tau. Cdc37 was found to co-localize with tau in neuronal cells and it was found to physically interact with tau from human brain. Moreover, Cdc37 levels significantly increased with age. Cdc37 knockdown reduced several phospho-tau species, an effect that was due to reductions in both phospho-tau and tau kinase stability, specifically CdK5 and Akt. Conversely, GSK3β and microtubule affinity regulating kinase 2 (Mark2) were unaffected by Cdc37 modulation. Cdc37 over-expression prevented phospho-tau clearance following Hsp90 inhibition, while Cdc37 suppression potentiated Hsp90-inhibitor-mediated phospho-tau clearance. Thus, Cdc37 can stabilize phosphorylated tau in two ways; by directly stabilizing phosphorylated tau via Hsp90 and by regulating the stability of distinct tau kinases. We propose that changes in Cdc37 neuronal levels or its activity could dramatically alter the kinome, leading to profound changes in the tau phosphorylation signature, altering its proteotoxicity.
Poster #17

**Constructing Lethal Factor – Anthrax Toxin Pore – Nanodisc Complexes for EM structural Analysis.**

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Membrane Protein insertion into lipid nanodiscs is not a straightforward task because this procedure often requires extensive detergent screening methods to solubilize membrane protein components prior to assembling the membrane protein - lipid nanodisc complex. We have developed a potentially novel method for inserting membrane proteins into lipid nanodisc that forgoes laborious detergent screens. To avoid large scale aggregation, we basically construct our membrane protein nanodisc complexes on an immobilized support. To do this, we first constructed immobilized N terminal domains of the Anthrax lethal factor using an E126C lethal factor mutant. This domain is specifically orientated through sulfhydryl linkages to achieve optimal affinity binding of the heptameric anthrax protective antigen prepore. Once bound, the soluble Anthrax Protective antigen toxin prepore is then converted to its pore translocon membrane insertion competent form with the hydrophobic tip exposed to solution. The His-tagged matrix scaffolding protein (MSP)-POPC-Cholate (detergent) micelle forms around the exposed Pore hydrophobic tip, the detergent is removed and the Lethal Factor-PA pore-nanodisc complex is removed from the beads after cleaving the covalent disulfide linkage. The preparations are pure (no vesicles or PA aggregates) and the initial images of this new purified complex has been constructed using single particle negative stain and cryo-electron microscopy.

Poster #18

**Luciferase as a Folding Sensor for Aging and Stress in C. elegans**

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An imbalance in protein homeostasis can result in severe molecular damage to the cell, dysregulation of key tissues leading to pathology, and susceptibility to neurodegenerative diseases. The collapse of proteostasis is an early molecular event in the progression of aging. Aging is marked by the accumulation of misfolded proteins and a diminished chaperone capacity. Adaptation and survival requires an ability to sense damaged proteins and to coordinate induction of protective stress response pathways, chaperones and clearance networks. To better understand the mechanistic details of the proteostasis network we developed tools to assess the chaperone capacity upon proteotoxic stress in the model organism C. elegans.

This proteostasis sensor reports on folding challenges in different tissues upon an imbalance of proteostasis in C. elegans and is based on the meta-stable protein luciferase, whose enzymatic activity allows a rapid and quantitative assessment of the folding state.

Using this sensor we can demonstrate that aging is the biggest proteotoxic challenge for protein folding and hence function. The modulation of the proteostasis background by knock down of
genetic regulators of proteostasis can delay or enhance the functional and conformational decline of the sensor and thus the proteome.

Poster #19

Hsp70 Inhibitors to Overcome Tamoxifen Resistance in Breast Cancer

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A prevalent problem in the use of chemotherapies for the treatment of human cancers is the potential for the development of resistance. While the exact nature of the mechanisms involved in resistance development vary based on cancer type as well as the nature of the therapeutics; some oncogenic factors have been established to have roles in the desensitization to common treatments. Previous data from our lab demonstrated Hsp70 inhibitors can reduce Akt levels, a major proliferation and survival factor also linked to estrogen receptor therapeutic based resistance. Using these Hsp70 ATPase-inhibiting compounds in a cell culture model of tamoxifen resistant breast cancer, we have identified compounds which can re-sensitize resistant cells. These compounds inhibit cytosolic and mitochondrial members of the 70-kDa heat-shock protein family. Thus, in addition to reducing Akt levels and possibly other oncogenic chaperone clients, these drugs may also interrupt mitochondrial function leading to reduced viability in the presence of tamoxifen. With these mechanisms in mind, we now have data that tamoxifen resistant MCF7 cells regained sensitivity to tamoxifen after brief treatments with Hsp70-family inhibitors. The resistant cells were grown in media containing tamoxifen. This media was replaced with media containing an Hsp70 inhibitor compound for four hours. After the four hours, the Hsp70 inhibitor media was removed and tamoxifen-containing media was reapplied to the cells. Multiple viability and cytotoxicity assays confirm the drop in viability and increased cytotoxicity following these treatments. These studies demonstrate that Hsp70 family members are potentially valuable targets for new cancer therapeutics.

Poster #20

Modeling Infectious Proteins in C. elegans

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Prions are self-propagating infectious protein aggregates, identified in mammals and fungi. They were thought to be unique amongst amyloidogenic proteins in that they are not only aggregation-prone but also transmissible. This assumption has been challenged by recent discoveries in vivo and in vitro that other intracellular protein aggregates of polyglutamine (polyQ), α-synuclein, Aβ or tau have the capacity to spread to neighboring cells and seed aggregation of soluble homologous proteins in a prion-like manner.
In order to establish a prion model in *Caenorhabditis elegans*, we generated transgenic animals stably expressing the prion domain (NM) of the yeast translation termination factor subunit Sup35p, as well as Sup35NM bearing a deletion in the oligorepeat region (RΔ2-5) or an extended oligorepeat region (R2E2). In yeast, a deletion of four of the five oligorepeats in Sup35NM leads to a strong decrease in spontaneous prion induction whereas an extension of this region leads to a more frequent prion formation. Similarly, in *C. elegans*, these proteins aggregate in an oligorepeat region length dependent manner: R2E2 is highly aggregation-prone, whereas RΔ2-5 remains mostly soluble in two different tissues, body wall muscle and intestine.

To investigate the infectious nature of prion vs. non-prion aggregates, we are injecting *in vitro* formed congeneric aggregates (Sup35NM and polyQ45 fibrils) into intestinal cells and analyze seeding and conversion of endogenously expressed soluble prion (RΔ2-5) and non-prion (polyQ33) proteins. The seeding of endogenous proteins is highly sequence-specific and conversion only occurs by injecting aggregates with homologous sequences acting as a template. In future studies we will compare conversion kinetics and aggregate characteristics of prion vs. non-prion aggregates. Also, the influence of the cellular folding environment on seeding and conversion dynamics will be tested.

**Poster #21**

**Internal Initiation of Degradation for Protein Processing by the Proteasome**

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The proteasome is the central protein degradation machine in eukaryotes, and it unfolds and degrades its substrates by translocating them through a narrow pore into the protease degradation chamber. For proteins to be degraded by the proteasome, two components are typically required: a ubiquitin modification that targets the substrate to the proteasome and an unstructured stretch of amino acids that serves as an initiation site. The proteasome can initiate degradation of a protein at a terminus or internally, although the mechanism of degradation when two polypeptide stretches are present in the pore is unknown. Degradation is processive and complete, but in some cases partially degraded proteins are released, and can take on new biological functions. One proposed mechanism for partial degradation, based on *in vivo* data from the Jentsch lab, posits that an internal initiation site, flanked by folded domains, leads to bidirectional degradation. If one of the flanking domains is sufficiently stable, degradation stalls in that direction, and a partially degraded protein is released. Using a fully purified system, we set out to determine whether internal initiation leads to partial degradation, and more generally how protein domains positioned on either side of a proteasome initiation site affect one another's degradation. We find that internal initiation leads to enhanced processing because stabilizing a domain on one side of the initiation site also protects the second domain from proteasomal degradation. These results suggest that in bidirectional degradation the two protein chains compete with one another for access to the proteasome.

**Poster #22**

**Maturation and Secretion of IGF-I and –II Depends the Activity of Human Glucose Regulated Protein 94**
Glucose-regulated protein 94 (GRP94) is one of the most abundant constituents of the endoplasmic reticulum (ER). GRP94 expression is up-regulated when cellular glucose tension drops, in response to hypoxia or excessive levels of reducing agents. GRP94 is a major calcium-binding protein of the ER and it is also linked to disposal of misfolded proteins by the ER-associated degradation pathway.

Even though GRP94 is ubiquitously expressed only very few proteins have so far been shown to be clients of GRP94. This list includes immunoglobulins, integrins, Toll-like receptors and plant CLAVATA proteins. Recently, we have demonstrated that GRP94’s chaperone activity is indispensable for maturation of Insulin-like Growth Factors (IGF) and without this interaction, mature IGF is not secreted and the required IGF signaling does not occur.

IGFs are the principal mediators of growth in childhood and activate major signaling pathways important for cell survival and proliferation throughout the lifespan. IGF deficiency leads to severe growth retardation. We have shown that biosynthesis of IGF-I/II is proportional to the activity of GRP94. Therefore, we hypothesized that variants of human GRP94 with reduced function cause primary IGF deficiency. We now focus on genomic analysis of human GRP94, aimed at relating allelic variations and mutations to serum IGF levels.

So far we have identified four mutations within GRP94 gene, alongside multiple SNPs (point mutations, deletions, insertions). K513N substitution in exon 13 creates a hypomorphic GRP94 variant with 30% reduced ability to support IGF production. Three other mutations are the P300L in exon 7 in the linker domain of GRP94 (found in patient with primary IGF deficiency); K442R in exon 12 and one codon deletion in the sequence Asp-Asp-Asp-Asp in exon 17. We are currently assessing the functional consequences of these and other variants on the activity of GRP94.

Poster #23
Conformationally Distinct Aggregation of Het-S is Associated With Prion Related Toxicity in Yeast

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Abnormal accumulation of amyloids in the brain can lead to neuronal toxicity causing illnesses such as Alzheimer’s, Huntington’s, Parkinson’s and prion diseases. The mechanism of this toxicity is unknown. Prion diseases are infectious and are characterized by the conversion of the normal, soluble form of the prion protein, PrPC, to a misfolded, aggregated, insoluble prion form, PrPSc. Prions also exist in yeast and fungi, where they are responsible for certain heritable traits and are sometimes associated with toxicity. One such prion is the [Het-s] prion in the filamentous fungus Podospora anserina (Coustou et al., 1997). The het-s locus exists as two alternate wild type alleles: Het-s (‘little’S) and HET-S (‘big’S). [Het-s] is the prion form of the HET-s protein. In the prion form, [Het-s] functions in heterokaryon incompatibility, that is, it causes cell death when vegetatively fused to a Podospora carrying the HET-'big’S allele (Balguerie et al., 2004). In contrast, the non-prion form, Het-s*, is not lethal in the presence of HET-S.
We have established a yeast system to explore the mechanism of [Het-s]/HET-S toxicity. As in Podospora, in our yeast system the prion [Het-s] is toxic when expressed together with HET-S. Using this yeast system we tested the hypothesis that aggregation of the HET-S protein is the cause of the toxicity. The prion domain of Het-s (amino acids 218-289) fused to GFP (PrD-GFP) forms a prion in yeast (Taneja et al., 2007), called [PrD+] here. However, in Podospora, [PrD+] is not toxic with HET-S, while we find that it is mildly toxic in yeast. The prion form of a larger fragment of Het-s (amino acids 157-289, called Tox here) is toxic with HET-S in both Podospora and yeast. We show that HET-S can be seeded to aggregate by either [TOX+] or [PrD+] prion. Despite the same level of HET-S aggregation in [TOX+] and [PrD+] cells, [TOX+] cells are much more toxic than [PrD+] cells, thus the aggregation of HET-S per se appears not to be the cause of toxicity. Rather, it appears that the types of HET-S aggregates seeded by [TOX+] and [PrD+] differ, and only the aggregates seeded by [TOX+] are largely associated with toxicity. This difference was detected because HET-S aggregates seeded by [TOX+], but not in [PrD+], are composed of detergent resistant trimers.

Poster #24

Development of High-Throughput Chemical Screens for Small Heat Shock Protein 27 (Hsp27)

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Small heat shock proteins, such as Hsp27, are a diverse family of ubiquitous molecular chaperones that contribute to cellular protein homeostasis. Specifically, Hsp27 is believed to prevent the aggregation of misfolded substrate proteins by binding and holding them in stable complexes. Thus, Hsp27 may be a therapeutic target in many neurodegenerative diseases, which are caused by protein aggregation. The chaperone activity of Hsp27 is controlled by homo-oligomerization, with larger oligomers generally considered to be more active. Normally, oligomerization is controlled by cellular stress and substrate availability; however, we hypothesized that chemical probes might be discovered that independently dictate the self-assembly of Hsp27 by binding to critical protein-protein interfaces. Towards this goal, we are developing two high-throughput chemical screens. The first screen relies on fluorescence and thermal shift experiments to reveal promising compounds in 384-well plates. The second platform involves NMR-based, fragment-screens. Initial results suggest that active compounds can control the self-assembly and chaperone functions of Hsp27 in vitro. We are continuing to analyze these compounds in several biochemical assays. We anticipate that these studies will provide a starting point for pharmaceutical design, while also elucidating the molecular roles of Hsp27 in neurodegenerative disease.

Poster #25

Nogo-A Knockdown Inhibits Hypoxia/Reoxygenation-Induced Activation of Mitochondrial-Dependent Apoptosis in Cardiomyocytes

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Programmed cell death of cardiomyocytes following myocardial ischemia increases biomechanical stress on the remaining myocardium, leading to myocardial dysfunction that may result in congestive heart failure or sudden death. Nogo-A is well-characterized as a potent inhibitor of axonal regeneration and plasticity in the central nervous system, however the role of Nogo-A in non-nervous tissues is essentially unknown. In this study, Nogo-A expression was shown to be significantly increased in cardiac tissue from patients with dilated cardiomyopathy and from patients who have experienced an ischemic event. Nogo-A expression was clearly associated with cardiomyocytes in culture and was localized predominantly in the endoplasmic reticulum. In agreement with the findings from human tissue, Nogo-A expression was significantly increased in cultured neonatal rat cardiomyocytes subjected to hypoxia/reoxygenation. Knockdown of Nogo-A in cardiomyocytes markedly attenuated hypoxia/reoxygenation-induced apoptosis, as indicated by the significant reduction of DNA fragmentation, phosphatidylserine translocation, and caspase-3 cleavage, by a mechanism involving the preservation of mitochondrial membrane potential, the inhibition of ROS accumulation, and the inhibition of cytochrome c release. Together, these data demonstrate that knockdown of Nogo-A may serve as a novel therapeutic strategy to prevent the loss of cardiomyocytes following ischemic/hypoxic injury.

**Poster #26**

**Hsp70 Chaperone Complex as a Pharmacological Target in Tauopathies**


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Molecular chaperones are a promising therapeutic target in the treatment of neurological diseases of protein misfolding. This is because of their ability to interact with “client” proteins that become misfolded and aggregate-prone. One of these clients, the microtubule associated protein tau, abnormally aggregates in numerous neurodegenerative diseases, including Pick’s disease, frontotemporal dementia, Parkinson’s disease and Alzheimer’s disease. The Hsp70 chaperone complex has been previously shown to regulate the ubiquitination and degradation of tau. Moreover, this has been done through pharmacological compounds that modulate the rate of ATP hydrolysis by Hsp70. The ATPase activity of Hsp70 has been shown to regulate the levels of tau in the following manner: compounds that increase the hydrolysis of ATP cause tau to accumulate, and compounds that decrease the rate of ATP hydrolysis cause the degradation of tau. Tau plays a crucial role in regulating the dynamic stability of microtubules during neuronal development and synaptic transmission. In tauopathies conformational changes in tau are associated with the initial stages of disease pathology. One of these changes is the loss of function of tau for the microtubules. Some conditions that can accomplish this are pharmacological compounds that destabilize the microtubules, hyperphosphorylation of tau, mutations in tau, and prefibrillar β-amyloid. Our new findings suggest that when the microtubules are destabilized Heat Shock Cognate 70 rapidly associates with tau. At this point we can treat with modulators of ATPase activity and direct tau towards degradation. One compound that was shown to inhibit the ATPase activity of Hsp70 proteins and reduce tau levels is methylene blue (MB). Furthermore, we found that treatment of MB in vivo is capable of
protecting neurons, reducing soluble tau levels and enhancing cognition in a mouse model of tauopathy. These data suggest that the Hsp70 chaperone complex may be a promising therapeutic target in the treatment of tauopathies.

Poster #27

Characterization of Promoter-Specific Effects Displayed by FKBP52 and β-Catenin in the Regulation of AR Function

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One of the most interesting topics in steroid hormone research is examining the influence of regulators of the steroid hormone signaling pathways. By identifying and understanding the relationship between these regulators on events of transcriptional regulation, receptor localization, and degradation events, we can target these regulators to enhance or decrease their influence on the steroid hormone receptors’ activities within the cell. Increased transcription by the Androgen Receptor (AR) has been implicated in several prostate cancer studies. Two proteins in particular, FKBP52 and β-Catenin, are of particular interest to our group. To date, the AR activators FKBP52 and β-Catenin have always been studied separately, but they have never been examined for potential interaction in signaling cascades promoting prostate cancer. β-Catenin is a 92 kDA protein which is a well characterized member of several pathways, from signal transduction to cellular adhesion. β-Catenin’s involvement in the Wnt signaling pathway appears to be important for understanding the development of prostate cancer. Wnt signaling involving β-Catenin is implicated both directly and indirectly in AR regulation. Another coactivator of the AR signaling pathway is FK506 binding protein 52 (FKBP52). It is an immunophilin that potentiates AR activity. Decreased levels of FKBP52 are implicated in androgen insensitivity syndrome and female infertility. Increased levels of FKBP52 are implicated in prostate cancer. β-Catenin and FKBP52 appear to coactivate AR in a synergistic fashion when transfected into FKBP52 knock out MEFs. We have found that β-Catenin and FKBP52 synergize using the synthetic steroid hormone regulated MMTV promoter, but this synergism is maximized when the endogenous AR probasin promoter is used. The promoter specificity of this synergism suggests that β-Catenin and FKBP52 exert their effects on AR signaling at the transcriptional level. We wish to explore other endogenous AR promoter reporters, as well. An understanding of the relationship between these proteins as well as other key components in the AR complex is critical towards our goal to develop better therapeutic targets for prostate cancer therapies.

Poster #28

Role of the J-Protein Heterodimer Pam18:Pam16 in Stimulation of the Hsp70-Driven Translocation of Proteins Across the Mitochondrial Inner Membrane

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The majority of mitochondrial proteins are encoded by nuclear DNA and synthesized in the cytosol, and must therefore be translocated into the mitochondrial matrix. This essential process is driven by an Hsp70-based “motor,” comprised of mitochondrial Hsp70 (termed Ssc1 in yeast) and several other essential proteins. Ssc1 resides on the matrix side of the inner membrane, where it binds polypeptides as they exit the translocon protein-conducting channel.
and drives their movement into the matrix. The ATPase activity of Ssc1 is stimulated by its specialized J protein partner Pam18, which forms a heterodimer with Pam16, an unusual cochaperone which contains a "J-like" domain with sequence and structural homology to J-domains. Pam16 lacks the invariant HPD motif characteristic of J proteins and does not stimulate the ATPase activity of Ssc1, but interaction between the "J-domains" of Pam16 and Pam18 is critical for heterodimer formation.

Despite its essential role in mitochondrial protein import, the physiological significance of the Pam18:Pam16 interaction is poorly understood. It is known that Pam16 plays an important role in tethering Pam18 to the translocon, and is also proposed to regulate the stimulatory activity of Pam18 via its interaction with the "arm" of Pam18. The "arm" is an 11 amino acid region N-terminal to the J domain, which wraps around the "J-domain" of Pam16 in the crystal structure. Here we have investigated the idea that the interaction between Pam18 and Pam16 serves a regulatory role in the stimulation of Ssc1 ATPase activity. The genetic and biochemical effects of altering the Pam18 arm are very similar to mutations which disrupt the J-domain:J-domain interface, which have been shown to affect association of Pam18 with the translocation apparatus. Overall, our data do not support a regulatory function of the heterodimer; rather, our results are consistent with the stability of the Pam18:Pam16 heterodimer being critical for Ssc1 stimulation and proper mitochondrial function.

**Poster #29**

**The ER Lumenal Chaperones Play a Unique Role in the Degradation of the Epithelial Sodium Channel**

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The Epithelial Sodium Channel (ENaC) is an ion channel located in the epithelial membrane of both the kidney and the lungs, where it tightly regulates sodium reabsorption. Because the control of salt and water homeostasis is of such critical importance to the body, even slight errors in ENaC function can lead to major health concerns, including hypertension, hypotension, pulmonary edema, and Cystic Fibrosis-like symptoms. ENaC is composed of three homologous subunits – α, β, and γ – that assemble in the endoplasmic reticulum (ER) to form the mature ENaC channel. However, heterotrimer formation is highly inefficient, oftentimes leading the otherwise functional subunits to be degraded by the protein quality control pathway known as Endoplasmic Reticulum Associated Degradation (ERAD). To date, only a few proteins are known to affect ENaC stability and assembly. To characterize the mechanism of ENaC degradation, a yeast ENaC expression system was developed (Kashlan et al., *J. Biol. Chem.*, 2007; Buck et al. *Mol. Biol. Cell*, 2010). Using this system, we have found that Jem1 and Scj1, two ER lumenal Hsp40 chaperones, play an essential role in ENaC degradation. Jem1 and Scj1 facilitate the ATP hydrolysis of the ER lumenal Hsp70 chaperone, BiP. Surprisingly BiP does not appear to play a role in ENaC subunit degradation, although it is an essential player in the degradation of other well-characterized, Hsp40 dependent ERAD substrates. To further investigate the role of ER lumenal chaperones in ENaC degradation, the involvement of the BiP nucleotide exchange factors (NEFs), Lhs1 and Sil1 was examined. We found that Lhs1 and Sil1 appear to play unique and variable roles in ENaC subunit degradation. These data lead to the hypothesis that the Hsp40s, Jem1 and Scj1, are working in combination with the NEFs, Lhs1 and Sil1, to facilitate ENaC degradation. To test this hypothesis a *lhs1∆jem1∆scj1Δ* deletion strain was created and the extent of αENaC degradation was assayed. We found that
the combined effect of deleting Lhs1, Jem1, and Scj1 further stabilized ENaC in comparison to the single deletions. Consistent with these data, the \textit{lhs1\textbackslash jem1\textbackslash scj1\Delta} strain is synergistically sensitive to agents that induce ER stress. Our results indicate that the ER luminal Hsp40s interact synthetically with the NEF Lhs1 to target ENaC for ERAD.

**Poster #30**

**Protein Folding in the Cell: Investigating the Influence of Codon Usage**

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\textit{In vivo}, proteins start to fold while they are still being synthesized by the ribosome. The process of protein folding \textit{in vivo} is coupled to the process of translation, which could alter the folding pathway, or in the most extreme case, the final folded structure of the encoded protein. The rate at which a protein is translated could thus have a dramatic effect on folding. Translation rates are determined in part by synonymous codon usage. Most amino acids can be encoded by more than one codon, but these codons are not used with the same frequency, and their corresponding tRNAs do not occur at equal concentrations. Common codons are typically translated faster than rare codons, and clusters of rare codons can cause significant pauses in translation. Here, we specifically address whether rare codon clusters can alter the final folded structure of a protein.

We have designed a synthetic construct in which the presence or absence of a stretch of rare codons might act as a switch between two alternative native states or folding pathways. The protein used in this study consists of three half-domains, connected by flexible linkers, which will associate with one another to form alternative structures based on the presence or absence of a stretch of rare codons in the mRNA. The interactions of the half-domains with each other will be dependent on the presence or absence of rare codon clusters at strategic locations, enabling us to control the final native state of the protein without altering the amino acid sequence.

**Poster #31**

**Rescuing Expression of CFTR F508del in HEK293 Cells by Altering the Synonymous Codons**

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Cystic fibrosis is a common lethal disease that occurs in about 1 in every 2000 births in Caucasians. Cystic fibrosis is caused by a loss in the expression of a chloride channel, CFTR. Of the patients with cystic fibrosis, 90% have a 3bp deletion, which eliminates codon 508 (F508del). This mutation alters CFTR folding such that CFTR does not get to the plasma membrane, but instead, is degraded by endoplasmic reticulum associated protein degradation (ERAD). While the F508del mutation is commonly responsible for cystic fibrosis, little is known on why the mutation alters the folding of CFTR or how it can be corrected by a rational approach. The results in this study demonstrate that the expression and localization of both wild type and F508del CFTR are dramatically dependent on which synonymous codons are used in the open reading frame of CFTR. There is a striking dependence on the synonymous codon usage on the total amount of CFTR expressed in the cell, the trafficking to the plasma...
membrane, and on the level of functional CFTR for both the wild type and mutant, F508del, forms of CFTR. Surprisingly, by optimizing the synonymous codons, we are able to express the F508del form of CFTR on the plasma membrane of HEK293 cells at levels that are comparable to that observed by expression of wild type CFTR using the native codons. These results have profound implications on the coupling of translation and folding and on the effect of codon usage on translation in the mammalian cell.

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**Poster #32**

**Investigation of [PSI+] Prion Variant Establishment in Yeast**

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Differences in the clinical pathology of the mammalian prion diseases are thought to be because of distinct conformations of the aggregated prion proteins, called prion strains. Here we examine [PSI+] prion variant establishment in yeast. The [PSI+] prion protein Sup35 is efficiently induced to take on the prion conformation following its transient overexpression in the presence of another prion, eg. [PIN+]. We establish that Sup35 can misfold into more than one variant type within a single cell. When these cells containing strong and weak variants were followed in pedigrees, establishment of the weak or strong variant phenotype occurred in daughters, sometimes in granddaughters and some in great granddaughters.

Moreover, some prions never stabilized, always giving rise to unstable [PSI+], strong [PSI+] and occasionally weak [PSI+] and this instability was not due the presence of [PIN+].

**Poster #33**

**GEI-11 Regulates Cholinergic Signaling and Restores Proteostasis in C. elegans Muscle Cells**

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The molecular response to proteotoxicity invokes cell-autonomous processes and inter-cellular signaling cascades that restore protein homeostasis, or proteostasis. To identify novel pathways that regulate proteostasis in animals, we have performed a reverse genetic screen in Caenorhabditis elegans to identify genes that suppress polyglutamine (polyQ) aggregation and toxicity in muscle cells. Here, we show that knockdown of gei-11 upregulates expression of acetylcholine receptors (AChR), increasing cholinergic function at the neuromuscular junction (NMJ) that leads to a calcium flux, activation of calcium-dependent calmodulins and kinases, and activation of HSF1. This results in the up-regulation of cytosolic chaperones and the suppression of misfolding of multiple endogenous metastable proteins, with a beneficial effect for muscle proteostasis. We propose that an imbalance in neuromuscular cholinergic activity activates a cell non-autonomous signaling cascade that leads to the protective induction of heat shock response and improvement of protein homeostasis in post-synaptic cells.
**Poster #34**  
**Spergualin Analogs as Specific Inhibitors of Hsp70-CHIP Protein-Protein Interactions**  
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The molecular chaperone, heat shock protein 70 (Hsp70), is an essential regulator of protein homeostasis that has roles in both primary folding and clearance of misfolded proteins. Thus, Hsp70 is thought to make triage decisions and direct the fate of its protein substrates. Several studies have suggested that it is the interaction between Hsp70 and a network of co-chaperone proteins that determines how its substrates are processed. For example, binding of Hsp70 to the E3 ubiquitin ligase, CHIP, is thought to guide Hsp70-bound substrates to the proteasome. However the mechanism governing this fate decision remains unresolved. We have recently discovered that the natural product, spergualin, directly inhibits the protein-protein interaction between Hsp70 and CHIP. To improve the potency and stability of this promising chemical probe, we developed a new, combinatorial synthetic route that facilitates access to spergualin analogs. These efforts have yielded a more stable analog that potently inhibits the Hsp70-CHIP interaction. We anticipate that these inhibitors will be useful for probing the roles of the Hsp70-CHIP complex in directing protein triage in vitro and in vivo.

**Poster #35**  
**Identification of Genes that Provide Cellular Protection Against a Thiol Cross-Linking Agent in Saccharomyces Cerevisiae**  
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Organic electrophiles represent an important class of xenobiotic and endogenously produced toxins that damage DNA and proteins. The adduction of electrophiles to these macromolecules interrupts biological processes such as DNA replication, protein folding and turnover. We have recently identified an electrophile (diethyl acetylenedicarboxylate, DAD) that is capable of cross-linking proteins by undergoing reaction with two thiol groups. Because of its unique, electrophilic properties, we sought to determine whether the defense mechanisms that provide protection against DAD are similar to those which protect against electrophiles that cannot cross-link thiol groups in proteins. To this end, we have screened a panel of Saccharomyces cerevisiae deletion mutants lacking various detoxification enzymes to determine their survival when exposed to DAD. We have identified several cytoprotective genes, including many in the glutathione transferase family, (e.g. GTTI, GTO1, and GTO2, all of which localize to different cellular compartments). Our results suggest that glutathione metabolic enzymes in multiple cellular compartments protect cells against damage by thiol cross-linking electrophiles. We predict that our current studies using a monofunctional electrophile, N-ethylmaleimide, will reveal that a similar group of genes provide protection against electrophiles not capable of cross-linking.

**Poster #36**  
**Cap Structures Reduce β-Helix Aggregation Propensity**  
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Examinations of β-sheets in protein crystal structures have revealed that edge β-strands are protected from solvent by a variety of mechanisms. Protection of unpaired hydrogen bond donors and acceptors in β-sheet edge strands could prevent intermolecular associations and therefore reduce aggregation of these proteins. We investigated the role of β-sheet capping mechanisms in the folding and aggregation properties of pertactin, an autotransporter protein from *Bordetella pertussis*. Pertactin, a right-handed β-helix protein, folds extremely slowly in vitro, yet is surprisingly resistant to aggregation. Examination of the pertactin crystal structure suggests two different strategies might prevent multimerization. The C-terminus has irregular β-helical structure, avoiding solvent exposure of both the hydrophobic core of the β-helix and its edge strands. The N-terminal rung of the β-helix contains three charged residues. Removal of the C-terminal cap or mutation of two of the three N-terminal charged residues led to increased aggregation and formation of soluble oligomeric fibers as observed by size exclusion chromatography and transmission electron microscopy. We are creating a construct that lacks both the C- and N-terminal caps, to examine whether these mutations will lead to even greater aggregation and fiber formation. Results from these studies will allow us to determine to what extent pertactin, and by extension other β-helical proteins, employ capping mechanisms to reduce/prevent aggregation.

**Poster #37**

**Mammalian UBR1 Ubiquitin Ligase Promotes Cellular Sensitivity to an Hsp90 Inhibitor**

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UBR1 and UBR2 ubiquitin ligases function in the N-end rule degradation pathway in lower and higher eukaryotic cells. In yeast, the UBR1 homologue also functions by N-end rule independent means to promote degradation of unfolded proteins generated via stress or with the small molecule inhibitor of Hsp90, geldanamycin. Based on these studies we examined the role of mammalian UBR1 in the degradation of protein kinase clients upon Hsp90 inhibition. Our findings show that protein kinase clients Akt and Cdk4 are still degraded in mouse UBR1-/- cells treated with geldanamycin, but that their levels recover within 12-18 hours, in contrast to wild-type cells. Similar findings were made for human BT474 breast cancer cells with knocked down UBR1. In this case, the ErbB2 oncogenic protein kinase levels recovered within 12-18 hours of geldanamycin treatment in the UBR1 knockdown cells. UBR1-/- cell viability was also greater compared to wild type cells in the presence of geldanamycin, and in a manner that was reversible upon transfection of a plasmid expressing UBR1. Further studies revealed that UBR1 levels are themselves sensitive to geldanamycin, suggesting that this ubiquitin ligase is also an Hsp90 client. Our findings suggest that UBR1 plays a role in determining the sensitivity of cancer cells to the chemotherapeutic effects of Hsp90 inhibitors.


**Poster #38**

**Ubr1, Ubr2 and San1 Contribute to Protein Kinase Quality Control Via Distinct Pathways**

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Ubr1, Ubr2 and San1 ubiquitin ligases function in the quality control of misfolded proteins. Ubr1 and Ubr2 function in the cytosol while San1 functions in the nucleus, although all act on a similar cohort of misfolded proteins. For example, each ubiquitin ligase acts on newly synthesized protein kinases whose folding was inhibited with the Hsp90 inhibitor, geldanamycin. To further investigate the pathways by which these ubiquitin ligases are specified, we analyzed a protein kinase, Ste11<sup>ΔN</sup>K444R, at steady state. Ste11<sup>ΔN</sup>K444R was tagged with GFP in order to visualize the kinase and to follow its fate upon geldanamycin treatment. Our studies revealed that Ste11<sup>ΔN</sup>K444R was prone to aggregation upon geldanamycin treatment, with one to two aggregates forming outside the nucleus in ~ 20% of treated cells. Aggregate formation was dose dependent and saturation was obtained at 15µM geldanamycin. We followed aggregate formation in cells deleted for ubiquitin ligases known to promote degradation of Ste11<sup>ΔN</sup>K444R in geldanamycin treated cells. Our results showed that Ubr1, Ubr2 and San1 had different effects on aggregate formation. In <i>ubr1</i>Δ cells, there was a two-fold increase in the number of aggregates, which formed with similar dose dependence as in wild type cells. In <i>ubr2</i>Δ cells there was suppression of aggregate formation at lower doses and a small increase in aggregate formation at higher drug doses compared with <i>ubr1</i>Δ cells. In addition, <i>ubr2</i>Δ partially suppressed the effect of deleting <i>UBR1</i>. We found that the suppression phenotype resulted from increased proteasome activity in <i>ubr2</i>Δ cells, which correlates with stabilized Rpn4, a transcription factor for proteasome subunits. Thus, Ubr1 and Ubr2 affect aggregate formation in cells by distinct mechanisms. In <i>san1</i>Δ cells there was slightly less aggregation than in <i>ubr1</i>Δ cells and also slightly more proteasome activity. Our findings show that Ubr1 has a distinct role in protecting against aggregation of misfolded protein kinases upon Hsp90 inhibition.


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**Poster #39**

**Utilizing Methylene Blue (MB) as a Chemical Tool to Elucidate Hsp70-Dependent Modulation of the Cellular Fate of the Microtubule Associated Protein Tau (Tau/MAPT)**

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The microtubule associated protein tau (tau/MAPT) is known to bind and stabilize microtubules. However, in several neurodegenerative diseases, classified as tauopathies, tau becomes hyperphosphorylated and prone to aggregation. Genetic studies have identified molecular chaperones, including Hsp70 and Hsp90, as important regulators of tau turnover. Based on these observations, our group has developed chemical modulators of Hsp70 that can promote degradation of tau. As a result of these efforts, methylene blue (MB) has been identified as a potent Hsp70-dependent promoter of tau degradation. We envisioned that MB might be used as a tool to understand the mechanisms by which the chaperones control the cellular fate of tau. First, we hypothesized that MB-mediated changes in Hsp70 leads to the observed decrease in cellular tau levels. To test this hypothesis, we explored the effect of MB in vitro. We found that MB irreversibly inhibits Hsp70 ATPase turnover by selectively oxidizing Cys306. Interestingly this cysteine residue is present only on the stress-inducible form of Hsp70 (Hsp72) and not the constitutively expressed form (Hsc70). Consistent with this data MB is unable to inhibit the ATPase turnover of Hsc70. Next, to demonstrate that oxidation of Cys306 on Hsp72 is responsible for the observed decrease in cellular tau levels, we will genetically test mutants of Hsp70 for their affects on tau levels and MB treatment. These studies will help to understand the molecular mechanism by which MB leads to changes in cellular tau levels, but they also
may have uncover a more general redox sensing property of Hsp72. Next, we hypothesized that MB-mediated changes in Hsp72 lead to subsequent changes in the tau-associated protein complexes which promote the degradation of tau. We aim to utilize MB to uncover early events in formation of this complex. Towards this aim, we tested the differential association of proteins with tau in response to MB treatment using quantitative spectral analysis. In doing so, we have identified a list of candidate proteins which include Hsp70, Hsp90, and HMGE that may be involved in the degradation of tau and responsiveness to MB treatment. Further study may identify novel protein-protein interactions central to tau degradation, which may serve as future therapeutic targets in the treatment of tauopathies.

Poster #40

The Yeast Bag-1 Homolog Snl1 Recruits ribosomes and Hsp70 to the ER/nuclear membrane

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Cells are exposed to a variety of environmental and physiological stresses including temperature, pH and nutrient availability. Cellular stress results in protein misfolding and altered cellular homeostasis. Hsp70, a highly conserved molecular chaperone, is present in all organisms and is key to maintaining proteins in their functional state thereby maintaining cellular homeostasis. Hsp70 assists in folding by iterative cycles of ATP binding, hydrolysis and nucleotide exchange tightly coupled to substrate binding and release. The multiple steps in this cycle are strictly regulated by co-chaperones, including nucleotide exchange factors (NEFs) that accelerate the rate of ADP/ATP exchange on Hsp70.

In yeast and human cells, three distinct cytosolic NEFs are present: Sse1 (Hsp110), Fes1 (HspBP1) and Snl1 (Bag-1). The NEFs in yeast functionally interact with the cytosolic Hsp70s, Ssa and Ssb. Snl1 is unique among the cytosolic NEFs as it is localized at the ER membrane with its Hsp70 binding domain exposed to the cytosol. In the course of characterizing Snl1, a number of abundant proteins were found to associate with both full length Snl1 and a soluble truncation mutant lacking the transmembrane domain, Snl1ΔN, but not with the other NEFs. These were identified as ribosomal proteins of the 40S and 60S subunits by mass spectrometry. Several lines of evidence indicate that Snl1 interacts independently with the ribosome and Hsp70. Components of the ribosome-associated complex (RAC; Ssb, Ssz1 and Zuo1) are not required for association; Snl1 binds to the ribosomal proteins and Hsp70 in a non-competitive manner; and interaction with ribosomal proteins, but not Hsp70, is sensitive to high salt. A series of deletions from the N-terminus of Snl1 were constructed and amino acid residues 50-60 were found to be required for ribosome binding but not Hsp70 interaction. This is the first evidence of a ribosome-associated NEF and suggests a novel role for enhancement of protein synthesis and folding at the ER/nuclear membrane.

Poster #41

Remodeling Protein Folding and Ca2+ Homeostasis in Gaucher’s Disease Patient-Derived Fibroblasts: A Novel Mechanism for Mutant Glucocerebrosidase Proteostasis

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Gaucher’s disease is caused by deficiency of lysosomal glucocerebrosidase (GC) activity and accumulation of GC substrate, glucosylceramide. A number of point mutations in GC encoding gene destabilize the enzyme native structure, resulting in protein misfolding and degradation. Particularly, the L444P GC variant, often associated with neuropathic manifestations of the disease, is severely destabilized and immediately degraded, resulting in complete loss of enzymatic activity. In addition, glucosylceramide accumulation causes Ca\(^{2+}\) efflux from the endoplasmic reticulum (ER) through ryanodine receptors (RyRs) in the neurons of Gaucher’s disease patients.

We hypothesized that excessive \([\text{Ca}^{2+}]_{\text{ER}}\) efflux impairs ER folding and studied how modulation of \([\text{Ca}^{2+}]_{\text{ER}}\) affects folding of L444P GC in patient-derived fibroblasts. We recently reported that RyRs blockers mediated \([\text{Ca}^{2+}]_{\text{ER}}\) modulation, recreating a "wild type-like" folding environment in the ER, more amenable to rescuing the folding of mutated L444P GC through proteostasis regulation (Wang et al. ACS Chem Biol 2010 DOI: 10.1021/cb100321m). Treating patient-derived fibroblasts with a RyRs blocker and a proteostasis modulator, MG-132, resulted in enhanced folding, trafficking, and activity of the severely destabilized L444P GC variant. Global gene expression profiling and mechanistic studies were conducted to investigate the folding quality control expression pattern conducive to native folding of mutated L444P GC, and reveal that the ER-lumenal BiP/GRP78 plays a key role in the biogenesis of this GC variant.

Because Ca\(^{2+}\) homeostasis modulation was observed to dramatically influence the ER folding environment in Gaucher’s disease cells, we considered a class of Ca\(^{2+}\) blockers that bind to L-type channels on the plasma membrane. Particularly, we investigated small molecule L-type Ca\(^{2+}\) channel blockers that can also inhibit \([\text{Ca}^{2+}]_{\text{ER}}\) efflux through the RyRs. We discovered that maximal rescue of L444P GC folding, trafficking and activity could be achieved using representative of this class of molecules that simultaneously i) counteract the disruptive effect of glucosylceramide accumulation on Ca\(^{2+}\) homeostasis, ii) induce upregulation of BiP expression, and iii) moderately induce the unfolded protein response, but, as opposed to previously discovered proteostasis regulators, do not induce cytotoxicity and prevent apoptosis induction.

**Poster #42**

**Determining the Role of Chaperones in Endoplasmic Reticulum-associated Degradation (ERAD): Distinct Requirements for ER and Luminal Substrates**

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In the event of protein misfolding, due to mutations or cellular stress, misfolded proteins can aggregate. Protein aggregation, particularly in the ER, is linked to several disease states. One mechanism that eliminates misfolded secretory proteins is known as endoplasmic reticulum-associated degradation (ERAD). The ERAD of certain integral membrane proteins has specifically been linked to human diseases such as cystic fibrosis and hyper/hypotension. One notable difference between some of the disease-causing proteins is the number of transmembrane domains they contain. In this study, the chaperones and components required for ERAD were examined using the native yeast protein sterile 6 (Ste6p), an ABC transporter protein with 12 transmembrane domains. A mutated form of Ste6p, called Ste6p\(^*\), has a premature truncation in nucleotide-binding domain 2 (termed NBD2\(^*\)) which creates a signal for degradation, making Ste6p\(^*\) an ERAD substrate. The goal of this study was to determine the chaperones required for the ERAD of substrates containing different numbers of transmembrane domains but that contain the same degradation signal, NBD2\(^*\).
To this end, a series of chimeric proteins were constructed from Ste6p*, each of which contains NBD2*. Chimera A contains two transmembrane domains with NBD2* in the cytosol, Chimera B contains only one transmembrane domain with NBD2* in the ER lumen, Chimera C contains a signal for a lipid-anchor, and Chimera D lacks ER targeting sequences. The rate of degradation for each of these chimeras was monitored in pdr5Δ yeast treated with MG132, a chemical inhibitor of the proteasome. Following treatment, all four chimeric proteins were significantly stabilized, suggesting that all of the chimeras are degraded via the proteasome, a hallmark of ERAD. The rates of degradation were then studied in yeast strains with temperature-sensitive mutant alleles encoding Ssa1p (a major cytosolic Hsp70) or Kar2p (the main ER-luminal Hsp70). The chimeras that contained their signal for degradation in the cytosol (Chimeras A, C, and D) showed stabilization in the ssa1-45 strain, while Chimera B was not stabilized. Conversely, Chimeras A, C, and D showed no stabilization in the kar2-1 strain while Chimera B was partially stabilized. Because all four chimeras are degraded via ERAD and exhibit expected Hsp70 dependence, future studies will examine the role of glycosylation in the degradation of these substrates, as well as the effect that altering transmembrane hydrophobicity has on retrotranslocation and ERAD efficiency.

**Poster #43**

**Poof-Be-Gone! Developing an Inducible Degradation System to Rapidly Direct Proteins to the Proteasome in Mammalian Cells**

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The ubiquitin-proteasome system (UPS) controls the concentrations of hundreds of proteins through their regulated degradation and thus affects numerous cellular processes including cell cycle control, signal transduction, and gene expression. The degradation signal or degron that targets proteins to the proteasome has two distinct components: a proteasome binding tag and an initiation region. The proteasome binding tag is typically a chain of ubiquitin molecules attached to the protein substrate. The proteasome binds the tag and then engages the substrate at the initiation region. Although both components of the degron are usually located on the same polypeptide, we have shown that they can function to target a protein for degradation in vitro when separated onto two different subunits of a protein complex. We will adapt this trans-targeting mechanism to construct a broadly applicable inducible degradation system. The goal is to develop a method to interfere in the UPS specifically and by inducing the degradation of proteins rather than inhibiting it. Initially, we hope to prove, in principle that it is possible to induce the degradation of individual proteins in vivo using a strategy based on some recent insights into the mechanism of substrate selection by the proteasome. If we are successful in this first step, we will adapt the methodology to make it progressively more suitable for therapeutic strategies. The long-range goal is to develop a therapeutic application to remove harmful, detrimental, and cancer-related proteins from human cells. These experiments are being performed in mammalian HEK293 cells. In combination with cell culture, we are using Fluorescence Activated Cell Sorting (FACS) instrumentation to test the effectiveness of this strategy.

**Poster #44**

**Using Yeast to Study TorsinA, an ER Luminal AAA⁺ ATPase Linked to the Human Neurological Disease Dystonia**
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Dystonia is a human genetic disease characterized by involuntary and sustained muscle contractions. The most severe type of dystonia is early-onset Torsion dystonia, which is associated with a 3-base pair deletion leading to the loss of a glutamate at the C-terminus of TorsinA (TorsinA\textsuperscript{△E}). The biological function of TorsinA and the reasons that the TorsinA\textsuperscript{△E} variant is associated with the disease are poorly understood. Moreover, the penetrance of the disease is highly variable, suggesting that genetic modifiers affect disease severity. Our objective is to develop a model in the yeast \textit{Saccharomyces cerevisiae} in order to study TorsinA’s cellular role, to find differences between the function of TorsinA and TorsinA\textsuperscript{△E} in the cell, and to identify genetic modifiers of TorsinA function. To this end, we introduced into yeast human TorsinA and TorsinA\textsuperscript{△E} expression vectors under the control of a strong constitutive promoter. Similar to TorsinA in humans, we found that TorsinA in yeast localizes to the endoplasmic reticulum (ER) and that both TorsinA and TorsinA\textsuperscript{△E} are glycosylated. We also observed that the glycosylation of TorsinA and TorsinA\textsuperscript{△E} is dependent on the ER Hsp70 chaperone Kar2/BiP, and on the ER Hsp40 co-chaperones Jem1 and Scj1. Further, we found that TorsinA\textsuperscript{△E} stability is modulated by Kar2/BiP function. We are currently testing the hypothesis that TorsinA stability is associated with its glycosylation state.

**Poster #45**

The p23 molecular chaperone and GCN5 acetyltransferase cooperatively modulate the stability of protein-DNA complexes.

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Chromatin and associated regulatory protein complexes are known to play an active role in the gene expression. Recent live cell studies have revealed that many regulatory proteins exist in a dynamic equilibrium within cells. We are interested in understanding the purpose of dynamic transcription complexes and the mechanisms that promote dissociation of structures employed to initiate transcription events. We found that the RNA and protein levels of Gcn5 and Hdac1 are selectively increased in p23 null mouse embryonic fibroblast (MEF) cells. As prior studies have shown that p23 affects DNA binding of a wide array of factors and given the functional overlap between p23 and Gcn5, we assessed whether Gcn5 and p23 cooperatively function to modulate DNA binding activities. Using HSF1, as a model system, we found that both p23 and GCN5 inhibit HSF1 binding activity. Interestingly, the impact of Gcn5 on HSF1 DNA binding was reliant on p23, as Gcn5 had no apparent effect on HSF1-DNA complexes in the absence of p23. We suggest that p23 is required to transiently disassemble the HSF1-DNA complex, and only after dissociation, Gcn5 is able to acetylate HSF1 and inhibit its DNA binding activity. In addition to HSF1, p23 and GCN5 modulate DNA binding activity of other transcription factors and proteins involved in DNA replication and telomere maintenance. Together, our studies suggest that p23 and Gcn5 work cooperatively to displace various proteins from their cognate DNA targets.

**Poster #46**
The Flexible Connection of the N-Terminal Domain in ClpB Supports Substrate Binding and Controls the Aggregate Reactivation Efficiency

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ClpB is a bacterial heat-shock protein from the AAA+ family of ATPases associated with various activities. ClpB reactivates aggregated proteins in cooperation with the DnaK chaperone system. The ClpB monomer contains two nucleotide-binding domains (NBDs) connected by a coiled-coil middle domain and a smaller N-terminal domain attached to the first NBD with a 15 aa-long unstructured linker containing a Gly-Gly motif. The active form of ClpB is a nucleotide-stabilized cylinder-shaped hexamer. The ClpB-mediated protein disaggregation is linked to translocation of substrates through the central channel in the hexameric ClpB. The channel entrance is surrounded by a ring of the N-terminal domains. The role of the N-terminal domain in aggregate reactivation is not fully understood. It was suggested that the domain’s mobility may control the efficiency of substrate insertion into the ClpB channel. We produced 7 variants of ClpB with modified sequence of the N-terminal linker. To increase the linker's length and conformational flexibility, we inserted up to 4 Gly next to the GG motif. To decrease the linker's length and flexibility, we deleted the GG motif and converted it into GP and PP. We found that none of the linker modifications affected the basal ClpB ATPase activity or its capability to form oligomers. However, all linker variants showed a lower aggregate reactivation rate than wt ClpB. The loss of the chaperone activity correlated with a lower aggregate binding capability of the ClpB linker variants. Unexpectedly, the observed functional defects occurred for the longer linkers containing multiple glycines as well as for the shorter ones with Gly/Pro substitutions. Molecular dynamics simulations showed that all modified linkers display different end-to-end distributions than the wt linker. For the linkers containing multiple glycines, a lower than expected degree of conformational flexibility may be due to the transient formation of a salt bridge-stabilized alpha-helical segment. We conclude that the flexible attachment of the N-terminal domain supports the chaperone activity of ClpB by controlling the efficiency of substrate binding. Moreover, the length and conformational flexibility of the linker region may have been optimized during evolution to achieve the most advantageous aggregate-removal rates.

Poster #47

In Vivo Functions of the Hsp90 Co-Chaperone Cpr7

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Heat Shock Protein 90 (Hsp90) is an abundant molecular chaperone protein present in prokaryotes and essential to eukaryotes. This protein is involved in the activation and stabilization of several transcription factors such as heat shock factor (HSF) and oncogenic kinases including Her-2. Hsp90 function is dependent on a battery of co-chaperones that are involved in Hsp90’s ATPase cycle as well as the stabilization and transfer of client proteins. One such co-chaperone is the cyclophilin Cpr7. This protein is a homologue of the human Cyp-40 protein and contains both tetratricopeptide repeat (TPR) and peptidyl prolyl isomerase (PPIase) domains. The deletion of CPR7 from S. cerevisiae results in a slow growth phenotype, while the deletion of its homologue, CPR6, has no apparent impact on cell growth. The growth defect resulting from the deletion of CPR7 cannot be recovered by overexpression of CPR6; however, it can be rescued by the overexpression of the essential gene CNS1, which encodes another
TPR-containing protein. In addition to its slow growth phenotype, the deletion of CPR7 results in decreased Hsp90 chaperoning functions. Although Cpr7 is needed for Hsp90 function, the exact mechanism in which it assists Hsp90 is vague. We found a unique connection that links Cpr7 function to Hsp90’s ATP induced conformational changes. In addition, we demonstrate that the deletion of CPR7 coupled with Hsp90 mutants lacking a majority of their charged linker led to an extreme growth defect or cell death. These Hsp90 charged linker mutants alone have no effect on cell growth. This suggests that Cpr7 may assist Hsp90 function through its modulation of Hsp90’s ATP induced conformational changes, possibly through its association with Hsp90’s charged linker.

**Poster #48**

**Activation of Wild-Type and Mutant Cystic Fibrosis Transmembrane Conductance Regulator Chloride-Channels by Auranofin**

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Cystic Fibrosis (CF) is a fatal genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). One of the therapeutic strategies for the treatment of CF is to develop drugs that repair the defects of different CFTR mutations in the cellular processing, folding and channel gating. We have recently identified Auranofin, a gold-based anti-rheumatic drug, as a novel modulator of CFTR channels. Auranofin activated CFTR mediated currents in the human colonic epithelial cells (T84) and Fisher rat thyroid epithelial cells (FRT) expressing wild-type CFTR and several CF-associated mutants. The Auranofin-stimulated transepithelial short-circuit current (Isc) was blocked by two specific CFTR inhibitors, Inh-172 and GlyH-101, indicating that Auranofin is an activator of wt-CFTR. Auranofin stimulated electrogenic Cl⁻ transport across the apical membrane in a concentration-dependent manner with an EC₅₀ of ~60μM and ~30μM, in T84 and FRT cells, respectively. Unlike forskolin, our data demonstrated that Auranofin did not increase cellular cAMP and cGMP levels in T84 and FRT cells. Thus, Auranofin is not an inhibitor of phosphodiesterase or an activator of adenylate cyclase. In addition, the PKA inhibitor H89, the PKC inhibitor BisX and cGMP-dependent protein kinase inhibitor KT5823 failed to abolish the Isc response to Auranofin, suggesting that activation occurs through a PKA-independent pathway. However, activation of CFTR by Auranofin was suppressed by staurosporine, a nonselective protein kinase inhibitor, and TBBt, a specific inhibitor of Casein kinase 2 (CK2). Moreover, the mutation of CK2 phosphorylation sites in CFTR abolished the channel activation, indicating that the effect of Auranofin on CFTR channel gating is regulated by CK2. By site-directed mutagenesis analysis of CFTR, the preliminary results suggest that channel opening might be attributed to the direct binding of Auranofin.

F508del-CFTR is the most common mutation in cystic fibrosis. Our data also revealed that Auranofin significantly increased the forskolin-stimulated Isc in temperature-corrected F508del-CFTR expressing FRT cells (27°C, 48hr) with an EC₅₀ of ~20μM. When F508del-CFTR-FRT cells were incubated with correctors, overnight at 37°C, Auranofin without forskolin directly stimulated Isc. When pretreated with a combination of correctors, this direct stimulating effect of Auranofin was enhanced 4-fold compared to the effect on cells treated with a single corrector. Although Auranofin was a weak activator of G551D-CFTR in the absence of forskolin, it significantly increased Isc of forskolin-stimulated G551D-CFTR by ~8–fold with an EC₅₀ of ~20 μM. A similar effect of Auranofin was also observed for the G1349D-CFTR mutant. Taken
together, these results indicate that Auranofin is an activator of wt-CFTR by a cAMP-independent mechanism and a potentiator of CF-associated CFTR mutants including F508del-CFTR, G551D-CFTR. (Supported by grants from the Cystic Fibrosis Foundation).