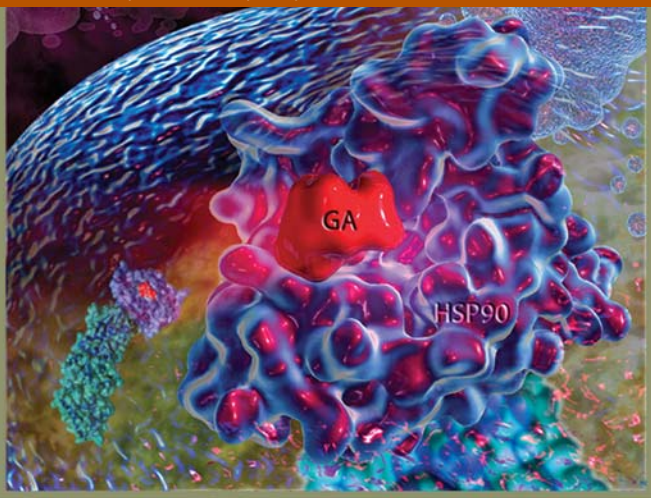
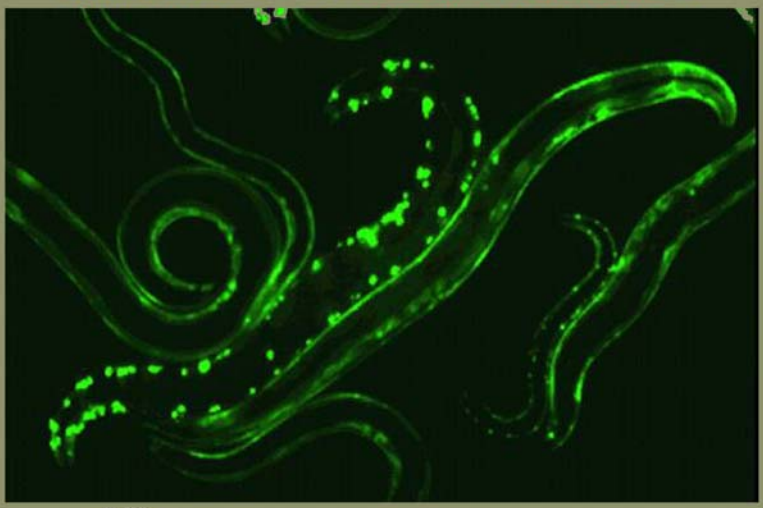
Abstracts of papers presented
at the 2010 meeting on

MOLECULAR CHAPERONES & STRESS RESPONSES

May 4–May 8, 2010





Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

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at the 2010 meeting on

MOLECULAR CHAPERONES & STRESS RESPONSES

May 4–May 8, 2010

Arranged by

F. Ulrich Hartl, *Max Planck Institute for Biochemistry, Germany*
David Ron, *New York University School of Medicine*
Jonathan Weissman, *HHMI/University of California, San Francisco*

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

This meeting was funded in part by the **National Institute on Aging;** the **National Heart, Lung and Blood Institute;** and the **National Institute of General Medical Sciences;** branches of the **National Institutes of Health;** and **Enzo Life Sciences, Inc.**

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Front cover, top: HSP90, a regulator of cell survival. Inhibition of HSP90 activity by drugs like geldanamycin (GA) destabilizes client proteins which ultimately lead to the onset of apoptosis. Dana Haley-Vicente, Assay Designs (an Enzo Life Sciences company).

Front cover, bottom: *C. elegans* expressing a proteotoxic polyglutamine-expansion protein. Richard Morimoto, Northwestern University.

Back cover, clockwise from top left:
Maltose binding protein (translucent), shown suspended in its molten globule state with chaperone protein SecB (yellow), destined for transport across the cellular membrane. Philipp Bechtluft, Arnold Driessen, Sander Tans and Graham Johnson.

Nuclear localization of Hsp10 in cultured human lung fibroblasts. The image shows Hsp10 in the cytoplasm and in the nucleus of most cells in a culture of the human lung fibroblast cell line, HLF-1. The chaperonin is considered a mitochondrial protein but here we demonstrate by immunohistochemistry that it also occurs in the cytoplasm and in the nucleus. Bar = 50 microns. Simona Corrao. University of Palermo.

Structure of the oligomeric small heat shock protein, alphaB-Crystallin, determined by solid-state NMR. The structure reveals that the C-terminus IXI motif from one unit in the oligomer binds in the substrate-binding groove of a neighboring until, suggesting an auto-inhibitory mechanism for chaperone activity. Rachel Klevit, University of Washington. Artwork prepared by S. Jehle.

Structure of the protein alpha-crystallin determined by electron microscopy. Johannes Buchner, Technische Universität München.

Several ribosomes (brown) are shown translating a messenger RNA (gray). As the elongating nascent protein (orange) emerges from the ribosome, it undergoes limited folding, but interactions with the exit tunnel and the surrounding area (shaded red) prevent the acquisition of stable tertiary structure. The ribosome effectively acts as a chaperone to prevent premature folding and misfolding of the nascent chain. After release from the ribosome, the free protein folds into its native structure (large orange structure). These conclusions are based on our single molecule force spectroscopy data probing the folding state of ribosome-associated nascent chains. Christian Kaiser, University California, Berkeley.

MOLECULAR CHAPERONES & STRESS RESPONSES

Tuesday, May 4 – Saturday, May 8, 2010

Tuesday	7:30 pm	**1** Chaperone Biochemistry and Protein Folding
Wednesday	9:00 am	**2** Chaperone Function in Disease and Development
Wednesday	2:00 pm	**3** Poster Session I
Wednesday	4:30 pm	Wine and Cheese Party *
Wednesday	7:30 pm	**4** Evolution and Regulation of Protein Folding Machines
Thursday	9:00 am	**5** Manipulating Chaperone Networks and Protein Folding Pathways
Thursday	2:00 pm	**6** Poster Session II
Thursday	7:30 pm	**7** Chaperones and Proteolysis
Friday	9:00 am	**8** Diseases of Protein Misfolding
Friday	2:00 pm	**9** Poster Session III
Friday	6:00 pm	Concert
	7:00 pm	Banquet
Saturday	9:00 am	**10** Quality Control and Protein Trafficking

Poster sessions are located in *Bush Lecture Hall*

* *Airslie Lawn*, weather permitting

Mealtimes at Blackford Hall are as follows:

Breakfast 7:30 am-9:00 am

Lunch 11:30 am-1:30 pm

Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

Printed on 100% recycled paper.

PROGRAM

TUESDAY, May 4—7:30 PM

SESSION 1 CHAPERONE BIOCHEMISTRY AND PROTEIN FOLDING

Chairperson: **J. Bardwell,** Howard Hughes Medical Institute, University of Michigan, Ann Arbor

WEDNESDAY, May 5—9:00 AM

SESSION 2 CHAPERONE FUNCTION IN DISEASE AND DEVELOPMENT

Chairperson: **E. Deuerling,** University of Konstanz, Germany

WEDNESDAY, May 5—2:00 PM

SESSION 3 POSTER SESSION I

WEDNESDAY, May 5—4:30 PM

Wine and Cheese Party

WEDNESDAY, May 5—7:30 PM

SESSION 4 EVOLUTION AND REGULATION OF PROTEIN FOLDING MACHINES

Chairperson: **J. Frydman,** Stanford University, California

THURSDAY, May 6—9:00 AM

SESSION 5 MANIPULATING CHAPERONE NETWORKS AND PROTEIN FOLDING PATHWAYS

Chairperson: **W. Balch,** The Scripps Research Institute, La Jolla, California

THURSDAY, May 6—2:00 PM

SESSION 6 POSTER SESSION II

THURSDAY, May 6—7:30 PM

SESSION 7 CHAPERONES AND PROTEOLYSIS

Chairperson: **B. Sauer,** Massachusetts Institute of Technology, Cambridge

SESSION 8 DISEASES OF PROTEIN MISFOLDING

Chairperson: **A. Dillin,** The Salk Institute, La Jolla, California

FRIDAY, May 7—2:00 PM

SESSION 9 POSTER SESSION III

FRIDAY, May 7—6:00 PM

CONCERT
Grace Auditorium

Soo Bae, cello

Praised by The New Yorker as "superb," and for "rich and romantic with crisp incisive technique" by The Strad, cellist Soo Bae was recognized in September 2006 by her home country of Canada as the top young cellist in the nation when she garnered First Prize in the Canada Council of the Arts Instrument Bank competition, resulting in a three-year loan of the ca. 1696 Bonjour Stradivari cello. This reaffirms her status among the premiere cellists of her generation, following top prizes awarded to her in July 2006 at the 6th Adam International Cello Festival & Competition in Christchurch, New Zealand and in March 2005 at the Concert Artists Guild International Competition.

Ms. Bae begins the 2007-08 season as featured soloist with the Asian Youth Orchestra for its 2007 Asia Tour of Japan, Hong Kong,and China performing Tchaikovsky's Rococo Variations under the baton of Okku Kamu. North American orchestral highlights in 07-08 include the New Hampshire Music Festival Orchestra, Scottsdale Symphony, Wartburg Symphony (IA), Ohio Valley Symphony, Toronto's Korean Canadian Youth Symphony, the National Arts Center Orchestra of Canada and the Christchurch Symphony in New Zealand. An avid chamber musician, she has participated in collaborative tours of both Europe and Asia and has appeared at numerous music festivals. she also tours regularly with the "Music from Marlboro" throughout the US. Born in Seoul, Korea, Soo Bae received her Bachelor of Music in 2001 from The Curtis Institute of Music and her Masters of Music degree and then the prestigious Artist Diploma from The Juilliard School where she serves as an assistant faculty under Joel Krosnick.

FRIDAY, May 7

BANQUET

Cocktails 7:00 PM Dinner 7:45 PM

SESSION 10 QUALITY CONTROL AND PROTEIN TRAFFICKING

Chairperson: **R. Hegde,** University of California, San Francisco

Proteostasis network responses to stress in yeast and human
Li Wang, William E. Balch, Andrew Dillin, Richard I. Morimoto, Hui Ge, Walter Newman, Peter Reinhart.
Presenter affiliation: Proteostasis Therapeutics Inc., Cambridge, Massachusetts. 197

PROTEIN STABILIZATION BY SELECTION

James Bardwell

University of Michigan, Dept. of Molecular Cellular and Developmental Biology, Howard Hughes Medical Institute, 830 N University Drive, Ann Arbor, MI, 48109

We have developed a powerful genetic method that allows selection for protein variants with enhanced stability by directly linking protein stability to antibiotic resistance. This has allowed us to better understand the relationship between protein stability and function to better understand protein folding in vivo and to address practical problems associated with protein instability.

TRAJECTORY OF ATP-INDUCED DOMAIN MOVEMENTS IN GROEL

Dan Clare[1], Daven Vasishtan[1], Maya Topf[1], Joel Quispe[2], George Farr[3], Art Horwich[3], Helen Saibil[1]

[1]Birkbeck College, Dept. of Crystallography, London, WC1 EHX, United Kingdom, [2]Scripps Research Institute, Dept. of Molecular Biology, La Jolla, CA, 92037, [3]Yale University School of Medicine, Dept. of Genetics and HHMI, New Haven, CT, 06520

ATP binding to chaperonins triggers a series of dramatic conformational changes that determine the sequence of events in substrate binding, encapsulation and eventual release from these double-ring complexes. Upon ATP binding to the central, equatorial domains of GroEL, the substrate-binding apical domains become extremely mobile, until they are captured by binding of the co-chaperonin GroES. We have used cryo electron microscopy, statistical analysis of single particle images and flexible domain fitting to resolve the structures in an ensemble of dynamic GroEL-ATP states. Complexes with ATP bound to one or both rings are observed. The results trace out a trajectory of domain movements that ultimately lead to GroES binding and formation of the folding chamber. These movements are very different from those predicted by molecular dynamics simulation of trajectories based on the two conformations observed by crystallography, and provide new insights into the process of substrate encapsulation.

ROLE OF MOLECULAR CHAPERONES IN SOD1-LINKED ALS

Arthur Horwich, Wayne Fenton, Krystyna Furtak, Maria Nagy, Urmi Bandyopadhyay, Navneet Tyagi

Yale School of Medicine, Genetics/HHMI, 295 Congress Ave, New Haven, CT, 06510

Mutations in the abundant cytosolic superoxide scavenging enzyme SOD1 account for ~2% of cases of ALS (Lou Gehrig's Disease), associated with misfolding and a toxic gain of function, resulting in motor neuron dysfunction and cell death. We have been modeling this system using a folding-defective form of human SOD1, G85R, that behaves as a misfolded monomer, unable to form the normal disulfide bond or to produce the normal homodimer. Mice transgenic for G85R SOD1-YFP develop soluble oligomers and aggregates of the fusion protein in spinal cord and succumb to motor paralysis. Hsc70 and, at later times, Hsp110, associate with the soluble misfolded G85R SOD1-YFP species in mutant spinal cord but not with SOD1-YFP in the cord of wild-type transgenics. Similarly, C.elegans transgenic for the mutant fusion are locomotor paralyzed compared with normal movement of a wild-type transgenic, associated with soluble oligomer formation and aggregation of the mutant fusion protein. We report on new studies profiling chaperone content in laser- dissected motor neurons, measuring turnover of mutant and wild-type SOD1-YFP in the transgenic mice, and reconstituting chaperone interactions in vitro.

CHAPERONIN-CATALYZED RESCUE OF ENTROPICALLY TRAPPED STATES IN PROTEIN FOLDING

Kausik Chakraborty[1,4], Jyoti Sinha[1], Manal Chatila[1], Qiaoyun Shi[1], Bernhard C Poschner[1], Martin Sikor[2], Guoxin Jiang[1], Don C Lamb[2,3], F-Ulrich Hartl[1], Manajit Hayer-Hartl[1]

[1]Max Planck Institute of Biochemistry (MPIB), Department of Cellular Biochemistry, Am Klopferspitz 18, Martinsried, 82152, Germany, [2]Center for Nanoscience, and Munich Center for Integrated Protein Science (CiPSM), Ludwig-Maximilians-Universität, Physical Chemistry, Department of Chemistry and Biochemistry, Butenandtstrasse 11, Munich, 81377, Germany, [3]University of Illinois at Urbana-Champaign, Department of Physics, 1110 W. Green St, Urbana, IL, 61801, [4] Institute of Genomics and Integrative Biology (CSIR), Proteomics and Structural Biology, Mall Road, Delhi, 110007, India

GroEL and GroES form a chaperonin nano-cage for single protein molecules to fold in isolation. Whether the isolation of folding-substrates is the sole determinant of efficient folding inside the cavity or the chaperonin nano-cage edits the folding-information encoded by the amino acid sequence is not understood. Here, we addressed this question using a double-mutant of the maltose binding protein, DM-MBP, as a substrate. We show that DM-MBP refolding is not limited by the formation of reversible aggregates and populates a kinetically-trapped state that is collapsed but structurally disordered. Introducing two long-range disulfide bonds into the DM-MBP sequence reduces the entropic barrier of folding and accelerates native state formation ~10-fold. Strikingly, steric confinement of the unfolded DM-MBP in the chaperonin cage closely mimics the effect of constraining disulfides on folding kinetics, in a manner mediated by negative charge clusters of the cage wall. These findings suggest that chaperonin dependence correlates with the tendency of proteins to populate entropically stabilized folding intermediates. The capacity to rescue proteins from such folding traps may explain the uniquely essential role of the chaperonin-cages within the chaperone network.

SINGLE MOLECULE ANALYSIS OF NASCENT PROTEIN FOLDING

Christian M Kaiser[1,4], Daniel H Goldman[2], Susan Marqusee[3,4], Carlos Bustamante[1,2,3,4]

[1]University of California, Berkeley, Department of Physics, 366 LeConte, Berkeley, CA, 94720, [2]University of California, Berkeley, Department of Chemistry, 420 Latimer Hall, Berkley, CA, 94720, [3]University of California, Berkeley, Department of Molecular and Cell Biology, 142 LSA #3200, Berkeley, CA, 94720, [4] University of California, Berkeley, QB3 Institute–Berkeley, Stanley Hall, Berkeley, CA, 94720

Most proteins in the cell must fold into specific three-dimensional structures to become biologically active. Insights into the mechanisms underlying protein folding have been gained largely from *in vitro* refolding experiments employing isolated full-length proteins. In the cell, however, proteins are synthesized by the ribosome in a vectorial manner. Hence, amino-terminal portions of the protein can participate in folding events while more carboxyl-terminal segments are still being synthesized. As translation proceeds, the elongating nascent protein traverses the polypeptide exit tunnel before emerging from the exit site located at the surface of the large ribosomal subunit. Interactions with components of the tunnel and the area around the exit site have been shown to influence the conformation of the emerging protein. Thus, it is likely that both the vectorial process of translation itself and the ribosomal environment affect the folding pathway of translating polypeptides. As soon as nascent proteins emerge from the ribosome, their folding transitions are guided by interactions with molecular chaperones, such as Trigger factor. How the concerted interplay between translating ribosomes and molecular chaperones affects the folding of newly synthesized proteins, thereby contributing to efficient folding in the crowded environment of the cell, has remained poorly understood. We have developed an experimental methodology to directly probe folding transitions of ribosome-associated nascent polypeptides by single molecule force spectroscopy. Using optical tweezers, we are able to mechanically "pull" on the nascent protein emerging from the ribosomal exit tunnel. In combination with a reconstituted *in vitro* translation system, we are capable of interrogating the conformational state of defined, incrementally longer nascent polypeptides in their natural context on the ribosome. This experimental system allows us to recapitulate the folding transitions during the earliest stages of protein biogenesis and to dissect how the ribosome and molecular chaperones contribute to the efficient folding of newly synthesized proteins.

This work was supported by grants from the National Institutes of Health (Career Development Award, Grant No. 5K99GM086516-02) and the California Institute for Quantitative Biosciences (QB3 Distinguished Postdoctoral Fellowship) to C.M.K.

TAMING THE BEAST: STRUCTURAL BIOLOGY OF A SMALL HEAT SHOCK PROTEIN

Rachel E Klevit[1], Stefan Jehle[1], Benjamin Bardiaux[2], Katja Dove[1], Barth von Rossum[2], Hartmut Oschkinat[2,3], Ponni Rajagopal[1]

[1]University of Washington, Biochemistry, Box 357742, Seattle, WA, 98195, [2]Leibniz-Institut für Molekulare Pharmakologie, Structural Biology, Robert-Rössle-Strasse 10, Berlin, 13125, Germany, [3]Freie Universität Berlin, Chemistry and Biochemistry, Takustraße 3, Berlin, 14195, Germany

The polydisperse, oligomeric structures of small heat shock proteins (sHSPs) have eluded investigation by standard structural biology approaches. A paradigm example is αB-Crystallin (αB), a ~ 580 kDa oligomer composed of ~28 subunits. We have used solution-state and solid-state NMR, chaperone-like activity assays, and SAXS (Small Angle X-ray Scattering) to determine a structure of αB and gain insight into its function. Similar to most sHSPs, αB has a conserved α-crystallin domain flanked by variable N- and C- terminal regions. Solid-state NMR of oligomeric αB reveals a highly curved α-crystallin domain dimer as the building block of the oligomer. A hybrid approach using SAXS and solid-state NMR was used to determine a structural model of αB: αB forms an oligomer with tetrahedral symmetry comprised of four units of three dimers organized in a triangular array. Solid state NMR data also show that residues from the C-terminal region, including the conserved IXI-motif, bind in the putative substrate-binding groove, suggesting an auto-inhibitory mechanism. NMR spectra reveal a modulation of this interaction by pH changes, providing a structural basis for the known pH-dependent activation of αB. Cardiac ischemia is associated with a drop in cellular pH from 7.5 to 6.9 and αB binds cardiac muscle proteins more strongly at the lower pH presumably to confer protection from stress denaturation. SAXS data collected at pH 7.5 and 6.5 reveal a large increase in the radius of gyration at the lower pH, consistent with dissociation of the C-terminal region from the substrate-binding groove.
To more fully understand the structural basis of αB function, we are using isolated α-crystallin domains as biochemical and structural models for sHSPs. Solution-state NMR studies on the α-crystallin domain dimer from αB (αB10.1) show that 1) the structure of the isolated domain is similar to its structure in the context of the oligomer and 2) similar to the oligomer, αB10.1 undergoes significant conformational changes over the narrow pH range from 7.5 to 6.9. In contrast, pH-induced conformational changes are not detected in a disease-associated mutant, R120G-αB, in either the α-crystallin domain or in the oligomer. To identify the basis of the pH-dependent behavior, we have designed and compared numerous mutant forms of αB. We will present structural and biochemical data on an engineered mutant that, like R120G-αB, does not display a pH-induced conformational change and has low activity in chaperone-like activity assays. Together, our observations suggest that conformational plasticity providing for adaptability to changes in environment is essential for αB function.

THREE-DIMENSIONAL STRUCTURE OF THE HSP104 AAA+ ATPASE

Francis T Tsai, Sukyeong Lee, Bernhard Sielaff, Jungsoon Lee

Baylor College of Medicine, Department of Biochemistry & Molecular Biology, One Baylor Plaza, Houston, TX, 77030

Yeast Hsp104 and bacterial ClpB belong to the large superfamily of ring-forming AAA+ machines that convert ATP into mechanical work. Hsp104 and ClpB are functional orthologs that recognize aggregated proteins as substrates, and remodel them in an ATP-dependent manner. In addition to its essential role in acquired thermotolerance development, Hsp104 is also essential for the inheritance, maintenance, and elimination of [*PSI*+], a yeast prion that increases translational read-through of nonsense codons. The ability to disaggregate stress-damaged proteins is strictly dependent on the M-domain that is a hallmark of the ClpB/Hsp104 family. While the three-dimensional structures of ClpB[1,2] and Hsp104[3,4] have been reported, the location of the M-domain is controversial and its function in protein disaggregation remains unclear.

To address this on-going controversy, we examined the structure of Hsp104 using a multi-pronged structural and biochemical approach. At this meeting, I will present unpublished, recent data from my lab suggesting that Hsp104 and ClpB are more similar in structure than previously proposed. Moreover, I will present biochemical data of an engineered Hsp104 hexamer that provides new mechanistic insight into the structure-function relationship of Hsp104.

References
1. Lee *et al.* Cell 115, 229-240 (2003).
2. Lee *et al.* Mol Cell 25, 261-271 (2007).
3. Wendler *et al.* Cell 131, 1366-1377 (2007).
4. Wendler *et al.* Mol Cell 34, 81-92 (2009).

RAPID FOLDING COMPLETION IN THE CAGE OR ESCAPING OUT OF THE CAGE; ALTERNATIVE FATES OF SUBSTRATE POLYPEPTIDE INTERACTING WITH GROEL/GROES INTERFACE REGION IN THE CRITICAL INTERMEDIATE OF CHAPERONIN-ASSISTED FOLDING

Fumihiro Motojima, Masasuke Yoshida

Kyoto Sangyou University, Life Science, Kamigamo Motoyama, Kyoto, 603-8555, Japan

In the chaperonin-assisted protein folding, substrate protein undergoes folding in the chaperonin cage made by a GroEL central cavity and a GroES lid. The classic mechanistic model assumes that the substrate protein in the cage is isolated from outside and exists as a free polypeptide. However, we found that a part of unfolded polypeptide in the cage is always protruding to outside through narrow space near the GroEL/GroES interface. This state is rate-limiting in the whole chaperonin-assisted folding reaction. Then, the whole polypeptide is released either into the cage or into the outside medium. The former gains native structure in the cage ten times more rapidly than spontaneous folding (in-cage folding), and the latter undergoes spontaneous folding in the outside medium. GroES remains bound to GroEL and the cage is intact during these events. Partition of the in-cage folding and the escape varies among substrate protein and is affected by hydrophobic interaction between polypeptide and GroEL wall. The mutant GroEL with a small in-cage folding yield produces less amount of native model substrate protein in *Escherichai coli* cell. These observations necessitate revision of the classic mechanistic model of chaperonin.

FUNCTIONS AND MECHANISMS OF CYTOSOLIC CHAPERONES IN PROTEIN HOMEOSTASIS

Elke Deuerling, Ansgar Koplin, Steffen Preissler, Yulia Ilina, Miriam Koch, Annika Scior

University of Konstanz, Biology, Universitaetsstr. 10, Konstanz, 78457, Germany

The yeast Hsp70/40 system Ssb/RAC binds to ribosomes and contacts nascent polypeptides to assist cotranslational folding. Here, we demonstrate that NAC, another ribosome-tethered system, is functionally connected to Ssb/RAC and the cytosolic Hsp70 network. Simultaneous deletions of genes encoding NAC and Ssb caused conditional loss of cell viability under protein folding stress conditions. Furthermore, NAC mutations revealed genetic interaction with a deletion of Sse1, a nucleotide exchange factor regulating the cytosolic Hsp70 network. Cells lacking Ssb or Sse1 showed protein aggregation which is enhanced by additional loss of NAC, however, these mutants differ in their potential client repertoire. Aggregation of ribosomal proteins and biogenesis factors accompanied by a pronounced deficiency in ribosomal particles and translating ribosomes only occurs in ssbΔ and nacΔssbΔ cells suggesting that Ssb and NAC control ribosome biogenesis. Thus, Ssb/RAC and NAC assist protein folding and likewise have important functions for regulation of ribosome levels. These findings emphasize the concept that ribosome production is coordinated with the protein folding capacity of ribosome-associated chaperones.

THE HEAT SHOCK RESPONSE: INTEGRATED NEURONAL NETWORKS FOR METABOLIC CONTROL, STRESS, AND AGING

Richard I Morimoto, Anat Ben-Zvi, Veena Prahlad, Laetitia Chauve

Northwestern University, Biochemistry, Molecular Biology and Cell Biology, Rice Institute for Biomedical Research, 2145 Sheridan Rd., Tech MG90, Evanston, IL, 60208

The stability of the proteome is of central importance to the health of the cell, and contributes significantly to the lifespan of the organism. Chronic expression of misfolded and damaged proteins associated with conformational diseases, exposure to stress, or aging results in proteostasis imbalance with devastating consequences on the stability of the proteome. Key to this balance are stress-inducible transcription factors including Hsf1 that regulate the heat shock response (HSR). Hsf1 has also been shown to be essential for multiple lifespan regulatory pathways and is regulated by the metabolic state of the cell through the activity of the NAD-dependent sirtuin, Sirt1. During development and aging in C. elegans, there is a sharp decline in the capacity of the proteostasis network in early adulthood coincident with the peak of fecundity. The collapse of proteostasis coincides with an age-dependent dampening of the inducibility of the HSR and unfolded protein response (UPR). This decline can be suppressed by activation of Hsf1 (or DAF-16) in early development revealing a strategy to restore the youthful proteostatic state. These studies provide an understanding of stress responses and Hsf1 function at a cellular and molecular level. In C. elegans, the HSR is regulated at the organismal level by cell non-autonomous control by the AFD thermosensory neurons. The HS signal requires active neurotransmission via specific neurohormones and neuropeptides to somatic tissues to regulate HS gene expression. This stress sensing mechanism appears specific to the environmental signal as animals deficient for the AFD-dependent HSR are fully responsive to cadmium activation of HS genes in an Hsf1-dependent manner. This suggests that different neuronal networks sense distinct environmental stress signals to control proteostasis. This is further supported by the observation that an imbalance of cholinergic signaling and overexcitation results in the collapse of proteostasis in post-synaptic muscle cells. These results, reveal that the HSR is organized at the systems level of the organism to sense the stress signal through active neuronal activity, and together with the metabolic state, sets the proteostasis network to ensure stability of the proteome and the health of the organism.

INSIGHTS INTO HSP90-CLIENT PROTEIN INTERACTIONS FROM NMR

H. Jane Dyson, Sung Jean Park, Maria A Martinez-Yamout

The Scripps Research Institute, Molecular Biology, 10550 North Torrey Pines Road, La Jolla, CA, 92037

The major function of the chaperone Hsp90 involves interactions with a relatively large set of proteins, termed client proteins. It is not clear in what state, whether folded, partly folded or alternatively folded, the client proteins participate in the interaction. We present NMR studies of the interactions between one client protein, the p53 DNA-binding domain, and fragments of Hsp90 of various sizes, comprising 1 and 2 domains of the protein, up to the full-length dimeric protein. Attenuation of the p53 resonances in the complex, together with the observed lack of such attenuation in the Hsp90 domain spectra have led us to suggest that the p53 DNA binding domain forms a "folded globule" state in the complex. This state is characterized by secondary structure indistinguishable from that of the free protein, but contains a manifold of states that are in intermediate exchange on the NMR time scale, thus causing resonance broadening and disappearance. Further evidence for the loose and flexible nature of the bound p53 client is provided by the fluorescence behavior of the dye 1-anilinonaphthalene-8-sulfonic acid (ANS), which shows increased emission and a blue shift in the presence of the complex. Hydrogen/deuterium exchange rates of the amide protons of the p53 DNA binding domain are also significantly increased in the presence of a low concentration of Hsp90, consistent with a loosened structure for the p53 DNA binding domain in the complex. Further NMR experiments using samples with specifically labeled methyl groups and employing saturation transfer techniques have allowed the dissection of the interaction sites between the Hsp90 domains and the client protein. We conclude that the interaction between Hsp90 and p53 is complex, and involves a structural change in the client protein to a loosened state. These observations have profound implications for the understanding of Hsp90-client proteins in general, and may provide a reason for the persistent difficulty that has been experienced in the crystallization of Hsp90-client protein complexes for structural studies.

THE HSP110 MOLECULAR CHAPERONE MODULATES DYNEIN ACTIVITY

Taras Makhnevych[1], Philip Wong[2], Oxana Pogoutse[3], Franco J Vizeacoumar[3], Yolanda Chong[3], Charles Boone[3], Andrew Emili[3], Walid A Houry[1]

[1]University of Toronto, Biochemistry, 1 King's College Circle, Toronto, M5S1A8, Canada, [2]Institute of Bioinformatics and Systems Biology, German Research Center for Environmental Health, Ingolstadter Landstrasse 1, Neuherberg, 85764, Germany, [3]University of Toronto, Molecular Genetics, 160 College Street, Toronto, M5S3E1, Canada

In order to characterize the cellular pathways and protein complexes that require Hsp70/Hsp110 molecular chaperones in the cell, we carried out a systematic pull-down analysis of all the cytoplasmic/nuclear Hsp70 (Ssa1, Ssa2, Ssa3, Ssa4, Ssb1, Ssb2, Ssz1) and Hsp110 (Sse1 and Sse2) chaperones in yeast using strains in which these chaperones were endogenously N- or C-terminally TAP-tagged. This allowed us to map a protein interaction network consisting of 1069 unique interactions between the 9 chaperones and 473 proteins. The network highlights roles for Hsp70/Hsp110 chaperones in 14 broad biological processes. Analysis of the generated chaperone-protein interaction network demonstrated that a number of Hsp70/Hsp110 chaperones co-purified with several microtubule motors including dynein. Careful analysis of the chaperone deletion phenotypes revealed a function for Sse1 in mitotic spindle morphology. We demonstrated that deletion of *SSE1* gene produced increased number of cells which were not able to properly position the nuclei at the bud neck in mitosis resulting in aberrant spindle morphology phenotypes. Imaging studies and biochemical analyses suggested that Sse1 is required for proper functioning of the cytoplasmic dynein motor, Dyn1. In the absence of Sse1, dynein was no longer concentrated at the plus ends of the cytoplasmic microtubules and, instead, was aggregating in the cytoplasm. The deletion of *SSE1* gene also affected dynein interaction with components of the dynactin complex. Altogether, our data demonstrate that the Sse1 chaperone is necessary for dynein function and suggest that Sse1 might modulate dynein conformational states during the motor functional cycle. Further data on the effect of Sse1 on dynein activity will be presented at the meeting.

THE ROLE OF SMALL HEAT SHOCK PROTEINS IN PROTEIN FOLDING DISEASES

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The Human and Drosophila small Heat Shock Protein (sHSP) family comprises 10 and 11 members respectively. Some members of these sHSP have been implicated directly or indirectly in several neurodegenerative and neuromuscular disorders. Several members are upregulated in brain amyloidosis and mutations of several HSPB proteins have been associated with muscular and neurological disorders. Comparing the members from both species reveals at least two functionally distinct classes: some members support HSP70 dependent (re)folding activities, whereas another subclass specifically prevents aggregation of (disease-associated) proteins, which is largely HSP70 independent. Both types of activities can lead to lifespan extensions in Drosophila. By a series of functional studies, we furthermore identified the Drosophila ortholog of the human HSPB8. HSPB8 and its Drosophila ortholog cooperate with members of the BAG-family of proteins to stimulate autophagy, hereby facilitating the clearance of other aggregation-prone proteins. Mutations in HSPB8 are associated with autosomal dominant distal hereditary motor neuropathy and Charcot-Marie-Tooth disease type 2L. Using Drosophila as a model, we now show that besides a toxic gain of function, mutations in HSPB8 lead to a partial loss of function that likely contributes to disease progression. Our data furthermore imply that putative age-related decline in small HSP expression or -function could play a role in the onset of other folding diseases and in ageing in general.

This work was financially supporter by Senter Novem (IOP genomics grants IGE03018 and IGE0700), the Prinses Beatrix Foundation (WAR05-0129) and the European Union (IRG 230908).

HOW DO INTRINSICALLY UNFOLDED CHAPERONES WORK?

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Hsp33, a highly conserved redox-regulated molecular chaperone, plays an important role in protecting bacteria against oxidative stress conditions that lead to protein unfolding. The specific activation of Hsp33 under oxidative stress conditions involves the oxidative unfolding of its own C-terminal redox sensor domain, making Hsp33 a member of a new class of chaperones, which are active in a partially natively unfolded state. This observation raises a number of intriguing mechanistic questions; how do natively unfolded chaperones discriminate between unfolded substrate proteins and their own unfolded regions? Moreover, how does the DnaK-system, which triggers substrate release from Hsp33 upon return to reducing non-stress conditions, discriminate between unfolded Hsp33 and unfolded substrate proteins? To address these questions, we combined biophysical and computational studies with mass spectrometry and cell biology. We first elucidated the peptide binding specificity of active Hsp33 using peptide arrays. This analysis allowed us to establish an algorithm that predicts Hsp33 binding sites in protein sequences with high accuracy. While we were unable to identify a strong binding sequence, we identified a distinct group of amino acids, negatively charged residues and cysteines, which are strongly disfavored to bind Hsp33. Curiously, these are the very same amino acids that are strongly enriched in Hsp33's unfolded redox switch domain as well as in most natively unfolded proteins. Chaperone assays confirmed that Hsp33 does not interact with natively unfolded proteins but requires at least some secondary structure elements for binding, explaining how intrinsically unfolded chaperones, like Hsp33, avoid binding their own unfolded regions. Furthermore, we found that Hsp33's linker region undergoes substantial refolding and stabilization upon binding of the substrate proteins. While these results explained how Hsp33 prevents self-recognition and interaction with the DnaK-system, they did not explain how the DnaK-system, which is known to bind substrate proteins in a largely unfolded conformation, recognizes the partially folded substrate proteins bound to Hsp33. Hsp33 might either unfold the substrate proteins directly upon its binding as part of an entropy transfer mechanism, or upon subsequent reduction of its disulfide bonds. In either case, our results experiments provide mechanistic insights as to how intrinsically disordered chaperones function in multi-chaperone networks to prevent aggregation and support refolding of proteins.

THE INTERPLAY BETWEEN CHAPERONES, PRION PROTEIN DYNAMICS AND THEIR CELLULAR CONTEXT IS CRUCIAL FOR PROTEIN-ONLY INHERITANCE

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Prions are a unique group of proteins that can adopt a spectrum of physical and corresponding functional states *in vivo*. The physiological consequences of this conformational flexibility, ranging from neurodegenerative disease in mammals to heritable traits in fungi, are determined not only by the identity of the alternately folded protein but also by the efficiency with which the alternate fold is replicated and spread. The assembly of alternately folded prion protein into aggregates is believed to be a central event in these processes, but the biochemical identity of the active species has remained elusive, as prion aggregates are heterogeneous in size and potentially function.

To explore the relationship between protein aggregation and phenotypic stability, we exploited the Sup35/[*PSI*$^{+}$] prion system in S*accharomyces cerevisiae*. Using a combination of mathematical modeling and in vivo analysis of aggregate dynamics in response to perturbations of the system, we have determined that non-heritable and heritable prion aggregates are interconvertible species and that the transition between these forms is regulated by the ratio of aggregated prion protein to the molecular chaperone Hsp104, the efficiency with which prion aggregates are remodeled by chaperones, and the cell division pedigree. Shifts in the aggregate size distribution, which are specified by either the conformation of the prion protein or changes in expression levels, create heterogeneity in the complement of prion aggregates present in individual cells due to a size threshold for transmission. This aggregate size-limited transmission has profound effects on prion biology by creating cell-to-cell phenotypic diversification that in extreme cases leads to prion loss. Together, our studies highlight how the interplay of prion protein biogenesis and the cellular context in which it occurs mediate the appearance, maintenance and spread of protein-based phenotypes.

CONSERVED CONFORMATIONAL STATES AND COCHAPERONE INTERACTIONS DEFINE THE HSP90 MOLECULAR CHAPERONE CYCLE.

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The ubiquitous and highly conserved Hsp90 molecular chaperone is the central player in a multi-component complex that promotes ATP-dependent rearrangement and activation of essential substrate proteins including nuclear receptors and cell cycle kinases. While much is known about the abundant targets of Hsp90, significant questions remain unanswered including how dramatic conformational rearrangements, energy from ATP hydrolysis and critical cochaperone interactions contribute to rearrangement and activation of substrates. Using single particle electron microscopy (EM), we have determined 3-D reconstructions of Hsp90:AMPPNP and Hsp90:ADP that establish a three-state cycle with nucleotide binding alone triggering dramatic conformational changes in Hsp90. Using key crosslinking methods we have trapped these conformational states in yeast and human Hsp90s, establishing a universal cycle and a unique, species-specific conformational equilibrium. More recently we have achieved a cryo-EM reconstruction of the human Hsp90:Hop complex that reveals Hop interaction sites and a novel Hsp90 client-loading conformation that is on-path to the closed, hydrolysis-active state. Additional reconstructions of human Hsp90:Hop:Hsp70 and Hsp90:AMPPNP:FKBP52 identify active roles for co-chaperones, stabilizing Hsp90 conformational states and contributing to client protein activation.

SRR: A NOVEL TYPE OF HSP90-RELATED SUPRA-DOMAIN ASSOCIATED WITH NEURODEGENERATION

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Protein supra-domains are defined as recurring arrangements of two or three domains present adjacent to each other along a polypeptide chain. Such combinations have novel functions beyond those of the individual partner domains that compose them, which can exist in isolation. Here, we describe a new type of large supra-domain (~380 residues) in which one of the component partners (~200 residues) appears to be incapable of existing in a context other than immediately adjacent to the C-terminus of the well-characterized Hsp90-like ATPase domain. We found that this supra-domain has a broad phylogenetic distribution, with examples in Archaea, Bacteria and Eukarya. There is strong selective pressure for this arrangement to occur as part of very large repeated regions. We identified multiple strategies of convergent evolution to attain such configurations. In humans, this supra-domain is present in triplicate at the N-terminus of the protein sacsin (4,579 residues), mutated in the neurodegenerative disorder known as Spastic Ataxia of Charlevoix-Saguenay, and thus we termed it "sacsin repeating region" (SRR). Biochemical characterization demonstrated that SRRs possess ATPase activity, which appears to be a requirement for sacsin function, as a disease-causing mutation leads to an alternate conformation completely incapable of hydrolyzing ATP. We also found evidence of a convergent evolutionary strategy to place SRRs in proteins containing C-terminal J-domains, which we demonstrated here to be capable of stimulating the intrinsic ATPase activity of Hsp70. Our sequence and biochemical analyses indicate that SRRs necessitate nucleotide hydrolysis for their function, provided by the common Hsp90 ATPase domain, which, when coupled to the unique adjacent sequence, may give rise to a novel activity related to protein quality control.

MECHANISM OF HSP40-FAMILY CO-CHAPERONES DNAJA1 AND DNAJA2 WITH HSC70

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Human Hsc70 is the key cytosolic chaperone of the Hsp70 family important for the folding and sorting of various proteins. Its activity depends on ATP binding and hydrolysis, which requires the Hsp40 family of co-chaperones including the major type I forms DnaJA1 and DnaJA2. These have a J domain at the N-terminus and a C-terminal domain, subdivided into a proximal part containing a Cys-Zinc finger and a distal C-terminal region with a dimerization interface. The J domain function of Hsc70 ATPase stimulation is well established, while the C-terminal domain binds substrate polypeptide. Previous work suggested that DnaJA1 and DnaJA2 have distinct profiles in the biochemical properties of polypeptide binding, stimulation of the Hsc70 ATPase, and ability to support folding. Interdomain coordination appeared to be critical for function, possibly for the transfer of substrate from the DnaJA to Hsc70 by a still undefined mechanism. Furthermore, how such coordination is supported by the internal structures of the C-terminal domains remains poorly understood. To address this, we constructed chimeric and internal deletion mutants of DnaJA1 and DnaJA2. The mutants were tested for the activities of their separate domains, and for the ability to functionally cooperate with the Hsc70 chaperone. Mutations at the proximal and distal regions within the DnaJA C-terminal domain had a moderately negative effect on stimulation of the Hsc70 ATPase although the J domains were intact. Variable effects on substrate binding were also observed. Interestingly, disruption of either proximal or distal structures significantly inhibited the ability to promote refolding of denatured polypeptide by Hsc70. The local mutations also altered the overall configuration of the C-terminal domains. Experiments in cell culture systems confirmed functional differences in the mutants. We propose a model in which functional elements throughout the DnaJAs contribute to their activity with Hsc70.

ATF4 POTENTIATES CELL DEATH UPON ENDOPLASMIC RETICULUM STRESS BY DECREASING AND REDUCING THE DURATION OF EIF2α PHOSPHORYLATION

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In response to a variety of insults, eukaryotic cells reduce mRNA translation through phosphorylation of Ser51 on the α-subunit of eukaryotic translation initiation factor-2 (eIF2α). Mutation of Ser51 to Ala prevents phosphorylation and translation attenuation in response to cellular stresses. Cells with homozygous Ser51Ala mutation (A/A eIF2α cells) display increased sensitivity to endoplasmic reticulum (ER) stress and oxidative stress due to the deficiency or absence of properly adaptive translational and transcriptional responses. Paradoxically, phosphorylation of eIF2α increases synthesis of the activating transcription factor ATF4. Since A/A eIF2α mutant cells and $Atf4^{-/-}$ cells exhibit similar defects in transcriptional adaptive responses, it was expected that they should display similar increased sensitivity to ER-stress-induced death. However, it is here shown that while $Atf4^{-/-}$ cells are more sensitive to oxidative stress than wild type cells, they are more resistant to cell death induced by the ER stress agents tunicamycin (Tm) or thapsigargin (Tg). During ER stress, $Atf4^{-/-}$ cells exhibit increased and extended eIF2α phosphorylation likely due to reduced expression of GADD34, a regulatory subunit of protein phosphatase 1 that dephosphorylates eIF2α. Furthermore, overexpression of GADD34 reduces eIF2α phosphorylation and increases the sensitivity of $Atf4^{-/-}$ cells to ER stress. These results suggest that $Atf4^{-/-}$ deletion protects cells during ER stress by prolonging eIF2α phosphorylation, in spite of the defective adaptive transcriptional response caused by $Atf4^{-/-}$ deletion.

DIRECT OBSERVATION OF CHAPERONE-INDUCED CHANGES IN A PROTEIN FOLDING PATHWAY

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Using optical tweezers and all-atom molecular dynamics simulations, we studied the effect of chaperone SecB on the folding and unfolding pathways of maltose binding protein (MBP). In the absence of SecB, we find that the MBP polypeptide first collapses into a molten globule-like compacted state and then folds into a stable core structure, onto which several α-helices are finally wrapped. Interactions with SecB completely prevent stable tertiary contacts in the core structure, but have no detectable effect on the folding of the external α-helices. It appears that SecB only binds to the extended or molten globule-like structure and retains MBP in this latter state. Thus during translocation, energy is required only to remove SecB and not to disrupt stable tertiary interactions [Bechtluft et al., Science 318, (2007)]. Currently we are employing this single molecule approach to investigate how the chaperone trigger factor affects protein folding pathways.

THE FUNCTION OF THE CHAPERONE TRIGGER FACTOR IN CO-TRANSLATIONAL FOLDING OF PROTEINS

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When emerging from the ribosomal exit tunnel, nascent polypeptide chains are welcomed by ribosome-associated chaperones, in bacteria represented by trigger factor (TF). TF interacts with most emerging polypeptide chains and has been shown to support the folding of many different polypeptides *in vivo*, but how TF exerts its effect is unclear. Experiments measuring the activities of model substrates including firefly luciferase and the tetrameric β-galactosidase *in vivo* and *in vitro* suggest that TF causes a delay in the folding process (Agashe et al., 2004. Cell 117(2): 199-209). However, it is not known yet whether this is a general mechanism of TF function valid for different substrates and how this can be correlated to its chaperone function. In the present study, we investigated the effect of TF on the folding of various *E. coli* proteins in a coupled *in vitro* transcription/translation system by monitoring the kinetics of disulfide bond formation as an indicator of tertiary structure formation. Substrate proteins were selected based on their dependence on various chaperones for folding as well as variable structural features. Indeed, TF postpones the formation of some disulfide bonds during synthesis such as the wildtype disulfide bond in β-lactamase, while the formation of others is not affected. Furthermore, tethering a protein distant enough to the ribosome to allow complete folding nevertheless seems to constrain its folding abilities, suggesting that ribosome proximity can interfere with *de novo* folding of some newly synthesized polypeptides.

DETERMINING OLIGOMERIC STRUCTURES OF THE HETEROGENEOUS SMALL HEAT SHOCK PROTEIN AB-CRYSTALLIN

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The molecular chaperone activity of the Small Heat Shock Proteins (sHSPs) renders them an important component of the cellular proteostasis network. Current models for their function dictate that they act to bind unfolding client proteins, holding them stable relative to insoluble aggregation, under conditions of cellular stress. Structurally the sHSPs are poorly understood, and few high-resolution structures exist for these oligomeric proteins. This stems primarily from their apparently dynamic quaternary and tertiary structure, and that many members coexist in multiple oligomeric forms. Here we describe the oligomeric structures of human αB-crystallin, an heterogeneous sHSP which populates a distribution of oligomers spanning between approximately 10 and 40 subunits.

Our approach to structure determination is based on a combination of ion mobility mass spectrometry (IM-MS), methyl-TROSY NMR, electron microscopy (EM), X-ray crystallography and molecular modelling. Combining MS and NMR experiments allows us to correlate the quaternary and tertiary dynamics of polydisperse αB-crystallin ensemble. This provides a rationale for building oligomeric models of the sHSP based upon the crystallographic protomer, and these models are restrained by both the IM and EM data. As such we obtain not only pseudo-atomic structures for the oligomeric states populated by αB-crystallin, but also an understanding of the remarkable polydispersity of this protein.

EIF2A PHOSPHORYLATION CONTROLS THE BALANCE BETWEEN ADAPTATION AND APOPTOSIS IN RESPONSE TO HYPERTONIC CONDITIONS

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Regulation of cell volume is of great importance because persistent swelling or shrinkage leads to cell death. Tissues experience hypertonicity in both physiological (kidney medullar cells) and pathological states (hypernatremia). Mild hypertonicity induces an adaptive gene expression program that leads to cell survival by induction of osmolyte transporters and the molecular chaperon HSP70, which helps to counterbalance the deleterious effect of macromolecular crowding due to the loss of water. Severe hypertonic conditions lead to cell death. We show that the balance between adaptive and apoptotic cellular responses to osmotic stress is controlled by phosphorylation of the translation initiation factor eIF2α, the master regulator of the stress response. Fibroblasts and cortical neurons deficient in eIF2α phosphorylation were protected from hypertonicity-induced apoptosis. A novel link is revealed between eIF2α phosphorylation and translation initiation of mRNAs encoding pro- and anti-apoptotic proteins at internal ribosome entry sites (IRESs). Stress-induced phosphorylation of eIF2α tips the balance against the anti-apoptotic XIAP and Bcl-xL proteins. This regulation is independent of the global decrease of protein synthesis and of the induction of molecular chaperons but dependent on the cytoplasmic accumulation of the IRES-transacting factor hnRNP A1, which inhibits translation of XIAP and Bcl-xL mRNAs. We propose that eIF2α phosphorylation during hypertonic stress promotes apoptosis by increasing the cytoplasmic accumulation of hnRNP A1. The involvement of stress granules (sites of storage of translationally arrested mRNAs) in this process will be discussed.

SYSTEMATIC FITNESS ANALYSIS OF POINT MUTANTS OF HSP90

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What does amino acid sequence conservation (or lack thereof) observed in phylogenetic alignments really mean? Hsp90 is highly conserved at the amino acid sequence level in phylogenetic alignments. For example, yeast and human Hsp90 are 45% identical. Are invariant positions in phylogenetic alignments of Hsp90 more likely to lead to a fitness defect if mutated compared to highly variable positions? How accurate are fitness predictions based on evolutionary conservation? To begin to address these questions we used traditional point mutagenesis of Hsp90 to measure the fitness of yeast harboring these variants. The results of these small-scale experiments indicated that amino acids that are conserved across tremendous evolutionary times can be mutated without measureable impacts on fitness. These results spurred us to generate fitness measurements for a large set of Hsp90 point-mutants. To accomplish this, we developed a hi-throughput approach utilizing the power of deep-sequencing to monitor the abundance of each point-mutant in a population of competing yeast over time. For Hsp90, the data from this approach has enabled us to generate quantitative relationships between the conservation of an amino acid across evolution and the experimental fitness of an amino acid substitution.

MAPPING HEAT SHOCK FACTOR 1 ACTIVITY IN THE LANDSCAPE OF SACCHAROMYCES CEREVISIAE GENE DELETION MUTANTS

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Cells must sense both internal and environmental conditions in order to respond appropriately to changing circumstances. These processes have been modeled as homeostatic, in which an ideal state is maintained, or, alternately, as acute stress responses, where dangerous conditions invoke protective programs. Heat shock factor 1 (HSF1p) is key transcriptional regulator of such a process, the heat shock response. High temperatures and misfolded proteins hyper-activate HSF1p and thereby up-regulate expression of protective proteins, such as chaperones. However, HSF1 is also essential for viability under normal conditions, because it plays roles in maintaining proteostasis. To explore the functions of HSF1p in both heat-shock-induced and basal contexts, we designed a fluorescent reporter to measure HSF1p activity in a collection of yeast deletion strains spanning the entire genome. Activity was measured at room temperature and after heat shock of 1 or 8 hours at 37 degrees. We observed broad ranges of both basal and induced activity in the mutant strains, with related classes of genes clustering together. Furthermore, we found a striking relationship between basal and heat-shock-induced HSF1p activity: a large set of strains with high basal HSF1p activity are especially sensitive to heat shock. Our study sheds light on the dual roles of HSF1p as both a stress responder and a fine-tuned homeostatic regulator.

HSP104 PROCESSING OF PRION VARIANTS

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In protein misfolding diseases chaperones are able to recognize aberrantly folded proteins and aide in the refolding or clearance of these proteins. Amyloidogenic yeast proteins configure into self-perpetuating conformations and their propagation is facilitated by chaperones. Hsp104p is an AAA ATPase which functions as a disaggregase and is a requirement for prion propagation in vivo. Hsp104p is known to function in the severing of Sup35p aggregates to propagate the [PSI+] prion in the cytosol and facilitate faithful transmission from mother cell to daughter cell. Hsp104 is also required to propagate the yeast prion called RNQ+. Interestingly the over-expression of Hsp104 cures [PSI+] but not [RNQ+]. Recently, we found that a mutation in Hsp104 altered the recognition of RNQ+ prion variants differently. Together this data suggests that Hsp104p recognizes misfolded structures differently. Employing variants of the [RNQ+] protein in combination with mutants of Hsp104p, the requirements for chaperone recognition and processing are being elucidated in vitro to determine the biochemical properties required for handling of different Rnq prion variants by Hsp104.

HSP70 ACTIVITY IS REDUCED BY CA^{2+}/CALMODULIN

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Hsp70 plays a role as a molecular chaperone such as inhibition of protein denaturation (holdase), promotion of protein folding and renaturation of denatured proteins (foldase). Orchardgrass Hsp70 possesses a well conserved ATP binding site, Hsp70 domain and signature of cytosolic Hsp70 (EEVD motif), and plays as an ATPase, holdase and foldase in a dose-dependently. We also identified that Hsp70 binds to Arabidopsis calmodulin-2 (ACaM2) in a presence of Ca^{2+} via a conserved CaM binding domain on CaM-overlay assay. Hsp70 binding to Ca^{2+}/CaM reduced intrinsic Hsp70 ATPase activity, but its activity is not changed by increasing the concentration of ACaM2. In addition, Ca^{2+}/CaM binding decreased Hsp70 foldase activity. Based on the protein structure of bovine Hsc70 which is a highest structural homolog of orchardgrass Hsp70, a CaM binding domain is located near the ATP binding site. Therefore, Ca^{2+}/CaM binds to Hsp70 in a 1 to 1 molar ratio, CaM covers the ATP binding pocket of Hsp70, and thus reduces Hsp70 chaperone activity through blocking of ATP hydrolysis.

CELASTROL INHIBITS HSP90 CHAPERONING OF STEROID RECEPTORS BY INDUCING FIBRILIZATION OF THE CO-CHAPERONE P23

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Hsp90 is an ATP-dependent molecular chaperone. The best characterized inhibitors of Hsp90 target its ATP binding pocket, causing non-selective degradation of Hsp90's client proteins. We found that the small molecule celastrol inhibits the Hsp90 chaperoning machinery by inactivating the co-chaperone p23, resulting in a more selective destabilization of steroid receptors compared to kinase clients. Our in vitro and in vivo results demonstrate that celastrol disrupts p23 function by altering its three-dimensional structure, leading to rapid formation of amyloid-like fibrils. This study reveals a unique inhibition mechanism of p23 by a small molecule that could be exploited in the dissection of protein fibrilization processes as well as the therapeutics of steroid receptor-dependent diseases.

SYSTEMATIC ANALYSIS OF FACTORS UNDERLYING THE REFOLDING ACTIVITY OF DNAK

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DnaK is a member of the ubiquitous Hsp70 family of molecular chaperones and it plays important roles in prokaryotic proteostasis. Like other Hsp70s, it consists of a nucleotide-binding domain (NBD), which hydrolyzes ATP, and a substrate-binding domain (SBD), which binds unfolded polypeptides. Nucleotide hydrolysis by the NBD controls substrate binding in the SBD and this important regulatory step is further tuned by the associated co-chaperones DnaJ and GrpE. In this work, we generated ~ 31 mutations in the NBD to identify residues that impact allosteric communication in the DnaK-DnaJ-GrpE system. Of particular importance, we sought to understand how the various factors influencing ATPase activity controlled the folding of a model substrate, firefly luciferase. In vitro, many of the DnaK mutants exhibited altered ATPase and luciferase folding activities and their abilities to be stimulated by co-chaperones were changed. Using this unique series of mutants, we found a positive correlation between ATPase rate and luciferase refolding activity, which has been predicted by previous studies. Moreover, the Vmax of stimulation by DnaJ was positively correlated with refolding efficiency. Conversely, the stimulation by GrpE or substrate was less predictive of refolding activity, while the kcat for DnaJ stimulation was inversely correlated. In addition to these general trends, a number of DnaK mutants showed unique properties. For example, mutants were identified that, remarkably, completely blocked ATP hydrolysis but permitted refolding activity. To understand the structural basis of these 'outlier' mutants, we explored their ability to dimerize and studied their structure by partial proteolysis. These studies revealed that several mutants formed stable dimers and were highly susceptible to trypsin digestion, compared to the wild type DnaK. Together, these findings suggested that either the oligomerization or increased structural flexibility facilitated their unique properties. Currently we are working to determine whether these mutations impact the heat shock response or lambda phage replication in vivo. These studies are beginning to reveal aspects of allosteric control in the DnaK system and they support a model of complex, dynamic interactions in the chaperone complex.

GENETIC INTERACTIONS BETWEEN RIBOSOME-BOUND FACTORS AND COMPONENTS OF MRNA DECAY PATHWAYS

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When newly synthesized polypeptides leave the ribosome through the ribosomal exit tunnel, they encounter a set of ribosome-bound factors that interact with the nascent polypeptide. In Saccharomyces cerevisiae, the ribosome-associated complex (RAC) consisting of the Hsp70 homologue Ssz1p and the Hsp40 homologue Zuotin together with the Hsp70 homologue Ssb1/2p forms a functional chaperone triade. However, despite the fact that RAC/Ssb is a chaperone complex, no folding substrates of RAC/Ssb have been identified so far. The precise function of RAC/Ssb on the ribosome therefore is still largely unknown. Besides RAC/Ssb, the nascent chain-associated complex (NAC) also makes contact to the emerging polypeptide. Yet, like for RAC/Ssb the role of NAC at the ribosomal exit remains elusive.

As RAC/Ssb and NAC are among the first factors to encounter newly synthesized polypeptides, these complexes would be ideally suited to act at the interface of protein synthesis, protein folding, recognition of faulty proteins, and co-translational degradation. Different scenarios are conceivable in which co-translational degradation of proteins becomes necessary, e.g. readthrough of stop codons or translation of mRNAs such as nonsense- or nonstop-mRNAs. For the degradation of aberrant mRNAs different degradation pathways are known. However, little is known about the fate of the resulting proteins. Ribosome-bound factors like RAC/Ssb and NAC are good candidates to be involved in these processes. For this reason we started to analyse the genetic interaction of different mRNA decay factors with RAC/Ssb and NAC. Indeed, initial results point into the direction that ribosome associated protein biogenesis factors can influence the stability of proteins deriving from such mRNAs.

A HIGH-THROUGHPUT FLUORESCENCE-BASED ASSAY FOR SCREENING INHIBITORS OF PROTEIN DISULFIDE ISOMERASE ACTIVITY

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Protein disulfide isomerase (PDI) is a widely expressed enzyme, broadly distributed in eukaryotic tissues. PDI is relatively abundant, being found in the lumen of the endoplasmic reticulum (>400 μM), where it catalyzes the formation and rearrangement of disulfide bonds of secreted proteins. PDI is also known to be secreted from a variety of cell types. In addition to serving as a redox catalyst and isomerase, PDI-mediated reductive cleavage of disulfide bonds at the cell surface is critical to the entry and subsequent infectivity of a number of disease-causing agents, including human immunodeficiency virus (HIV), diphtheria toxin and *Chlamydia trachomatis*. PDI inhibitors, through blocking reductive cleavage of disulfide bonds associated with these pathogens, can prevent infectivity. A homogenous fluorescence-based assay was devised based upon PDI-catalyzed reduction of insulin in the presence of dithiothreitol, resulting in the formation of insulin aggregates which bind avidly to a novel red-emitting fluorogenic dye. Relative to analogous turbidometric assays of PDI activity, the fluorescence-based assay provides a vastly improved assay signal window, improved lower detection limit, and superior Z'-score (>0.8). Intra-plate and inter-plate CVs using the assay are typically 3-4%. Concentration-response plots were employed to determine the effects of bacitracin on PDI activity. These experiments were performed at constant enzyme and substrate concentrations while systematically varying bacitracin concentration. The IC50 of the PDI inhibitor was determined to be 309 ± 27 μM. The described assay has been specifically developed for use on a fluorescence microplate reader and can potentially be applied to high-throughput screening of PDI inhibitors from chemical libraries.

THE YEAST NOT4 E3 LIGASE CONTRIBUTES TO PROTEASOME ASSEMBLY THROUGH REGULATION OF RP CHAPERONES

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The S. cerevisiae Not4 RING finger E3 ubiquitin ligase is a subunit of the conserved Ccr4-Not complex, which plays a role in diverse cellular processes including transcription and RNA degradation. The first substrate identified for this E3 is a chaperone, namely the nascent-associated-polypeptide complex or NAC, termed EGD in yeast. Our earlier studies show that ubiquitination of the EGD complex by Not4 is important for its association with the ribosome and the proteasome. This finding has suggested that Not4 may play a role in the quality control of nascent peptides. Recently we determined that Not4 is important for ubiquitin turnover, and for structural and functional integrity of the proteasome. Indeed, the deletion of Not4 leads to accumulation of poly-ubiquitinated proteins and reduced free ubiquitin, and consistently is synthetically lethal with the deletion of the Ubp6 or Doa4 deubiquitinating enzymes, which also lead to reduction of free ubiquitin. Furthermore, purification of the proteasome in the absence of Not4 reveals functional and salt-resistant interaction between regulatory particle (RP) and core particle (CP), instability of free RP, and reduced RP in higher order complexes lacking CP. The RING domain of Not4, not essential for Not4 association with the Ccr4-Not complex, is critical for appropriate control of proteasome assembly. However, association of Not4 within the Ccr4-Not complex also contributes to proteasome integrity. Our most recent results suggest that Not4 is important for functional integrity of the proteasome through its importance for the appropriate balance of proteasome chaperones.

PI3K-MTOR REDUCES STRESS RESISTANCE BY ATTENUATING HSP70 TRANSLATION

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Protein translation is a key regulated cellular process that links nutrients with diverse biological processes. It has been known that some cellular proteins continue to be synthesized under conditions where global translation is severely compromised. Under stress conditions such as starvation or heat shock, general translation is inhibited, yet some mRNAs are induced as a stress response. One prominent example is the selective translation of heat shock proteins (HSP). Though the transcriptional regulation of HSP genes have been well established, no precise mechanism has been demonstrated to explain how these up-regulated mRNAs are efficiently translated in mammals.

Here we show that the stress-induced preferential translation of Hsp70 mRNA is negatively regulated by PI3K-mTOR signaling. This pathway plays a pivotal role as a key sensor of the cellular environment through regulation from nutrients, energy and stress conditions. The chaperone-mediated remodeling of mTOR and the pathway's regulation is clearly affected under multiple stress conditions. Our results show that the translation of *Hsp70* mRNA after heat shock is severely suppressed in cells lacking tuberous sclerosis complex 2 (TSC2). It seems that the hyperactive mTOR signaling in *TSC2*-/- cells disrupts the bi-functional switch between cap-dependent and a cap-independent translational mechanism. By further reducing the PI3K-mTOR signaling by siRNA-mediated knockdown or pharmacological inhibitors, HSP70 translation is further enhanced as well as stress resistance overall.

This study may have critical implications for the pathologies associated with mTOR dysregulation. Unrestrained mTOR activity in mammals is associated with the occurrence of disease states including inflammation, cancer and diabetes. By contrast, decreased mTOR signaling by genetic or pharmaceutical approaches (e.g. rapamycin) has been shown to extend lifespan in a variety of organisms. Interestingly, a robust stress response is required for life span extension in these organisms. Our ongoing research may provide a plausible mechanism for the molecular connection between nutrient signaling and the cellular stress response.

DIVERSITY OF HSP-CHAPERONES IN THE EARLY EUKARYOTE *PERKINSUS MARINUS*

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Study of Hsp-chaperones (Hsp) in the last few years revealed a multitude of roles for these molecules in physiology and pathology, expanding considerably the field of chaperonology. For example, Hsps were detected in the extracellular space and in circulation, and found to interact with immune system cells. These findings opened a new perspective for understanding autoimmunity and inflammation. Likewise, the realization that Hsps can cause or aggravate disease lead to the identification of the chaperonopathies as a new group of pathological conditions, prompting new strategies for studying pathogenetic mechanisms and developing therapeutic means. It has also become clear that understanding the chaperoning systems of primitive organisms can provide clues for elucidating the function of Hsps in higher eukaryotes, including human. Here, we report the results of our studies on the chaperoning system of the protistan *Perkinsus marinus* (Pm), which diverged early from an ancient lineage leading to the Dinozoa. Pm, a facultative intracellular a parasite of the oyster *Crassostrea virginica*, has adapted to withstand stress from the host's defenses as suggested also by our findings. We have searched the Pm genome, using human sequences as queries and the chaperonomics protocol described earlier, and found about 60 genes encompassing virtually all the main chaperoning families: Hsp90, Hsp70, Hsp60, CCT, Hsp40, Hsp10, sHsp, prefoldin, mitochondrial chaperones, Hip, Hop, and NAC. Iterative searches with the Pm sequences as queries revealed other related gene-candidates for future analysis, e.g., candidates related to Hsp90, 3; Hsp70, 12; Hsp60, 5; Hsp10, 2; mitochondrial Hsps, 2; prefoldin, 5. In addition, several gene-candidates related to Hsp40 are now under study. Initial phylogenetic analysis of the Hsp70 proteins showed that the newly identified gene-candidates cluster with the Pm Hsp70 genes previously found. Other chaperone genes were also found, e.g., co-chaperone hscB, tubulin-specific chaperone B, chaperone-binding protein, GrpE, and a cytochrome c oxidase copper chaperone. Many of the genes have ESTs. Additional chaperonomics analyses are under way to define more precisely the Pm Hsp genes, which will be followed by experimental testing to identify genes that are induced by stressors typically affecting Pm, such as changes in temperature, salinity, and iron concentration; and exposure to host factors (oyster plasma and hemocyte extracts). The data thus far reveal a diversity and complexity of Hsp-chaperone families that is remarkable, considering that Pm is a very ancient unicellular organism.

CHAPERONES IN DISEASE: QUANTITATIVE CHANGES IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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The chaperonopathies include inflammatory diseases among which COPD is one of the most important due to its frequency and impact on human health and economy. Previously, we showed that levels of the chaperones Hsp60 and Hsp10 in human airway epithelium declined from normal through metaplasia to dysplasia/cancer. In contrast, we found in COPD patients a positive correlation between increased Hsp60 and Hsp10 levels in epithelium and disease severity, but these levels showed no correlation with FEV1 values or cigarette-pack/year consumption. To clarify this apparent discrepancy and to unveil the role of Hsp-chaperones in COPD, we measured Hsp10,27,40,60,70,90 and Hsp transcription factor HSF-1 in four groups of bronchial biopsies from patients with mild-moderate or severe COPD, all smokers, in comparison to smoker and non-smoker, age-matched, healthy controls. We also correlated Hsp levels with neutrophil and activated neutrophil numbers in lamina propria (a hallmark of COPD severity). We used immunohistochemistry and RT-PCR and applied the pertinent non parametric statistical tests (Kruskal-Wallis and Mann-Whitney U) to evaluate results and compare groups. In the bronchial epithelium and lamina propria Hsp10,40, and 60 proteins were significantly increased in the severe COPD group compared to control smokers and non-smokers but no significant differences were observed for Hsp27,70,90, and HSF-1 levels between the four groups. Levels of HSF-1 mRNA were significantly higher in the severe COPD group compared to control smokers while Hsp10,40 and 60 mRNA levels were not changed in the four groups, suggesting a prevalent post-transcriptional regulation of Hsp proteins production. In all smokers the number of immunostained Hsp60 positive cells in the lamina propria significantly correlated with numbers of neutrophils and activated neutrophils expressing myeloperoxidase, suggesting a link between Hsp60 and the oxidative/nitrosative stress mediated by the activated neutrophils typically populating the bronchial mucosa in COPD. This correlation was also true for all COPD patients. No other correlations were found between inflammatory cells and Hsp levels or any other standard clinical or pathological parameter. Our results show for the first time that Hsp-chaperones are involved in COPD and open the road to investigate their role in pathogenesis (e.g., by initiating-perpetuating inflammation and/or airway remodeling) and to develop therapeutic means in which chaperones would be central players, either as targets for antichaperone compounds or as cytoprotective agents (chaperonotherapy), depending on whether the Hsp-chaperones play pro- or anti-pathogenetic roles.

HSP10 BEYOND MITOCHONDRIA: NOVEL LOCATIONS PREDICT AS YET UNDESCRIBED ROLES

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The impact of cigarette smoke stress (a form of oxidative stress, OS) on human lung fibroblasts and epithelial cells, particularly its effect on mitochondrial chaperonins expression, has not been extensively characterized despite the growing importance of these molecules in various pulmonary diseases. We studied the effects of non-lethal doses of cigarette smoke extract (CSE) on the expression of Hsp60 and Hsp10 in human lung fibroblasts (HFL-1 line) and epithelial cells (16HBE line). We focused on the identification of mitochondrial chaperonins in extra-mitochondrial sites, such as cytosol and nucleus, using subcellular fractionation analyses (SFA), western bloting (WB), and immunocytochemistry (ICC). Database searches and chaperonomics were carried out to identify and compare the proteins and genes of interest, and bioinformatics was applied to gather "in silico" structural data useful for interpreting and complementing the "in vitro" results. ICC and SFA showed differences in the subcellular localization of the two chaperonins in both cell lines: Hsp60 was localized preferentially to mitochondria in contrast to Hsp10, which was found in the cytosol at high levels, and in the nucleus. WB with nuclear fractions confirmed the presence of Hsp10 in the nucleus of both cell lines. Experiments showed that exposure to CSE caused variations in the levels of nuclear Hsp10, suggesting a link between exposure to exogenous OS and a cell response, which likely involves as yet undescribed Hsp10 roles distinct from the its typical intramitochondrial functions. Comprehensive databases and literature searches revealed that Hsp10 can display a complex array of functions related to its three different locations: cytoplasmatic, mitochondrial, and extracellular. Extracellular Hsp10 has been described as EPF (Early Pregnancy Factor) because it appears to be a key marker and functional factor during the establishment of pregnancy. Here, we provide data showing for the first time the presence of Hsp10 in the nuclear compartment of both epithelial and stromal human lung cell lines, and indicating that intranuclear Hsp10 levels are affected by OS due to an exogenous stressor like cigarette-smoke. These findings open new paths toward elucidation of the roles of intranuclear Hsp10 in the stress response. Bioinformatics and structural analyses also provided support to the notion that Hsp10 is not restricted to mitochondria and can localize elsewhere, e.g., the nucleus, even if the typical Hsp10 lacks DNA-binding motifs or nuclear import signals. The questions now are by what mechanism Hsp10 becomes a resident of the nucleus and what are its functions there.

A DISTINCT CLASS OF NUCLEAR RECEPTOR ALTERNATE SITE MODULATORS (NRAMS) THAT TARGET A NOVEL ANDROGEN RECEPTOR REGULATORY MECHANISM INVOLVING FKBP52 AND B-CATENIN

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Drugs that target novel surfaces on the androgen receptor and/or novel AR regulatory mechanisms are promising alternatives for the treatment of hormone refractory prostate cancer (HRPC). The 52 kDa FK506 Binding Protein (FKBP52) has been shown to be an important positive regulator of AR in cellular and whole animal models and represents an attractive target for the treatment of prostate cancer. We have identified a surface region on the androgen receptor (AR) hormone binding domain that, when mutated, displays a greater dependence on FKBP52 for normal function. Interestingly, the BF3 surface is also hypothesized to participate in the interaction of AR with the co-activator β-catenin, and co-expression of FKBP52 and β-catenin act in synergy to up-regulate both hormone-dependent and hormone-independent AR function. In addition, we have developed a series of small molecules that effectively inhibit the FKBP52 regulation of AR function and the synergistic up-regulation of AR function by FKBP52 and β-catenin. Surface plasmon resonance studies have confirmed that these inhibitors disrupt the regulation of AR by FKBP52 and β-catenin through interaction with the AR hormone binding domain. We have demonstrated that these novel compounds do not compete with hormone for binding the hormone binding pocket, nor do they compete with coactivator peptide for binding AF2. In addition, we have shown that these compounds inhibit AR function by preventing hormone-dependent dissociation of the Hsp90-FKBP52-AR complex which results in less hormone-bound receptor in the nucleus. Preliminary assays in early and late stage prostate cancer cells have demonstrated our novel compounds inhibit both prostate specific antigen expression and androgen-dependent proliferation. In summary, we have identified a putative FKBP52 interaction surface on the AR hormone binding domain and identified a series of small molecules that inhibit AR function by targeting that surface. This class of compounds, now termed NR alternate site modulators (NRAMs), would be useful in hormone-resistant prostate cancer, where coregulator overexpression and other mechanisms result in receptor activation in the absence of exogenous ligands. In addition, we have identified a novel functional interaction between FKBP52 and β-catenin that has clear implications in prostate cancer progression to the hormone refractory state.

UNFOLDING OF THE METASTABLE LINKER REGION AT THE CORE OF HSP33'S ACTIVATION AS A CHAPERONE

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An increasing number of redox-regulated proteins have been identified that use reactive oxygen species (ROS) as posttranslational modulators of their activity. One redox-regulated protein is the highly conserved, general chaperone Hsp33. Hsp33 is specifically activated under oxidative stress conditions that lead to protein unfolding (*i.e.*, hypochlorous acid stress) and protects bacterial cells against oxidative protein aggregation. Stress sensing in Hsp33 occurs via its C-terminal redox switch domain, which consists of a zinc center that responds to the presence of oxidants, and an adjacent metastable linker region that responds to unfolding conditions. Both regions are compactly folded in inactive Hsp33 and undergo substantial unfolding upon activation. This mechanism of activation makes Hsp33 a member of a growing group of chaperones, which are functional in a partially natively unfolded state. Here we show that single mutations in Hsp33's N-terminus are sufficient to either partially (Hsp33-M172S) or completely (Hsp33-Y12E) abolish the posttranslational regulation of Hsp33's chaperone function. Both mutations appear to work predominantly via the destabilization of Hsp33's linker region without affecting zinc coordination, redox sensitivity, or substrate binding of Hsp33. We found that the M172S substitution causes moderate destabilization of Hsp33's linker region, which seems sufficient to convert the redox-regulated Hsp33 into a temperature-controlled chaperone. The Y12E mutation leads to the constitutive unfolding of Hsp33's linker region thereby turning Hsp33 into a constitutively active chaperone. Our results demonstrate that the unfolding of Hsp33's linker region plays the central role in Hsp33's activation process while the zinc center acts as redox-sensitive toggle that adjusts the thermostability of the linker region to the redox status of the cell. These results further imply that stress conditions that act mainly on destabilizing Hsp33's linker without affecting the redox status of the zinc center, might be sufficient to activate Hsp33. This conclusion would not only explain recent findings, which showed that Hsp33 expression significantly increases bacterial resistance towards oxidative stress-independent stresses, but would entail that Hsp33 might be a much more versatile chaperone than previously anticipated. *In vivo* studies confirmed that even mild overexpression of the constitutively active Hsp33 mutant inhibits bacterial growth, providing important evidence that the tight functional regulation of Hsp33's chaperone activity plays a vital role in bacterial survival.

REGULATION OF THE HEAT SHOCK RESPONSE BY TISSUE-SPECIFIC NETWORKS IN *CAENORHABDITIS ELEGANS*

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The heat shock response (HSR) is orchestrated by inducible activators such as HSF-1 and feedback inhibitors including molecular chaperones such as Hsp70. The regulation of the HSR has been extensively investigated at the molecular level; however, much less is understood at the cellular and organismal level. To address this, we have employed genome-wide RNAi screens in *C.elegans* to identify new regulatory pathways for the HSR essential for its activation and repression. We have identified 13 genes that when knocked-down, block activation of the HSR reporter (*C12C8.1p::GFP* (*hsp70*)), and 55 genes that induce the reporter in the absence of stress in a tissue-specific manner. Included among these hits were *hsf1*, *hsp1* (*hsp70*), and *daf21* (*hsp90*). All 55 of the negative regulators induce the HS reporter in an HSF-1 dependent manner and nearly all induce another HS reporter, *hsp16.2*. The identified regulators are enriched in self-interactions and cluster into distinct networks that correlate with the tissue-specific reporter induction. To better understand the structure and function of the underlying regulation we performed an epistasis analysis, revealing complexity of the genetic pathways involved in the regulation of the HSR in metazoan organism.

MIMICKING CHAPERONE FUNCTION: PROTEIN FOLDING IN ISOLATION

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One of the key features of molecular chaperones is their ability to bind nonnative proteins and thus suppress their unspecific aggregation. To understand the mechanistic features of this process we set up a minimal system mimicking this function by immobilizing the test proteins via a polyhistidine-tag on a matrix. This allows retaining their flexibility to regain their native state. Additionally, the polypeptide chains are spatially separated, which decreases unfavorable interactions.

We compared the folding properties of isolated, matrix-attached proteins to those in solution and find striking differences in the folding and association kinetics. Interestingly, compared to folding in-solution, the matrix-assisted folding was more efficient for different proteins and seems to be comparable with chaperone-assisted protein folding.

FOLDING AND AGGREGATION OF THE INTRINSICALLY DISORDERED TAU PROTEIN ARE SELECTIVELY REGULATED BY CHAPERONES

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In many diseases of aging, aberrant protein production is a common feature. Interestingly, the majority of these proteins are clients of the chaperone network. Many disease-associated clients that accumulate with age have intrinsically unstructured hydrophilic regions, such as the microtubule associated protein tau; however other disease-associated clients are the more typical globular proteins with a hydrophobic core such as the kinase Akt. Our recent work on tau and Akt suggests that while both types of proteins can interact with the chaperone network and be stabilized or degraded they may be processed through the chaperone pathway in different directions. One primary question that has emerged from these studies is why intrinsically unstructured proteins are clients of the chaperone machinery at all. We speculate that it is this akward interaction with the chaperones that may accidentally lead to the misfolding and production of toxic aggregated intermediates of these disordered proteins that ultimately result in neuronal death. We now have evidence that an altered tau conformation known as MC1, which is thought to initiate the progression of Alzheimer's disease pathology, can be facilitated by molecular chaperones. This conformation is formed when the N-terminus of tau folds back on itself and interacts with the microtubule-binding domain. Thus, chaperones seem to be facilitating misfolding of tau. Moreover, we find that chaperones prevent heparin-induced tau fibrillarization, but produce other distinct amorphous aggregates. However, despite these seemingly deleterious biochemical effects, we have found that manipulating the activity of these chaperones with small molecules or by applying them exogenously to the brains of transgenic tau mice can improve neuronal function. Thus, manipulation of chaperones can have a beneficial role in models of neurodegenerative disease. We also show that chaperones do participate in the pathogenesis of disordered proteins, particularly tau, which pathologically accumulates in ~70% of all neurodegenerative diseases.

MODULATING AMYLOIDS BY CHEMICAL DISAGGREGASES

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Protein misfolding is associated with several devastating neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and Huntington's disease (HD). In many cases, protein misfolding is closely linked to the formation of amyloids, which are characterized by a cross-beta structure and an unusual stability. Using the yeast prion [PSI+], we investigate the mechanisms, by which small molecules modulate amyloid formation and prion stability. We identified that the small molecule epigallocatechin gallate (EGCG) blocked prion formation, eliminated pre-existing prions and solubilized amyloid proteins by disrupting intra- and inter molecular contacts. Thus, EGCG remodels amyloids in a manner reminiscent of molecular chaperones or protein remodeling factors. Notably, we also found that EGCG transforms one amyloid conformation into a different one. Moreover, we described how combining two small molecules, that antagonize prion formation by distinct mechanisms, synergized to directly inhibit and reverse the formation of diverse prion strains.

A NOVEL *HSP104* MUTANT PROPAGATES CRYPTIC [*PSI*+]

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The AAA+ ATPase Hsp104p is required for the efficient propagation of yeast prions in vivo as well as the recovery of yeast from severe heat stress. Hsp104p has been proposed to disaggregate large aggregates and resolubilize aggregated and misfolded proteins by threading polypeptides through its central pore, but the mechanism of substrate recognition is largely unknown. We have identified and characterized a novel Hsp104 mutant whose defect is specific to the [*PSI*+] prion. Yeast with this mutant appear [*psi*-] phenotypically and harbor no detectable Sup35 aggregates as assessed by a variety of biochemical assays. Interestingly however, this mutant propagates a cryptic form of [*PSI*+], as the [*PSI*+] phenotype appears in progeny from a cross to [*psi*-] cells expressing wild type Hsp104. Furthermore, this mutant dominantly cures a weak [*PSI*+] variant and inhibits [*PSI*+] induction when Sup35 is overexpressed. On the other hand, cells harboring this mutant *hsp104* allele can recognize most substrates, as it retains full functionality in thermotolerance. In addition, this mutant does not generally inhibit propagation of prion and prion-like aggregates, as both the [*RNQ*+] prion and non-toxic polyQ103-GFP maintain aggregates. Due to its ability to process other known substrates normally, we hypothesize that this mutant is defective specifically in the recognition and processing of [*PSI*+] aggregates. This defect is due specifically to its interaction with Sup35 in the [*PSI*+] state as the mutant cannot be rescued by the overexpression of its chaperone co-factors and is reduced in its ability to functionally interact with the prion-forming domain of Sup35 in vitro. We propose that this defect stems from its decreased ATPase activity and suggest that various levels of activity are required to resolve different substrates. This may also explain why the overexpression of Hsp104 cures only [*PSI*+] among the well-characterized yeast prions. We are using this mutant to elucidate the role of Hsp104p in the propagation of variants of [*PSI*+] and investigate the mechanism of substrate recognition.

CHAPERONE ASSISTED DEGRADATION OF MISFOLDED CYTOPLASMIC PROTEIN REQUIRES THE UBIQUITIN LIGASE UBR1

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Protein quality control and subsequent elimination of terminally misfolded proteins occurs via the ubiquitin proteasome system. Tagging of misfolded proteins with ubiquitin for degradation depends on a cascade of reactions involving an ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin ligases (E3). While ubiquitin ligases responsible for targeting misfolded secretory proteins to proteasomal degradation (ERAD) have been uncovered and characterized, little is known about E3 enzymes responsible for elimination of misfolded cytoplasmic proteins in yeast.
For finding novel factors in the protein quality control in the cytoplasm we have studied requirements for degradation of ER import defective mutated derivatives of carboxypeptidase yscY (ΔssCPY*, ΔssCPY*-GFP, ΔssCPY*Leu2myc). Previously we were able to show that for degradation of such mislocalized proteins the E2s Ubc4 and Ubc5, the cytoplasmic Hsp70 Ssa chaperone machinery and the co-chaperone Ydj1 are needed. Now we were able to show that for this chaperone-assisted degradation of a misfolded cytoplasmic protein the E3 ligase of the N-end rule pathway Ubr1 is required. Presently we are characterizing the binding properties of Ubr1 to the misfolded proteins, which seem to be independent of known binding regions within the enzyme.

A PROTEIN'S FOLDING STATE CAN BE DERIVED FROM ITS PRIMARY SEQUENCE USING A SIMPLE METHOD FOR COMPUTING OPTIMAL BACKBONE BURIAL.

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The hydrophobic effect is known to be a major determinant of protein tertiary structure. I have developed a simple, quantitative method for predicting optimal backbone burial in protein structures from patterns of sequence hydrophobicity. The optimum generated by the method matches well with the crystal structures of many proteins. By analyzing the sequences and structures of proteins such as myoglobin, UBC9, and VHL, I show that this method allows one to determine whether a primary sequence is that of a folded protein, a temperature-sensitive mutant, or a natively unstructured protein.

THE MOLECULAR MECHANISM OF IGG ANTIBODY QUALITY CONTROL

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Prerequisite for antibody secretion and function is the assembly into a defined quaternary structure, composed of two heavy and two light chains in the case of IgG. Unassembled heavy chains are actively retained in the endoplasmic reticulum (ER) *via* the interaction of the molecular chaperone BiP with the antibody C_H1 domain until they associate with light chains. Our mechanistic analysis of this critical quality control step revealed that, unlike all other antibody domains studied, the C_H1 domain of the heavy chain is an intrinsically disordered protein in isolation. It adopts the typical immunoglobulin fold only upon interaction with its native partner, the C_L domain. Structure formation proceeds *via* a trapped intermediate and can be accelerated by the ER-specific peptidyl-prolyl isomerase cyclophilin B. The molecular chaperone BiP modulates C_H1/C_L association by recognizing incompletely folded states of the C_H1 domain within the assembly pathway and directly competes for C_L binding to C_H1. *In vivo* experiments demonstrate that requirements identified for folding the C_H1 domain *in vitro*, including association with a folded C_L domain and isomerization of a conserved proline residue, are essential for antibody assembly and secretion in the cell. Our data provide an explanation for the high fidelity of antibody quality control and extend the view on chaperone controlled protein assembly mediated by intrinsic structural features of the client proteins.

SELF-ASSOCIATION OF UNFOLDED OUTER MEMBRANE PROTEINS AND ITS PREVENTION BY CHAPERONES

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We have investigated the self-association propensities of aqueous unfolded (U_{AQ}) forms of eight outer membrane proteins, OmpA, OmpW, OmpX, PagP, OmpT, OmpLa, FadL and Omp85. We found that high urea concentrations maintain all of these OMPs in monomeric forms and that OmpA and OmpX remain monomeric upon dilution to 1M urea. A pH screen showed that basic pH supports the least amount of U_{AQ} OMP self-association, consistent with earlier studies showing that basic pH was optimal for better folding efficiencies. The addition of KCl increased in U_{AQ} OMP self-association, although the magnitudes of the responses were varied. Urea can be used to tune the amount of U_{AQ} OMP self-association and our results show that low urea concentrations may be useful in optimizing folding conditions. Ongoing work with periplasmic chaperones investigates their abilities to prevent U_{AQ} OMP self-association and defines one of their functional roles.

THE INTERPLAY BETWEEN DEGRADATION AND REPAIR MACHINERY IN NUCLEAR PROTEIN QUALITY CONTROL

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The management of misfolded proteins is a key aspect of cellular physiology. Cells utilize compartment specific protein quality control (PQC) systems that function to repair or degrade misfolded proteins. Eukaryotic PQC systems have been characterized in many cellular compartments including the endoplasmic reticulum, cytoplasm, mitochondria, and nucleus. In the ER and cytoplasm, both protein synthesis compartments, there is strong interplay between the repair and destruction PQC pathways. However, little is understood about the interplay between destruction and repair machinery in a compartment that isn't directly involved protein biosynthesis like the nucleus. The budding yeast Saccharomyces cerevisiae is an ideal organism to study the interaction between the repair and degradation pathways in the nucleus because multiple chaperone-mediated repair pathways have been characterized in the yeast nucleus and the first nuclear PQC degradation system, mediated by the ubiquitin ligase San1, was discovered in yeast. Unlike the ER and cytoplasm, we found that San1 does not utilize protein chaperones for substrate recognition, but instead recognizes its substrates through a unique flexible interface. In the process of examining the genetic interactions between San1 and chaperones with known nuclear localization (such as Hsp70, Hsp90, Hsp104, Hsp26, and Hsp42), we did find that Hsp90 and San1 have a synthetic genetic interaction that is exacerbated by expression of toxic, aggregation-prone, misfolded nuclear proteins. Furthermore, by examining the degradation of San1 substrates in the absence of Hsp90 function, we found that Hsp90 may be negatively regulating San1-mediated degradation, and this may be the result of competition between San1 and Hsp90 for misfolded protein substrates in the nucleus. This is the first example where the repair and degradation arms of PQC compete rather than collaborate.

DENATURED PROTEINS FACILITATE THE FORMATION OF THE FOOTBALL-SHAPED GROEL-GROES COMPLEX

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Controversy exists over whether GroEL forms a GroEL-$(GroES)_2$ complex (football-shaped complex) during its reaction cycle. We have previously revealed the existence of the football-shaped complex in the chaperonin reaction cycle using a fluorescence resonance energy transfer (FRET) assay (Sameshima, T., Ueno, T., Iizuka, R., Ishii, N., Terada, N., Okabe, K. and Funatsu, T. (2008) *J. Biol. Chem.* 283, 23765-23773). Although denatured proteins alter the ATPase activity of GroEL and the dynamics of the GroEL-GroES interaction, the effect of denatured proteins on the formation of the football-shaped complex has not been characterized. In this study, a FRET assay was used to demonstrate that denatured proteins facilitate the formation of the football-shaped complex. The presence of denatured proteins was also found to increase the association rate of GroES to the trans-ring of GroEL. Furthermore, denatured proteins decrease the inhibitory influence of ADP on ATP-induced association of GroES to the *trans*-ring of GroEL. From these findings it is concluded that denatured proteins facilitate the dissociation of ADP from the *trans*-ring of GroEL and the concomitant association of ATP and the second GroES.

ANALYSIS OF TERNARY HSP90•COFACTOR COMPLEXES BY ANALYTICAL ULTRACENTRIFUGATION

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Hsp90 is a highly conserved ATP-driven molecular machine that is required for the activation and stabilization of a variety of client proteins. Substrates are involved in various cellular processes, ranging from steroid hormone receptors over transcription factors to an ever growing number of protein kinases. In order to activate substrates, Hsp90 has to hydrolyze ATP in a cyclic reaction and the hydrolysis energy is supposed to be transmitted to the substrate proteins via conformational changes in the Hsp90 dimer. The ATPase cycle is fine-tuned by co-factors which support or suppress hydrolysis, thereby accelerating or slowing down the turnover of the chaperone. As such, Hop/Sti1 was found to interact through its TPR domain with the very C-terminal MEEVD motif and with additional binding sites in the N- and M-domain of Hsp90. Interaction inhibits the N-terminal dimerization and prevents ATP hydrolysis. Other cochaperones have been described to inhibit the ATPase activity as well. Cdc37 was found to prevent N-terminal dimerization by inserting between the N-domains whereas p23/sba1 binds to an ATP-bound dimerized state, thereby decelerating hydrolysis. In contrast, Aha1 which binds to the N- and M-domains is the only known activator so far. For growth factor maturation, the assembly of multi-protein complexes have been described. Nevertheless, the mechanistic details of cofactor assembly on the Hsp90 scaffold leading to substrate activation remain elusive to date. Here, we use analytical ultracentrifugation to analyze the ability of Hsp90 cofactors to simultaneously form ternary complexes on the Hsp90 scaffold.

CHARACTERIZATION OF A NOVEL TRANSCRIPTION FACTOR INVOLVED IN OXIDATIVE STRESS RESISTANCE OF *ESCHERICHIA COLI*

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Reactive oxygen species arise continuously during aerobic metabolism and are the core of antimicrobial responses. In all cases, reactive oxygen species can damage macromolecules, cause oxidative stress when increasingly accumulated, and harm or kill cells. The extremely bactericidal reactive oxygen species hypochlorous acid (HOCl) is produced by the host immune system and observed in the gut and mucosal barrier epithelia. We use the gram-negative bacterium *Escherichia coli* as a model organism to study how bacteria withstand HOCl-stress. Using a library screen, we identified a novel transcription factor, YjiE, that confers HOCl-resistance. YjiE belongs to the LysR-type family of transcription factors, which typically bind to DNA as dimers or tetramers. We characterized YjiE *in vitro* and *in vivo* in order to understand its regulation. Purified YjiE binds to DNA and forms remarkable large oligomeric species. As shown by analytical ultracentrifugation and transmission electron microscopy, YjiE is a dodecamer in solution and forms hexagonal ring-like structures. These high oligomeric structures are maintained in the presence of low concentrations of HOCl. However in *E. coli* lysates we also observed smaller oligomers (dimer – hexamer). Considering that YjiE is a LysR-type transcription factor, such smaller oligomers are likely the active species. We found that the observed dodecameric structures are extremely stable and can only be dissociated in the presence of arginine, which is known to prevent intermolecular interactions. The smaller oligomers resulting from arginine treatment are currently characterized regarding DNA-binding activity and in *in vitro* transcription assays. Our work suggests that YjiE is a novel HOCl-responsive transcription factor. Further research should lead to a model that describes the regulation of the transcription factor upon exposure to reactive oxygen species.

FUNCTIONAL ANALYSIS OF HTPG, THE *E. COLI* HOMOLOG OF HSP90

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The Hsp90 family represents one of the most abundantly expressed and highly conserved families of molecular chaperones. The eukaryotic Hsp90 is known to control the stability and the activity of more than 200 client proteins, including receptors, protein kinases and transcription factors, and to work in cooperation with several cochaperones. Despite the high degree of homology between the eukaryotic Hsp90 and the prokaryotic homolog, HtpG, the function of HtpG remains elusive. To better understand the role of *E. coli* HtpG we looked for conditions in which HtpG confers a phenotype to the cell. We found that overexpression of HtpG elicits a temperature sensitive phenotype under certain stress conditions. Using this in vivo assay, we screened for HtpG mutants generated by in vitro random mutagenesis. We have purified some of the mutant proteins and are using in vitro assays to test whether these residues are important for the activities of HtpG, including prevention of aggregation of client substrates, ATPase and reactivation of model substrates in conjunction with the DnaK chaperone system of *E. coli*. It is anticipated that this study will lead to a better understanding of the role of HtpG in *E. coli* and more generally to the Hsp90 chaperone family.

UNRAVELING THE ROLE OF THE LON PROTEASE AND THE SECB CHAPERONE IN PRESECRETORY PROTEIN QUALITY-CONTROL

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We have analyzed the role of molecular chaperones and proteases in the quality-control of presecretory proteins in *Escherichia coli*, and shown significant overlaps between the chaperone SecB and the ATP-dependent protease Lon, both in vivo and in vitro. Through genetic suppressor analysis, we have isolated mutations in the lon gene, which specifically alleviated the cold-sensitive phenotype of a *secB* null strain. Further genetic experiments demonstrated that the damaging action of Lon in the absence of SecB relied on its protease activity. In comparison with its respective single mutants, the double *secB lon* mutant strongly accumulated aggregated presecretory proteins at physiological temperature, suggesting that the chaperone and the protease share substrates in vivo. Such a phenomenon was not observed with mutations in the *clpP* or *hslV* protease encoding genes. Reconstitution of the system in vitro revealed that the main SecB-Lon presecretory substrates identified were indeed highly sensitive to specific degradation by Lon, when compared to a cytosolic model protein. The Lon-sensitivity exhibited by SecB substrates was further confirmed by pulse-chase analyses. Finally, in vitro experiments demonstrated that Lon-sensitive presecretory proteins were significantly protected by SecB, thus facilitating their subsequent transfer to the sec translocase at the inner membrane. A model summarizing the role of Lon and the chaperone in preprotein quality-control will be presented.

CONTRIBUTION OF ENVIRONMENT AND GENETIC BACKGROUND TO AGGREGATION TOXICITY.

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Chronic expression of aggregation-prone proteins in conformational diseases results in the accumulation of damaged or misfolded proteins and cellular dysfunction. Because toxicity is modulated by aging, environmental conditions, and genetic background, and only a subset of cells is strongly affected despite the often broad expression of the mutant protein, this dysfunction is dependent on the specific cellular and genetic environment. Our previous studies in *C. elegans* models implicated components of proteostasis networks, such as heat shock factor 1 (HSF1) and molecular chaperones, and life-span regulators, such as DAF-16, in regulation of aggregation toxicity. Furthermore, we found that toxic phenotypes due to the expression of polyQ or mutant SOD1 proteins in muscle or neuronal cells of *C. elegans* can be strongly modulated by the presence in the same cell of metastable proteins, encoded by the endogenous mildly destabilizing temperature-sensitive mutations. We hypothesize that these genetic interactions are mediated by competition for components of the proteostasis networks, leading to the general compromise of the protein folding environment. We are using *C. elegans* models to probe how variation in the genetic background and environmental conditions affects the ability of an individual to respond to proteotoxic challenge and to characterize the pathways that channel the phenotypic expression of aggregation toxicity.

HSP12 IS AN INTRINSICALLY UNFOLDED STRESS PROTEIN WHICH FOLDS UPON MEMBRANE ASSOCIATION AND MODULATES MEMBRANE FUNCTION

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Hsp12 of *S. cerevisiae* is up-regulated several hundred-fold in response to stress. Our phenotypic analysis showed that this protein is important for survival of a variety of stress conditions including high temperature. In the absence of Hsp12, we observed changes in cell morphology under stress conditions. Surprisingly, in the cell, Hsp12 exists both as a soluble cytosolic protein and associated to the plasma membrane. The *in vitro* analysis revealed that Hsp12, unlike all other Hsps studied so far, is completely unfolded, but in the presence of certain lipids, it adopts a helical structure. The presence of Hsp12 does not alter the overall lipid composition of the plasma membrane but affects its organization and increases membrane stability.

COUPLED CHAPERONE ACTION IN THE BIOGENESIS OF FORM I HEXADECAMERIC RUBISCO

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After folding, many proteins must assemble into oligomeric complexes to become biologically active, but little is known about chaperone-mediated assembly reactions. Form I Rubisco, a complex of 8 large (RbcL) and 8 small (RbcS) subunits, catalyzes the fixation of atmospheric CO_2 in photosynthesis. The limited catalytic efficiency of Rubisco has sparked extensive efforts to re-engineer the enzyme with the goal to enhance agricultural productivity. Recently, we described the role of $RbcX_2$ as a specific assembly chaperone of cyanobacterial Rubisco (Saschenbrecker et al, 2007). We have now analyzed the formation of form I Rubisco by in vitro reconstitution and cryo-electron microscopy (Liu et al, 2010). We show that RbcL subunit folding by the GroEL/ES chaperonin is tightly coupled with assembly mediated by the chaperone $RbcX_2$. RbcL monomers remain partially unstable and retain high affinity for GroEL until captured by $RbcX_2$. Cryoelectron microscopic analysis of a trapped $RbcL_8$-$(RbcX_2)_8$ complex at ~9 Å resolution shows that $RbcX_2$ acts as a molecular staple in stabilizing the RbcL subunits as dimers and facilitates $RbcL_8$ core assembly. Specifically, $RbcX_2$ captures the flexible C-terminal segment of one RbcL subunit in its central binding cleft and binds another subunit via its peripheral binding region. A further function of $RbcX_2$ is to stabilize $RbcL_8$ in a conformation close to that in the $RbcL_8S_8$ holoenzyme, competent for RbcS association. Upon binding of RbcS subunits, the $RbcL_8$-$(RbcX_2)_8$ complex undergoes an allosteric structural change that results in the dissociation of $RbcX_2$ and formation of stable Rubisco holoenzyme. The strategies employed by $RbcX_2$ in achieving substrate specificity and efficient product release may be more generally relevant in the formation of complex oligomeric structures when folding is closely coupled to assembly.

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ROLE OF THE UNFOLDED PROTEIN RESPONSE IN INFLAMMATION

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Innate immunity is required for host defense, but dysregulation and prolonged activation can trigger chronic inflammatory diseases such as obesity and atherosclerosis, two leading health concerns in the US. In atherosclerotic lesions as well as in mouse models of obesity, activation of the unfolded protein response (UPR) has been reported. However, precise characterization of the relationship and mechanisms underlying UPR activation and inflammatory gene regulation remain unknown. Studies have reported activation of the IRE1a and PERK signaling pathways of the UPR by proinflammatory mediators as well as in the initiation of inflammation, suggesting that UPR may play a role in the pathogenesis of these inflammatory disorders, but the underlying mechanisms of this complex interplay remain uncharacterized. Our study will elucidate the regulatory function of UPR in inflammation and identify the mechanisms underlying the cross-talk between these two pathways. Significantly, this may identify potential UPR-targeted therapeutics in the treatment of inflammatory disorders.

ATPASE DOMAIN AND INTERDOMAIN LINKER PLAY A KEY ROLE IN AGGREGATION OF MITOCHONDRIAL HSP70 CHAPERONE SSC1

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The co-chaperone Hep1 is required to prevent the aggregation of mitochondrial Hsp70 proteins. We have analyzed the interaction of Hep1 with mitochondrial Hsp70 (Ssc1) and the determinants which make Ssc1 prone to aggregation. The ATPase and the peptide binding domain (PBD) of Hsp70 proteins are connected by a linker segment that mediates interdomain communication between the domains. We show that the ATPase domain and the interdomain linker represent the minimal Hep1 binding entity of Ssc1. Moreover, the ATPase domain in combination with the interdomain linker is crucial for aggregation of Ssc1. In the absence of Hep1, the ATPase domain with interdomain linker had the tendency to aggregate, in contrast to the ATPase domain with mutated linker segment or without linker, and in contrast to the PBD. Bacterial DnaK, the closest homolog of Ssc1, and a Ssc1 chimera, in which a segment of the ATPase domain of Ssc1 was replaced by the corresponding segment from DnaK, did not aggregate in Δhep1 mitochondria. The propensity to aggregate appears to be a specific property of the mitochondrial Hsp70 proteins. In conclusion, we suggest that interdomain communication makes Ssc1 prone to aggregation. Hep1 counteracts aggregation of Ssc1 by binding to the aggregation-prone conformer.

DISULFIDE BOND FORMATION IN LPTD (IMP) IS ESSENTIAL FOR LPS ASSEMBLY ON THE CELL SURFACE OF ESCHERICHIA COLI

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The cell surface of most Gram-negative bacteria contains lipopolysaccharide (LPS), which is responsible for maintaining the impermeability of the outer membrane (OM). Biogenesis of LPS involves synthesis at the inner membrane, transport across the cell envelope, and assembly at the cell surface. Here we probed the biogenesis and function of disulfide bonds of the Escherichia coli OM protein LptD, which is thought to mediate the last steps of LPS assembly at OM. Oxidation is essential for LptD function, not stability, yet no particular cysteine in LptD is essential. Two disulfide bonds between non-consecutive cysteines (C31-C724 and C173-C725) covalently link the N- and C- termini of LptD but either bond (C31-C724 or C173-C725) individually suffices for function and cell viability. The oxidase DsbA facilitates but is not required for LptD oxidation, likely because non-enzymatic oxidants can also oxidize LptD. Finally, even though disulfide bonds form between non-consecutive cysteines, the disulfide isomerase DsbC is not required either. Therefore, even though disulfide bond formation in LptD is essential, no particular cysteine residue in LptD is essential, and neither is the disulfide oxidase DsbA or the disulfide isomerase DsbC. LptD's oxidation thus seems overbuilt for standard laboratory conditions, perhaps implying that this redundancy could be beneficial under certain environmental conditions.

STRUCTURE-BASED VIRTUAL SCREENING OF HSP90 INHIBITORS AND POLYPYRROLE NANOTUBE FET PLATFORM AS A NOVEL TOOL FOR SENSING HSP90 INHIBITOR 17-AAG

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To identify novel classes of Hsp90 inhibitors[1], we utilized structure-based virtual screening of a chemical database with docking simulations in the N-terminal ATP-binding site of Hsp90[2], in vitro ATPase assay of yeast Hsp90[3], and cell-based Her2 degradation assay in a consecutive manner. As a result, five compounds were identified as novel Hsp90 inhibitors which exert anti-proliferative effect on MCF-7 breast cancer cells. Three inhibitors share the same scaffold of 3-phenyl-2-styryl-3H-quinazolin-4-one structure[4], and the other two compounds contain novel scaffolds of pyrimidine-2,4,6-trione and 4H-1,2,4-triazole-3-thiol[5]. Their binding modes to Hsp90 ATP binding pocket were analyzed using AutoDock program combined with molecular dynamics (MD) simulations. These results exemplify the usefulness of the structure-based virtual screening with molecular docking in drug discovery. We also demonstrate a novel tool for drug discovery, utilizing carboxylated polypyrrole nanotube (CPNT) field-effect transistor (FET) platform[6]. CPNTs were fabricated, immobilized on electrode, and functionalized with Hsp90. The resulting platform paved a way for rapid, sensitive, and specific detection of 17-AAG binding to Hsp90 in real-time, showing its potential as a convenient and sensitive interaction detector in a wide range of biological and pharmaceutical research area.

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SINGLE-MOLECULE CHARACTERIZATION OF THE FOOTBALL-SHAPED GROEL-GROES COMPLEX USING ZERO-MODE WAVEGUIDES.

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GroEL is an *Escherichia coli* chaperonin, which is composed of two heptameric rings. GroEL interacts with its cofactor GroES and assists protein folding in an ATP-dependent manner. It has been generally believed that an asymmetrical 1:1 GroEL-GroES complex (bullet-shaped complex) is only a functional form in the reaction cycle. Contrary to the belief, we have revealed that a symmetrical 1:2 GroEL-GroES complex (football-shaped complex) can be formed in the presence of denatured protein. However, the dynamics of GroEL-GroES interaction including a football-shaped complex is still unclear. To address this issue, we investigated the GroEL-GroES interaction using a single-molecule assay with zero-mode waveguides. We demonstrated that the first binding GroES does not always dissociate from the football-shaped complex prior to the second binding GroES. We also found the existence of two parallel pathways in the interaction cycle. These findings bear on the mechanism of protein folding mediated by GroEL. The implications for the cycle are discussed.

HSP90 MUTATION RESULTS IN ACTIVATION OF MULTIPLE GENES THAT REGULATE DEVELOPMENTAL PATHWAYS IN *SACCHAROMYCES CEREVISIAE*

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The molecular chaperone Hsp90 is a global cellular regulator critical for the folding and activation of diverse client proteins, including multiple kinases and transcription factors. Numerous studies demonstrate that inhibition of Hsp90 dramatically reduces both the accumulation and/or activity of client proteins. In contrast, we found that *Saccharomyces cerevisiae* expressing a mutant form of Hsp90 accumulate elevated amounts of Hbt1, a protein required for polarized morphogenesis. Increased Hbt1 expression was due to an approximate 10-fold increase in *HBT1* mRNA expression in the Hsp90 mutant cells. Increased *HBT1* expression was linked to derepression of Adr1, a transcription factor involved in regulating the transcriptional changes that accompany depletion of glucose. We used microarray analysis to determine the additional transcriptional changes occur in Hsp90 mutant cells. Approximately 150 genes exhibited a 4-fold or greater increase in expression in the Hsp90 mutant cells. In contrast, less than 10 genes were strongly down-regulated. Although some of the upregulated genes expressed are indicative of a general stress response, most of the upregulated genes are normally expressed at high levels only under conditions such as sporulation, starvation or stationary phase. Together, these results indicate that Hsp90 regulates transcriptional regulatory pathways that reflect the growth state of the cell. In particular, Hsp90 appears to play a major role in preventing transcription of genes not required for optimal growth under normal laboratory conditions.

A RIBOSOME-ANCHORED CHAPERONE NETWORK THAT FACILITATES EUKARYOTIC RIBOSOME BIOGENESIS

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Molecular chaperones assist cellular protein folding as well as oligomeric complex assembly. In eukaryotic cells, several chaperones termed CLIPS are transcriptionally and physically linked to ribosomes and are implicated in protein biosynthesis. Here we show that a CLIPS network comprising two ribosome-anchored J-proteins, Jjj1 and Zuo1, function together with their partner Hsp70 proteins to mediate the biogenesis of ribosomes themselves. Jjj1 and Zuo1 have overlapping but distinct functions in this complex process involving the coordinated assembly and remodeling of dozens of proteins on the ribosomal RNA. Both Jjj1 and Zuo1 associate with nuclear 60S ribosomal biogenesis intermediates and play an important role in nuclear rRNA processing leading to mature 25S rRNA. In addition, Zuo1, acting together with its Hsp70 partner SSB, also participate in maturation of the 35S rRNA. Our results demonstrate that, in addition to their known cytoplasmic roles in de novo protein folding, some ribosome-anchored CLIPS chaperones play a critical role in nuclear steps of ribosome biogenesis.

A CHAPERONE PROTEIN MODULATES THE HOMEOSTATIC REGULATION OF THE UNFOLDED PROTEIN RESPONSE

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The unfolded protein response (UPR) is an intracellular signaling pathway that maintains proper function of the endoplasmic reticulum (ER), counteracting variable stresses that impair folding of proteins entering the secretory pathway. In that capacity, the UPR is at the center of many normal physiological responses and pathologies.Although the UPR is thought to be a homeostat that finely tunes ER protein folding capacity and ER abundance according to need, the mechanism by which the core ER stress sensor Ire1 is activated in response to unfolded proteins and the role that the ER chaperone protein BiP plays in Ire1 regulation have remained unclear. Here we show that the UPR matches the response output to the magnitude of the stress by regulating the duration of Ire1 signaling. BiP binding to Ire1 serves to desensitize Ire1 to low levels of stress and promotes its deactivation when favorable folding conditions are restored to the ER. Using quantitative dynamic measurements guided by careful computational modeling, we demonstrate that, mechanistically, BiP achieves these functions by sequestering inactive Ire1 molecules, thereby providing a barrier to oligomerization and activation, and a stabilizing interaction that facilitates de-oligomerization and deactivation. Thus, BiP binding to or release from Ire1 is not instrumental for switching the UPR on and off as previously posed. By contrast, BiP ensures an appropriate response to restore protein folding homeostasis to the ER by modulating the sensitivity and dynamics of Ire1 activity.

IS CHAPERONIN FUNCTION MEDIATED BY WATER?

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There have been several hypotheses regarding the mechanism of chaperonin function. Here, I will describe a novel hypothesis that chaperonins affect protein folding by an indirect mechanism, i.e. by affecting the water surrounding the protein during refolding within chaperonins. While much work has gone into understanding the role of water in the bulk, water in confined biological spaces (such as the ribosome exit tunnel or chaperonins) is still poorly understood. It is natural to consider that there will be deviations from bulk-like behavior, but how? What are the differences, how pervasive are the changes? And how does the change in water impact protein folding and function. I will present results from recent simulations and theory that suggest that non-bulk-like deviations play a role in protein folding and function in the cell. For example, simulations of water inside of the a chaperonin has predictive properties for chaperonin function. Also, simulations of the water inside ribosomes leads to specific predictions to the nature of the nascent peptide at its earliest stages of folding. I will conclude with a discussion of how this nature of water changes may suggest a general change in how we should consider water in vivo.

PHOSPHO-REGULATION OF THE HSP90 CHAPERONE MACHINERY

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Hsp90 is a highly conserved molecular chaperone which contributes to the activation or conformational maturation steps of hundreds of specific client proteins. This activation process is strictly regulated at several levels. Intrinsically, the ATPase activity of Hsp90 is decelerated as a result of the slow conformational changes leading to the ATP hydrolysis competent state. In eucaryotes, Hsp90 associates with a distinct set of co-chaperones, some of which function as inhibitors or activators of the ATPase activity. Recently, it was shown that some of these co-factors target distinct conformational intermediates of Hsp90. In addition, posttranslational modifications of Hsp90 such as acetylation, nitrosylation and, importantly, phosphorylation provide an additional level of regulation. Here, we report a comprehensive analysis of yeast Hsp90 phosphorylation including identification of the sites modified in vivo by a combination of phosphopeptide enrichment and high-resolution mass spectrometry. The mechanistic consequences of Hsp90 phosphorylation were analyzed by phospho-mimetic Hsp90 mutants in vivo and in vitro. Our results show that the effect of phosphorylation on Hsp90 function depends strongly on the position of the phospho-sites in the protein structure and that distinct steps in the chaperone cycle of Hsp90 are affected.

GLOBAL FUNCTIONAL MAP OF THE YEAST P23 COCHAPERONE SBA1 REVEALS EXTENSIVE NUCLEAR MOLECULAR CHAPERONE ACTIVITIES

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In parallel with evolutionary developments, the Hsp90 molecular chaperone system shifted from a simple prokaryotic factor into an expansive network that includes a variety of cochaperones. We have taken high-throughput genomic and proteomic approaches to better understand the abundant yeast p23 cochaperone Sba1. Our work uncovered an unexpected Sba1 network that displayed considerable independence from known yeast Hsp90 (Hsp82) clients. Our data also revealed a broad nuclear role for Sba1, contrasting with the historical dogma of restricted cytosolic activities for chaperones. Validation studies demonstrated that Sba1 was required for proper Golgi function, had a role in ribosome biogenesis and was necessary for efficient DNA repair from a range of mutagens. Importantly, we found that the roles were conserved since mammalian p23 also affected these same pathways. Our high-throughput and corroborative studies demonstrate that the Sba1/p23 cochaperone serves a broad physiological network and functions both in conjunction with and sovereign to Hsp82/Hsp90.

To understand how a p23 cochaperone intersects with a nuclear protein we followed up on the synthetic lethal genetic interaction between sba1Δ and gcn5Δ. Normally, GCN5 encodes for a histone acetyltransferase protein. Surprisingly, the connection does not involve chromatin events. Rather, we found that p23 and Gcn5 form a regulatory circuit that coordinately regulates the DNA-bound state of diverse proteins. Using Heat Shock Factor 1 (HSF1) as a model target, we observed that both p23 and Gcn5 inhibited HSF1 DNA binding activity. p23 initiated disassembly of DNA-bound HSF1 while Gcn5 prolonged the dissociated state by acetylating a lysine residue within the DNA binding domain. In addition, p23 can control the enzymatic activity of Gcn5, which likely serves to close the regulatory circuit. Notably, p23 and Gcn5 affected the DNA binding activities of a variety of proteins including other transcription factors and proteins required for DNA replication and telomere maintenance. Our data support a model in which p23 and Gcn5 constitute a general system for modulating protein DNA dynamics across the genome.

HSP90 TRANSFORMS THE PHENOTYPIC MANIFESTATION OF GENETIC VARIATION IN *S. CEREVISIAE*

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Species can remain unaltered for long evolutionary periods, but can also undergo rapid diversification. However, mechanisms that either promote stasis or facilitate change remain enigmatic. The protein chaperone Hsp90 has been hypothesized to do both. Hsp90 facilitates maturation of metastable regulators of growth and development, but is expressed at a higher level than is normally required to fulfill these functions. This reservoir of Hsp90 can promote stasis by buffering genetic variation, allowing it to accumulate without phenotypic consequences. When environmental stress compromises the protein folding capacity of the organism, phenotypes arising from this variation are revealed. Hsp90 can also facilitate change by potentiating variation. In this case, new mutations depend on the reservoir of Hsp90 chaperone activity to produce their phenotypes. Such traits disappear when environmental stress compromises that reservoir. This hypothesis has been controversial because the adaptive value of Hsp90 buffered and potentiated variation is unclear. Indeed, only one such variant is known to be beneficial and certain buffered traits appear monstrous. Here, we establish that the interface between Hsp90 and environmental stress profoundly influences the phenotypic manifestation of natural genetic variation. In ecologically diverse strains of *Saccharomyces cerevisiae* a vast array of traits were buffered or potentiated by Hsp90. These traits were frequently beneficial to growth. We mapped hundreds of quantitative trait loci for such traits and dissected the causative polymorphisms for four (three coding, one regulatory). A simple change in growth temperature elicited the same broad phenotypic changes as Hsp90 inhibition, demonstrating the strong link Hsp90 provides among protein homeostasis, environmental stress, and the manifestation of genetic variation. Across the many tens of thousands of polymorphisms present in 48 ecologically diverse strains, selective manipulation of the reservoir of Hsp90 chaperone activity and changes in growth temperature each improved the correlation between genotype and phenotype, strongly arguing that Hsp90 has influenced genome evolution in this organism.

AVOIDING STRESS: UNCOVERING AN ESSENTIAL BEHAVIORAL CIRCUIT

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All organisms must be capable of adaptive responses that promote survival in stressful environments. While much attention has been paid to stress responses acting at a cellular level, little is known of whole organism responses to stress. In *C.elegans*, dispersal is a highly adaptive behavioral solution to the imposition of environmental stress. Experimentally, dispersal is observed as evacuation of the bacterial lawn. Reasoning that artificial inactivation of basic cellular processes might simulate the effects of environmental stress, we screened a library of ~3000 essential genes in search of gene knockdowns that elicit the dispersal response – despite the absence of an actual environmental stressor. We found that perturbation of many fundamental cellular processes elicits a robust dispersal response. These processes include transcription, translation, DNA repair, mitochondrial function, pH homeostasis, and protein homeostasis. Much of the protein homeostasis machinery (Hsp4, Hsp6/Hsp60, Hsp90, the CCT complex and the proteasome) acts upstream of dispersal behavior. Through examination of GFP stress reporters, we found that many of these gene knockdowns activated known cellular responses. A subset of gene knockdowns failed to activate any of our reporters, possibly indicating the discovery of previously unknown stress response pathways. An additional targeted screen implicated the hedgehog signaling pathway, nuclear hormone receptors, neuropeptides and G-protein coupled receptors (GPCRs) in dispersal behavior. These molecules may perform endocrine functions in stress adaptation. Epistasis analysis showed that insulin signaling and multiple FOXO transcription factors are differentially required within specific functional classes for dispersal. Distinct genetic requirements within protein homeostasis indicate that different protein folding and turnover pathways control dispersal behavior through a complex combination of mechanisms. Experiments with genetic lines in which RNAi is restricted to specific tissues showed that essentially any major tissue can produce an endocrine stress signal. We propose that dispersal behavior is a programmed component of stress adaptation that may be just as critical to organism survival as chaperone activity and other cellular responses under noxious environmental conditions.

HSP110 CHAPERONES CONTROL CLIENT FATE DETERMINATION IN THE HSP70/HSP90 CHAPERONE SYSTEM

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The heat shock protein 70 (Hsp70) molecular chaperone plays a central role in protein homeostasis, governing folding, transport and quality control of substrate polypeptides. Hsp70 works in conjunction with other chaperone machines, including Hsp90, in a substrate specific manner. The Hsp110 chaperone Sse1 is required for the activity of many Hsp90 client proteins in budding yeast, and functions as a nucleotide exchange factor (NEF) for cytosolic Hsp70. However, the precise roles Sse1 plays in client maturation through the Hsp70/Hsp90 chaperone system are not fully understood. We find that upon pharmacological inhibition of Hsp90, a model protein kinase, Ste11ΔN, is rapidly degraded while heterologously expressed glucocorticoid receptor (GR) remains stable. Hsp70 binding and acceleration of nucleotide exchange by Sse1 was required for productive maturation of GR and mating pathway signaling through endogenous Ste11, as well as to promote Ste11ΔN degradation. Overexpression of another functional NEF only partially compensated for loss of Sse1 in facilitating GR maturation and Ste11ΔN degradation, while the paralog Sse2 fully restored both activities. Sse1 was required for ubiquitinylation of Ste11ΔN upon Hsp90 inhibition, providing a mechanistic explanation for its role in substrate degradation. Hsp110 chaperones may play a global role in regulating protein degradation as ubiquitinylation of misfolded newly synthesized proteins was likewise impaired in *sse1*Δ cells. Sse1/2 co-purified with Hsp70 and other proteins comprising the “early-stage” Hsp90 complex, and was absent from “late-stage” Hsp90 complexes characterized by the presence of Sba1/p23. Together these findings support a model in which Hsp110 chaperones contribute significantly to the decision made by Hsp70 to fold or degrade a client protein.

MANAGING PROTEOMIC FOLDING PLATFORMS THROUGH PROTEOSTASIS

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The cell exploits the composition and dynamics of protein homeostasis (proteostasis) networks[1-3] to generate and maintain the proteomic profiles in cytosol, and for folding and function of proteins trafficking the exocytic and endocytic pathways in complex cell, tissue and host environments. It is now apparent that in folding disease and during aging, protein misfolding challenges the proteomic composition of the cell and tissue, compromising human health and longevity. A major goal for biologists is to understand the composition, protein-protein interactions and signaling mechanisms that direct the operation of cell that preserves a physiological proteomic platform. The capacity of small molecules (proteostasis regulators/pharmacological chaperones) to harness the activity of a tunable range of PN components to protect biology from a wide range of folding diseases highlights the potential of managing proteostasis to benefit human health.

[1](Science (2008) 319:916; [2]Ann. Rev. Biochem. (2009) 78:959; [3]FEBS Lett. (2009) 583:2639)

BIP BINDING TO THE ER-STRESS SENSOR IRE1 MODULATES THE HOMEOSTATIC REGULATION OF THE UNFOLDED PROTEIN RESPONSE

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The unfolded protein response (UPR) is an intracellular signaling pathway that maintains proper function of the endoplasmic reticulum (ER), counteracting variable stresses that impair folding of proteins entering the secretory pathway. As such, the UPR is thought to be a homeostat that finely tunes ER protein folding capacity and ER abundance according to need. The mechanism by which the core ER stress sensor Ire1 is activated in response to unfolded proteins and the role that the ER chaperone protein BiP plays in Ire1 regulation have remained unclear. We show that the UPR matches the response output to the magnitude of the stress by regulating the duration of Ire1 signaling. BiP binding to Ire1 serves to desensitize Ire1 to low levels of stress and promotes its deactivation when favorable folding conditions are restored to the ER. We propose that, mechanistically, BiP achieves these functions by sequestering inactive Ire1 molecules, thereby providing a barrier to oligomerization and activation, and a stabilizing interaction that facilitates de-oligomerization and deactivation. Thus BiP binding to or release from Ire1 is not instrumental for switching the UPR on and off as previously posed. By contrast, BiP ensures an appropriate response to restore protein folding homeostasis to the ER by modulating the sensitivity and dynamics of Ire1 activity.

EXPLORING THE REGULATION OF PROTEIN HOMEOSTASIS USING GENETIC INTERACTION MAPS

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Proper protein folding in the cell requires a delicate balance of diverse processes ranging from protein translation and degradation to control of membrane lipid content. As imbalance in these processes leads to stress and disease, it is essential to develop a comprehensive understanding of how they are coordinated to allow efficient protein folding. To do this, we have developed a strategy to (1) identify important players in a given process using a genetic screen, and then (2) make quantitative pairwise measures of genetic interactions among these players to create an organizational map. In yeast, this strategy has led to a remarkable array of functional information, including the identification of a conserved transmembrane protein complex and a mechanism for insertion of tail-anchored proteins. We are now extending this approach to metazoans using RNAi to identify genes that when compromised lead to protein misfolding. Toward this end, we have developed high-density lentiviral shRNA libraries that are easily adapted to allow knockdown of pairs of genes. Coupled with deep sequencing, these libraries allow measurement of genetic interactions in a high-throughput, simplified system that can be tailored to the study of any process of interest.

TARGETED DISRUPTION OF ANDROGEN RECEPTOR REGULATION BY THE IMMUNOPHILIN FKBP52

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Steroid hormone receptors require the ordered assembly of various chaperone and cochaperone proteins in order to reach a functional state. The final stage in the receptor maturation process requires the formation of a mutimeric complex consisting of an Hsp90 dimer, p23, and one of several large immunophilins. Studies conducted previously demonstrated that the large immunophilin FKBP52 acts to potentiate glucocorticoid, androgen, and progesterone receptor signaling pathways. Although we have made progress in identifying regions of functional importance on FKBP52, the site of interaction between FKBP52 and the receptors remains to be elucidated. The aim of these studies was to identify and characterize FKBP52-specific inhibitors that would not only serve as tools for the pharmacological analysis of FKBP52-receptor interactions, but may also lead to novel drugs with significant therapeutic potential. A modified receptor-mediated reporter assay in yeast was used to screen a natural compound library for FKBP52 inhibitors, which led to the identification of multiple hits. Structure activity relationship studies using the yeast-based assays led to the characterization of fifteen additional inhibitors, some of which display dramatically increased potency. Surface plasmon resonance studies confirmed that these inhibitors disrupt FKBP52 function through interaction with the AR hormone binding domain. In addition, scintillation proximity binding and fluorescence polarization assays demonstrated that the compounds bind to a previously unrecognized regulatory surface on the AR LBD termed BF3. Interestingly, mutations within the BF3 surface lead to increased dependence on FKBP52 for normal function. Preliminary assays in LNCaP cells demonstrated that the compounds inhibit Prostate-Specific Antigen expression and androgen-dependent proliferation. In summary we have identified molecules that inhibit FKBP52 regulation of AR function and represent an exciting new approach for the treatment of prostate cancer. In addition, these studies provide new insights into FKBP52-AR interactions.

THE CONFORMATIONAL DYNAMICS OF THE MITOCHONDRIAL HSP70 CHAPERONE

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Heat shock proteins 70 (Hsp70) are ubiquitous and conserved family of ATP-dependent molecular chaperones involved in plethora of cellular processes. They bind unfolded substrates in their substrate binding domain in a reaction regulated by nucleotides bound to nucleotide bound domain and modulated by interacting cochaperones. In mitochondria, a member of the Hsp70 family is involved in translocation of proteins into the organelle, their subsequent folding and also in biogenesis of Fe-S clusters. The conformational changes underlying its ATP-dependent cycle and their dynamics are key to understanding its numerous functions, yet are poorly understood. Here we used fluorescence spectroscopy to analyze, in real time and at single molecule resolution, the effects of nucleotides and cochaperones on the conformation of Ssc1, a major mitochondrial Hsp70 of yeast. The results of this analysis and their implications will be presented.

USING YEAST TO UNDERSTAND THE MECHANISMS THAT REGULATE HUMAN HEAT SHOCK FACTOR 1

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Hereditary protein conformational disorders are often associated with the expansion of polyQ repeats in the expressed proteins. These expansions lead to protein misfolding, neuronal toxicity and eventually cell death. While a definitive cure for these diseases has not been found, promising research has shown that enhanced expression of protein chaperones, mediated through the activation of the transcription factor HSF1, can suppress polyQ-dependent protein misfolding and prevent neurotoxicity. As such, pharmacological activation of HSF1 is likely to become an important part in the treatment of protein conformational disorders. However, our understanding of the biochemical mechanisms that regulate HSF1 activity remain limited. Our previous data show that human HSF1, when expressed in *Saccharomyces cerevisiae*, is constitutively maintained in a repressed state and unable to complement for the loss of the essential yeast HSF. Interestingly, many of the proteins previously linked to HSF1 repression in mammalian cells are conserved in yeast. Therefore, we hypothesize that gaining an understanding of the mechanisms by which human HSF1 is repressed in yeast will further our understanding of HSF1 regulation in metazoans. To explore these mechanisms we have utilized a variety of biochemical, genetic, and pharmacological approaches to identify novel hHSF1 repressor proteins. Among the negative regulators of HSF1 that we have identified are several protein kinases not previously linked to HSF1 repression. We have also developed a high-throughput screen to identify novel small molecule activators of human HSF1 in yeast and have identified HSF1A, a unique molecule that activates HSF1 in metazoan cells and promotes the expression of Hsp70 and other protein chaperones in an HSF1-dependent manner. In addition, we show that HSF1A ameliorates protein misfolding and cell death in polyQ-expressing neuronal precursor cells and protects against cytotoxicity in a fly model of polyQ-mediated neurodegeneration. We show that HSF1A interacts with components of the TRiC/CCT complex, suggesting a potentially novel regulatory role for this complex in modulating HSF1 activity.

ACTIVATION OF THE IRE1 ENDORIBONUCLEASE THROUGH A NOVEL LIGAND BINDING POCKET

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Protein folding in the endoplasmic reticulum (ER) is matched to the everchanging flux of proteins through the secretory pathway by the unfolded protein response (UPR) – a tripartite intracellular signaling pathway that transcriptionally and translationally regulates ER protein folding pathways. Signaling within the most conserved branch of the UPR is initiated by the sequence-specific cleavage of HAC1/XBP1 mRNA by the ER-stress induced kinase-endoribonuclease IRE1. IRE1 activation is directly linked to the ER folding environment through a cascade that is initiated by the stress-induced oligomerization of IRE1's lumenal domain. The stress signal is propagated across the ER membrane to the cytosol through autophosphorylation of the IRE1 kinase domain that allows for the high affinity binding of ADP to the conventional nucleotide binding cleft of the kinase and subsequent stabilization of the active dimeric/oligomeric conformation of the endoribonuclease domain. We have discovered a novel ligand binding pocket in the cytosolic domain of yeast IRE1 that can be targeted to selectively modulate IRE1 signaling. A co-crystal structure of IRE1 cytosolic domain engaged by both ADP and the flavonol quercetin reveals quercetin binding to an unanticipated ligand binding site at the dimeric interface of IRE1's Kinase-Extension-Nuclease domain. Ligand binding to this pocket enhances IRE1 endoribonuclease activity in vitro and in vivo and potentiates the effect of ADP binding at the conventional nucleotide binding site. Rational mutagenesis of IRE1 supports the functional relevance of the structural model of IRE1 activation through occupancy of this novel binding site and suggests the possibility for developing tools to explore the engagement of this pocket by endogenous ligand and other small molecules that may tune the IRE1 branch of the UPR independent of the state of the ER lumen.

GENETIC MODULATION OF PROTEIN AGGREGATION TOXICITY

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Protein misfolding and aggregation is known to be a major contributor to neuropathogenic disease development. The causality between intracellular protein aggregation and the intrinsic thermodynamic stability of proteins has been shown by multiple studies. Indeed, single point mutations in the genes coding for e.g. superoxide dismutase and Alzheimer's Aß peptide can trigger the formation of unfolded or alternately folded states and consequently affect the aggregation properties of these proteins dramatically. Alternately, extension of polyQ-stretches in proteins beyond a critical threshold leads to the formation of toxic species and intracellular aggregates, correlating to the development of a neuronal phenotype. The connection between protein aggregation and cellular damage is still unclear and the cellular factors and networks, directly governing this process are yet to be resolved. Using a novel aggregation-prone protein system, our study investigates how cellular proteins interact to affect aggregate and toxic species formation *in vivo*. Furthermore, we aim to identify cellular processes which are harmed by the occurrence of aggregates or aggregation intermediates.

THE ROLE OF MEKK1/JNK PATHWAY IN ENDOPLASMIC RETICULUM (ER) STRESS INDUCED APOPTOSIS

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Stress in the Endoplasmic Reticulum (ER) prompts cells to mount a transcriptional response, which is collectively known as the Unfolded Protein Response (UPR). While significant advances have been made regarding the pathways leading to the induction of ER chaperones and quality control genes, the pro-apoptotic branch of the UPR remains poorly understood. A better understanding of this latter branch may be particularly significant in understanding many degenerative diseases that manifest only after the death of ER-stressed cells. To study this pro-apoptotic branch of the UPR, we devised a facile assay system in which the ER of the developing Drosophila eye disc cells were overloaded with mutant Rhodopsin-1 that serves as a model for Retinitis Pigmentosa. The condition activated ER stress reporters, induction of pro-apoptotic genes reaper and hid, massive apoptosis and a partial ablation of the adult eye structure. Consistently, the elimination of these pro-apoptotic genes suppressed apoptosis caused by ER-stress. We used this apoptosis phenotype as a basis to perform a genetic screen and found Drosophila Mekk1, a MAP KKK family member, as a strong modifier of this phenotype. Specifically, apoptosis induced by misexpression of mutant Rh-1 was aggravated by overexpression of Mekk1, while the loss-of function mutant of Mekk1 suppressed the cell death. On the other hand, Mekk1 did not affect cell death caused by polyglutamine proteins, which form aggregates in the cytoplasm. In cultured cells, Mekk1 was required to activate JNK and p38 signaling pathways. Consistently, loss-of JNK (basket) function also blocked ER-stress-induced apoptosis in Drosophila eye discs. While showing a strong effect on apoptosis, Mekk1 did not affect the level of mutant Rh-1 expression or ER-stress markers, supporting the idea that it acts as a signaling molecule in the cytoplasm without directly affecting misfolded proteins in the ER. Additional epistasis experiments placed Mekk1/JNK upstream of the pro-apoptotic genes, reaper and hid. These findings indicate that the Mekk1/JNK genes constitute parts of a pro-apoptotic UPR branch that mediates ER-stress-triggered apoptosis.

MANIPULATING THE FOLDING AND FATE OF MISFOLDED RHODOPSIN-ASSOCIATED RETINAL DEGENERATION WITH HDAC INHIBITORS: FROM BENCH TO BEDSIDE

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Rhodopsin associated retinal degeneration, known as retinitis pigmentosa (RP), is a protein conformational disorder. The clinically common mutation, P23H, results in a misfolded protein fraction that is predominantly retained in the ER. We have previously shown that pharamacological chaperones can significantly improve the efficiency of folding and trafficking of this protein. As part of our ongoing studies, we have manipulated the cellular folding environment with histone deacetylase inhibitors (HDIs) including valproic acid (VPA) and trichostatin A (TSA). At low concentrations both HDIs resulted in the 50-80% increase in the folded rhodopsin. With increasing concentrations, however, the yields of folded P23H rhodopsin were significantly reduced, while the yields of WT rhodopsin increased. Further, the retinas of P23H mice treated with VPA had approximately 30-40% increase in the amounts of folded rhodopsin, which was statistically significant over the placebo treated animals. There was no substantial effect of VPA on the littermate controls or C57/B6 mice. In the retinas of S344ter opsin rats treated with VPA, the outer nuclear layer (ONL) had 2-3 rows of photoreceptor nuclei. In contrast, animals treated with PBS had only 1 row of nuclei in the ONL of the same retinal region. Separate quantitative data showed that the ONL thickness in treated animals was significantly higher than the controls. Finally, we treated RP patients with VPA, 250 mgs 2-3 times per day. The visual fields (VF) of 5 of 7 patients increased with treatment. In one case, the improvement in functioning retinal area was confirmed at two time points (23 and 27 weeks). Of the 13 eyes examined, 9 eyes had improved visual field on treatment, two eyes had decreased visual field and two eyes experienced no change with an overall average increase of 11%. Assuming typical loss in VF area without treatment, this increase in VF was statistically significant ($p<.02$). An average decrease in logMAR scores was seen in these 13 eyes, which was significant ($p<.02$) assuming no loss in acuity without treatment. Collectively, this work provides evidence that by manipulating the cellular folding environment with HDIs in both cellular and mice models, it is possible to develop rational therapies for PCDs like rhodopsin-associated RP.

SEQUENCES FLANKING POLYQ TRACTS IN DISEASE-ASSOCIATED PROTEINS MODULATE THEIR ABILITY TO FORM AGGREGATES IN *C. ELEGANS*

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A number of neurodegenerative diseases are caused by the toxic gain-of-function of proteins with expanded polyglutamine (polyQ) tracts. However, the underlying mechanisms of toxicity are less well understood. One intriguing aspect of polyQ disorders is that despite the often ubiquitous expression of disease-associated polyQ proteins in neurons, these proteins differentially affect neuronal subtypes, resulting in unique disease pathologies. This points to the sequences flanking the polyQ tract driving important elements of toxicity, rather than the polyQ tract alone being responsible for disease. To elucidate the impact of sequences flanking the polyQ tract in disease associated proteins, we have expressed three such proteins (Huntingtin, the human Androgen Receptor, and Ataxin-3) with and without expanded polyQ tracts in the genetic model system C. elegans. We previously showed that when polyQ peptides are translationally fused to YFP and expressed in different tissues of C. elegans the resultant chimeric protein displays polyQ length- and age-dependent aggregation/toxicity. Our new models that take into account the effect of flanking protein sequences have revealed that different polyQ-containing proteins yield unique aggregation patterns and biophysically distinct aggregates in C. elegans. This is likewise the case even when comparing different disease-associated N-terminal fragments of Huntingtin, the protein affected in HD patients. With the availability of C. elegans genetic tools, such as genome-wide RNAi, we are molecularly dissecting the mechanisms underlying this effect of flanking protein sequences on polyQ protein aggregation.

MODULATION OF THE EFFECTS OF A CHAPERONE PROTEIN ON A YEAST PRION BY THE GET PATHWAY AND THE UBIQUITIN SYSTEM

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Cross-β protein aggregates (amyloids) are associated with a variety of neurodegenerative disorders such as Alzheimer, Parkinson, Huntington and Creutzfeldt-Jacob diseases. Yeast [*PSI*$^+$] is a self-perpetuating (prion) amyloid-based isoform of the translation termination factor eRF3 (Sup35) of *Saccharomyces cerevisiae* that serves as a model for studying amyloid formation and propagation. [*PSI*$^+$] is eliminated by overproduction of the disaggregating chaperone Hsp104. A screen for mutants antagonizing excess Hsp104-dependent elimination of [*PSI*$^+$] has produced mutant alleles of the genes *GET2* and *TOM1*. Deletion of each of these genes also antagonized the Hsp104-mediated prion loss. Neither mutations nor deletions of *GET2* and *TOM1* influenced the Hsp104 function in thermotolerance, indicating that their effects are specific to the interaction of Hsp104 with amyloid aggregates.

Get2 is a component of the GET (Guided Entry of Tail-Anchored proteins) pathway responsible for the trafficking of tail-anchored (TA) proteins to the ER membrane. Deletions of other components of the GET pathway also counteracted [*PSI*$^+$] elimination by excess Hsp104. Disruption of the GET pathway leads to accumulation of aggregated TA proteins (and some GET proteins) in the cytoplasm. Preliminary data suggest that aggregates of TA and GET proteins interact with amyloids and/or co-chaperones, thus restricting the ability of Hsp104 to promote prion disaggregation.

Tom1 is a HECT domain ubiquitin ligase (E3 enzyme). Our previous data (Allen et al., 2007 JBC 282:3004-3013) implicated the ubiquitin conjugating (E2) enzyme Ubc4 in modulating the effects of excess Hsp104 on [*PSI*$^+$]. The role of Ubc4 or Tom1 in the direct ubiquitination of Sup35 has not been supported. Possibly, components of ubiquitin system influence a prion via ubiquitination of the auxiliary factors involved in prion propagation.

Overall, our data demonstrate that chaperone effects on prions are modulated by other aggregated and misfolded proteins present in the yeast cell. As both GET and ubiquitination pathways are involved in the response for the metal, redox and temperature stresses, our results point to the possible mechanisms mediating effects of environmental factors on amyloids.

INTERACTION BETWEEN AAA ATPASE P97/VCP AND THE ADAPTOR UBX OF FAF1

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p97/VCP, a member of AAA(+) ATPase, is involved in diverse cellular processes including endoplasmic reticulum-associated degradation (ERAD), membrane fusion, transcriptional activation, cell-cycle and apoptosis as one of the most abundant proteins in the cell. It interacts with various adaptors for activity in different biochemical contexts. Among these adaptors are UBX and UBD domains. Fas-associated factor 1 (FAF1), a multifunctional protein that is associated with Fas-mediated apoptosis, NF-kB signaling and endoplasmic reticulum-associated degradation (ERAD), contains a UBX domain at the C-terminal, and is reported to interact with p97/VCP. The crystal structures of UBX alone and bound to the N-terminal domain of p97/VCP have been determined and details will be described.

THE UNFOLDED PROTEIN RESPONSE NOT BY UNFOLDED PROTEINS

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Aberrancy of protein folding in the endoplasmic reticulum (ER) is known to trigger a cellular signaling pathway to induce various genes functioning in the ER. An ER-located transmembrane protein Ire1 is a key component of this cellular response, namely the unfolded protein response (UPR) or the ER stress response. By using yeast system, we and others previously revealed molecular steps by which unfolded proteins activate Ire1. BiP, which is a major ER-located molecular chaperone, binds to and represses Ire1. Upon accumulation of unfolded proteins in the ER, BiP dissociates from Ire1, and Ire1 forms high-order homo-oligomers. Unfolded proteins then directly interact with the Ire1 oligomers, which leads to full induction of the UPR. However, it has not been clear how other stress stimuli, including depletion of inositol from medium, activate Ire1. Our analyses of yeast Ire1 mutants presented here indicate that Ire1 neither forms high-order homo-oligomers nor directly associates with unfolded proteins upon its activation under some stress conditions. BiP dissociation also seems unnecessary. We then propose “aberrancy of membrane” activates Ire1 by a mechanism which is different from that for unfolded proteins. Considering also a wide variety of UPR target genes, the UPR is less likely to be only “by and for” unfolded proteins.

DEFINING A MECHANISTIC ROLE FOR YEAST HSP40 SIS1P IN THE PRION LIFE CYCLE THROUGH TARGETED MUTATIONAL ANALYSIS

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Prions are, in simplest terms, infectious proteins. These pathological entities arise from normal, functional proteins by spontaneously assuming alternate conformations and engaging in a self-templating mode of replication. In mammals, the formation and persistence of prions and related amyloid proteins have devastating consequences, being largely incurable and typically lethal. Countless investigations have indicated that a complex and essential relationship exists between cellular chaperone machinery and the transition between various physical stages of prion growth and development. Our current interest focuses on the chaperones that drive the prion "life cycle", specifically members of the Hsp40, Hsp70 and Hsp100 families. Numerous reports have indicated vital roles for the Ssa group of Hsp70s as well as Hsp40 cochaperone partners like Sis1p and Ydj1p. Past genetic screens have yielded chaperone mutants that have significant effects on various yeast prions. One notable mutant identified in previous work is the L483W mutant of SSA1, which is of considerable interest due to its apparent inability to stably host the yeast prion *[PSI+]*. In order to identify mutants of the SIS1 gene that negate the weakened *[PSI+]* phenotype exhibited by the L483W (SSA1-21) mutant and to ultimately uncover mechanistic details of the relationship between proteins of the Hsp70 and Hsp40 families relative to yeast prions, a series of Sis1p truncations and amino acid substitutions were analyzed through a genetic screen in conjunction with both wild type and Ssa1p mutants. Through this analysis, we show that the dimerization domain of Sis1p is essential for weakening *[PSI+]* in the L483W mutant and for eliminating *[PSI+]* in cells overexpressing Hsp104p. Results also indicate that the glycine/methionine-rich (G/M) region of Sis1p operates in an essential capacity to confer viability to the cell in the presence of the prion. The latter of these results is shown to be independent of soluble Sup35p levels and represents one of only a few conditional lethality mutants in yeast known to be contingent upon a prion.

LUCIFERASE AS A FOLDING SENSOR FOR ACUTE AND CHRONIC STRESS CONDITIONS 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. 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 it would be valuable to have tools to assess the chaperone capacity upon proteotoxic stress.
My goal is to develop such folding sensors that report on proteostasis in *C. elegans*. Firefly luciferase has been widely used as a model substrate to study protein folding, because of its rapid and sensitive bioluminescent activity that depends entirely on proper folding. De novo folding as well as refolding after chemical or thermal denaturation of this labile protein requires the assistance of chaperones, making luciferase an ideal model substrate and reporter to assess proteostasis.
Indeed, I could observe a decline in luciferase activity upon proteotoxic challenges induced either by the expression of aggregation prone proteins such as polyQ or mutant SOD1 or by exogenous stressors such as heat or oxidative stress. However, the most dramatic effect can be observed upon aging, indicating that the chaperone capacity decreases along the lifespan.
To combine the functional read-out of the folding sensor with a visual assessment of its folding state, a *C. elegans* line expressing luciferase fused to YFP was created. The expression of luciferase-YFP using different promoter constructs allows for visualization and biophysical inspection of the luciferase moiety upon proteotoxic challenges in various tissues. I could indeed observe a shift from a soluble expression pattern of luciferase-YFP under control conditions to the formation of aggregates upon exogenous stressors, mirroring the loss of function under these conditions in e.g. body wall muscle cells. In addition, luciferase has proven to be also an excellent tool to assess inter-compartmental proteostasis reporting on the folding capacity of specific organelles upon compartment-specific or cellular folding perturbations.

SPECTROSCOPIC IMAGING ANALYSIS OF THE DISAGGREGATION PROCESS OF MUTANT SOD1

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Accumulation of misfolded protein causes several neurodegenerative disorders, and protein aggregation is closely related to cytotoxicity. Here, we examine aggregation and disaggregation of amyotrophic lateral sclerosis (ALS)-linked mutant Cu/Zn superoxide dismutase 1 (SOD1). A significant portion of mutant SOD1 in the aggregate is mobile and can be exchanged with soluble cytosolic SOD1. However, little is known about how SOD1 exchanges between the aggregate and soluble forms, or the biological relevance of this exchange in terms of the toxicity of mutant SOD1. To elusidate the dynamics of SOD1 aggregation and disaggregation in human cultured cells, fluorescence correlation spectroscopy (FCS) and fluorescence resonance energy transfer (FRET), which can analyse the oligomerization/aggregation state, were employed. These analysis revealed that soluble oligomers and soluble aggregates of mutant SOD1 are disaggregated into cytosol during the recovery of proteasome activity. Surprisingly, the cellular toxicity by mutant SOD1 was observed during the disaggregation process rather than the aggregation process. Thus, the toxic characteristics of soluble mutant SOD1 oligomers may differ between the disaggregation and accumulating processes.

MAMMAL-RESTRICTED STRUCTURAL ELEMENTS PREDISPOSE HUMAN RET TO FOLDING IMPAIRMENT BY HSCR MUTATIONS

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The protein folding and maturation of human RET is adversely affected by a range of missense mutations found in patients with Hirschsprung's disease (HSCR), a complex multigenic disease. Here, we present the crystal structure of the two amino-terminal cadherin-like domains (CLD(1-2)) from human RET which adopt a clam-shell arrangement distinct from classical cadherins. Surprisingly, CLD1 structural elements and disulphide composition are unique to mammals indicating structural diversity within higher and lower vertebrate RET CLD regions. We identify two unpaired cysteines that predispose human RET to maturation impediments in the endoplasmic reticulum and show how "misfolding" mutations, found in patients with HSCR disease, are correctly folded and surface localised when present in human RET without the unpaired cysteines. This finding was applied to establish a quantitative cell-based RET maturation assay that offers a biochemical correlate of HSCR disease severity. Our findings provide a key conceptual framework and means of testing and predicting genotype-phenotype correlations in HSCR.

INVESTIGATING THE MECHANISM OF [*PSI*$^{+}$] CURING BY HSP104 OVEREXPRESSION

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In most cases, proteins adopt a single three-dimensional fold, specifying a unique function in the cell. Prions, however, are an exception to this rule, adopting more than one fold *in vivo*. Despite this conformational flexibility, prion forms are self-replicating, a mechanism which allows each functional or phenotypic state to be stably propagated. This self-replication is mediated through an oligomeric complex of prion state protein, which acts as a template for the conversion of newly made protein to the prion form. Efficient self-replication can only occur, however, if these prion complexes are fragmented to generate additional templates and to ensure their efficient transmission. In yeast, Hsp104 directs prion self-replication by fragmenting prion complexes through its disaggregase activity. Upon deletion of Hsp104, prion complexes increase in size and decrease in mobility, leading to prion loss or curing by decreasing both the rate of prion self-replication and the rate of transmission to daughter cells.

While all known yeast prions are dependent upon Hsp104 for propagation, the Sup35/[*PSI*$^{+}$] prion is unique in that excess Hsp104 also induces loss of the prion form although the molecular mechanism by which this occurs is currently unknown. To gain insight into this process, our focus has been to investigate the *cis* and *trans* requirements for [*PSI*$^{+}$] curing by assessing the changes in prion complex dynamics that accompany Hsp104 over-expression. Our analyses indicate that excess Hsp104 induces prion loss by inhibiting the activity of this chaperone. This effect is mediated through a sequence element in the Sup35 protein that is conserved among prionogenic homologues of Sup35 in fungi but not in those incapable of adopting the prion form. We hypothesize that upon overexpression, Hsp104 interacts non-productively with aggregates due to limitations of an as yet unidentified co-factor that directly binds to Sup35.

INDEPENDENT EVOLUTION OF THE CORE DOMAIN AND ITS FLANKING SEQUENCES IN SMALL HEAT SHOCK PROTEINS

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Small heat shock proteins (sHsps) are molecular chaperones involved in the maintenance of protein homeostasis and in protein folding diseases as well as cancer. In this protein family, a conserved core domain, the so called α-crystallin or Hsp20 domain, is flanked by highly variable non-conserved, sequences that are essential for the chaperone function of these proteins. The analysis of more than 8,700 sHsp sequences revealed a broad variation of the primary sequences within the superfamily as well as phyla-dependent differences. Especially the number of sHsps per genome, the amino acid composition and the length distribution of the different sequence parts vary significantly.
The reconstruction of the evolutionary tree for the sHsp superfamily unraveled an unconventional evolutionary concept, in which the conserved α-crystallin domain and its flanking sequences evolved independently of each other. Interestingly, the flanking regions fall into several subgroups indicating that they were remodeled several times in parallel, but independent of the evolution of the α-crystallin domain.
Conclusions
The evolutionary history of sHsps is set apart from that of other protein families in that two, exon-boundary-independent evolutionary strategies are combined: the evolution of the conserved α-crystallin domain and the independent evolution of the N- and C-terminal sequences. This scenario allows variability in specific small parts of the protein and thus promotes functional and structural differentiation of sHsps, which is not reflected in the general evolutionary tree of species.

ACTIVATION OF MISSENSE MUTANT CBS ENZYME *IN VIVO* BY PROTEASOME INHIBITORS AND TREATMENTS THAT INDUCE HSP70

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Missense mutant proteins, such as those produced in individuals with genetic diseases, are often misfolded and subject to processing by intracellular quality control systems. Previously, we have shown using a yeast system that enzymatic function could be restored to I278T cystathionine beta-synthase (CBS), a cause of homocystinuria, by treatments that affect the intracellular chaperone environment. Here, we extend these studies and show that it is possible to restore significant levels of enzyme activity to 17 of 18 (94%) disease causing missense mutations in human cystathionine β-synthase (CBS) expressed in S. cerevisiae by exposure to either ethanol, proteasome inhibitors, or deletion of the Hsp26 small heat shock protein. All three of these treatments induce Hsp70, which is necessary but not sufficient for rescue. In addition to CBS, these same treatments can rescue disease-causing mutations in human p53 and the methylene tetrahydrofolate reductase gene. These findings do not appear restricted to S. cerevisiae, as proteasome inhibitors can restore significant CBS enzymatic activity to CBS alleles expressed in fibroblasts derived from homocystinuric patients and in a mouse model for homocystinuria that expresses human I278T CBS. These findings suggest that proteasome inhibitors and other Hsp70 inducing agents may be useful in the treatment of a variety of genetic diseases caused by missense mutations.

THE HTID TUMOR SUPPRESSOR, A DNAJA3 PROTEIN, ACTS AS A GUARDIAN OF HOMEOSTASIS OF SIGNALING NETWORKS

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The last two decades of cancer research has proven the complexity of the processes leading to cancer and confirmed the functional diversity and multiple cellular actions of the proteins involved in these processes. The evolutionarily conserved tid (tumorous imaginal discs) gene, encoding DNAJA3 protein family members, was originally identified and confirmed as a tumor suppressor by gene therapy using the fruit fly Drosophila melanogaster (Dm). The tid genes encode three splice forms - long (L), intermediate (I) and short (S), encoding three cytosolic and three mitochondrial proteins. The distinct hTid molecules interact with diverse physiological partners in diverse cellular compartments (1-3 and literature cited here). In general, two modes of action of the Tid proteins can be discerned: i) interaction with cytosolic molecules, such as the APC tumor suppressor (2) or the IKB Inhibitor of NF?B signaling (4) or ii) binding to receptors mediating signal transduction, e.g. Ptc) (1) and ERBB-2 (5). Here we show that hTid binding to diverse ligands takes place simultaneously in the cells. The partners build topologically determined complexes with further molecules, components of signaling cascades determining cell polarity, pattern formation and cell cycle regulation. The interactions seem to be essential for the homeostasis of the aforementioned signaling, the off/on status. We show that the three forms are differentially expressed in normal tissues. In general, the expression profile is common for diverse cells. This pattern changes in diverse cancers of epithelial origin, such as basal cell carcinomas (BCCs) (1), colon cancers (3) and breast tumors associated with HER-2 overexpression. With regard to the latter our data suggest an association of hTidL elevation with well differentiated status of the tumors and its drastic drop down in metastasizing tumors with poor prognosis. The expression profiles determined in normal and tumor tissues suggest that the concentration of the distinct htid splice forms is precisely regulated at both physiological and non physiological conditions.

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STRUCTURAL ANALYSIS OF THE G PROTEIN B_1 SUBUNIT (GB_1) AND THE CYTOSOLIC CHAPERONIN CONTAINING THE TAILLESS-COMPLEX POLYPEPTIDE 1 (CCT) PROVIDES INSIGHT INTO THE MECHANISM OF GB_1 FOLDING - AN IMPORTANT INTERMEDIATE IN THE ASSEMBLY OF THE G PROTEIN BΓ DIMER

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A complex forms between the G protein β_1 subunit ($G\beta_1$) and the cytosolic chaperonin containing the tailless-complex polypeptide 1 (CCT) during the folding of nascent $G\beta_1$, enabling $G\beta_1$ to assemble into the G protein heterotrimer and act in downstream signaling. We have isolated the $G\beta_1$-CCT complex from insect cells over-expressing human recombinant Gβ1 protein with an antibody-affinity tag. The complex was subjected to structural determination by cryo-electron microscopy in an effort to determine $G\beta_1$-CCT interactions and the mechanism by which the $G\beta_1$ subunit is folded into its native state. Reconstructions of cryo-EM images reveal that $G\beta_1$ forms a globular structure within the CCT folding cavity. $G\beta_1$ was mostly folded and bound to the apical domains of two adjacent CCT subunits, CCTβ and CCTζ. The $G\beta_1$ binding sites on CCTβ were identified through mutagenesis and co-immunoprecipitation. A hydrophobic groove between two helices and another hydrophobic loop of CCTβ contribute significantly to the $G\beta_1$ binding. By elucidating the mechanism of Gβγ assembly, we can identify therapeutic targets in the assembly process that could be exploited to control the amount of Gβγ being made by the cell and thus the level of G protein signaling.

GENETIC DISSECTION OF CFTR QUALITY CONTROL MECHANISMS IN *C. ELEGANS*

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Most cases of Cystic Fibrosis (CF) are caused by the ΔF508 mutation in the ABC transporter protein CFTR. In the endoplasmic reticulum (ER), $CFTR^{\Delta F508}$ mis-folds into a kinetically trapped, reversible structure which is degraded through a ubiquitin-dependent process termed ER-associated degradation (ERAD). The genes involved in $CFTR^{\Delta F508}$ recognition, processing, and degradation are poorly understood. To better understand these mechanisms, we have modeled ΔF508-dependent defects in the nematode *C. elegans* using the closely related *C. elegans* ABC transporter *pgp-3*. Like CFTR, *PGP-3* is trafficked to the apical plasma membrane of polarized epithelial cells. Introduction of the ΔF508 mutation destabilizes the protein and blocks apical membrane localization. Like human $CFTR^{\Delta F508}$, $PGP3/CFTR^{\Delta F508}$ stability and localization are rescued by mutants that produce high levels of the chemical chaperone glycerol, suggesting some ΔF508-dependent quality control mechanisms are conserved between *C. elegans* and humans. Currently, we are investigating the specific post-transcriptional mechanisms impacted by the ΔF508 mutation. Furthermore, we are performing forward and reverse genetic screens to identify genes that promote instability of the $PGP3/CFTR^{\Delta F508}$ protein. Our *C. elegans* model represents the first high-throughput genetic animal model system for CF studies. The genes identified in our screens may provide new drug targets for CF therapies and could illuminate novel modifier loci that influence CF onset, severity, or progression.

HSF1 AND HSF2 DIFFERENTIALLY CONTROL DISTINCT TRANSCRIPTOME IN MAMMALS

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HSF1 and HSF2 are related members of the HSF family known to regulate the stress response. Mouse knockout experiments have shown that HSF1 is a maternal factor which severely alters oocyte meiosis and provokes female infertility (1, 2). In contrast, $Hsf2^{-/-}$ females are fertile and can produce offspring (3-5). In most tissues and cells lines (e.g. lung, liver, heart), HSF1 and HSF2 are co-expressed and accumulating data have recently shown these factors are able to interact to regulate common target genes (6, 7). Nevertheless, we found that oocytes contain 100 fold more transcripts encoding HSF1 than HSF2 and previous attempts to detect HSF2 protein in oocytes were unsuccessful. These observations suggest that these factors might control different transcriptome depending on germinal or somatic cellular context.
These data prompted us to perform genome wide comparative transcriptomic analysis using wild-type, $Hsf1^{-/-}$ and $Hsf2^{-/-}$ oocytes. We used 200 oocytes per genotype (4-5 ng of RNA) to generate cDNAs that were hybridized to NimbleGen microarrays (that had 42600 transcripts represented on the array). Using a 1.5-fold differential threshold comparing knockout to wild type oocytes, we identified 1665 genes regulated by HSF1, 959 regulated by HSF2 and 130 regulated by both factors (p-value ≤ 0.05).
We compared our results with a previous HSF1 microarray experiment performed in somatic cells (MEFs) by Trinklein and al. (8). 93% of the genes regulated by HSF1 in oocytes and fibroblasts are different suggesting that maternal function of HSF1 in oocyte differs from its function in somatic cells. Moreover, GO term analysis revealed enrichment in different processes between oocytes and fibroblasts strengthening the hypothesis that HSF1 controls distinct transcriptome in oocytes and somatic cells.
Regarding HSF2 transcriptome, it is interesting to highlight that, despite the absence of any phenotype in $Hsf2^{-/-}$ oocytes, HSF2 regulates around 1000 genes. Thus, although it is dispensable, HSF2 may play a role in oocyte which remains to be identified.

All these data will help us to better understand the role played by HSF1 and HSF2 in this particular germ cell, oocyte.

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ROLES OF HEAT SHOCK PROTEINS IN ENDOPLASMIC RETICULUM ASSOCIATED DEGRADATION (ERAD) PROCESS FOR OVEREXPRESSED OR MUTATED PENDRIN

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Pendrin is an 82 kDa membrane transporter for monovalent anions such as I^-, Cl^-, and HCO_3^- in thyroid follicular cells, inner ear epithelia, and renal cortical collecting ducts. Mutations in *SLC26A4* gene which produces Pendrin are considered as major causes for Pendred syndrome characterized by inherent sensorineural hearing impairment and thyroid goiter. Recent studies revealed that overexpression or some mutations of *SLC26A4* gene perturbs the folding of Pendrin and causes its retention in endoplasmic reticulum (ER) followed by endoplasmic reticulum associated degradation (ERAD). However, the specific mechanisms of ERAD process for Pendrin are still unknown. It has been shown that treatment of thyroid cells with 17-AAG, an Hsp90 inhibitor, inhibits efflux of I^-, suggesting the potential roles of Hsp90 or other chaperones in regulation of Pendrin or other I^- exporters. When 17-AAG was treated to human embryonic kidney cell line (HEK293) transiently expressing Pendrin, Pendrin protein levels increased. This result implies that Hsp90 might be involved in regulation of folding or degradation of Pendrin. We will also discuss about the roles of other chaperones in regulation of the stability and membrane translocation of Pendrin.

FUNCTIONAL ANALYSIS OF NADPH-DEPENDENT THIOREDOXIN REDUCTASE C (ATNTRC) AS A REDOX CHAPERONE IN CHLOROPLAST

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AtNTRC, an electron donor of a chloroplast 2-Cys Prx, was induced by heat- and cold-shocks in the light, and overexpression of AtNTRC (AtNTRC^{OE}) in Arabidopsis (*Arabidopsis thaliana*) led to an acquired thermotolerance, whereas a knockout mutant (*ntrc1*) showed heat sensitive phenotype. Biochemical and transmission electron microscopic analyses showed that the recombinant and native AtNTRC proteins exhibit diverse quaternary structures. High molecular weight (HMW) complex of AtNTRC was revealed to display a higher chaperone activity but a lower disulfide reductase activity; in contrast, the low molecular weight (LMW) AtNTRC exhibited a higher disulfide reductase activity but a lower chaperone activity. Heat treatment of recombinant AtNTRC induced HMW complex formation which is facilitated by addition of NADPH, and C-terminal thioredoxin domain is required for the HMW complex formation. Analysis of AtNTRC^{OE} and *ntrc1* also showed that AtNTRC confer freezing tolerance on plants. Recombinant AtNTRC exhibited a cryoprotection activity for MDH and LDH, and it also bound to RNA and DNA protecting them from RNase A and metal catalyzed oxidation damages, respectively. Proteomic analyses of WT, NTRC^{OE} and *ntrc1* indicated many proteins involved in chloroplast biogenesis, metabolism and stress response are up- or down-regulated. These results indicate AtNTRC functions not only as an electron donor for 2-Cys Prx but also as a protector for macromolecules in chloroplast. [Supported by EB-NCRC & BK21 program]

HEAT DEPENDENT FUNCTIONAL SWITCH OF ARABIDOPSIS THIOREDOXIN DOMAIN CONTAINING PROTEIN (ATTDX)

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We found that Arabidopsis AtTDX, a heat-stable and plant-specific thioredoxin (Trx)-like protein, exhibits multiple functions, acting as a disulfide reductase, foldase chaperone, and holdase chaperone. The activity of AtTDX, which contains 3 tetratricopeptide repeat (TPR) domains and a Trx motif, depends on its oligomeric status. The disulfide reductase and foldase chaperone functions predominate when AtTDX occurs in the low molecular weight (LMW) form, whereas the holdase chaperone function predominates in the high molecular weight (HMW) complexes. Because deletion of the TPR domains results in a significant enhancement of AtTDX disulfide reductase activity and complete loss of the holdase chaperone function, our data suggest that the TPR domains of AtTDX block the active site of Trx and play a critical role in promoting the holdase chaperone function. The oligomerization status of AtTDX is reversibly regulated by heat shock, which causes a transition from LMW to HMW complexes with concomitant functional switching from a disulfide reductase and foldase chaperone to a holdase chaperone. Overexpression of AtTDX in Arabidopsis conferred enhanced heat shock resistance to plants, primarily via its holdase chaperone activity. [This research was supported by BK21 and EB-NCRC programs]

INTERMEDIARY COMPLEXES DURING THE HSP90 CYCLE

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The molecular chaperone Hsp90 is required for the activation of cellular substrates, including protein kinases, transcription factors and steroid hormone receptors. In particular steroid hormone receptors define at which stages of the activation cycle Hsp90-cochaperones associate with Hsp90. The but the molecular events, which lead to the entry of one co-chaperone and the exit of another co-chaperone during the chaperone cycle are still enigmatic.
In order to precisely describe these events, we set out to study the Hsp90 chaperone cycle by fluorescently labeling individual components of the hetero-oligomeric protein complex. We used analytical ultracentrifugation in combination with other biochemical tools to reconfigurate the chaperone cycle. We identified novel complexes, which are required for the progression of the chaperone cycle as potential intermediary organizations.

MULTIPLE ER-RETENTION/RETRIEVAL SIGNALS IN TRANSMEMBRANE SEGMENTS REGULATE SURFACE EXPRESSION AND DEGRADATION OF NAV1.8 THROUTH CALNEXIN-DEPENDENT PATHWAY

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The voltage-gated sodium channel (Nav) 1.8 contributes substantially to the rising phase of action potential in small dorsal root ganglion neurons. Nav1.8 is majorly localized intracellularly and its expression in the plasma membrane is regulated by exit from the endoplasmic reticulum (ER). Previous work has identified an ER-retention/retrieval motif in the first intracellular loop of Nav1.8 which prevents its surface expression. Here we demonstrate that the transmembrane segments of Nav1.8 also contain ER-retention/retrieval signals that cause Nav1.8 to be retained in the ER. The acidic amino acid-containing motifs are critical in determining the functional signals. In addition, we show that the ER-retention/retrieval signals in the transmembrane segments result in increased degradation of proteins through calnexin-dependent ER-associated degradation (ERAD) pathway. Thus our results reveal a critical role of transmembrane segments in the surface expression and degradation of Nav1.8.

A NEWLY IDENTIFIED RIBOSOME-ASSOCIATED CHAPERONE COMPLEX REGULATES THE CELLULAR METABOLISM OF TAIL-ANCHORED MEMBRANE PROTEINS

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Hundreds of membrane proteins possess a single carboxyl-terminal transmembrane domain (TMD), thus termed as tail-anchored (TA) proteins, play various but important roles in multiple cellular processes, such as cell apoptosis, protein translocation and vesicular transportation. Endoplasmic reticulum (ER) specific TA proteins are post-translationally targeted to their destination by a cytosolic factor termed TRC40 through a TMD-dependent capturing mechanism. How the highly hydrophobic TMDs of TA proteins are effectively captured by TRC40 upon their release into the crowded cytosol was unknown. Recently in our lab, we identified a conservative ribosome-associated chaperone complex comprising of three different proteins, Bat3, Ubl4A and TRC35, respectively, that specifically recognizes and shields the TMDs of TA proteins, and transfers the TA substrates to TRC40 for targeting to the ER. Depletion of the Bat3 complex from the cytosol caused non-TRC40 factors to compete for the TMD of TA substrates, while the efficient capture by TRC40 is restored by adding back purified Bat3 complex. Meanwhile, we observed that TA proteins are efficiently poly-ubiquitinated in the absence of either membranes (RMs) or TRC40, suggesting that under stressing conditions, TA substrates that fail to target to the ER will be degraded through the ubiquitin-proteasome pathway. Complete deletion of the TMD or hydrophilic mutations made in the TMD of TA substrates abolished the poly-ubiquitination, indicating the putative E3 ligase (s) are recruited by the TMD itself or TMD-selective binding factor (s). Since two of the three components of Bat3 complex, the Bat3 and Ubl4A contain ubiquitin-like (UBL) domain, we speculate that Bat3 complex could perform the scaffold or adaptor role in connecting the TA proteins to the putative E3 liagse(s). In fact, depletion of Bat3 complex from the cytosol specifically led to reduced poly-ubiquitination of TA proteins. Therefore, the Bat3 chaperone complex acts as the triage factor of TA membrane proteins at the ribosome, facilitating their efficient targeting to the ER or directing them to the ubiquitin-proteasome pathway depending on an unknown regulating mechanism.

HEAT SHOCK PROTEIN GP96 (GRP94, HSP90B1) COMES OF AGE: THE CLIENT, THE CO-CHAPERONE AND THE MECHANISM OF ACTION

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gp96 (grp94 and HSP90b1), the HSP90 paralogue in the endoplasmic reticulum, was discovered three decades ago and has been implicated in controlling protein homeostasis in the secretory pathway. However, the fundamental questions of gp96 as a molecular chaperone remain unanswered. Using a conditional gp96 knockout mouse (Yang et al. Immunity, 2007), we have uncovered new functions of gp96 and have revealed its novel mechanism of action. These discoveries include: (a) gp96 is a master chaperone for Toll-like receptors (TLRs); (b) gp96 is critical for the folding of a majority of integrins; (c) gp96 selectively regulates T and B lymphopoiesis but not myelopoiesis; (d) gp96 is dispensable for the folding of immunoglobulin in vivo; (e) gp96 is essential for the assembly of the platelet glycoprotein Ib-IX-V complex and loss of gp96 results in a disorder that is clinically and hematologically indistinguishable from Bernard-Soulier syndrome; (f) gp96 regulates the intestinal homeostasis; (g) gp96 folds TLRs in a manner that is dependent on its intrinsic ATPase activity and a TLR-specific co-chaperone in the endoplasmic reticulum. We will provide a comprehensive update on the function and mechanism of gp96, arguably the last frontier of HSP90 biology.

CHARACTERIZATION OF PURIFIED MYOCILIN: GLAUCOMA AS A PROTEIN MISFOLDING DISEASE

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Mutations in the myocilin protein cause an inherited form of open angle glaucoma, a prevalent neurodegenerative disorder usually associated with increased intraocular pressure. Myocilin forms part of the trabecular meshwork extracellular matrix, which is presumed to regulate intraocular pressure. Missense mutations, which cluster in the olfactomedin (OLF) domain of myocilin, render the protein prone to aggregation in the endoplasmic reticulum of trabecular meshwork cells, causing cell dysfunction and death. Cell biological studies have demonstrated temperature-sensitive secretion of myocilin mutants, but difficulties in expression and purification in the past decade have precluded extensive biophysical characterization of wild-type myocilin and disease-causing mutants in vitro. We have overcome these limitations by purifying from E. coli wild-type and glaucoma-causing mutant forms of the myocilin OLF domain fused to maltose binding protein (MBP). Monomeric fusion proteins can be isolated in solution. To determine the relative stability of wild-type and mutant OLF domains, we developed a stability assay without removal of MBP. The assay provides the first direct evidence that mutated OLF is folded but less thermally stable than wild-type. We tested the ability of chemical chaperones to stabilize mutant myocilin. Several compounds stabilize the mutants to near or better than that of wild-type OLF. Our work lays the foundation for detailed structure/function investigations. In the long term, we hope to identify tailored small molecules capable of stabilizing mutant myocilin and promoting secretion to the extracellular matrix, to better control intraocular pressure and ultimately delay the onset of myocilin glaucoma. This therapy would be the first of its kind for glaucoma.

EFFICIENT HSV-1 INFECTION REQUIRES THE CELLULAR HEAT SHOCK RESPONSE

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Inhibition of molecular chaperones by drugs, siRNA depletion or expression of dominant-negative chaperones impairs HSV-1 infection. For example, the ATPase activity of Hsc70 is required for the formation of viral replication centers, efficient virus production and for the nuclear transport of the HSV-1 capsid protein UL6. Additionally, Hsp90 ATPase activity is required for the nuclear import of the HSV-1 polymerase and the major capsid protein and production of infectious virus. The heat shock response is regulated by the transcription factor heat shock factor 1 (HSF-1). In this study, we investigated whether the cellular heat shock response is induced during HSV-1 infection. When activated during heat stress, HSF-1 trimerizes, becomes hyperphosphorylated and upregulates the transcription of heat shock responsive genes such as hsp70. Hyperphosphorylation of HSF-1 can be detected as a mobility shift by SDS-PAGE in cells heat shocked at 42°C. Interestingly, HSV-1 infection at 37°C is sufficient to induce a mobility shift of HSF-1 in infected fibroblast cell lines suggesting activation of the heat shock response. Hsp70 protein levels do not, however, increase during HSV-1 infection, raising the possibility that the inducible heat shock response is only partially activated during infection. A small molecular inhibitor of the heat shock response, KNK437, inhibits the induction of chaperones such as Hsp70 during heat stress. We found that KNK437 inhibits replication compartment formation, gene expression and inhibits production of progeny virus. Furthermore, HSF-1 does not undergo a change in mobility in infected cells treated with KNK437 suggesting that the target of KNK437 may be HSF-1. Our data suggest that the inducible heat shock response may be required for HSV-1 infection at the level of early gene expression; however, the virus may inactivate other aspects of the heat shock response. This is reminiscent of other examples in which DNA viruses like herpesviruses manipulate cellular response pathways by activating some components and inactivating others in order to create environments conducive to their own replication.

INTERPLAY BETWEEN ERDJ PROTEINS IN REGULATING BIP FUNCTIONS

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BiP, the mammalian Hsp70 orthologue in the endoplasmic reticulum (ER), participates in most functions of this organelle. It takes part in the maturation of unfolded nascent proteins and is also involved in selecting the ultimately misfolded proteins and directing them to the ER associated degradation (ERAD) machinery. How BiP distinguishes between substrates having these contradictory fates remains a question. Some ER-localized DnaJ-like (ERdj) proteins bind directly to substrates and can recruit BiP to them. We hypothesized that the apparently opposing functions of BiP might be regulated by distinct ERdj proteins. Our previous data showed that unassembled Ig γ heavy chains (HC), which have very long half-lives in plasmacytoma cells, are associated with ERdj3 (Shen and Hendershot, MBC, 2005). We did not observe any association of these HC with ERdj4 but did find small but detectable levels of ERdj5 bound to them. When ERdj3 levels were decreased with shRNA, the HC turned over more rapidly and were associated with components of the ERAD machinery. In addition, there was an increase in the association of the HC with both ERdj4 and ERdj5. Over-expressing ERdj4 in transient transfection experiments resulted in a faster degradation of the HC and of a non-secreted Ig LC that also binds to BiP. Finally, we co-expressed an ERdj3 mutant that remains stably bound to substrates due to an inability to interact with BiP to determine if ERdj3 must be released from the substrate to promote degradation. We found that this mutant diminished the turnover of the NS-1 LC. Based on these observations, we hypothesize that ERdj3 assists in folding and stabilization of γ HC before they assemble with LC. Releasing ERdj3 from the substrate allows ERdj4 and/or ERdj5 to bind and targets the unfolded protein for degradation. These studies provide insights into how ERdj proteins allow BiP to function both in folding and degradation of secretory pathway substrates.

AN ARCHAEAL MODEL OF HUMAN CHAPERONOPATHIES: THE GROUP II CHAPERONINS

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Group II chaperonins occur in archaea and eukaryotes and their subunits are similar. Here we focus on the lone chaperonin subunit (Cpn-Pf) of the archaeon *Pyrococcus furiosus* and those from humans found to have disease-associated mutations, CCT4, CCT5, and the CCT8-related BBS6 (MKKS), BBS10, and BBS12. Amino acid identities and similarities between Cpn-Pf and CCT4 and CCT5 are 40-60 percent, i.e., the same as between CCT4 and CCT5; similarities between Cpn-Pf and BBS6, 10, and 12 are low due to extra segments in the latter three. Structurally, the archaeal and eukaryotic chaperonins have equivalent domains (equatorial, intermediate, and apical) and, functionally, play similar roles in protein folding via essentially the same mechanism. However, the process is considerably simpler in archaea than in eukaryotes, particularly in *P. furiosus* because it has only one type of subunit to build the chaperoning machine. We, therefore, are developing an archaeal model for investigating the functional effects of mutations and post-translational modifications in the chaperonin molecule that would reproduce those observed in human chaperonopathies, which have not yet been elucidated due to the complexity of the mechanisms and the multiplicity of subunit types of eukaryotes. We found that the alpha helix, 21GRDAQRMNILAARIVAETIR40, in the N terminal segment of Cpn-Pf was essential for double-ring formation, which was necessary for ATPase and chaperoning activities. Deletion of the helix formed by 21GRDAQRMNIL30 resulted in the limited assembly of assorted tetramers, dimers, and monomers. Site-directed mutagenesis of each residue in this helix revealed that Arg22, Asn28, and Ile29 are critical for oligomerization. Two antiparallel alpha helixes from adjacent molecules form the interaction interface. Arg22 together with Glu37 from an adjacent helix constituted a stable ion pair, and one such ion pair located at each end of the helix doublet locked the two helices, stabilizing the oligomer. Arg26 may also have the potential to form an ion pair with Glu37. Although no interaction partners for Asn28 and Ile29 were found, their mutations (i.e., N28A, N28Q, N28K and I29A, I29L, I29K) caused oligomer dissociation, indicating that any change at these two residues can result in excess flexibility to the helix, decreasing the probability of formation of the stabilizing ion pairs. This is the first report of a comprehensive mutational analysis of the oligomerization pathway of group II chaperonins. Mutations at equivalent residues on the human orthologs will provide insights into their assembly and clues for understanding chaperonopathies at the molecular level.

CHARACTERIZATION OF THE CONFORMATIONAL CHANGES IN THE CHAPERONE CYCLE OF THE ER RESIDENT HSP70 BIP

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The endoplasmic reticulum (ER) of eukaryotic cells is a unique chemical environment for protein folding. Immunoglobulins (Ig) are an important class of proteins which are folded and assembled in the ER of B-cells by specialized chaperone complexes. The Hsp70 member BiP (heavy chain binding protein) and its Hsp40 co-chaperone ERdJ3 are key players in this process.
To elucidate the dynamics and conformational prerequisites for substrate processing by BiP, an IgG domain and a peptide derived from this domain were used as endogenous model substrates. The thermodynamics and kinetic parameters for their interaction and the conformational changes during the chaperone cycle of BiP were analyzed by single molecule FRET experiments, revealing the domain communication of BiP during the chaperone cycle. Besides the modulation of the domain orientations in BiP by nucleotides, the effects of bound substrates as well as the influence of ERdJ3 on BiP conformations were analyzed.

MODULATION OF DBC2 FUNCTION BY THE MOLECULAR CHAPERONE HSP90

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Deleted in Breast Cancer 2 (DBC2/ RhoBTB2) is a tumor suppressor gene from human chromosome 8p21.3: a chromosomal region that was commonly found deleted in a screen of breast cancer samples. DBC2/ RhoBTB2 is a member of the atypical RhoBTB subfamily. It contains an N-terminal Rho-like GTPase domain, a linker region containing a PEST motif, followed by a tandem pair of BTB (broad complex/bric-a-brac/poxvirus/zinc finger) domains, a putative RING domain and a Ser-rich C-terminus. DBC2 has been demonstrated to interact with and be ubiquitinated by Cullin 3 and reported to lack GTP binding activity. DBC2 was a protein that was found to specifically co-adsorb from cell lysate with Cdc37. The specific interaction of DBC2 with the Hsp90 chaperone machinery was confirmed by reconstitution of the interaction with DBC2 generated de novo by coupled transcription/ translation in reticulocyte lysate. Domain dissections of DBC2 indicated that DBC2 interacted with the Hsp90 chaperone machine, primarily through its Rho domain, and retains the capability to bind GTP. GTP binding shows hallmarks of being an Hsp90 dependent process as it is reduced by the Hsp90-specific inhibitor geldanamycin. Ubiquitination of DBC2 was confirm by the specific adsorption of higher molecular weight adducts of DBC2 by immobilized ubiquitin-binding domains. LC/MS analysis of DBC2 pull downs indicates that DBC2 specifically interacts with the Cullin 3 E3 ligase, as well as components of the Hsp90 chaperone machinery. Over-expression of DBC2 in MCF7 cells, a cell line that does not express DBC2, induces cell death. However, Hela cells, which normally express DBC2 are immune to the effects of DBC2 over-expression. In Hela cells endogenously expressed DBC2 turns over very rapidly, and is immunoadorbed from cell lysates as a high molecular weight adduct in a complex with a high molecular weight adduct of Hsp90. Our studies suggest that Hsp90 modulates DBC2 activity and is involved in the mechanism of regulation of DBC2's tumor suppressor function. (This work was support by a grant from OCAST: HR-06 070).

EVIDENCE FOR A FUNCTIONAL ROLE OF THE N-TERMINAL DOMAIN OF HEAT SHOCK PROTEIN 27 (HSP27) IN ITS CHAPERONE ACTIVITY

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Cells under stress accumulate misfolded and aggregated proteins which can be toxic and may lead to disease states. The induction of small heat shock protein (sHsp) expression enables cells to defend against protein aggregation. Hsp27 forms dynamic oligomeric complexes that can dissociate into binding competent dimers depending upon the phosphorylation state of the protein[1]. Hsp27 consists of a highly conserved α-crystallin domain which is flanked by a short C-terminal extension and a non-conserved N-terminal domain. We are using spectroscopic tools to examine the role of the N-terminal domain in the recognition and binding of substrate. Residues along the N-terminal domain were replaced with cysteines, spin labeled, and analyzed for changes in EPR lineshape and solvent accessibility. EPR data, in the absence of phosphorylation, has revealed a sterically packed and solvent-inaccessible environment for the N-terminal domain. Upon phosphorylation of Hsp27, EPR spectra of spin labels report a transition to a more solvent-exposed environment suggesting the exposure of the N-terminal domain. In contrast, phosphorylation does not change the environment of the α-crystallin domain. Binding of Hsp27 to destabilized T4 lysozyme (T4L) mutants results in a distinct transition in the environment of the N-terminal domain from solvent-exposed to solvent-inaccessible. We propose that the interaction between the N-terminal domain of Hsp27 and substrate in its non-native state is a critical event for chaperone activity.

1. Shashidharamurthy, R., Koteiche, H.A., Dong, J., Mchaourab, H.S. (2004) J. Biol. Chem. 280, 5281-5289

SUBSTRATE RECOGNITION BY *E. COLI* CLPB AND YEAST HSP104, MOLECULAR CHAPERONES THAT RESCUE PROTEINS FROM AN AGGREGATED STATE

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ClpB of prokaryotes and Hsp104 of yeast are AAA+ (ATPases Associated with various cellular Activities) proteins and members of the Clp/Hsp100 family of molecular chaperones. They are essential for the survival of cells during extreme stress and function by disaggregating and reactivating proteins damaged by stress conditions. *In vivo* and *in vitro*, they act in conjunction with the DnaK/Hsp70 chaperone system. However, *in vitro* they are able to perform some protein remodeling activities in the absence of the DnaK/Hsp70 system, when mixtures of ATP and ATP gamma S are used as the nucleotide source. Structural studies have shown that ClpB/Hsp104 exists as a hexameric ring with each protomer consisting of an N-terminal domain followed by two AAA+ nucleotide-binding domains, the first of which is interrupted by the insertion of a long coiled coil middle domain.

To gain insight into substrate recognition by ClpB/Hsp104 when acting independently of the DnaK/Hsp70 system, we have compared protein unfolding by ClpB/Hsp104 using GFP fusion proteins containing N- or C-terminal peptides of different lengths and sequences as substrates. Although ClpB and Hsp104 share high sequence homology, our experiments show that they differ in substrate preference. To further explore substrate recognition, we have engineered chimeras by swapping domains of Hsp104 and ClpB. Several chimeras have been purified and characterized *in vitro*. Results suggest that some variants recognize the preferred substrates of Hsp104 and others the preferred substrates of ClpB.

DISSECTING THE ROLE OF DNAK AND COORDINATION OF ATP HYDROLYSIS FOR CONTROLLING THE DISAGGREGATION ACTIVITY OF CLPB

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The AAA+ chaperone ClpB mediates the reactivation of aggregated proteins in cooperation with the DnaK chaperone system (KJE), an activity that is crucial for the survival of eubacteria during severe stress. KJE has an essential function during the initial phase of the disaggregation process, the mechanistic requirement for this activity is, however, not well understood. Here, we have analyzed in vivo the binding of DnaK/DnaJ and ClpB chaperones to their physiological substrates: stress-induced and polar localized protein aggregates. By using fluorescent chaperone fusion proteins we demonstrate that DnaJ, DnaK and ClpB bind in a hierarchic manner to protein aggregates. The interaction of ClpB with aggregated proteins strictly requires initial coating of aggregates by DnaJ/DnaK, providing a rationale for the requirement of KJE in ClpB-mediated protein disaggregation. In agreement with a crucial role of KJE in targeting ClpB to substrates, we demonstrate that switching ClpB substrate specificity allows for KJE-independent substrate disassembly by ClpB. This ClpB only mediated "disaggregation activity" requires ATP hydrolysis and allows for dissecting the role of coordinated ATP hydrolysis for ClpB activity.

CRYSTAL STRUCTURE OF THE MAMMALIAN CYTOSOLIC CHAPERONIN CCT IN COMPLEX WITH TUBULIN

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Protein folding in the cell is assisted by a large group of proteins termed molecular chaperones, one of the most important members being the chaperonins or Hsp60s (Heat Shock Proteins of 60 kDa). The eukaryotic cytosolic chaperonin CCT (chaperonin containing TCP-1), also know as TRiC) is the most complex of all chaperonins, an oligomeric structure built by two identical rings, each composed of single copies of eight different 60kDa subunits called α, β, γ, ζ, ε, δ, θ and η. This macromolecular complex of 1 MDa has crucial relevance in several essential biological processes, emerging as a key molecule due to its role in the folding of many important molecules including actin, α and β tubulins. An electron density map at 5 Å resolution has enabled us to build the CCT complex and to dock one of its well-known substrates inside the cavities. Here we present the crystal structure of this protein machine in complex with tubulin, providing information about the molecular mechanism by which this macromolecular complex aids the tubulin folding process. Our data provide new important insights into the function of this molecular machine.

A HIGH-THROUGHPUT RNAI SCREEN IDENTIFIED SUMO AS A REGULATOR OF THE HEAT SHOCK FACTOR HSF-1

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The heat shock transcription factor HSF-1 has important roles in stress tolerance, aging, innate immunity, cancer, and protein diseases. Activation of HSF-1 leads to heightened expression of target genes such as heat shock proteins (HSPs) that promote protein homeostasis and cellular adaptation to stress. Because active HSF-1 mediates resistance to a wide variety of proteotoxic stressors, drugs that upregulate HSF-1 may be therapeutic for a variety of protein misfolding diseases, such as Huntington's disease. However, recent studies suggest that HSF-1 activity may also be involved in undesirable phenotypes, including cellular transformation to a cancerous state. While global regulation of HSF-1 might be undesirable, identification of regulators of HSF-1 may allow us to find more nuanced ways to control its activity. To identify such HSF-1 regulators, we conducted a high-throughput RNAi screen for regulators of an HSF-1-dependent reporter transgene in *Caenorhabditis elegans*. Our screen covered 17,540 genes and identified 43 regulators of an HSF-1-dependent reporter. RNAi inhibition of these genes did not similarly affect an HSF-1-independent reporter, suggesting that they specifically regulate HSF-1-dependent phenotypes. One gene identified by our screen was *smo-1*, which encodes the sole *C. elegans* SUMO homolog. SUMO is a small ubiquitin-like molecule used to post-translationally modify proteins. Repression of *smo-1* led to hyper-activation of the HSF1-dependent reporter, but only following activation of the reporter by heat shock. Consistent with this phenotype, *smo-1(RNAi)* also improved whole-animal thermotolerance. Sequence analysis of HSF-1 revealed a consensus site for sumoylation at the N-terminus, and recent studies from other labs confirm that HSF1 is post-translationally sumoylated. These results suggest that SUMO may directly modify HSF-1 in *C. elegans* in response to specific activating conditions and that such modifications inhibit HSF-1-regulated gene expression and stress resistance *in vivo*. Our findings have implications for sumoylation as a future target for therapies intended to regulate HSF-1.

ANALYSIS OF THE STRESS TRANSDUCER, PERK, IN SCIATIC NERVES OF THE CMT 1B NEUROPATHY MOUSE.

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Charcot-Marie-Tooth 1 disease is a common inherited neuropathy. This pathology is characterized by the loss of myelin sheath integrity in the peripheral nervous system (PNS) resulting in slowed nerve conduction velocity, hind limb muscular atrophy, and postural abnormalities. It is caused by mutations in a wide range of genes; one of them is Myelin Protein Zero (MPZ), which is expressed by Schwann cells, the myelin forming glia of the PNS. MPZ encodes the most abundant protein of peripheral myelin and is required to compact its structure allowing fast conduction. When MpzS63Δ, a mutant causing CMT1B in humans, is expressed in mouse with wild type alleles, it produces a demyelinating neuropathy that mimics the corresponding human disease. MpzS63Δ is correctly expressed and translated but it does not reach the myelin sheath being retained in the endoplasmic reticulum (ER). The ER accumulation of unfolded proteins is generally followed by induction of the Unfolded Protein Response (UPR), an adaptive mechanism aimed to relieve ER stress. P0S63Δ accumulation triggers a dose dependent, UPR with increased phosphorylation of eIF2α, Atf6 cleavage, Ire-1 induced Xbp-1 splicing and Chop induction. Genetic ablation of Chop restores motor capacity and ameliorates electrophysiological and morphological abnormalities in S63Δ mice, suggesting that the UPR is pathogenetic and maladaptive. Since Chop is downstream of the Perk/eIF2α pathway, we studied the effects of Perk ablation in normal and S63Δ mice. Preliminary behavioral, morphological and biochemical data from these mice suggest that Perk is maladaptive in S63Δ nerves.

PROGRESSION OF ENDOPLASMIC RETICULUM STRESS IN WAKE ACTIVE NEURONS WITH AGING

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Fragmentation of wakefulness and sleep are expected outcomes of advanced aging. We hypothesize that metabolically active wake neurons are susceptible to age related changes in the unfolded protein response and that dysfunction with age might contribute to the observed wake impairments. In this series of experiments we sought to more fully characterize age-related changes in wakefulness and then, in relevant wake neuronal populations, explore functionality and endoplasmic reticulum homeostasis. We report that old mice (22-24 month old) show greater sleep/wake transitions in the active period with markedly shortened wake periods, shortened latency to sleep at the end of subjective night, and less wake time is observed in the subjective day in response to a novel social encounter. Consistent with sleep/wake instability and reduced social encounter wakefulness, we find that noradrenergic and orexinergic wake neurons in the aged mice display reduced c-fos with enforced wakefulness and endoplasmic reticulum dyshomeostasis with reduced BiP, persistent p-PERK and nuclear translocation of CHOP. We further hypothesize that transgenic mice with reduced BiP, like aged mice, would display wake impairments correlated to decreased neuronal activity and increased ER stress. Data from 12 month old BiP (+/-) and wild-type littermates will also be presented.

HEAT SHOCK MOLECULAR CHAPERONES PLAY A VITAL ROLE IN THE PROTEASOMAL DEGRADATION OF THE IRF-1 TUMOR SUPPRESSOR PROTEIN

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Interferon regulatory factor 1 (IRF-1) is a tumor suppressor protein that plays a central role in DNA damage and antiviral responses. There is currently a dearth of information concerning the IRF-1 proteome and how its structure and interactome relates to its function. We recently demonstrated the association of IRF-1 with members of the molecular chaperone family including Hsp70 and Hsp90. Like other Hsp90 client proteins, treatment with inhibitors such as 17AAG induces the proteasomal degradation of IRF-1. Conversely, upregulation of Hsp90 results in the accumulation of transcriptionally active nuclear, rather than cytoplasmic IRF-1 protein. Hsp70, which binds to sites in the N- and C-terminus of IRF-1, mediates the effects of Hsp90. The C-terminal Hsp70 binding site on IRF-1 is a discrete Pro-Leu rich motif encompassing a co-signature LXXLL repressor motif. Mutation of the C-terminal Hsp70 binding motif is sufficient to render IRF-1 refractive to 17AAG induced degradation, suggesting that binding of Hsp70 plays a key role in IRF-1 regulation.

Although the E3-ubiquitin ligase CHIP (C-terminus of Hsc70 interacting protein) can form a complex with IRF-1 through its association with Hsp70, we have also identified a direct interaction between these two proteins that is sufficient to signal IRF-1 ubiquitination. The interaction between CHIP and an Arg-Lys-Ser rich domain that forms a novel multi-protein binding interface on IRF-1 (Mf2-domain) is required to form a high affinity interaction; however, the CHIP:IRF-1 interface is complex and also involves additional weaker points of contact. CHIP, together with ubiquitin E2 enzymes of the UbcH5 and UbcH6 families, can efficiently ubiquitinate IRF-1 in the absence of Hsp70. Furthermore, both full length IRF-1 and the isolated Mf2 domain pull down CHIP from cells in a stress regulated manner. Thus, upon exposure to heat stress or heavy metals, soluble IRF-1 protein levels decrease and it is found in complex with CHIP. Further, formation of the IRF-1:CHIP complex correlates with increased IRF-1 ubiquitination. Our data suggests that IRF-1 function is highly regulated through the concerted actions of members of the chaperone family and that CHIP is an E3-ubiquitin ligase for IRF-1 under some stress conditions.

CHAPERONING CELL-CELL INTERACTIONS: GRP94 CLIENTS GOVERN CELL-CELL INTERACTION AND CELL MORPHOGENESIS IN THE *DROSOPHILA* MIDGUT

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GRP94, the endoplasmic reticulum Hsp90, is a metazoan-restricted chaperone essential for early development in mammals, yet dispensable for mammalian cell viability. This dichotomy suggests that GRP94 is required for the functional expression of secretory and/or membrane proteins that enable cells to function in the context of tissues. To explore this hypothesis, we have identified the *Drosophila* ortholog of *GRP94*, *Gp93*, and report that *Gp93* is an essential gene in Drosophila. Loss of zygotic *Gp93* expression is late larval lethal and causes prominent defects in the larval midgut, the sole endoderm-derived larval tissue. *Gp93*-null larvae display pronounced defects in the midgut epithelium, most prominently in the copper cell region. The copper cell region contains copper cells, the *Drosophila* equivalent of mammalian parietal cells, in register with interstitial cells. In *Gp93*-null larvae, the copper cells display highly aberrant apical membrane morphology, reduced gut acidification function, defects in the septate junctions (paracellular cell-cell interaction sites), and depressed gut motility. The defects in copper cell structure/function accompanying loss of Gp93 expression resemble those reported for mutations in *labial*, an endodermal homeotic gene required for copper cell specification and morphogenesis, and α-spectrin, thus suggesting an essential role for Gp93 in the functional expression of secretory/integral membrane protein-encoding lab protein target genes and/or integral membrane protein(s) that interact with the spectrin cytoskeleton, and which serve critical roles in cell morphogenesis, intercellular interactions, and epithelial membrane specialization.

STRUCTURAL DYNAMICS OF THE HEXAMERIC DISAGGREGASE CLPB

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The bacterial AAA+ chaperone ClpB cooperates with the DnaK (Hsp70) chaperone system in the solubilization and refolding of aggregated proteins. DnaK acts at initial stages of the disaggregation reaction, enabling ClpB to extract single unfolded proteins from an aggregate via ATP driven threading through its axial pore. ClpB harbors an N-terminal domain of unknown function, two AAA domains and a ClpB-specific middle domain (M-domain) that is essential for protein disaggregation. While the basic principle of ClpB-mediated protein disaggregation has been analyzed, central aspects of the disaggregation reaction are still poorly understood including the organization and structural dynamics of the ClpB hexamer. While hexameric models of ClpB based on cryo electron microscopy have been reported, such models are different in various central aspects and also do not provide a structural rationale for the unique disaggregation activity of ClpB.
We analyzed the conformational changes occuring within ClpB upon oligomerization, ATP binding and ATP hydrolysis by proton-deuterium exchange experiments. We found that the M-domain showed dramatic structural changes upon ClpB oligomerization, suggesting that it is an integral part of hexameric ClpB assemblies. Upon ATP binding further structural changes are observed in the conserved motif 2 but not motif 1 of the M-domain, indicating that M-domain respond to nucleotides in a spatio-specific manner. Further analysis of ClpB variants harboring mutations in either AAA-1 or motif 2 of M-domain reveals an interdependent communication between both domains. The implications of these findings on the mechanism of protein disaggregation will be discussed.

FLUORESCENCE CORRELATION SPECTROSCOPY FOR THE STUDY OF ER STRESS SENSOR, IRE1α.

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IRE1, an ER-localized transmembrane protein, plays a central role in the unfolded protein response. Upon ER stress, IRE1 senses the accumulation of unfolded proteins in the ER, and transfers signal from the ER to the cytosol. Recent studies indicate that the luminal domain of yeast Ire1 senses the unfolded proteins via a two-step mechanism. In the first step, BiP dissociation allows yeast Ire1 to make stable self-associate (or cluster). In the second step, direct association of unfolded proteins with the luminal domain of clustered Ire1 leads to its activation on the cytosolic effector domain. However, it has been unclear whether a similar mechanism is applicable to mammalian IRE1α.

In this study, to elucidate the dynamic association mechanism of mammalian IRE1α, fluorescence correlation spectroscopy (FCS) was employed in living cells. FCS can analyse the association state of fluorescent molecules from diffusion measurement. This analysis revealed that upon ER stress IRE1α makes a transient and relatively small complex. Because the complex-formation was not detected in D123P mutant that is defective in self-association, this complex would represent the self-associate of IRE1α. Such a transient self-association in mammalian IRE1α is different from the stable cluster-formation of yeast Ire1. This implies the difference between the two sensors on the mechanism of ER stress sensing.

HSP27 LOCALIZES TO THE PENUMBRAL REGION OF ABETA(Aβ) RICH PLAQUES IN MOUSE MODELS OF ALZHEIMER'S DISEASE(AD)

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by neurofibrillary tangles, senile plaques, and cerebral amyloid angiopathy. Massive reactive gliosis with increased expression of the small heat shock proteins (sHsp) Hsp27 and αB-crystallin is found in association with diffused plaques. Strong association between sHsp immunoreactivity and severity of the AD-specific changes has been reported. However, the source of the normally intracellular chaperone has remained unclear. It has been suggested that it may accumulate passively after its release from dead or dying cells. Here we studied the expression pattern of Hsp27 and αB-crystallin in 2 different mouse models (APP PS1 double transgenic mice and Tg2576 transgenic mice). The transgenic mice models accumulate plaques but show no cell death and are useful in delineating between passive release of chaperones and an act of cellular defense against protein aggregation. Immunostaining confirmed the induction of Hsp27 in plaques ridden areas of brains in transgenic specimens but showed a low basal expression in corresponding age-matched non-transgenic specimens. Interestingly, Hsp27 immunoreactivity showed a halo like pattern around the plaques. Costaining of the sections for Hsp27 and markers for neurons and glial cells showed no clear correlation with NeuN positive neurons and GFAP microglia with induced Hsp27. Thus, our results suggest that elevation of Hsp27 around the plaques is an active phenomenon, which needs further work to determine the cellular source.

DOES THE ER CONTAIN DISTINCT SUBREGIONS TO SUPPORT BOTH OXIDATIVE FOLDING AND REDUCTIVE UNFOLDING?

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The endoplasmic reticulum (ER) is an organelle composed of a continuous membrane system in which apparently opposite processes, such as protein folding and unfolding, take place. How proteins can either be properly folded and after passing ER quality control be transported, or if they fail to do so, be unfolded and targeted for retrotranslocation is not currently understood, especially for non-glycosylated substrates, which are handled by the ER Hsp70 BiP and its associated co-chaperones. There is evidence for the existence of an ER quality control compartment, but whether it is a constitutive or a transient structure is not known. Additionally, whether non-glycosylated BiP substrates are targeted to such ER compartments has not been tested. Since it has been reported that some proteins involved in endoplasmic reticulum associated degradation (ERAD) are enriched in the smooth ER, we are investigating whether proteins involved in protein folding and those involved in protein unfolding and degradation segregate differentially to the rough or smooth ER. We are particularly interested in how the ER DnaJ (ERdj) proteins, which regulate BiP's ATPase activity as well as its interaction with substrates, localize within the ER. We are currently measuring the localization of several chaperones, co-chaperones as well as proteins involved in ERAD by subcellular fractionation and determining whether inducing ER stress changes their localization. In addition, we have observed an interaction between endogenous ERdj proteins and retrotranslocon components by co-immunoprecipitation, which is in agreement with a role for some ERdj proteins in the later stages of ERAD. These studies will help us determine whether BiP substrates are targeted to different ER subdomains for folding or unfolding and whether the ERdj proteins play a role in this process.

UPREGULATION OF PROANGIOGENIC FACTORS BY THE UNFOLDED PROTEIN RESPONSE

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Tumor cells encounter an environment that has a decreased supply of oxygen and nutrients and increased accumulation of acidic by-products, which impinge on the folding capacity of the endoplasmic reticulum resulting in an increased amount of unfolded proteins. Cancer cells adapt to this microenvironment by activating cellular stress pathways including the Unfolded Protein Response (UPR). The UPR is a complex transcriptional/translational adaptation that assists in decreasing the unfolded proteins accumulated in the ER by transiently inhibiting protein translation, upregulating expression of ER chaperones and increasing the degradative capacity of the cell. To characterize the UPR in a solid tumor model we performed microarray analysis in Daoy, a human medulloblastoma cell line treated with a conventional UPR inducer thapsigargin. Increased expression of the expected UPR targets was observed, as were several proangiogenic factors including VEGF, FGF2, angiogenin and IL-8. Using real time PCR, we confirmed that these transcripts were elevated in several rat and human cancer cell lines, as well as mouse embryonic fibroblasts. Gene ontology analyses of the microarray data revealed significant enrichment of genes associated with regulation of angiogenesis. 19 genes annotated to angiogenesis were positive regulators. Of these, 14 genes have a greater than 2 fold increase in expression on UPR activation. To better characterize the role of the UPR, we first focused on VEGF. Real-time PCR analyses revealed that the UPR increases VEGF transcription rate and in some cell lines induction of VEGF with a conventional UPR inducer was even greater than that achieved with hypoxia, a well known inducer of VEGF. XBP-1(S), an UPR-inducible transcription factor binds to two regions on the VEGF promoter, also, ATF4, another UPR-inducible transcription factor binds to a regulatory region in the VEGF gene. We have also shown that VEGF mRNA stability is increased in response to UPR activation via AMP and p38MAP kinases. These results indicate that the UPR plays a significant role in regulating VEGF expression and is likely to be important and have widespread implication in angiogenesis and tumor growth.

THERMOSENSORY NEURONS REGULATE PROTEIN FOLDING IN *C. ELEGANS* MODELS OF PROTEIN MISFOLDING DISEASE.

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The heat shock response is a highly conserved response of all cells to stress and protein misfolding and in isolated cells, is autonomously initiated by protein damage. We showed previously that within the metazoan *C. elegans* the heat shock response is cell non-autonomously regulated by the thermosensory neuronal circuitry. Although affecting only 2 of the 959 adult cells of *C. elegans*, mutations of the thermosensory (AFD) neurons alone were able to impair the transcriptional induction of heat shock proteins (HSPs) throughout the organism. Our studies suggested a model whereby neuronal signaling inhibited the cell autonomous response to heat shock allowing for the integration of the stress response throughout the organism. We are currently investigating how neuronal regulation influences protein folding homeostasis in *C. elegans*. We find that mutations in the thermosensory circuitry affect the aggregation of YFP-tagged polyQ proteins in a tissue specific manner. While theremosensory neurons do not significantly change polyQ aggregation within muscle cells, the aggregation of polyQ repeat containing polypeptides within the intestine is significantly rescued in the AFD mutants in an HSF-1-dependent manner. Our studies are thereby consistent with the prediction that neuronal signaling within an organism inhibits the cell autonomous response to protein misfolding, and integrates the cellular responses to stress across the organism. We are using genetic, cell biological and biochemical studies to understand how neuronal function regulates protein folding. We anticipate that these studies will shed light on why in neurodegenerative diseases such as Huntington's disease, cells often fail to induce this protective response, leading to cellular dysfunction and death.

A NUCLEAR-BASED QUALITY CONTROL MECHANISM FOR CYTOSOLIC PROTEINS

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Intracellular quality control systems monitor protein conformational states. Irreversibly misfolded proteins are cleared through specialized degradation pathways. Their importance is underscored by numerous pathologies caused by aberrant proteins. In the cytosol, where most proteins are synthesized, quality control remains poorly understood. Stress-inducible chaperones and the 26S proteasome are known mediators but how their activities are linked is unclear. To better understand quality control of cytosolic proteins, a panel of model misfolded substrates was analyzed in detail. Surprisingly, their degradation occurs not in the cytosol but in the nucleus. Degradation is dependent on the E3 ubiquitin ligase San1p, known previously to direct the turnover of damaged nuclear proteins. San1p, however, is not required for nuclear import of substrates. Instead, the Hsp70 family proteins Ssa1p and Ssa2p are needed for efficient import and degradation. These data reveal a new function of the nucleus as a compartment central to the quality control of cytosolic proteins.

THE PHYSIOLOGICAL UNFOLDED PROTEIN RESPONSE IN MAMMALS

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Unfolded protein response (UPR) is a key cellular defense mechanism associated with many human "conformational" diseases, including cancer, neurodegeneration, obesity and diabetes. In spite of its fundamental and clinical importance, little is known about UPR activation under physiological and pathological conditions. Here we have developed a sensitive Phos-tag-based system that allows for direct visualization of the early initiating events in UPR signaling in various *in vivo* conditions. Using this powerful tool, we characterized UPR activation in diverse tissues under various physiological and pathological conditions including fasting-refeeding, obesity, and plasma cell differentiation. Strikingly, our data challenges the notion that plasma cell differentiation and obesity are associated with overt UPR activation; rather, we reveal two novel modes of non-canonical UPR activation *in vivo*.

MTOR LINKS PROTEIN QUALITY AND QUANTITY CONTROL BY SENSING CHAPERONE AVAILABILITY

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A proper balance between synthesis, maturation and degradation of cellular proteins is crucial for cells to function properly. The costly process of protein synthesis is tightly coupled to energy status and nutrient levels by the mammalian target of rapamycin complex 1 (mTORC1), whereas the quality of newly synthesized polypeptides is largely maintained by molecular chaperone and ubiquitin-proteasome systems. Little is known about how cells sense both the quantity and quality of translational products to maintain intracellular homeostasis.

Here we demonstrate that cells are able to distinguish moderate reductions in the quality of intracellular proteins from severe protein misfolding by differentially regulating mTORC1 signaling. This order-of-severity sensing mechanism relies on molecular chaperones. Reduction of chaperone availability enhances mTORC1 signaling, whereas depletion of chaperone availability under severe stress conditions suppresses mTORC1 signaling. We found that molecular chaperones regulate mTORC1 assembly by coordinating with nutrients. Analogous to the chaperone-mediated regulation of heat shock factor 1 (HSF1), this remodeling mechanism enables mTORC1 to rapidly detect and respond to environmental cues, while sensing intracellular protein misfolding. The tight linkage between protein quality and quantity control provides a plausible mechanism about how protein misfolding fuels metabolic dyshomeostasis.

A SELECTION SYSTEM FOR THE IDENTIFICATION OF FACTORS INVOLVED IN PROTEIN FOLDING

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We have reported a method to link protein folding to antibiotic resistance (1). We found that heterologous proteins inserted into TEM-1 β-lactamase will generate high levels of penicillin resistance if the heterologous protein is well folded. In the absence of efficient folding, the inserted protein is prone to degradation, separating β-lactamase into two parts. As a result, the host shows low level of resistance to PenV.

We reasoned that one should be able to select for a folding enhancing host by plating cells on increasing concentration of β-lactam antibiotics. To minimize the possibility of obtaining mutations that merely increase intrinsic PenV resistance of the host (e.g. by altering cell wall permeability), a second system was developed utilizing another easily selectable phenotype: DsbA mediated resistance to cadmium. Similar to the β-lactamase system, a guest protein is inserted into a permissive site of DsbA. It is noted that those two selection systems are independent of each other. They only have the inserted protein in common; the two markers (β-lactamase and DsbA) and the principles of Penicilin and cadmium resistance are very different. Therefore, the host would be likely to show both increased PenV and $CdCl_2$ resistance only if the folding of the guest protein is enhanced.

Together, this dual system should be able to effectively select for a strain that improves the folding or stability of poorly folded proteins. A host strain was co-transformed with the β-lactamase plasmid and the DsbA plasmid containing a destabilized variant of the protein Im7. The resulting strain was mutagenized by EMS and selected for PenV resistance. Colonies with higher PenV resistance were screened for $CdCl_2$ resistance. A number of strains that increase both penicillin and cadmium resistance were obtained; their characteristics will be described.

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REGULATION OF THE BACTERIAL ENVELOPE-STRESS RESPONSE

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E. coli DegS is a protease, which can bind peptide signals exposed in misfolded outer-membrane porins (OMPs). This event activates cleavage of a transmembrane regulator (RseA) and ultimately transmits a signal that activates gene expression of stress genes in the cytoplasm. We will discuss the detailed allosteric mechanisms that are responsible for OMP-peptide and substrate-mediated activation of DegS. We also will discuss an independent regulatory mechanism, involving the RseB protein, that prevents cleavage of RseA unless a second stress signal is detected.

AAA PROTEASES AND MITOCHONDRIAL QUALITY CONTROL

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Mitochondria are multifunctional, reticulated organelles intimately embedded in cellular physiology. Dysfunction of mitochondria has severe cellular consequences and is linked to aging and neurodegeneration in human.

Various intraorganellar proteases limit mitochondrial damage and prevent the accumulation of misfolded polypeptides with possibly deleterious effects on cellular integrity. These include AAA proteases, conserved ATP-dependent proteases in the inner membrane, which exert versatile activities within mitochondria: 1) they conduct protein quality control surveillance and degrade misfolded and damaged inner membrane proteins; 2) they regulate mitochondrial biogenesis acting as processing peptidases; and 3) independent of their proteolytic functions, they mediate the ATP-dependent membrane dislocation of proteins. A mutational analysis of AAA domains of AAA protease subunits revealed that a coordinated ATP hydrolysis within AAA ring complexes is required for maximal force generation by these proteolytic machines.

On an organellar level, fusion and fission of mitochondria contributes to the maintenance of mitochondrial activities and allows the selective removal of damaged mitochondria. Increasing evidence points to an intimate link of AAA protease function to mitochondrial dynamics and the dynamin-like GTPase OPA1, mutated in dominant optic atrophy. OPA1 is required for mitochondrial fusion, regulates cristae morphogenesis, and protects cells against apoptosis. Stress conditions and mitochondrial dysfunction induce degradation of OPA1 isoforms and trigger the fragmentation of the mitochondrial network allowing their autophagic degradation.

MECHANISM OF PROTEIN DISAGGREGATION BY THE ClpB/DnaK BI-CHAPERONE SYSTEM

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The bacterial AAA+ protein ClpB solubilizes and reactivates aggregated proteins in cooperation with DnaK and DnaJ (KJ). KJ act at initial stages of the disaggregation process, enabling ClpB to extract unfolded polypeptides from aggregates via substrate threading through its central channel. We show that this activity of KJ is essential to target ClpB to aggregates both in vitro and in vivo. However, substrate unfolding and threading can be rendered independent of KJ if the ClpB N-domain is replaced by that of V. cholerae ClpV, thereby generating a chimera which disassembles VipAB tubules. This system now allows to analyse the correlation between threading and ATPase activity in absence of the DnaK ATPase.
To gain further insight into the disaggregation process we mapped the conformational changes within ClpB throughout the ATPase cycle by HD exchange and mutant analysis. Dramatic conformational changes were observed within the coiled coil (middle) domain upon oligomerisation, suggesting that it becomes integral part of the core of the hexameric ring structure. Upon ATP binding further structural changes occurred in particular in conserved motif 2 of the M-domain. Further analysis of ClpB variants harboring mutations in either motif 2 or the first ATPase domain reveals an interdependent communication between both domains.
Conserved residues of the middle domain including Y503 are essential for cooperation with KJ to mediate access of substrates to the central pore of ClpB. The mutant phenotypes observed in vitro correlate to aggregate-targeting/disaggregation defects in vivo.

DISSECTION OF THE ER-ASSOCIATED PROTEIN DEGRADATION BY *IN VIVO* PHOTOCROSSLINKING

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Misfolded proteins in the lumen of the endoplasmic reticulum (ER) are eliminated by a pathway called ER-associated protein degradation (ERAD) or retro-translocation. Luminal proteins selected for ERAD (ERAD-L substrates) are moved across the ER membrane, poly-ubiquitylated and released into the cytosol for proteasomal degradation. The mechanism of retro-translocation of ERAD-L substrates is largely unknown but requires a complex containing the ubiquitin ligase Hrd1p and three other membrane proteins (Hrd3p, Usa1p, and Der1p). We have developed an in vivo photo-crosslinking assay to identify the binding partners of an ERAD-L substrate along the ERAD pathway. We find that the substrate interacts initially with Hrd3p and the luminal lectin Yos9p and is then transferred to the ubiquitin ligase Hrd1p. Hrd1p-crosslinking requires an intact degradation signal and is affected by mutations in certain ERAD components. Usa1p is needed at a subsequent step in which Hrd1p undergoes oligomerization. Surprisingly, ubiquitination is required prior to the function of Usa1p. Our results define a pathway of ERAD-L and indicate that Hrd1p is its central component.

E3 UBIQUITIN LIGASES REQUIRED FOR CHAPERONE-DEPENDENT CYTOPLASMIC QUALITY CONTROL

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Selective destruction of misfolded proteins can occur by dedicated quality control QC ubiquitination pathways. The E3 ligases responsible for these pathways are tasked with recognition of structural features shared by many misfolded substrates. For such QC pathways to be useful biologically useful, they must allow the selective destruction of misfolded variants of normally-stable proteins, but be sufficiently general to mediate the degradation of many diverse proteins. Furthermore, they should alleviate the physiological effects of proteotoxic stress. It is known that chaperone-dependent ubiquitination of misfolded proteins occurs in the eukaryotic cytosol, but the E3s involved have remained unclear.

Using a variety of approaches in yeast, we have discovered that the ubiquitin ligases Ubr1 and San1 are required for destruction of misfolded proteins that originate in the cytosol. These E3s function to detect and eliminate a wide variety of cytoplasmic misfolded proteins, by providing parallel but independent pathways for QC ubiquitination. In both cases, Hsp70 chaperones and specific co-chaperones are required for successful ubiquitination of cytoplasmic substrates, and damaged proteins are selectively targeted. In each case, a role in cytoplasmic QC for these E3s is surprising. The San1 E3 is a nuclear ligase required for degradation of misfolded proteins in that compartment. Our date indicate that misfolded cytoplasmic substrates are delivered to the nucleus in a chaperone-dependent manner. The highly conserved Ubr1p ligase is traditionally known for its involvement in the N-end rule, by which proteins with specific destabilizing N-termini are subject to rapid degradation. The QC function of Ubr1p is distinct from the N-end rule function, and targets misfolded proteins in the cytosol. Phenotypic studies indicate that both Ubr1 and San1 can serve to eliminate proteotoxic stresses, and that the Urb1 ligase may be central to alleviation of cellular stresses that occur in the cytoplasmic compartment. We have developed an in vitro assay to directly study the action of these ligases on misfolded proteins, and the details of the chaperone requirement in each pathway. Our current model is that cytoplasmic proteins are subject to both biochemical and cell biological versions of chaperone-mediated triage, thus ensuring their degradation by a number of available degradation mechanisms both in and separate from their cytoplasmic place of origin.

CHAPERONE-ASSISTED SELECTIVE AUTOPHAGY (CASA) IS ESSENTIAL FOR MUSCLE MAINTENANCE

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How are biological structures maintained in a cellular environment that constantly threatens protein integrity? Here we elucidate proteostasis mechanisms affecting the Z-disk, a protein assembly essential for actin anchoring in striated muscles, which is subjected to mechanical, thermal and oxidative stress during contraction. Based on the characterization of the Drosophila melanogaster co-chaperone Starvin (Stv), we define a conserved chaperone machinery required for Z-disk maintenance. Instead of keeping Z-disk proteins in a folded conformation, this machinery facilitates the degradation of damaged components, such as filamin, through chaperone-assisted selective autophagy (CASA). Stv and its mammalian ortholog BAG-3 coordinate the activity of Hsc70 and the small heat shock protein HspB8 during disposal that is initiated by the chaperone-associated ubiquitin ligase CHIP and the autophagic ubiquitin adaptor p62. Impaired CASA results in Z-disk disintegration and progressive muscle weakness in flies, mice, and men. Our findings reveal the importance of chaperone-assisted degradation for the preservation of cellular structures, and identify muscle as a tissue that highly relies on an intact proteostasis network, thereby shedding light on diverse myopathies and aging.

SELECTIVE TARGETING OF MISFOLDED PROTEINS IN THE ENDOPLASMIC RETICULUM FOR LYSOSOMAL DEGRADATION

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Accumulation of misfolded proteins in the cell is associated with a variety of diseases including liver diseases, cystic fibrosis and neurodegeneration. Misfolded proteins in the endoplasmic reticulum (ER) are typically degraded by ER associated degradation (ERAD), which involves retrotranslocation of the protein from the ER to the cytosol and degradation by the proteasome. However, some misfolded proteins are poorly recognized by the ERAD system, necessitating their disposal by other pathway(s) that are not well characterized.

To understand these alternate pathways, we created a fluorescent protein-tagged misfolded variant of the prion protein that is efficiently targeted to the ER, but does not appear to undergo ERAD, which we call PrP^{misf}. Using time-lapse live cell imaging in combination with other FP-tagged markers, we have analyzed the fate of this misfolding PrP variant. We observe that in steady state conditions, PrP^{misf} is localized primarily to ER and can also be found in lysosomes. However upon treatment with ER-stressors, the entire ER-localized population of PrP^{misf} is synchronously segregated from ER-resident proteins and transported to lysosomes via the Golgi apparatus.

These findings establish a simple and robust system to study selective sorting and lysosomal targeting of misfolded ER-retained proteins. We are currently using this model to identify the key factors and molecular mechanisms involved in the selective recognition, segregation, and transport of misfolded proteins from the ER to lysosomes. We are addressing the role of ER-stress response in triggering this degradation pathway. The findings from this study may provide novel insights into the regulation of degradation pathways utilized by other disease-related proteins that transit the secretory pathway.

PARKIN IS A TARGET OF THE UNFOLDED PROTEIN RESPONSE AND PROTECTS CELLS FROM ER STRESS-INDUCED MITOCHONDRIAL DAMAGE AND CELL DEATH

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Endoplasmic reticulum (ER) stress has been implicated in the pathogenesis of several neurodegenerative diseases. In Parkinson's disease (PD), ER stress markers are significantly increased in postmortem brains, and neurotoxins used to induce the degeneration of dopaminergic neurons in vivo can trigger ER stress. Here we show that parkin, which is mutated in the majority of autosomal recessive parkinsonism, is transcriptionally regulated by the unfolded protein response (UPR) pathway and prevents ER stress-induced damage of mitochondria. Up-regulation of parkin under ER stress is mediated via the PERK/ATF4 branch of the UPR by binding of ATF4 to a specific site within the parkin promoter. In addition, we show that c-Jun can bind to the same site within the parkin promoter, but acts as a transcriptional repressor of parkin gene expression. Parkin-mediated protection of cells from ER stress-induced cell death is independent of the proteasome and does not decrease the level of ER stress. Instead, parkin can suppress mitochondrial dysfunction and damage induced by ER stress. Our study emphasizes an important role of ER and mitochondrial stress in the pathogenesis of PD and indicates that parkin plays a role in the interorganellar crosstalk between the ER and mitochondria to promote cell survival under stress.

A SURPRISING TWIST ON THE MITOCHONDRIAL UPR AND LONGEVITY

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Mitochondria have been the cornerstone of longevity research in yeast, worms, flies and rodents to connect rates of metabolism with longevity. The initial finding that mitochondrial reduction during a specific time in the worm's life cycle could be uncoupled from mitochondrial metabolic activity suggested that mitochondria might establish the rate of aging in a manner that is independent of previously anticipated modes, such as generation of reactive oxygen species. In our search for this signaling mechanism we report that key tissues are essential for establishing and maintaining the prolongevity cue from altered mitochondria. Additionally, we find that the mitochondrial unfolded protein response is essential and specific for the ETC longevity pathway. Finally, we find that mitochondrial perturbation in one tissue can be perceived and acted upon in distal tissues that have not undergone mitochondrial perturbation. This last finding indicates that mitochondrial stress in one cell can be communicated to unaffected cells in a multicellular organism.

ATOMIC STRUCTURES OF AMYLOID-LIKE FIBRILS AND OF TRUNCATED ALPHAA AND ALPHAB CRYSTALLINS

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To understand the structural basis of protein aggregation and of chaperone action in reversing aggregation, we have determined crystal structures for some 60 fibril-like microcrystals of segments of aggregating proteins, and also of small heat shock proteins of the alpha crystallin class. The fibril-like structures have common features, consisting of pairs of tightly interdigitating beta sheets, with a dry interface. Several fibril-forming segments crystallize in more than one polymorph, illuminating the atomic basis of amyloid polymorphism.

The alpha crystallins show unusual polymorphic interfaces, essentially encoding polydisperse intermolecular contacts. One interface is domain swapped, in two different ways. Another shows three distinct antiparallel beta interactions, with ratchet-like character. These polymorphic interfaces ensure polydisperse solutions of alpha-crystallin, preventing crystallization even at its high concentration in the eye lens, thereby preventing light scattering in the lens.

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TRANSTHYRETIN: SYSTEMIC AMYLOID PRECURSOR, NEURONAL AMYLOID INHIBITOR: A PROBLEM IN PROTEIN CHEMISTRY SOLVED BY ORGANISMAL BIOLOGY

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Mutant and wild type transthyretin (TTR) molecules are the amyloid precursors in the systemic amyloidoses Familial Amyloidotic Polyneuropathy and Cardiomyopathy and Senile Systemic (Cardiac) Amyloidosis. TTR transgenic models express many of the features of the respective diseases. The molecular mechanisms of TTR aggregation, fibril formation and cytoxicity for cultured target cells have been defined in detail as have the similar processes related to Aβ, the toxic protein in human Alzheimer's disease (AD). We previously reported that crossing a mouse strain over-expressing the wild type human transthyretin gene (that develops age dependent systemic deposition) with the well characterized APP23 transgenic model of human AD profoundly suppresses the neuropathologic and abnormal behavioral features seen in APP23 mice expressing only the human AD-associated transgene. Further the AD-like neurodegenerative disease is accelerated when the APP23 AD gene is expressed on the murine TTR knockout background. Surface plasmon resonance revealed that recombinant human and murine TTR interact with $A\beta_{1\text{-}40/1\text{-}42}$. Our recent data indicate that the majority of hippocampal and cortical neurons from human AD brains and APP23 mice stain with an antibody to TTR (not seen in control brains) indicating that those neurons contain TTR. We now report that primary neurons from APP23 mice transcribe 10 times as much TTR mRNA as do those from control WT B6 mice. They also synthesize and secrete TTR protein. Both characteristics are even more prominent in primary neurons from the APP23 x hTTR transgenic animals. Confocal images of primary neurons from mice expressing both the human AD gene and TTR show intracellular co-localization of TTR and Aβ or its precursors. Co-immunoprecipitation experiments of APP23 cortical brain extracts with anti-TTR and anti-Aβ antibodies show that the two proteins intimately interact *in vivo*. The data suggest that the biologically protective effect of TTR in the APP23 murine model of human AD is related to the direct interaction of TTR and some form of Aβ. We have also shown that TTR inhibits seeding of Aβ nuclei *in vitro* and the cytotoxicity produced by Aβ oligomers in tissue culture. The relevance of the interaction to disease pathogenesis is further suggested by the induction of TTR transcription in neurons in response to the introduction of the Aβ precursor by lentiviral infection. The data are consistent with the systemic amyloid precursor TTR serving a chaperone-like function for some form of Aβ as part of a transcriptionally regulated anti-amyloidogenic neuronal proteostatic response.

EVIDENCE ON THE ABILITY OF MOLECULAR CHAPERONES TO BIND AND NEUTRALIZE PREFORMED TOXIC OLIGOMERS AND MOLECULAR INSIGHT INTO THEIR MECHANISM OF ACTION

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The formation of protein misfolded oligomers is a constant challenge for any living organism and a causal event of many diverse human diseases. It is increasingly recognized that early oligomers, rather than mature fibrillar aggregates, are the pathogenic species in protein deposition diseases. We have designed protocols to form two types of oligomers from the HypF-N protein. The first is toxic when added to cultured cells and injected into rats' brains, whereas the second type is benign. We have also used established protocols to form toxic oligomers from the Aβ peptide associated with Alzheimer's disease. We have tested the effect of five molecular chaperones, namely human Hsp70, human αB-crystallin and the three known extracellular chaperones (human clusterin, aptoglobin and α2-macroglobulin) on the toxicity of pre-formed oligomers. All five chaperones were found to be effective in suppressing or decreasing remarkably the toxicity of both HypF-N and Aβ oligomers, whereas they were found to have no effect on the non-toxic HypF-N oligomers. Such an effect is chaperone-dependent as control proteins such as lysozyme and BSA were found to leave the toxicity of preformed oligomers unaffected. The suppression of the oligomers toxicity by the chaperones was monitored by all the measurables of cell viability. This means that while HypF-N toxic oligomers cause an entry of extracellular calcium into the cytosol, an increase of the cellular ROS levels, a permeabilization of the cell membrane to the oligomers and to the calcein probe and mitochondrial dysfunction, the five tested chaperones suppressed all these events. To gain molecular insight into the interaction between the chaperones and the pre-formed oligomers we have focused on HypF-N oligomers and αB-crystallin. We found that the ThT-binding of the preformed HypF-N oligomers was not affected by αB-crystallin, indicating that the oligomers are not disassembled by the chaperone. αB-crystallin did not change the pyrene fluorescence at three critical positions within preformed HypF-N oligomers (involved in the difference between toxic and non-toxic oligomers), indicating that the chaperone did not cause a structural reorganization of the oligomers. Analyses performed with SDS PAGE and intrinsic fluorescence of the soluble and insoluble fractions indicated binding between the HypF-N oligomers and αB-crystallin, suggesting that the chaperone suppresses toxicity by shielding the reactive surfaces on the oligomers. Overall, the data present evidence on the ability of chaperones to bind and neutralize preformed toxic oligomers and offer molecular insight into their mechanism of action.

COMPARTMENTALIZATION OF AGGREGATED PROTEINS DETERMINES THEIR TOXICITY

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Neurodegenerative diseases constitute a class of illnesses marked by pathological protein aggregation in the neurons of affected individuals. Although these disorders are invariably characterized by the death of highly specific sub-populations of neurons, protein aggregation occurs in all cells, which indicates that disease states arise only when aggregates manifest a toxic gain-of-function in particular cellular environments. The precise determinants of this toxic effect are not well defined. The starting point for uncovering the origins of neurodegenerative disease pathology must therefore be a thorough understanding of the protein folding quality control system that eukaryotic cells employ to mitigate the potentially toxic consequences of aggregation. Here we show that an essential factor in preventing aggregate-induced toxicity in human cells is the proper compartmentalization of soluble and insoluble aggregates. We find that the toxicity of ALS-linked aggregates of mutant superoxide dismutase 1 (SOD1G93A) and the enhanced toxicity of a mutant glutamine-expanded Huntingtin is due to their localization in the JUNQ, a central cellular quality control compartment that is normally a destination for soluble misfolded proteins to be refolded or degraded. By localizing to the JUNQ, these poorly soluble proteins retain Hsp70, an essential chaperone, thereby impairing the solubility of other JUNQ substrates. Enhancing the sequestration of toxic SOD1G93A in an insoluble compartment, the IPOD, alleviates the harmful effects of SOD1 aggregation on cell viability. Our results underline the importance of spatial organization of cellular quality control in avoiding unproductive and harmful interactions between toxic aggregates and essential chaperones. We propose that diverting toxic aggregates from the JUNQ to the IPOD sequestration compartment as a potential strategy for alleviating the toxicity underlying neurodegenerative diseases.

A COMMON MECHANISM FOR AGGREGATION OF ALS-CAUSING SUPEROXIDE DISMUTASE-1 MUTANTS

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The copper-zinc superoxide dismutase-1 (SOD1) is a highly structured protein and, a priori, one of the least likely proteins to be involved in a misfolding disease. However, more than 140, mostly missense, mutations in the SOD1 gene cause aggregation of the affected protein in familial forms of amyotrophic lateral sclerosis (ALS). The remarkable diversity of the effects of these mutations on SOD1 properties has suggested that they promote aggregation by a variety of mechanisms. Experimental assessment of surface hydrophobicity using a sensitive fluorescent-based assay, revealed that diverse ALS-causing mutations provoke SOD1 amyloidogenesis by increasing their propensity to expose hydrophobic surfaces. These findings could not be anticipated from analysis of the amino acid sequence. Our results uncover the biochemical nature of the misfolded amyloidogenic precursor and reconcile the seemingly diverse effects of ALS-causing mutations into a unifying mechanism. Furthermore, the method we describe here will be useful for investigating and interfering with aggregation of various proteins and thereby provide insight into the molecular mechanisms underlying many neurodegenerative diseases.

INTERACTOME ANALYSIS OF DE NOVO-DESIGNED B-AGGREGATING POLYPEPTIDES IN MAMMALIAN CELLS

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Protein misfolding and aggregation have the potential to seriously damage living cells, and can be involved in neurodegenerative diseases such as Alzheimer's and Huntington's. Several artificially designed peptides with alternating hydrophobic and hydrophilic residues have previously been described. These β-peptides form stable amyloid structures and are toxic to cells. This system has allowed us to study amyloid aggregation in cells without any interference from loss of function effects that complicate studies on naturally occurring amyloidogenic proteins. It has long been postulated that amyloids exert their toxicity at least in part through aberrant interactions with other components of the proteome.

We have tested this possibility by expressing myc-tagged versions of such β-peptides in HEK293T cells followed by immunoprecipitation and analysis of the interacting partners using quantitative mass spectrometry. Using this approach, we have found more than 100 interactors of a toxic β-peptide. Analysis of the physical properties of these interactors shows that they contain significantly more predicted regions of intrinsic disorder than the average protein found in the lysate, suggesting that the β-peptides are able to target a portion of the proteome which is already in a partially unfolded state.

We have furthermore used a pulsed-SILAC technique to show that the β-peptides preferentially interact with proteins early during their lifetimes, perhaps before they have completed correct folding. There is, however, a subset of proteins which interact with the β-peptides later during their lifetimes, presumably after they have folded. These proteins are particularly enriched in predicted disorder, emphasizing the importance of disorder for binding to amyloid. We also show that chaperones, particularly Hsp110, interact strongly with the β-peptides early during the aggregation process, and in amounts correlating to the toxicity of the β-peptide.

Taken together, these results suggest that amyloidogenic peptides interact with an unstable portion of the proteome; either with proteins which have yet to properly fold, or with proteins that contain intrinsically disordered regions. This second portion of the interactome also tends to be involved in a large number of cellular interactions, and contains components of many important regulatory pathways. Loss of function of such a segment of the proteome could lead to the collapse of key cellular functions and thus to severe toxicity.

CYTOSOLIC STRESS RESPONSE PROTECTS AGAINST TOXICITY OF INFECTIOUS AND MUTANT PRION PROTEINS

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A common pathological denominator of different neurodegenerative disorders, such as Alzheimer's disease or Prion diseases, is the formation of aberrant protein conformers and the occurrence of pathognomonic proteinaceous deposits. However, there is an ongoing debate about the nature of the toxic protein species and how they selectively damage neuronal populations. Interestingly a previous study in transgenic mice revealed that the heat shock factor (HSF) 1, the major transcriptonal regulator of the cytosolic heat shock response (HSR), can modulate neuronal survival in a mouse model of infectious prion diseases (Steele et al, PNAS, 105, 2008). We have now established a novel cell culture model to specifically study the impact of infectious and mutant prion proteins on the regulation of the cytosolic HSR and to analyze whether the HSR might be protective against toxic effects of aberrant PrP conformers. Neither PrP^{Sc} nor neurotoxic PrP mutants induced the HSR, however, they lowered the threshold for an induction of the HSR and sensitized cells to stress-induced cell death. Moreover, expression of an constitutively active HSF1 or the overexpression of cytosolic HSP70 alleviated the toxic effects of infectious PrP^{Sc} and neurotoxic PrP mutant. These data emphazise a protective role of the HSR in neurodegenerative disorders and suggest that infectious and mutant prion proteins might activate similar intracellular signaling pathways.

GENERATION AND CHARACTERIZATION OF LUCIFERASE MUTANTS AS SENSORS OF PROTEOME STRESS

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Since proteins are responsible for most biological functions, the physiology of a cell, tissue, organ or organism depends on the proper quality of the proteome. Proteins are protected against misfolding by molecular chaperones, which constitute a central element of the cellular machinery of protein quality control. Terminally misfolded proteins are degraded by the proteolytic arm of quality control. The proper balancing of folding, refolding and degradation is critical in maintaining protein homeostasis (or proteostasis). The disturbance of proteostasis is associated with numerous diseases, including neurodegenerative conditions such as Huntington's and Parkinson's disease. To measure and compare the status of the protein quality control system in different conditions, we generated a series of proteostasis sensors. These proteins are increasingly destabilized versions of firefly luciferase, a protein known to require molecular chaperones for folding and refolding. The mutant proteins were employed to compare the folding capacity of different mammalian cell lines. We showed further that the gradual collapse of the proteostasis network during aging results in the aggregation of GFP-tagged versions of the sensor proteins in C. elegans. In summary, we have generated a set of widely applicable proteostasis sensors from a single reporter protein, which can be used to study the dysregulation of proteostasis in different disease states.

STI1 COORDINATION OF HSP70 AND HSP90 IS CRITICAL FOR CURING OF *S. CEREVISIAE* [*PSI*$^{+}$] PRIONS BY HSP104

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Propagation of all known yeast prions requires the disaggregating activity of the hexameric AAA+ chaperone Hsp104. Intriguingly, overproduction of Hsp104 "cures" cells of the [*PSI*$^{+}$] prion, while having little to no effect on other yeast prions. Previous studies demonstrated that Hsp104's ability to cure [*PSI*$^{+}$] is independent of activities related to thermotolerance and disaggregation, as the amino-terminal domain of Hsp104 is required for curing but dispensable for disaggregation. Also, earlier evidence suggests that the Hsp70 mutant Ssa1-21 impairs [*PSI*$^{+}$] by a similar mechanism. Here we confirm this link by finding deletion of *STI1*, which suppress Ssa1-21 impairment of [*PSI*$^{+}$], also blocks Hsp104 curing of [*PSI*$^{+}$]. Hsp104's TPR interaction motif was dispensable for curing, but cells expressing Sti1 mutants defective in Hsp70 or Hsp90 interaction cured less efficiently and the Hsp90 inhibitor radicicol abolished curing. These results imply Sti1 acts in curing through Hsp70 and Hsp90 interactions, possibly by coordination of their activities. Accordingly, strains lacking constitutive or inducible Hsp90 isoforms cured at reduced rates. We confirm an earlier finding that elevating free ubiquitin enhances curing, but it did not overcome inhibition of curing caused by Hsp90 defects, suggesting Hsp90 is important for the contribution of ubiquitin to curing. We also find curing associated with cell division. Our findings identify Sti1 and Hsp90 as crucial for efficient curing of [*PSI*$^{+}$] by overexpressed Hsp104, point compellingly to a role for Hsp70 in prion curing, and provide evidence supporting the earlier suggestion that destruction of prions by protein disaggregation does not adequately explain the curing.

DIFFERENTIAL IMPACT OF TETRATRICOPEPTIDE REPEAT PROTEINS ON THE STEROID HORMONE RECEPTORS

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Tetratricopeptide repeat (TPR) motif containing co-chaperones of the chaperone Hsp90 are considered control modules that govern activity and specificity of this central folding platform. Here, we compared the influence of the TPR proteins FK506 binding proteins 51 and 52, protein phosphatase-5, C-terminus of Hsp70 interacting protein, cyclophillin 40, hepatitis-virus-B X-associated protein-2, and tetratricopeptide repeat protein-2 on all six steroid hormone receptors in a homogeneous mammalian cell system. To be able to assess each cofactor's effect on the transcriptional activity of on each steroid receptor we employed transient transfection in a reporter gene assay. In addition, we evaluated the interactions of the TPR proteins with the receptors and components of the hsp90 chaperone heterocomplex by coimmunoprecipitation. In the functional assays, corticosteroid and progesterone receptors displayed the most sensitive and distinct reaction to the TPR proteins. Androgen receptor's activity was moderately impaired by most cofactors, whereas the Estrogen receptors' activity was impaired by most cofactors only to a minor degree. Second, interaction studies revealed that the strongly receptor-interacting co-chaperones were all among the inhibitory proteins. Intriguingly, the TPR-proteins also differentially co-precipitated the heterochaperone complex components Hsp90, Hsp70, and p23, pointing to differences in their modes of action. Thus, the results of this comprehensive study provide important insight into chaperoning of diverse client proteins via the combinatorial action of (co)-chaperones.

CELLULAR PRION PROTEIN MEDIATES TOXIC SIGNALING OF Aβ OLIGOMERS

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Formation of aberrant protein conformers is a common pathological denominator of different neurodegenerative diseases, such as Alzheimer's disease or prion diseases. Increasing evidence indicates that soluble oligomers are associated with early pathological alterations in these entities. Moreover, oligomeric assemblies of different disease-associated proteins may share common structural features. However, little is known about possible co-factors and signaling pathways implicated in neuronal cell death. Using a novel cell culture assay we show that expression of the cellular prion protein (PrP^{C}) sensitizes cells to toxic effects of soluble amyloid-β (Aβ) oligomers, secreted by transfected cells or prepared from synthetic peptides. Both the unstructured N-terminal domain and the C-terminal glycosylphosphatidylinositol anchor of PrP^{C} are required to mediate toxic effects of Aβ. Interestingly, the same domains of PrP^{C} are required to mediate toxicity induced by the scrapie prion protein (PrP^{Sc}). Moreover, adverse effects of both Aβ and PrP^{Sc} could be blocked by an NMDA receptor antagonist and an oligomer-specific antibody. Our study emphasizes that PrP^{C} is involved in toxic signaling of pathogenic protein conformers associated with different neurodegenerative diseases.

SINGLE-CELL STUDIES OF *SACCHAROMYCES CEREVISIAE*: ANALYSIS QUALITY CONTROL MECHANISMS OF THE SECRETORY PATHWAY

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Cellular quality control processes such as ER associated degradation (ERAD) and the Unfolded Protein Response (UPR) occur at varying timescales and impact protein concentrations and intracellular localization[1-4]. We investigated the UPR during heterologous protein expression in *S. cerevisiae* by evaluating single-cell level protein expression, trafficking, and dynamics in vivo using fluorescent protein (FP) variants and confocal microscopy. Our experimental system includes live cell imaging of ER luminal (BiP, PDI, Scj1) and membrane proteins (Sec61, Doa10, Hrd1) expressed as FP fusions under the endogenous promoters. Using this system, we can continuously monitor protein trafficking during protein translocation, protein folding/maturation, and ERAD in order to: analyze localization effects of proteins involved in ERAD; examine organelle dynamics under various environmental conditions to initiate ERQC; and confirm the existence of cellular variability during UPR activation. In addition, we have quantified the expression, localization, and activity of heterologous proteins (i.e. G-protein coupled receptors (GPCRs)) and identified specific mechanisms that play a role in trafficking of GPCRs. By understanding these interactions and verifying the results with Western analysis and pulse-chase, we have been able improve GPCR trafficking and localization in *S. cerevisiae.*

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AGGREGATION OF P53 AS A MECHANISM OF TUMOUR SUPPRESSOR INACTIVATION AND ONCOGENIC GAIN-OF-FUNCTION

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It remains largely unexplained why the most aggressive p53 missense mutations possess dominant-negative activity and why they often display gain of oncogenic activity. Since p53 is active as a tetramer, the current view is that dominant-negative effects arise from the formation of inactive mixed tetramers that incorporate both mutant and wild type p53. Gain-of-function is believed to result from a poorly defined conformational perturbation of the wild type by mutant p53 within the tetramer. Here we show, both in cultured cells and in murine and human tumours, that the dominant-negative activity of p53 mutants is not tetramer-dependent but instead results directly from the increased aggregation propensity of these mutants. Upon aggregation, mutant p53 is able to induce misfolding and aggregation of wild-type p53 into cellular inclusions. Further, we show that aggregation of p53 induces heat-shock response and is responsible for the inactivation by co-aggregation of its paralogues p63 and p73. Suppression of the aggregation propensity in mutant p53 completely abrogates these gain-of-function effects and fully restores wild type activity. Finally, using existing literature we classify patient data by the propensity of the mutant to form aggregates and find a significant difference in loss-of-heterozygosity and clinical outcome. Aggregating p53 mutants associate with poorer therapeutic response and long-term survival and tumor progression is also less dependent on loss-of-heterozygosity. The poorer clinical outcome of patients with aggregating mutations suggests that inhibition of p53 aggregation might be a valuable therapeutic strategy.

A DYNAMIC PICTURE OF HSP90 ACTIVE CYCLE

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The conformational changes throughout the Hsp90 ATPase cycle are controversial. We set out to obtain a dynamic picture of Hsp90 throughout its active cycle and upon binding of co-factors. NMR spectroscopy is the method of choice for studying protein dynamics, but we had to overcome the problem that Hsp90's molecular weight is one order of magnitude above the traditional NMR size limit.

We made human Hsp90beta accessible for an NMR approach by combining advanced NMR technology and an assignment strategy based on its isolated domains. We focussed on Isoleucine side chains, which are ubiquitously present all over Hsp90 and result in higher intensity than the usual amide spectra. This allowed us to annotate key elements in Hsp90 such as the ATP binding pocket, the ATP lid or the N-terminal dimerisation helix and to monitor their changes in the full-length protein by NMR.

We mapped conformational changes in Hsp90 upon binding of nucleotide, drugs and co-factors. This resulted in a comprehensive picture of Hsp90 throughout its active cycle. The results will be presented.

APJ1: SPECIALIZED CYTOSOLIC/NUCLEAR J PROTEIN INVOLVED IN SUMO-TARGETED UBIQUITIN MEDIATED PROTEOLYSIS IN YEAST

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J proteins are obligate cochaperones of Hsp70s. Each contains a highly conserved J domain that plays a critical role in the stimulation of Hsp70's ATPase activity, thus stabilizing their interactions with client proteins. However, regions outside the J domain are structurally diverse. We have been using *Saccharomyces cerevisiae* as a model system to understand the evolution and specialization of J-protein function. The yeast cytosol/nucleus contains three J-proteins (Ydj1, Apj1 and Xdj1) having a domain structure very similar to that of DnaJ (often called Class I J-proteins). The absence of only one of these three, the very abundant Ydj1, causes an obvious growth defect. To understand J-protein specificity we have sought to identify the *in vivo* function of Apj1.

Our data supports the hypothesis that Apj1 is involved in the SUMO (Small Ubiquitin Like Modifier) pathway, specifically in SUMO-targeted ubiquitin degradation, which has dedicated E3 ubiquitin ligases that recognize SUMO modified substrates and target them for ubiquitin mediated proteolysis. First, a deletion of *APJ1* has a synthetic growth defect when combined with a deletion of either *SLX5* or *SLX8* (*S*ynthetic *L*ethal of [*X*] unknown function), which encode known subunits of the Slx5-Slx8 SUMO-targeted ubiquitin ligase (STUbL) complex. Secondly, SUMO-conjugated proteins accumulate in *apj1Δ slx5Δ* cells. Thirdly, a known target of the Slx5:Slx8 STUbL complex, Mot1 (Modifier of Transcription), is affected in *apj1*Δ cells, as in *slx5*Δ cells. Together, our preliminary results suggest that Apj1 co-operates with the Slx5:Slx8 STUbL complex in recognition and/or degradation of proteins in yeast and thus has a role in protein quality control. Interestingly, no genetic interaction was observed between *slx5*Δ and a deletion of either *YDJ1* or *XDJ1*, suggesting that Apj1 performs a specialized function in this pathway, not carried out by other Class I J proteins. Experiments are in progress to determine the basis of this specificity of Apj1 function.

THE ROLE OF ER STRESS RESPONSE IN ASTROCYTE DIFFERENTIATION

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The endoplasmic reticulum (ER) stress response is a defense system for dealing with the accumulation of unfolded proteins in the ER lumen. This system is termed the unfolded protein response (UPR). In mammalian cells, the three major ER stress transducers of the UPR are PERK, IRE1, and ATF6. These transducers sense unfolded proteins in the ER lumen and transduce signals to the nucleus. We identified Old Astrocyte Specifically Induced Substance (OASIS) as a novel ER stress transducer, which is especially expressed in astrocytes in central nervous system, and involved in the ER stress response. OASIS has the transmembrane domain that allows it to associate with the ER, and it also possess the transcription activation domain and the bZIP domain. However, the function of OASIS in astrocytes has remained unclear. We generated *Oasis* deficient mice to examine the roles of OASIS in astrocytes. Although the primary cultured astrocytes prepared from E18.5 *Oasis* deficient mice did not show the significant morphological differences, the BrdU corporation assay showed that the proliferation of *Oasis* deficient astrocytes were progressed compared with wild type astrocytes. We examined the expression levels of astrocyte differentiation markers using the neural precursor cells (NPCs) prepared from E14.5 mice. The expression levels of *Gfap* and *S100β* which are the markers of mature astrocytes, were decreased in *Oasis* deficient cells during astrocyte differentiation. In E18.5 mice cerebral cortex, the number of GFAP and S100β positive cells was decreased in *Oasis* deficient mice. In contrast, the number of Nestin and bLBP, which are the markers of immature astrocytes, positive cells was increased in *Oasis* deficient mice. Taken together, the maturation from NPCs to astrocytes was inhibited, and immature astrocytes, which are exhibited higher proliferation potential, were increased in *Oasis* deficient mice.

THE E2 UBE2W AND THE DEUBIQUITINATING ENZYME ATAXIN-3 REGULATE THE UBIQUITIN LIGASE CHIP

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The chaperone and ubiquitin proteasome systems comprise the two major pathways to prevent the accumulation of misfolded proteins. The ubiquitin ligase (E3) CHIP sits at the interface of these pathways and is a critical component of the cellular response to proteotoxic stress. The mechanisms regulating the activity of CHIP, however, remain poorly understood. Here we describe a mechanism for the activation and inactivation of CHIP that relies on the addition and removal of ubiquitin to CHIP. We identify a novel ubiquitin conjugating enzyme (E2), Ube2w, that monoubiquitinates CHIP, a modification that is essential for CHIP function in cells. We also describe a novel mechanism that couples the completion of substrate ubiquitination with the inactivation of CHIP: We show that the deubiquitinating enzyme (DUB) ataxin-3, limits the length of polyubiquitin chains added to CHIP substrates and deubiquitinates CHIP in response to completion of substrate ubiquitination. Together these data describe a novel regulatory mechanism that regulate CHIP function and provide new insight into the functionality of DUB/E3 pairs.

EXPLORING THE SEQUENCE DETERMINANTS OF AMYLOID STRUCTURE USING POSITION-SPECIFIC SCORING MATRICES

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Protein aggregation results in beta-sheet–like assemblies that adopt either a variety of amorphous morphologies or ordered amyloid-like structures. These differences in structure also reflect biological differences; amyloid and amorphous beta-aggregates have different chaperone affinities, accumulate in different cellular locations and are degraded by different mechanisms. Further, amyloid function depends entirely on a high intrinsic degree of order. Here we experimentally explored the sequence space of amyloid hexapeptides and used the derived data to build Waltz, a web-based tool that uses a position-specific scoring matrix to determine amyloid-forming sequences. Waltz allows users to identify and better distinguish between amyloid sequences and amorphous beta-aggregates and allowed us to identify amyloid-forming regions in functional amyloids.

CONFORMATIONAL FLEXIBILITY OF THE GRP94 LID REVEALED BY STRUCTURES OF INHIBITOR-PROTEIN COMPLEXES

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GRP94, the endoplasmic reticulum hsp90, participates in the conformational maturation of proteins destined for surface display or export, including all of the Toll-like receptors, IgGs, insulin-like growth factors, and some integrins. Despite having over 50% sequence identity with cytoplasmic Hsp90 and similar structures, the two paralogs have not been shown to complement each other in vivo, suggesting that mechanistic differences between the two exist. We have shown earlier that the N-terminal regulatory domain of GRP94 contains a unique ligand-dependent switch that leads to an opening, rather than a closing, of the “lid” elements when nucleotides occupy the ATP binding pocket. This “extended open” conformation appears to be incompatible with both the “closed” lid and the formation of the N-terminal dimer interface that were observed upon ATP binding to Hsp90. To further probe the conformational states available to GRP94 and its mechanistically distinct lid, we have now determined the structure of the N-terminal domain of GRP94 in complex with PUH54, a novel inhibitor that binds with high affinity to GRP94 but only weakly to cytoplasmic Hsp90. The structure of the GRP94-PUH54 complex reveals a novel lid state for GRP94 that, for the first time, places it on a trajectory towards a conformation that closes over the ATP binding pocket. This conformation has implications both for the mechanistic cycle of GRP94 and also for the design of specific inhibitors for GRP94.

THE ROLE OF ADIPOCYTE XBP1 IN OBESITY

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Endoplasmic reticulum (ER) stress has been reported to be present in liver and adipose tissue of obese mice and suggested to link obesity to insulin resistance and type 2 diabetes. To alleviate the stress in ER and maintain its homeostasis, the unfolded protein response (UPR) is considered to be activated. The haploinsufficiency of X-box binding protein 1 (XBP1), a key transcription factor of UPR, resulted in impaired insulin signaling. However, the tissue-specific function of XBP1 in obesity hasn't been investigated. Here we will present the data for these studies.

BSD2 - A REDOX-REGULATED CHAPERONE DEDICATED TO RUBISCO

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Expression of Ribulose 1,5 bisphosphate carboxilase/oxygenase (Rubisco) is tightly regulated by light-induced redox changes. We describe a gene-specific chaperone that is associated with nascent synthesis of the large subunit (LSU) polypeptide in a redox-sensitive manner. In land plants and green algae, Rubisco holoenzyme consists of eight large and eight small subunits (L8S8). We previously showed that oxidative stress leads to a dramatic arrest in the synthesis of its LSU, and a complete cessation of holoenzyme assembly. In the absence of assembly partners, the free small subunits (SSU) are therefore rapidly degraded. Interestingly, the N-terminus of Rubisco LSU is structured as a hidden RNA Recognition Motif (RRM), since it is buried and masked in the mature assembled protein. However, oxidizing conditions induce conformational changes that lead to exposure of this domain, promoting its RNA binding activity and causing ribosome stalling. The LSU-encoding *rbcL* transcript remains associated with the ribosomes, but shifts from heavy to light polysomes, as well as to monosomes, supporting that regulation occurs during translation elongation. We propose that during nascent synthesis of Rubisco LSU chains, the growing chain interacts with a dedicated chaperone that blocks the RNA binding activity of the N-terminal RRM domain, until assembly takes place. This novel regulatory mechanism is ancient and conserved throughout photosynthetic organisms, as it is also observed in land plants and in purple bacteria (*Rhodospirillum rubrum*), that express the primitive form II of Rubisco. This form consists of only two LSU chains and lacks the SSU. We propose that under oxidizing conditions the nascent LSU chains fail to interact with their dedicated chaperones, resulting in exposure of the RRM that binds any RNA in its vicinity. A novel DnaJ-like protein that is required for expression of Rubisco was identified in the chloroplasts of maize bundle sheath cells (BSD2). The partial similarity with DnaJ is by virtue of its four zinc finger domains. We identified the BSD2 homolog from the green alga *Chlamydomonas reinhardtii* and show that under normal conditions multimers of this protein are associated with *rbcL* loaded polysomes. Exposure to oxidizing conditions, or introducing a nonsense mutation in the *rbcL* coding region change its polysomal distribution, providing support to the association of BSD2 with nascent LSU chains. Altogether we describe a novel mechanism for chaperone-assisted control of Rubisco expression.

FRUIT RIPENING PROGRAM IS CHARACTERIZED BY DOWN-REGULATION OF SMALL HEAT SHOCK PROTEIN AND CYCLOPHILIN CHAPERONE GENES

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Protein synthesis is assisted and sustained in living cells by a number of factors including heat shock protein (HSP) and cyclophilin (CyP) chaperones. These molecular chaperones bind to nascent as well as mature proteins. The discovery of HSPs was realized through their identification as immediate responsive genes/proteins to heat stress, and later shown to bind denatured or unfolded proteins upon heat stress to protect structural conformation and prevent irreversible aggregation of specific proteins. The program of ripening (senescence) is the terminal stage of fruit development, which, in tomato, is associated with a ~10-fold reduction in de novo protein synthesis. However, during the initial stages of ripening the HSP and CyP transcripts persist, and are undetected thereafter. We are exploring the role(s) of these chaperones in fruit development and ripening, and during abiotic stresses. With this aim in mind, we have created novel transgenic tomato fruit that have longer life and are relatively better adept at withstanding heat shock stress. One of these transgenic plants overexpresses a yeast S-adenosylmethionine decarboxylase (SAMDC) gene in a ripening-specific manner, and accumulates higher polyamines i.e. spermidine and spermine. The latter protect plants against abiotic stresses, and enable constitutive and sustained increase in transcripts of small HSPs and CyP during ripening. We are analyzing the tomato sHSP17.6 gene promoter and mRNA sequence for the presence of cis acting elements. Specific heat shock elements (HSEs) were found in the sHSP17.6 promoter region as well as downstream in mRNA coding sequence. Also apparent are DNA-binding sites for transcription factors such as WRKY, MYB and CBF. We are experimentally testing direct and indirect interactions between HSEs, stress related transcription factors and polyamine-responsive elements (PREs). These studies should throw further light on our understanding of crosstalks among transcriptional factors regulating fruit development and ripening and, in particular, the role of chaperone genes.

RIBOSOME-ASSOCIATED PEROXIREDOXINS SUPPRESS OXIDATIVE-STRESS INDUCED *DE NOVO* FORMATION OF THE [*PSI*+] PRION IN YEAST

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Peroxiredoxins are ubiquitous antioxidants which protect cells against oxidative stress. We show that the yeast Tsa1 and Tsa2 peroxiredoxins co-localize to ribosomes where they function to protect the Sup35 translation termination factor against oxidative stress-induced formation of its heritable [*PSI*+] prion conformation. In a *tsa1 tsa2* [*psi*-] [*PIN*+] strain, the frequency with which the [*PSI*+] prion forms *de novo* is significantly elevated. The Tsa1 and Tsa2 peroxiredoxins, like other 2-Cys peroxiredoxins, have dual activities as peroxidases and chaperones, and we show that the peroxidase activity is required to suppress spontaneous *de novo* [*PSI*+] prion formation. Molecular oxygen is required for [*PSI*+] prion formation since growth under anaerobic conditions prevents prion formation in the *tsa1 tsa2* mutant. Conversely, oxidative stress conditions induced by exposure to hydrogen peroxide elevates the rate of *de novo* [*PSI*+] prion formation leading to increased suppression of all three termination codons in the *tsa1 tsa2* mutant. Altered translational fidelity in [*PSI*+] strains has been suggested to provide a mechanism which promotes genetic variation and phenotypic diversity. In agreement with this idea, we find that prion formation provides yeast cells with an adaptive advantage under oxidative stress conditions, since elimination of the [*PSI*+] prion from *tsa1 tsa2* mutants renders the resulting [*psi*-] [*pin*-] cells hypersensitive to hydrogen peroxide. These data support a model where peroxiredoxins function to protect the ribosomal machinery against oxidative damage, but when these systems become overwhelmed, [*PSI*+] prion formation provides a mechanism for uncovering genetic traits which aid survival during oxidative stress conditions. We are currently investigating the mechanism of [*PSI*+] prion formation in the *tsa1 tsa2* mutant and will present our recent data describing the role of oxidative stress in prion formation.

SLOWING BACTERIAL TRANSLATION SPEED ENHANCES EUKARYOTIC PROTEIN FOLDING EFFICIENCY

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The mechanisms for *de novo* protein folding differ significantly between bacteria and eukaryotes, as evidenced by the often observed poor yields of native eukaryotic proteins upon recombinant production in bacterial systems. Polypeptide synthesis rates are faster in bacteria than in eukaryotes, but the effects of general variations in translation rates on protein folding efficiency have remained largely unexplored. By employing *E. coli* cells with mutant ribosomes whose translation speed can be modulated, we have found that reducing polypeptide elongation rates leads to enhanced folding of diverse proteins of eukaryotic origin, including luciferase from the firefly *P. pyralis*, the green fluorescent protein from *A. victoria* and the telomere-binding protein Cdc13 from *S. cerevisiae*. These results suggest that in eukaryotes, protein folding necessitates slow translation rates. We have performed controls to rule out that the observed results are a consequence of increased protein degradation during slow translation or increased amino acid misincorporation during fast translation. In order to ascertain that a slow translation regime did not trigger the bacterial stress response, we analyzed steady-state levels of the main chaperones involved in protein folding in *E. coli*. We found no major differences in the chaperone levels between cultures grown under slow and fast regimes. We found that this generalized reduction in translation speed does not adversely impact the folding of the endogenous bacterial proteome, which confirmed our earlier observations that protein folding in bacteria appears to be largely uncoupled from protein synthesis. Utilization of this strategy has allowed the production of native eukaryotic multi-domain proteins previously unattainable in bacterial systems and may constitute a general alternative to the production of aggregation-prone recombinant proteins.

This work was supported by a grant from the Welch Foundation (H-1648 to J.M.B.).

CHAPERONIN-CATALYZED RESCUE OF ENTROPICALLY TRAPPED STATES IN PROTEIN FOLDING

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GroEL and GroES form a chaperonin nano-cage for single protein molecules to fold in isolation. Whether the isolation of folding-substrates is the sole determinant of efficient folding inside the cavity or the chaperonin nano-cage edits the folding-information encoded by the amino acid sequence is not understood. Here, we addressed this question using a double-mutant of the maltose binding protein, DM-MBP, as a substrate. We show that DM-MBP refolding is not limited by the formation of reversible aggregates and populates a kinetically-trapped state that is collapsed but structurally disordered. Introducing two long-range disulfide bonds into the DM-MBP sequence reduces the entropic barrier of folding and accelerates native state formation ~10-fold. Strikingly, steric confinement of the unfolded DM-MBP in the chaperonin cage closely mimics the effect of constraining disulfides on folding kinetics, in a manner mediated by negative charge clusters of the cage wall. These findings suggest that chaperonin dependence correlates with the tendency of proteins to populate entropically stabilized folding intermediates. The capacity to rescue proteins from such folding traps may explain the uniquely essential role of the chaperonin-cages within the chaperone network.

FUNCTIONAL CONSEQUENCES OF NUCLEOTIDE BINDING TO THE PROTEASOMAL ATPASES

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Protein degradation by the eukaryotic 26S proteasome or the homologous archaeal PAN-20S proteasome complex is a multistep process that requires ATP hydrolysis by the proteasome-associated AAA ATPase complex. However, the mechanisms by which these hexameric ATPases utilize ATP to promote protein breakdown and activate proteasomal function are poorly understood. Although PAN contains six identical ATPase subunits, we found that it exhibits three types of binding sites: 2 high affinity conformations (Kd=0.2uM), 2 with lower affinity (Kd=60uM), and 2 with conformations that fail to bind ATP. In fact, PAN never bound more than four of any type of nucleotide (ADP, ATP, or the nonhydroylyzable analog ATPγS), even at high concentrations. ATP binding to the high and lower affinity sites has distinct functional consequences on the proteasome. With two ATPγS molecules bound, PAN maximally stimulates opening of the gated channel for substrate entry into the 20S proteasome and has a high affinity for the 20S as well as for protein substrate. However, the binding of 4 ATPγS reduces PAN's ability to stimulate gate opening as well as its affinity for the 20S and substrates. These functional consequences of nucleotide binding are conserved in the mammalian 26S proteasome, since gate opening exhibited nearly identical multiphasic dependence on ATPγS concentration as found for the PAN-20S complex. Because ATP binding drives the association of the C-termini of the ATPase with the 20S and only two ATPase subunits bind ATP for maximal function it's likely that only two ATPases' C-termini dock into the 20S at any time and in a predictable pattern. This observation suggests how the hexameric ATPase ring associates with the heptameric 20S proteasome to regulate substrate degradation.

AN EARLY ROLE FOR HSP70 IN THE FORMATION OF AGGRESOMES

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During times of limited proteasomal function, aggresome formation is the active process by which cells sequester misfolded proteins towards the microtubule-organization center and away from the rest of the functioning proteome. Recent evidence has suggested that this aggregation process may be beneficial. However, very little is known about the molecular mechanisms that facilitate aggresome formation and only a few of the major mediators of aggregation are known. For example, genetic studies have suggested a role for heat shock protein 70 (Hsp70) in aggresome formation, but the details of this process remain uncertain. To specifically explore the roles of Hsp70 without changing its cellular concentration, we have developed membrane-permeable, chemical tools that stimulate or inhibit the ATPase activity of this chaperone. These probes are designed to explore the timing of Hsp70's actions and to understand how nucleotide-dependent structural transitions impact aggresome formation. Using both yeast and mammalian cells expressing the polyQ expanded exon 1 fragment of Huntingtin protein (Htt), we pharmacologically manipulated the ATPase activity of Hsp70. Fluorescence microscopy experiments showed that this activity is only important during early stages of Htt aggregation. Moreover, stimulating this activity caused an accumulation of toxic structures, consistent with a role for soluble, oligomeric polyQ in proteotoxicity. To understand whether these toxic structures are associated with specific protein-protein interactions, we performed immunoprecipitations of Htt and identified compound-dependent interacting proteins by tandem mass spectrometry. These studies revealed a number of chaperones, including Hsp26, which differentially interact with Htt during aggregation. Together, these biochemical and chemical genetic studies are beginning to reveal the hierarchy of proteins downstream of Hsp70 that facilitate aggresome formation.

A CELLULAR FUNCTION OF THE BACTERIAL HSP70 C-TERMINUS INDEPENDENT OF INTERDOMAIN ALLOSTERY AND CO-CHAPERONE INTERACTION

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The ubiquitous family of Hsp70 molecular chaperones preserve core functional properties throughout phylogeny, such as the allosteric regulation of a substrate-binding domain (SBD) through dynamic coupling to an ATPase domain, and interaction with co-chaperone proteins that further modulate Hsp70 activity and provide links to specific functional roles. In eukaryotic Hsp70s, a C-terminal sequence motif mediates interaction with Chip, Hip and Hop proteins to coordinate Hsp70 function with other chaperone networks and degradation machinery. However, these proteins do not exist in bacteria, leading us to ask why many bacterial Hsp70s contain C-terminal sequence beyond the structured SBD β-domain and helical lid. Analysis of a multiple sequence alignment of 739 diverse Hsp70s reveals two sub-families based on patterns of C-terminal conservation: one group displays sequence resembling closely the eukaryotic protein-protein interaction motif, and surprisingly, a second group displays a previously undescribed conservational pattern distinct from eukaryotes. We explored the functional roles of these conserved sites within bacterial Hsp70s by mutation of the predominant *E. coli* Hsp70, DnaK. While all variants including C-terminal truncations are able to support lambda phage replication, in the context of a more stringent *in vivo* assay for DnaK function that we have developed, truncations and point mutations that alter the conserved C-terminal motif lead to loss of function. Purified DnaK constructs harboring the deleterious C-terminal mutations do not alter substrate binding, interdomain allostery or interaction with the co-chaperones DnaJ and GrpE *in vitro*, suggesting that the C-terminus is involved in a previously uncharacterized functional role.

ILLUMINATING PRECISE QUANTIFICATION IN YEAST-BASED ANTIPRION DRUGS SCREENING BY USING FRAP AND SDD-AGE TECHNIQUES

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Yeast cells can be used to screen candidate drugs against mammalian prions since yeast prion exhibits clear mechanistic analogies but stringent species barrier with mammalian prion PrP. Although the coloration reporter system of [*PSI*$^+$]/[*psi*$^-$] yeast cells is a convenient tool to identify the ability of the tested molecules, there are a number of false positives including, in particular, compounds interfering with the white/red reporter system but not prion aggregation. In addition, SDS-PAGE analysis, which is used as a traditional quantification method in secondary screening by Bach et al., cannot detect the relative size of polymers which play an important role in the aggregation of Sup35p. In this study, an improved semi-denaturing detergent-agarose gel electrophoresis (SDD-AGE) technique, which could get more molecular weight information, were used to quantify the effects of tested compounds on Sup35p aggregation at protein level. Moreover, with the expression of Sup35p-GFP (NGMC) the fluorescent foci and fluorescence recovery after photobleaching (FRAP) were used to quantify the effects of tested compounds on Sup35p aggregation by confocal microscope realtimely in the living yeast cell. Using these two methods, effects of two known active antiprion compounds GuHCl and phenanthridine on Sup35p aggregation was precisely quantified at protein and cell levels. This result indicated that fluorescent assay and SDD-AGE analysis are of great feasibility to quantify the curing effects of candidate antiprion compounds in the yeast-based screening system.

DIFFERENT CHARACTERS OF STRESS-DEPENDENT [*PSI*$^+$] INDUCED BY SODIUM CHLORIDE, MAGNESIUM CHLORIDE, SODIUM FORMATE AND SODIUM ACETATE

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Early researches on yeast prion have found that [*PSI*$^+$] phenotype could transiently appear in an *SUP35* repeat-expansion mutant [*psi*$^-$] strain at stressful conditions, such as high salt concentrations. In this study, we investigated [*PSI*$^+$] phenotype change with native Sup35p under different conditions of sodium chloride, magnesium chloride, sodium formate and sodium acetate. Moreover, the Sup35p status changes in vivo were further quantified with fluorescence recovery after photobleaching (FRAP) technique and semi-denaturing detergent-agarose gel electrophoresis (SDD-AGE) analysis at cell and protein levels.

Interestingly, although all tested salt stresses could result in different intensity of stress-dependent [*PSI*$^+$] phenotype and present dose-dependent relationship in [*psi*$^-$] yeast strains, the characters of Sup35p aggregation state in these stress induced cells were diversified. Fluorescent assay showed that fluorescent foci could appear only in sodium formate treated Sup35p-GFP (NGMC) [*psi*$^-$] cells. FRAP results indicated all those four salt stresses induced Sup35p aggregate to a certain extent in [*psi*$^-$] cells and the size of aggregates had increased with a time-dependent relationship. The aggregate sizes of Sup35p under 1M magnesium chloride and sodium formate conditions were bigger than those under 1M sodium chloride and sodium acetate conditions. SDD-AGE results indicated that SDS-insoluble Sup35p polymers, which might act as “seeds”, obviously presented only in magnesium chloride and sodium formate treated [*psi*$^-$] cells. However, it seemed that those SDS-resistant Sup35p polymers are apparently different from that in [*PSI*$^+$] cells. In addition, the influence of [*PIN*$^+$] on Sup35p polymerization could not be readily excluded.

THE EFFECTS OF CHAPERONES AND HETEROLOGOUS PRION INTERACTIONS ON PRION FORMATION AND PROPAGATION

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Folding proteins into their correct conformation is necessary for cellular vitality. Dysregulation of protein homeostasis can lead to protein aggregation and amyloid formation that is associated with numerous neurodegenerative disorders. In yeast, however, prions can "misfold" to form a self-propagating aggregate that is innocuous. The protein Sup35 is thought to convert to a prion form when changing conditions make its prion state, [*PSI*+], confer selective growth advantages. While a number of molecular chaperones affect the formation and propagation of the [*PSI*+] prion, another prion, [*RNQ*+], formed by Rnq1p, regulates the epigenetic switch of Sup35p. This is thought to occur by a direct physical interaction between the [*RNQ*+] prion and Sup35p, but the mechanism is unclear. Using a reporter for the [*RNQ*+] prion status, we previously identified a number of missense mutations in Rnq1p that greatly reduced the induction of [*PSI*+], but did not affect the propagation of [*RNQ*+]. With these Rnq1p mutants, we conducted a screen for second-site suppressors using a Sup35p chimera. We identified mutations in this construct that rescued the [*PSI*+] induction defect of the Rnq1p mutants. We hypothesize that the amino acid residues around these mutations define the interface between the [*RNQ*+] prion and Sup35p. We hope that further analysis will provide evidence that the [*RNQ*+] prion acts to cross-seed the formation of [*PSI*+]. This work will help elucidate how one aggregated protein can facilitate the conformational change of another protein. Strikingly, though, once the [*PSI*+] prion is propagating, the [*RNQ*+] prion has no effect. Instead, once prions are established, chaperones play a crucial role.

One of the best-characterized chaperones in yeast is Hsp104p. The deletion of Hsp104p cures all known prions. While over-expression of Hsp104p also cures the [*PSI*+] prion, it has been shown to not affect [*RNQ*+]. Using the same reporter protein we developed, we can screen for chaperones that, when over-expressed, affect the propagation of [*RNQ*+]. Interestingly, it is suggested that chaperones have varied effects on different prion conformations, also called prion strains or variants. With this work, we hope to provide insight into how molecular chaperones recognize and affect prion variants differently.

THE MYCOBACTERIAL PROTEASOME DEGRADATION PATHWAY

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In eukaryotes, proteasomes represent the main degradation route for cellular proteins, however, in bacteria, the occurrence of the 20S proteasome is limited to the group of actinobacteria, amongst them the highly pathogenic *Mycobacterium tuberculosis* (*Mtb*). *Mtb* uses a tagging system functionally analogous to eukaryotic ubiquitination, termed pupylation, to recruit substrates for proteasomal degradation. The pupylation-degradation pathway contributes to the virulence of *Mtb* supporting its persistance and proliferation inside the host macrophages. We have reconstituted and characterized the pupylation pathway that leads to conjugation of the prokaryotic ubiquitin-like protein (Pup) to proteasomal substrate proteins. We show that Pup is rendered coupling-competent by deamidation of its C-terminal glutamine through the action of a previously uncharacterized protein Dop (deamidase of Pup). Then, PafA catalyzes formation of an isopeptide bond between the glutamate and a substrate lysine. The latter step turns over one ATP to ADP per coupled Pup, suggesting that Pup is activated for conjugation via phosphorylation of its C-terminal glutamte. We have also investigated the events downstream of the pupylation pathway, which lead to degradation of pupylated substrate proteins by the mycobacterial proteasome complex. We demonstrate that the proteasomal ATPase Mpa alone can recruit and unfold pupylated substrates, but that for degradation, physical interaction between Mpa and the proteasome is required. Initially, Pup is recognized by the coiled-coil domains of Mpa. Subsequently, the pupylated substrate is engaged into the Mpa-pore via the flexible N-terminal segment of Pup, suggesting that this segment functions as an unfolding initiation site. This assigns a dual role to Pup, serving to tether the substrate to the proteasome complex in analogy to ubiquitin, but unlike ubiquitin additionally serving also to initiate unfolding. As a result, unlike ubiquitin that is recycled at the regulatory particle, Pup is degraded along with the substrate protein.

MAMMALIAN UBR1 AND UBR2 UBIQUITIN LIGASES PROMOTE DEGRADATION OF HSP90 CLIENTS.

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Quality control processes maintain protein homeostasis by regulating polypeptide folding and degradation. Nascent and misfolded polypeptide folding is promoted by molecular chaperones, but rapid degradation ensues when they are inhibited. This degradation has clinical application such as when oncogenic kinases are degraded upon Hsp90 inhibition with geldanamycin. Mammalian CHIP is one ubiquitin ligase that promotes such degradation, but knocking out CHIP results only in limited breakdown of the quality control system indicating the presence of other ubiquitin ligases. Recent studies from our lab found that S. cerevisiae Ubr1 and Ubr2 ubiquitin ligases are involved in the cytosolic protein quality control. We therefore wanted to test whether the same quality control pathway is conserved in a mammalian system.

We used an in vivo geldanamycin-dependent degradation assay to see the effect of mouse UBR1 and UBR2 (homologs of yeast Ubr1) in the clearance of misfolded proteins. Using UBR1-/-, UBR2-/- and UBR1-/-UBR2-/- mouse embryonic fibroblasts we observed that there was reduced degradation of several Hsp90 client protein kinases, including Akt and Cdk4 compared to wild type cells. Strikingly there was an increase in the cellular levels of CHIP, a chaperone-associated ubiquitin ligase in the UBR knock out cells. In addition, we observed that UBR1 and UBR2 knockout cells were more resistant to geldanamycin with respect to cell viability. This phenotype was similar to that observed in yeast. These findings suggest that UBR1 and UBR2 promote cytosolic quality control in a conserved manner in a mammalian system.

CHARACTERIZATION OF AHA1'S INTERACTIONS WITH THE HSP90 CHAPERONE MACHINE AND CLIENT PROTEINS

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The activator of Hsp90 ATPase, Aha1, is a putative Hsp90 co-chaperone that is so named, because it activates Hsp90 catalyzed ATP hydrolysis. Genetic studies have implicated Aha1 as being a general stimulator of Hsp90 function, and studies in vitro indicate that Hsp90 and Aha1 directly interact. Here we have examined the interactions of Aha1 with Hsp90 and its co-chaperone components under quasi-physiological conditions in reticulocyte lysate. Formation of stable complexes between Hsp90 and Aha1 required the addition of molybdate to reticulocyte lysate. The molybdate-stabilized interaction was labile in the presence of 0.5 M NaCl, a property common to complexes formed between Hsp90 and its co-chaperones. Pull-down assays indicate that Aha1 is present in Hsp90 complexes that also contain FKBP52 and p23. However, Aha1 was found to be absent from Hsp90 complexes containing p60/HOP, HIP, Hsp70 or Cdc37. Furthermore, while Aha1, like its smaller yeast homologue Hch1, restores v-Src activity in yeast strains deficient in Hsp90 function, no interaction of Aha1 and newly synthesized v-Src was detected and over-expression of Aha1 in reticulocyte lysate did not stimulate the activity of v-Src. In addition, no Aha1 was detected in Hsp90 complexes formed with newly synthesized progesterone hormone receptor and AKT, and over-expression of Aha1 in reticulocyte lysate reduced Hsp90-dependent renaturation of thermally denatured firefly luciferase. However, an interaction between over-expressed Aha1 and AKT was detected in HeLa cells. Overexpression of Aha1 or suppression of Aha1 expression via shRNA in cultured cells had no effect on phosphotyrosine levels, suggesting that Aha1's effect on v-Src activity in yeast is indirect. A concentration-dependent increase in ANS fluorescence was observed upon its titration into a solution of recombinant Aha1p or its C-terminal START domain, but not its N-terminal domain, indicating the presence of a hydrophobic binding pocket. Aha1's lipid binding specificity and interactions with novel client proteins will be discussed. (This work was supported by a grant from the OCAST: HR03-076).

QUANTITATIVE HIGH-THROUGHPUT ANALYSIS OF HSP90 CLIENTS AND COCHAPERONES

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Hsp90 is an evolutionary conserved molecular chaperone involved in cellular stress response and protein homeostasis. In addition, it has an important role in e.g. signal transduction, chromatin remodeling and cell cycle. In contrast to other cellular chaperones, Hsp90 does not appear to be a general chaperone for de novo folding. Rather, it stabilizes a wide variety of client proteins and keeps them poised for activation. Multiple high-throughput studies in various organisms have uncovered many new Hsp90 clients, but several aspects of the clients remain elusive. How does Hsp90 recognize its client proteins? Are there sequence or structural features that characterize client proteins? How do various cochaperones contribute to Hsp90 specificity?

We have utilized and further developed a luminescence and ELISA-based LUMIER assay to study protein-protein interactions (PPIs). The enhanced LUMIER assay facilitates quantitative measurement of PPIs in mammalian cells in high-throughput format. Its high sensitivity and linear range allows quantitative analysis of the effects of point mutations that cause subtle changes in protein interaction profiles. Furthermore, the assay is compatible with signaling-dependent events or small-molecule inhibitors.

We have used the assay to study the association of Hsp90 with a large set of functionally important human proteins (kinases, transcription factors, cellular quality control proteins), and uncovered hundreds of new Hsp90 client proteins. Our results also establish how alternative splicing, posttranslational modifications, single-nucleotide polymorphisms and clinically relevant disease mutations can influence Hsp90 clientele. Furthermore, interaction assays with known and novel Hsp90 cochaperones have allowed us to construct quantitative interaction maps that reveal how Hsp90 clientele is shaped by both general cochaperones and client-specific adaptor proteins.

A NOVEL ATP DEDEPENDENT CONFORMATIONAL CHANGE OF P97 ND1 FRAGMENT REVEALED BY CRYSTAL STRUCTURES OF DISEASE RELATED MUTANTS

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Mutations in human p97, a type II AAA+ protein, have been implicated in IBMPFD (inclusion body myopathy associated with Paget's disease of the bone and frontotemporal dementia). Previous crystallographic analyses showed that wild type p97 are invariably bound with ADP in the D1 domain. Here, we show the crystal structures of ND1 fragment of the disease-related p97 mutants, for the first time, with ATPγS bound in the D1 domain. In the present structure, the N domain undergoes rotational and translational movement. This conformational change has been confirmed to be driven by the bound nucleotide forms instead of caused by mutations, as the movement is reversible by ADP. Small angle X-ray scattering experiments further suggest that wild type p97 can undergo similar conformational change as that observed in the mutants. P97 is known to have pre-bound ADP in its D1 domain. We found that the amount of pre-bound ADP in D1 domain is consistently lower in mutants. Nucleotide binding affinity of the D1 domain in mutants, as determined by ITC experiments, showed slightly lowered Kd values towards ADP than in wild type p97. Interestingly, titration with ATPγS displayed a unique biphasic exothermal profile which was only observed in the mutants, which could only be fitted by a two-site model suggesting that mutants allow the binding of ATPγS to both empty and ADP-prebound D1 sites. However, in the case of wild type p97, ATPγS can only get into the empty D1 sites and nucleotide exchange is prohibited in the sites with ADP-prebound.
We proposed that IBMPFD mutations altered the nucleotide binding behavior of the D1 domain during the ATP cycle thus allowing a uniform and symmetrical binding of all D1 sites within the hexamer for ATPγS. As a result, all the N domains in a p97 hexamer could undergo a concerted conformational change as supported by the present crystal structures and the biphasic titration profile from ITC experiments for the mutants. In the wild type scenario, there is a tight control over the timing of nucleotide exchange among the D1 domains within the hexamer and the pre-bound ADP is not easily exchangeable. ATPγS is only allowed to get into the D1 empty sites suggesting an asymmetric hexamer in the presence of ATPγS. We believe that this asymmetric property in nucleotide states of D1 domains in various subunits of a p97 hexamer is essential for the function of p97. External stimulus such as ATP hydrolysis in D2 domain or interaction with adaptor proteins may be needed to trigger the nucleotide exchange at the ADP-prebound sites. The present work also shed light into how the mutations might affect the function of p97 leading to the disease.

HIGH MANNOSE-BINDING LECTINS INDUCED BY ER STRESS REGULATE THE ERAD AND SECRETION OF PLASMA GLYCOPROTEIN MUTANTS

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High mannose-type oligosaccharides, cotranslationally introduced to a nascent polypeptide by N-glycosylation, play a critical role in protein quality control (folding, transport and degradation). The fates of newly synthesized glycoproteins are thought to be determined through interactions with intracellular lectins such as EDEM (ER-degradation enhancing α-mannosidase-like protein), ERGIC-53 and VIP36 that recognize high mannose-type glycans and are induced by ER stress. EDEM has been reported to recognize misfolded glycoproteins and facilitate the ERAD of misfolded glycoproteins. Cargo receptors (ERGIC-53 and VIP36) are known to recognize only a limited number of folded glycoproteins and transport their cargoes selectively through the secretory pathway. Antithrombin (AT) and α1-antitrypsin (α1-AT) are plasma glycoproteins that function as proteinase inhibitors. Three AT mutants (AT ΔE, AT ΔF, AT P-stop) and two α1-AT mutants (α1-AT NHK, α1-AT Z) are known to be degraded through the ERAD pathway. Here we examined if these lectins participate in the quality control of misfolded glycoproteins, using these three AT and two α1-AT mutants as model proteins. Co-immunoprecipitations of EDEM with three AT mutants were observed, but not with AT Wt. Co-transfection of EDEM accelerated the degradation of AT P-stop. However, to our surprise, it delayed the degradation of AT ΔE and acclerated the secretion of AT ΔF without degradation. These results suggest that EDEM regulates the degrdation and secretion of AT mutants. Overexpression of ERGIC-53 induced a marked increase in the secretion of both α1-AT NHK and α1-AT Z, but not α1-AT Wt, three AT mutants or AT Wt. In contrast, overexpression of VIP36 resulted in an accelerated secretion of three AT mutants but not AT Wt, two α1-AT mutants or α1-AT Wt. Thus, both ERGIC-53 and VIP36 were shown to participate selectively in the quality control of specific glycoproteins regardless of their mutations. These results suggest that high mannose-binding lectins induced by ER stress are involved in the quality control of misfolded glycoproteins and regulate the ERAD and secretion of those mutants.

ATP-INDEPENDENT PROTEIN REFOLDING BY PH-TRIGGERED CHAPERONE BINDING AND RELEASE

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Molecular chaperones are typically either ATP-dependent or rely heavily on their ATP-dependent chaperone counterparts in order to promote protein folding. We describe a mechanism by which the tiny (9.7 kDa) acid stress chaperone HdeA is capable of independently facilitating the refolding of acid-denatured proteins in the bacterial periplasm, which lacks both ATP and ATP-dependent chaperone machines. Acid rapidly activates HdeA by partial unfolding and dimer dissociation. The partially unfolded HdeA monomer can then adaptively recognize a broad range of substrate proteins, in part using hydrophobic regions of the now exposed dimer interface. This allows HdeA to stably bind substrates at low pH and prevent their irreversible aggregation. pH neutralization subsequently triggers the slow release of substrate proteins from HdeA, keeping the free concentration of aggregation-sensitive intermediates below the threshold where they begin to aggregate. This provides a straightforward and ATP-independent mechanism that allows HdeA to facilitate protein refolding following extreme acid stress. Unlike previously characterized chaperones, HdeA appears to facilitate protein refolding by using a single substrate binding-release cycle. Rather than relying on cellular ATP stores and co-chaperones, this cycle is entirely regulated by the external pH and is therefore energy-neutral for the bacteria.

AN INITIAL STEP OF DENATURED PROTEIN RECOGNITION BY HSC70 AND DJA1

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Protein folding is a prominent chaperone function of the Hsp70 system. We reported that the mammalian Hsc70 system with either type 1 DnaJ protein, DjA1 or DjA2, and a nucleotide exchange factor efficiently mediate the refolding of an unfolded protein. Many studies have examined chaperone interactions with peptide substrates or a few proteins that do not form aggregates, and various models were postulated to explain the folding by the Hsp70 system. However, the system plays a prominent role in the folding of aggregation-prone unfolded proteins. The direct measurement of the kinetics and stoichiometry of chaperone binding is obviously lacking.

To solve this issue, we developed a surface plasmon resonance (SPR) technique to monitor the initial processes of chaperone bindings to an unfolded protein. The substrate protein on a chip showed conformational transitions that slowly led to reduced chaperone binding. Some portion of the coupled protein was restored to the native conformation by the Hsc70 system as revealed by the enzymatic activity. Surprisingly, the SPR measurement revealed that the multiple Hsc70 proteins and a dimer of DjA1 initially bind independently to an unfolded protein. The SPR method delineated the essential features of chaperone binding; that multiple molecules of Hsc70 and a dimer of DjA1 independently bind to an unfolded protein at the initial step. The association rate of the Hsc70 was faster than that of DnaJ protein in a folding-compatible condition. The Hsp70 binding involved a conformational change that corresponds to the rate of ATP hydrolysis. The DjA1 binding occurred in a bivalent manner, and this coincides with the structural observations that the canonical DnaJ proteins are homodimers. Furthermore, an experiment with a peptide array scan revealed that the binding sites for Hsc70 and DjA1 are distinct and that there were 5 sites for Hsc70 and 2 sites for DjA1. All of these findings consistently incorporated the nature of individual chaperones that are known but not adequately considered in the folding models of unfolded proteins.

UBC9 IS A NOVEL REGULATOR OF XBP1, A TRANSCRIPTION FACTOR CONTROLLING MAMMALIAN ER STRESS RESPONSE

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In the endoplasmic reticulum (ER), newly synthesized secretory and transmembrane proteins are correctly folded with the assistance of ER chaperones. If cells synthesize proteins in excess of the folding capacity of the ER, unfolded or misfolded proteins are accumulated in the ER, and cause cell death (ER stress). To cope with ER stress, cells activate the cytoprotective mechanism called the ER stress response or unfolded protein response (UPR).
XBP1 is a transcription factor essential for mammalian ER stress response. In the absence of ER stress, XBP1 mRNA is expressed as pre-mRNA, from which a negative regulator pXBP1(U) is translated. Upon ER stress, XBP1 pre-mRNA is converted to mature mRNA by the mechanism called cytoplasmic splicing, resulting in production of an active transcription factor, pXBP1(S). During the recovery phase from ER stress, pXBP1(U) enhances degradation of pXBP1(S). Thus, expression of XBP1 is regulated by two mechanisms – cytoplasmic splicing and degradation by the proteasome, though the underlying mechanism has remained to be clarified. Here, we identified UBC9 as a novel regulatory factor of XBP1 expression. UBC9 was found to bind to XBP1, and a leucine zipper motif of XBP1 is essential for binding. Overexpression of UBC9 in HeLa cells increased XBP1 expression and enhanced transcriptional induction in response to ER stress. From these results, we propose that UBC9 is a positive regulator of XBP1 expression.

ACCURATE PREDICTION OF DNAK-PEPTIDE BINDING VIA MOLECULAR MODELING AND EXPERIMENTAL DATA.

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Molecular chaperones are essential elements of the protein quality control machinery that governs translocation and folding of nascent polypeptides, refolding and degradation of misfolded proteins, and activation of a wide range of client proteins. The prokaryotic heat-shock protein DnaK is the E. coli representative of the ubiquitous Hsp70 family, which specializes in the binding of exposed hydrophobic regions in unfolded polypeptides. Accurate prediction of DnaK binding sites in E. coli proteins is an essential prerequisite to understand the precise function of this chaperone and the properties of
its substrate proteins. In order to map DnaK binding sites in protein sequences, we have developed an algorithm that combines sequence information from peptide binding experiments and structural parameters from homology modelling. We show that this combination significantly outperforms either single approach. The final predictor had a Matthews correlation coefficient (MCC) of 0.819 when assessed over the 144 tested peptide sequences to detect true positives and true negatives. To test the robustness of the learning set, we have conducted a simulated cross-validation, where we omit
sequences from the learning sets and calculate the rate of repredicting them. This resulted in a surprisingly good MCC of 0.703. The algorithm was also able to perform equally well on a blind test set of binders and non-binders, of which there was no prior knowledge in the learning sets. The algorithm is freely available at http://limbo.switchlab.org

EVALUATING THE ROLE OF MICRORNAS IN PROTEOSTASIS USING *C. ELEGANS*

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Multiple cellular pathways influence the proteostasis network that maintains proper folding of the proteome. Dysregulation of expression of genes in these pathways may contribute to protein misfolding and aggregation. We propose that microRNAs provide a mode of regulation of genes influencing proteostasis. To determine whether microRNAs regulate these pathways, a deletion of Dicer, which is essential for the processing of all precursors of microRNAs, was crossed into a *C. elegans* strains expressing polyQ in the body wall muscle cells. Deletion of *dcr-1* leads to an enhancement of polyQ aggregation suggesting that microRNAs play a significant role in proteostasis. Using a bioinformatics program called targetscan, we are surveying the 3'UTR of the genes that modify aggregation to identify binding sites for specific microRNAs. Mutations or deletions in *mir-124*, *mir-355* or *let-7* enhance polyQ aggregation. Our goal is to demonstrate that specific microRNAs provide a mode of regulation that can influence the level of target genes in cellular pathways that influence proteostasis.

UNDERSTANDING THE ROLE OF PDI

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From a screen for mutants which are physiologically linked to the unfolded protein response, we identified a novel allele of protein disulfide isomerase (PDI). This is the first time a mutant of PDI has been isolated from a forward genetic screen. Pdi1p is an essential protein in *Saccharomyces cerevisiae* involved in the formation of disulfide bonds in secretory and cell-surface proteins, but its in vivo role in oxidative protein folding is unclear. The PDI mutant contains a mutation in the a' domain, and exhibited a defect in folding of various endogenous proteins. We sought to elucidate the role of Pdi1p in protein folding in the ER, and its involvement in other aspects of protein quality control.

PARTITIONING OF AGGREGATION-PRONE PROTEINS INTO INTRACELLULAR INCLUSIONS IS DETERMINED BY QUALITY CONTROL MACHINERY AND NOT INHERENT AGGREGATION PROPENSITY

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The accumulation of intracellular protein aggregates is associated with aging, cell death and a number of degenerative diseases. To date, the properties of proteins that determine their aggregation propensity has mainly been studied in vitro, an environment devoid of the quality control machinery that allow cells to maintain proper proteostatsis. Here, we study the intracellular behavior of a panel of mutants of the Aβ peptide, which have previously been shown to have a broad range of intracellular aggregation propensities. We find that none of these peptides show evidence of aggregating when expressed under normal conditions, and than all are sequestered in an insoluble inclusion under conditions of proteasome inhibition that alter cellular proteostatsis. Our study illuminates how cells manage different kinds of aggregation-prone species under different conditions of protein folding stress.

CO-TRANSLATIONAL ASSEMBLY OF G PROTEIN BΓ DIMERS BY PHOSDUCIN-LIKE PROTEIN 1 AND THE CYTOSOLIC CHAPERONIN COMPLEX

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Phosducin-like protein 1 (PhLP1) and the cytosolic chaperonin complex (CCT also called TRiC) have been shown to be essential chaperones in the assembly of the G protein βγ dimer. Gβ initially binds CCT and is folded into a quasi-native conformation, but requires PhLP1 to release from CCT. Once released, Gγ binds Gβ in the PhLP1-Gβ complex. The status of Gγ prior to association with Gβ, whether it is free in the cytosol or bound to a chaperone, is unknown. Recent work from our laboratory suggests that Gγ associates with the Gβ-CCT complex co-translationally through a coiled-coil interaction between the N-termini of Gγ and Gβ. This interaction appears to stall Gγ translational until PhLP1 can associate with the complex and release Gβ from CCT. Evidence for such a co-translational mechanism of Gβγ assembly includes: i) inhibition of Gγ translation in the absence of PhLP1, ii) enhancement of Gγ translation is the absence of Gβ, iii) requirement of the Gγ N-terminal residues that form the coiled-coil with Gβ for PhLP1-dependence of Gγ translation, and iv) association of both PhLP1 and CCT with translating ribosomes. Co-translational assembly allows hydrophobic residues on the small 71 amino acid Gγ to make immediate contacts with their hydrophobic partners on Gβ, thus avoiding problems of aggregation and degradation in the cytosol. These findings suggest an elegant mechanism by which these two proteins, which are unstable in their monomeric form, can associate into a stable complex as Gγ is being synthesized.

A NOVEL ER-LOCALIZED DNAJ PROTEIN, DNAJB12, PARTICIPATES IN ER QUALITY CONTROL.

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Endoplasmic reticulum (ER) is a cellular compartment where newly synthesized secretory and membrane proteins are folded and modified. Proteins that failed to be correctly folded are pulled out or retrotranslocated from the ER to the cytosol, and polyubiquitinated for degradation by the proteasome, which is known as ER-associated degradation (ERAD). Especially for membrane proteins, folding and ERAD are facilitated by cytosolic or ER luminal Hsp70 and Hsp40 family proteins. Mammalian cystic fibrosis transmembrane conductance regulator (CFTR) is one of the most widely used model proteins for exploration of quality control of membrane proteins. As for folding of CFTR, a cytosolic Hsp40 family protein Hdj2 is reported to play an important role. Recently, yeast Hlj1, which is an Hsp40 family protein anchored on the ER membrane, is involved in the degradation of CFTR artificially expressed in yeast cells. However, mammalian Hsp40 family protein contributing to the degradation of CFTR has not been identified so far.
Here we show that DNAJB12 plays an important role in the degradation of a malfolded membrane protein in mammalian cells. DNAJB12 is an Hsp40 family protein and is evolutionarily conserved from *S. pombe* to human. Our analysis of its expression revealed that the mRNA of DNAJB12 is ubiquitously expressed in mouse tissues. Further we show that DNAJB12 is a type-II transmembrane protein located on the ER with its J-domain facing the cytosolic side. This topology predicts that DNAJB12 may be able to corporate with cytosolic Hsc70 for the degradation of CFTR. Indeed, we have found that DNAJB12 is associated with Hsc70 via its J-domain in vivo. Remarkably, overexpression of DNAJB12 accelerated the degradation of CFTR, whereas knockdown of this protein resulted in the increased expression of CFTR. Furthermore, the DNAJB12-dependent degradation of CFTR was inhibited by a proteasome inhibitor. These findings suggest that DNAJB12 plays an important role in ERAD of mammalian ER membrane proteins.

MOLECULAR MECHANISM FOR NASCENT CHAIN-MEDIATED *XBP1U* MRNA TARGETING ONTO MEMBRANE

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On decoding genetic code of mRNA, ribosome forms mRNA-ribosome-nascent chain (R-RNC) complex transiently, which should be, in principle, able to couple protein function with its own mRNA. However, almost all proteins acquire their function after released from ribosome and folded. Furthermore, duration of R-RNC complex is much shorter as compare with entire lifetime of each protein, which makes it difficult for nascent chain to affect its own mRNA. One exception is our previous finding that XBP1u protein recruits its own mRNA onto membrane as a state of nascent chain, which promotes cytoplasmic splicing of *XBP1u* mRNA by endoplasmic reticulum (ER) transmembrane protein IRE1α upon ER stress conditions. In our model, a highly hydrophobic region in XBP1u, HR2 is responsible for attaching the protein onto membrane and the C-terminal 53 residues downstream of HR2 is required for coupling the membrane localization of XBP1u with that of its own mRNA. Our model predicts XBP1u R-RNC complex needs to be targeted onto membrane before completion of the synthesis of XBP1u. However, XBP1u has only 53 amino acid residues after HR2. In addition, HR2 will probably need to be exposed out side of the ribosome tunnel for the interaction of HR2 with the membrane. Thus, it remained unclear how R-RNC is targeted to the membrane before the termination of the XBP1u synthesis. Here we propose novel mechanism to perform this process, metazoan cells have evolved.

CELLULAR FUNCTIONS OF THE HSP40 PROTEINS DNAJA1 AND DNAJA2

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Proteins in the cell are maintained in a dynamic balance of folding, degradation and localization. The human cytosolic chaperone Hsc70 is thought to have a central role in this balance. The Hsp40/DnaJ family of co-chaperones are essential to stimulate the ATPase and substrate binding activities of Hsc70. Recent work has examined the biochemical properties of DnaJA1 (Hdj2) and DnaJA2, the main Type I Hsp40 proteins in humans. It was proposed that the DnaJAs have similar yet partially specialized characteristics which cooperate with Hsc70 in different situations. The function of the DnaJA co-chaperones in the cellular context was next addressed. Both DnaJA1 and DnaJA2 were active in HeLa cell models of polypeptide folding, with DnaJA1 generally showing the greatest activity. Mutants of the DnaJAs were used to explore their requirements for function, and their internal structures were found to be notably important. Intriguingly, in another model of chaperone-dependent HERG potassium channel folding at the endoplasmic reticulum of HEK293 cells, the DnaJAs were found to inhibit trafficking and maturation of the channel. Instead, the polypeptide was retained at the endoplasmic reticulum and degraded by proteasomes, most likely involving the Hsc70-dependent CHIP E3 ubiquitin ligase. Thus, the DnaJAs together with Hsc70 are key factors in the cellular regulation of protein destinies.

STRUCTURAL AND THEORETICAL STUDIES INDICATE THAT THE CYLINDRICAL PROTEASE CLPP SAMPLES EXTENDED AND COMPACT CONFORMATIONS

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The highly conserved ClpP protease consists of two heptameric rings that interact by the interdigitation of an α-helix - β-strand handle domain motif to form a tetradecameric cylinder. We previously proposed that protease dynamics results in the temporary unstructuring of interacting pairs of handle domains, opening transient equatorial side pores that allow for peptide egress. Here, we report the structure of an Escherichia coli ClpP mutant in which each opposing pair of protomers is linked by a disulfide bond. This structure resembles the compact ClpP structure of Streptococcus pneumoniae, Mycobacterium tuberculosis, and Plasmodium falciparum ClpP, rather than the active, extended structure seen for all previously determined E. coli ClpP structures. The structural data, along with normal mode analysis, support a model whereby the ClpP cylinder switches dynamically between an active extended state required for substrate degradation and an inactive compact state required for peptide product release.

FORMATION OF NON-TOXIC ABETA FIBRILS BY SMALL HEAT SHOCK PROTEIN (SHSP) UNDER STRESS CONDITION

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Small heat shock protein (sHsp) is a molecular chaperone with a conserved alpha-crystallin domain that normally forms large oligomers (9-50mer) in their native state. It has been shown that the oligomer dissociates to prevent protein aggregation under stressed conditions. Since sHsps have been found too be upregulated and co-localized with amyloid beta (Ab) in senile plaques of Alzheimer's disease patients, sHsps are considered to be involved in the disease. Previous studies showed that sHsp prevent Ab aggregation in vitro. However, it remains to be demonstrated how the quaternary structure of sHsp influence on Ab aggregation. In this study, we have examined Ab aggregation in the presence of sHSP from fission yeast Schizosaccharomyces pombe (SpHsp16), which is homologous with other sHsps found in AD brain, and more importantly, shows clear temp-dependent transition between oligomer and dimer (J. Biol. Chem. (2005) 280, 32586).

It has been shown that SpHsp16 dissociates into dimer under heat shock condition (50 deg) while it forms oligomer at a normal temperature (30 deg). Ab fibrillation in the presence of with an equimolar (monomer conc.) amount of SpHsp16 at 30˚C or 50˚C for 24 hr was examined by transmission electron microscopy (TEM). When Ab was incubated with SpHsp16 at 30 deg, no mature amyloid fibrils were formed, indicating that Ab fibrillation was inhibited by oligomeric SpHsp16. In contrast, significant amount of amyloid fibrils were observed in samples incubated with SpHsp16 at 50˚C. Thioflavin T (ThT) fluorescence analysis suggests a difference in their inner structures. Interaction between Ab molecules and SpHsp16 of both oligomeric and dimeric structures was confirmed with co-immunoprecipitation.

Cell toxicity of Ab fibrils formed in the presence of dimeric SpHsp16 on rat PC12 cells was examined with MTT assay. Interestingly, its cell toxicity was much lower than that of "normal" Ab fibrils formed without SpHsp16. Immuno-histochemical study showed that this low toxicity could be explained by weak affinity of the fibril to the cell surface.

In summary, our results showed that oligomeric SpHsp16 inhibits Ab fibrillation, and dimeric SpHsp16 induces formation of low toxic novel fibrillar amyloid which has different characteristics with well-known Ab amyloid fibrils formed without a molecular chaperone.

THE UNFOLDED PROTEIN RESPONSE TRANSDUCER IRE1A IS A KEY REGULATOR OF HEPATIC STEATOSIS

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The endoplasmic reticulum (ER) is the cellular organelle responsible for protein folding and assembly, lipid biosynthesis, and calcium storage. The Unfolded Protein Response (UPR) is an adaptive intracellular stress response to the accumulation of unfolded or misfolded proteins in the ER. In this study, we demonstrated that the most conserved UPR sensor IRE1α, an ER transmembrane protein kinase/endoribonuclease, is required to maintain hepatic lipid homeostasis by preventing hepatic lipid accumulation and maintaining lipoprotein secretion. To elucidate the physiological role of IRE1α-mediated signaling in the liver, we generated hepatocyte-specific *Ire1α* null mice by utilizing albumin promoter-controlled Cre recombinase-mediated deletion. Hepatocyte-specific deletion of *Ire1α* caused defective induction of genes encoding functions in ER-to-Golgi protein transport, oxidative protein folding, and ER-associated degradation of misfolded proteins. In response to ER stress, mice with hepatocyte-specific *Ire1α* deletion display profound hepatic steatosis. Further investigation revealed that IRE1α modulates expression of key metabolic transcription factors of the CCAAT/enhancer binding protein and peroxisome proliferator-activated receptor families and is required for efficient secretion of apolipoproteins upon disruption of ER homeostasis. The identification of IRE1α as a key regulator of hepatic steastosis may have important implications for human fatty liver diseases as well as metabolic syndrome.

ASSOCIATION WITH ENDOPLASMIC RETICULUM PROMOTES GADD34 TURNOVER

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GADD34 (product of a growth arrest and DNA damage-inducible gene) is expressed in response to a variety of cell stresses. Biochemical and cell biological showed that GADD34 protein was present in both membrane (primarily endoplasmic reticulum) and cytosol and proteasome inhibition preferentially enhanced the GADD34 levels protein associated with the endoplasmic reticulum (ER). Structure-function analyses identified an evolutionarily conserved ER-localization sequence near the N-terminus of GADD34 and highlighted key residues in an amphipathic helix as critical for membrane binding. Besides ER localization, yet other mutations on this region highlighted an unexpected ability of GADD34 to be localized at mitochondria. Both soluble and membrane-associated GADD34 effectively scaffolded the catalytic subunit of protein phosphatase-1 (PP1) and the eukaryotic translation initiation factor, eIF2α and promoted eIF2α dephosphorylation. The redistribution of GADD34 to cytosol was accompanied by significant reduction in its rate of degradation by the proteasome, resulting increased protein translation and misfolding. Together, the data hint a potential mechanism by which GADD34 trafficking between distinct subcellular compartments may regulate the cellular levels of the protein and control protein translation.

MOLECULAR MECHANISMS OF THE HRD LIGASE IN ER PROTEIN QUALITY CONTROL

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Environmental or cellular insults constantly interfere with accurate folding and maturation of proteins. Since misfolded polypeptides tend to accumulate and may form toxic aggregates, protein quality control (PQC) mechanisms have evolved to ensure structural integrity of the cellular proteome. In the early secretory pathway, terminally misfolded or unassembled proteins are cleared from the endoplasmic reticulum (ER) in a process known as ER-associated protein degradation (ERAD). Aberrant proteins are first recognized and then retrotranslocated across the ER membrane into the cytosol for destruction by the ubiquitin-proteasome proteolytic system. In the yeast *Saccharomyces cerevisiae* glycoproteins with luminal lesions are removed by the HRD ligase, a large multiprotein complex embedded in the ER membrane that includes the E3 ubiquitin ligase Hrd1p. The ER luminal subcomplex contains the major substrate receptors Hrd3p and Yos9p. Hrd3p, a type I membrane protein, recruits potential substrates based on the presence of misfolded domains whereas Yos9p recognizes a mannose$_7$-glycan structure specific for defective proteins. Only the recognition of this bipartite signal triggers the degradation of misfolded polypeptides. Here we will show data that Hrd3p and Yos9p might exhibit functions beyond substrate recognition. Our *in vitro* data suggest that Hrd3p and Yos9p prevent denatured proteins from aggregation and ensure their solubility as a prerequisite for efficient retrotranslocation into the cytosol.

TARGETING HSP27 ABROGATES IL-6 SIGNALLING PATHWAY AND PROLONGS TIME FOR CASTRATED RESISTANT PROSTATE CANCER

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Introduction and Objectives: Androgen withdrawal is the most effective form of systemic therapy for men with advanced prostate cancer. Unfortunately, androgen-independent progression is inevitable, and the development of hormone-refractory disease and death occurs within 2 to 3 years in most men. The understanding of molecular mechanisms promoting the growth of androgen-independent prostate cancer cells is essential for the rational design of agents to treat advanced disease. We previously reported that Hsp27 level correlates with castrated resistant prostate cancer (CRPC). However, the mechanism by which Hsp27 mediates CRPC remains elusive.
Material and methods: LNCaP and C4-2 cells were treated or not with IL-6 and submitted to western blot, co-IP and immunofluorescence analysis. Cells were transfected with Hsp27 WT and Hsp27 siRNA and tested for their ability to modulate STAT3, Jak2, Erk expression and phosphorylation by western blot and STAT3 and PSA transactivation by luciferase assay.
complexes, STAT3 dimerization, nuclear translocation and tranactivation. Since, STAT3 phosphorylation was correlated with AR activation in ligand independent manner, Hsp27 knockdown abrogates IL-6 inducing PSA activation/expression in vitro and in vivo. Finally, Hsp27 knockdown using OGX-427 delayed time to CRPC in LNCaP xenograft model.εResults: Here we report that Hsp27 is highly expressed and phosphorylated in androgen independent C4-2 cell line compared to androgen dependent cell line LNCaP. IL-6 induced Hsp27 phosphorylation via p38 kinase and triggers a rapid formation of Hsp27/STAT3 complexes in a time dependent manner. Hsp27 knockdown using siRNA or antisense (OGX-427 currently in phase I clinical trial in CRPC) abrogate IL-6 signalling pathway. Hsp27 knockdown inhibits the ability of IL-6 to induce STAT3 phosphorylation on tyrosine and serine and STAT3/Jak-2 and STAT3/PKC
Conclusion: Taken together, these results support an important function of Hsp27 in CRPC and specifically in IL-6 signalling pathway and support further the rational of targeting Hsp27 in CRPC.

MECHANISMS OF MEMBRANE PROTEIN INSERTION INTO THE ER

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Our laboratory is working to develop a molecular level understanding of the pathways of secretory and membrane protein biosynthesis and metabolism. We are especially interested in the regulatory machinery controlling protein entry and insertion into the endoplasmic reticulum (ER), the site where nearly all secreted and membrane proteins are first made. Recently, we discovered a new pathway for membrane protein insertion into the ER used by a large class of 'tail-anchored' membrane proteins. In this pathway a cytosolic ATPase we named TRC40 selectively recognizes its membrane protein substrates, delivers them to the ER, and facilitates their insertion into the membrane. We have reconstituted these steps in vitro and are now using this system to identify novel machinery essential for regulating the efficient and proper biogenesis of tail-anchored proteins. Our ongoing studies reveal that substrate capture by TRC40 requires an additional protein complex that we have now purified and identified. Functional analysis reveals that this complex is acting as a 'pre-targeting' factor that directly binds tail-anchored substrates at the ribosome and transfers them to TRC40. Mechanistic and structural studies of the components of this membrane protein insertion pathway will be presented.

A NOVEL FUNCTION OF XBP1U PROTEIN IN THE UNFOLDED PROTEIN RESPONSE: THE ROLE IN MRNA-TETHERING TO THE ER MEMBRANE

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Accumulation of unfolded proteins in the endoplasmic reticulum (ER) by various ER stresses triggers the cytoplasmic splicing of *XBP1u* (unspliced) mRNA in metazoans or *HAC1u* mRNA in yeast by the transmembrane kinase/endoribonuclease IRE1, resulting in activation of the unfolded protein response, which maintains ER homeostasis. Under unstressed condition, whereas *HAC1u* mRNA in yeast is translationally repressed by stable base-pairing between 5'-untranslated region and its intron, *XBP1u* mRNA in mammals is translated and unstable XBP1u is produced. Since ER stress sensor IRE1 could be located in the ER membrane, *HAC1u/XBP1u* mRNAs should be recruited to the ER membrane in spite of nonsecretory proteins. Recently we reported that the nascent XBP1u polypeptide recruits its own mRNA to the membrane mediated through the HR2 hydrophobic region of XBP1u, which enhances the efficiency of cytoplasmic splicing of *XBP1u* mRNA under ER stress. Our data showed that the C-terminal 53-amino acid region of XBP1u protein, which locates to the downstream of HR2, is sufficient for tethering *XBP1u* mRNA to the membrane. However, if *XBP1u* mRNA-tethering to the membrane occurs during translation, 53-amino acid residues seem to be slightly short for the tethering because HR2 should be exposed outside of the ribosome tunnel. We hypothesize that decreasing the rate of polypeptide synthesis at the C-terminus of XBP1u or polypeptide synthesis by polyribosome may contribute to the efficient tethering of mRNA to the membrane. Here we present and discuss a novel mechanism of membrane-association of XBP1u mRNA.

INTERPLAY BETWEEN PROTEIN SYNTHESIS, FOLDING AND MISFOLDING.

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It has been firmly established that the information necessary for proteins to fold is contained within their amino acid sequence. However, since the process of translation is vectorial, nascent proteins emerge gradually from the ribosome as incomplete chains that are susceptible to misfolding. Ribosome movement along an mRNA is variable and may be influenced by tRNA availability and the physical-chemical properties of the anticodon-codon interaction. Thus, the speed at which a polypeptide is synthesized is not uniform and depends, at least partially, on its coding sequence, which determines which specific isoacceptor tRNAs are utilized. Recent experiments from our laboratory suggest that translation speed and local folding events of the nascent protein are intimately coupled and require precise coordination. We wish to further understand how genetically encoded information may regulate translation speed and thus protein folding pathways. We have exploited differences in tRNA availability and protein folding efficiencies between bacteria and eukaryotes as tools to study these relationships. We have been capable of predictably accelerating and decelerating translation speed by engineering sequences that encode identical proteins, yet contain systematic variations along wobble positions of synonymous codons. Furthermore, we find that subtle manipulation of translation speeds along particular mRNA segments by these means can lead to significant increases in the folding efficiencies of these proteins upon recombinant production in bacteria. *We believe our findings provide insight into how the genetic code, although nearly universal in its amino acid encoding properties, is utilized differently between bacteria and eukaryotes to regulate local protein folding events during protein biogenesis.* Additionally, our results may provide further insight into how so-called “silent” polymorphisms may result in human disease and on how physiological and disease-related variations in tRNA concentrations impact cellular proteostasis, implicated in a wide variety of human diseases.

This work was supported by an award from the Pew Scholars Program in the Biomedical Sciences to J.M.B.

THE ROLE OF UDP-GLC:GLYCOPROTEIN GLUCOSYLTRANSFERASE 1 IN GLYCOPROTEIN MATURATION

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An endoplasmic reticulum (ER) quality control system assists in efficient folding and disposal of misfolded proteins. N-linked glycans are critical in these events because their composition dictates interactions with molecular chaperones. UDP-glucose: glycoprotein glucosyltransferase 1 (UGT1) is a key quality control factor of the ER. It adds glucoses to N-linked glycans of non-glucosylated substrates that fail a quality control test, supporting additional rounds of chaperone binding and ER retention. How UGT1 functions in its native environment is poorly understood. The role of UGT1 in the maturation of glycoproteins at basal expression levels was analyzed. Prosaposin was identified as a prominent endogenous UGT1 substrate. A dramatic decrease in the secretion of prosaposin was observed in ugt1-/- cells with prosaposin localized to large juxtanuclear aggresome-like inclusions, indicative of its misfolding and the essential role that UGT1 plays in its proper maturation. A model is proposed that explains how UGT1 may aid in the folding of sequential domain containing proteins such as prosaposin.

ROLE OF CYCLOPHILIN B IN ER-ASSOCIATED PROTEIN DEGRADATION

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It is generally believed that formation/disassembly of intra- and inter-molecular disulfide bonds and cis/trans isomerization of prolyl bonds in nascent polypeptides are rate-limiting steps for protein maturation in the endoplasmic reticulum (ER). Disulfide bonds reduction and prolyl bonds isomerization might also be crucial to insure the *partial* unfolding of terminally misfolded polypeptide chains that must be dislocated across the ER membrane for degradation operated by cytosolic proteasomes. The crucial intervention of several ER-resident oxidoreductases (PDIs) in polypeptide maturation is well documented and PDIs involvement in disposal of folding-defective proteins (ERAD) has also been established. In contrast, a role of peptidyl prolyl cis/trans isomerases (PPIs) in conformational maturation of nascent polypeptides or in protein quality control in the ER lumen of living cells is not yet supported by experimental data. Several tests aiming at understanding the role of PPIs by genetic manipulation or by pharmacologic inactivating their activity failed to reveal detectable phenotypes *in vivo*. This questions the involvement and the relevance of PPIs in protein quality control in the living cell.

Here we show the potent inhibitory effect of Cyclosporine A (CsA) on disposal from the ER of soluble, misfolded polypeptides (ERAD-Ls substrates). CsA is a cyclic undecapeptide produced by the fungus *Tolypocladium inflatum gams* used in the clinics as an immunosuppressant to reduce the risk of graft rejection. CsA specifically inactivates immunophilin members of the PPIs family. We identify cyclophilin B as the ER-resident target of CsA that regulates disposal from the ER of a sub-class of ERAD-Ls substrates containing cis-prolines.

TRANSCRIPTION FACTORS TFE3 AND MLX REGULATE EXPRESSION OF GOLGI-RELATED GENES IN MAMMALIAN GOLGI STRESS RESPONSE

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When increased production of secretory proteins overwhelms the capacity of the endoplasmic reticulum (ER) and the Golgi apparatus, eukaryotic cells expand their capacity to sustain secretory function. The capacity of the ER is enhanced by the mechanism called the ER stress response, but the mechanism regulating Golgi capacity (the Golgi stress response) has remained unclear. Here, we found that transcription of Golgi-related genes, including Golgi-specific glycosylation enzymes, was upregulated when mammalian cells were treated with a Golgi stress-inducing agent, monensin. This transcriptional induction is commonly regulated by a novel cis-acting element called the Golgi apparatus stress response element (GASE). We isolated a bHLH-ZIP protein, MLX, as a GASE binding protein, and found that overexpression of MLX suppressed transcriptional induction mediated through GASE, whereas another bHLH-ZIP protein, TFE3, is essential for GASE-mediated transcriptional induction. These findings suggested that the mammalian Golgi stress response is controlled by an elaborate network of bHLH-ZIP transcription factors.

PROTEOSTASIS NETWORK RESPONSES TO STRESS IN YEAST AND HUMAN

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Age-associated neurodegenerative diseases associated with protein misfolding, such as Huntington's, Parkinson's, and Alzheimer's diseases, generate patterns of neuronal stress responses that allow a systems-based approach for the identification of novel drug targets, disease progression biomarkers, and the effect of putative therapeutics on normalizing network-level alterations. We have developed a novel searchable protein-protein interaction database (PN-Explorer) utilizing data obtained across five species from yeast to human. By assembling an interactome network using physical interaction data and overlaying gene expression datasets, we have identified sub-networks that are co-expressed under various stress conditions. Furthermore, analyzing transcriptional data from a range of human post-mortem brain tissues, we have identified both positive and negative network responses associated with neurodegeneration. These data uncover similarities and differences in the proteostasis network in response to aging and age-onset diseases, distinct in qualitative and quantitative ways from network changes associated with the aging process. Such differences can be used to identify novel pathways and targets for therapeutic intervention, and should also provide insights into disease-progression biomarkers.

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VISITOR INFORMATION

EMERGENCY	CSHL	BANBURY
Fire	**(9) 742-3300**	**(9) 692-4747**
Ambulance	**(9) 742-3300**	**(9) 692-4747**
Poison	**(9) 542-2323**	**(9) 542-2323**
Police	**(9) 911**	**(9) 549-8800**
Safety-Security	**Extension 8870**	

Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Doctor MediCenter 234 W. Jericho Tpke., Huntington Station	**631-423-5400** (**1034**)
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400** (**1039**)

Free Speed Dial
Dial the four numbers (********) from any **tan house phone** to place a free call.

GENERAL INFORMATION

Books, Gifts, Snacks, Clothing, Newspapers
BOOKSTORE 367-8837 (hours posted on door)
Located in Grace Auditorium, lower level.

Photocopiers, Journals, Periodicals, Books, Newspapers
Photocopying – Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar
Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room

Messages, Mail, Faxes

Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking

June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m. Two tennis courts open daily.

Russell Fitness Center

Dolan Hall, west wing, lower level
PIN#: **Press 64345 (then enter #)**

Concierge

On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones

Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

Take Syosset Taxi to Syosset Train Station ($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
US Airways	800-428-4322