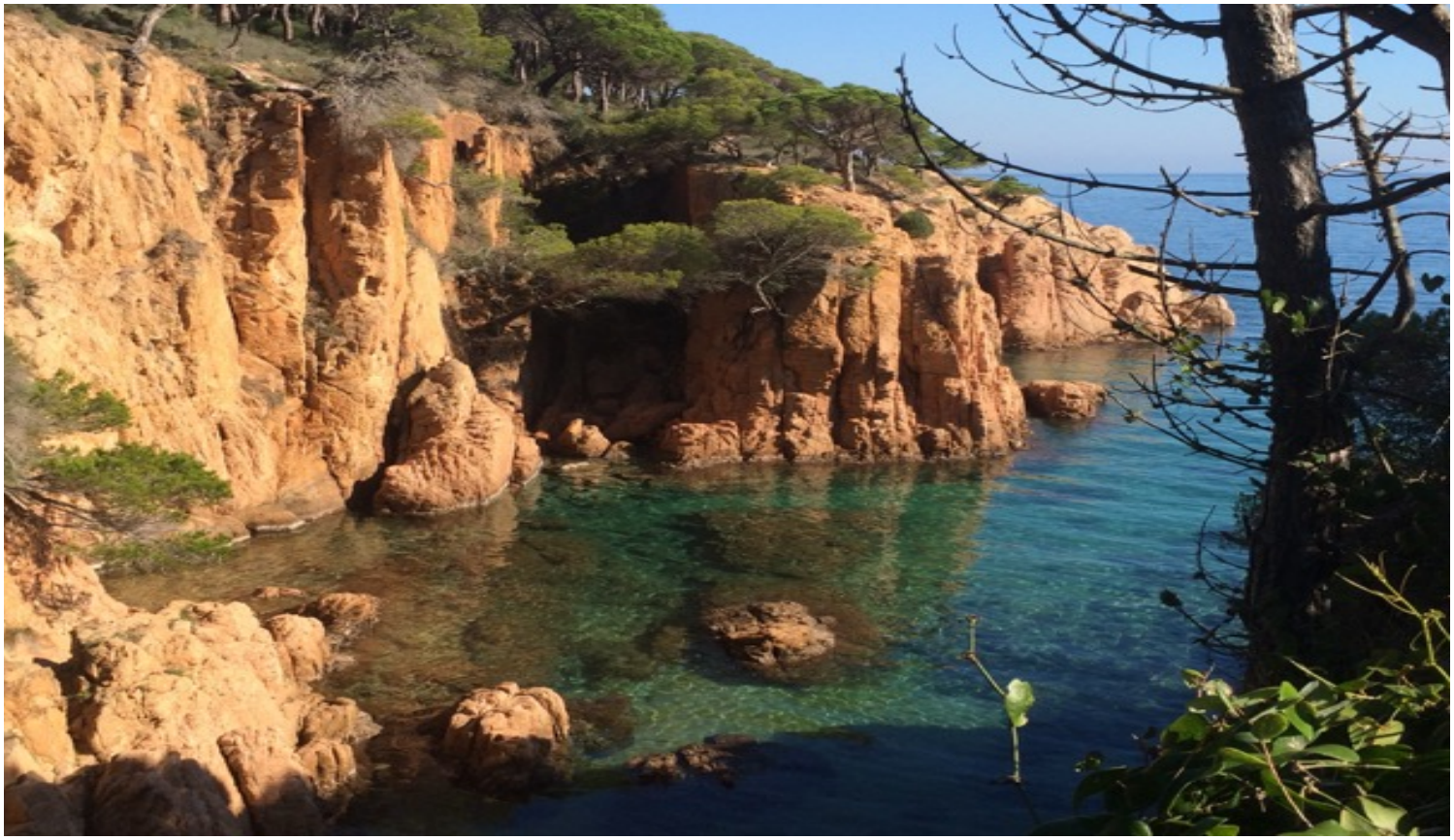
EMBO Conference

Protein Quality Control: Success and failure in health and disease

14th to 19th May 2017

Sant Feliu de Guixols, Girona, Spain



Organising Committee: A. Bertolotti MRC LMB, Francis Crick Avenue, Cambridge, CB2 0QH, UK
UK Tel: 0044 1223 267 051 aberto@mrc-lmb.cam.ac.uk
B Bukau (Germany), P. Murphy (UK), F Birnie (UK)

PROTEIN QUALITY CONTROL: SUCCESS AND FAILURE IN HEALTH AND DISEASE
14TH – 19TH May 2017: SCIENTIFIC PROGRAMME

SUNDAY 14TH MAY

From 14:00		Arrival and Check-in
15:00 – 17:30		Registration
17:30 – 19:00		Welcome Reception
19:00 – 20:00		Dinner
Opening Session, Chair: Anne Bertolotti		
20:00 – 21:00	Helen Saibil	Keynote Lecture 1 - Advances in the structural biology of chaperones and amyloids
21:00 – 22:00	Jonathan Weissman	Keynote Lecture 2 - Systematically mapping of mammalian stress pathways through CRISPRi and CRISPRa

MONDAY 15TH MAY

Quality control at the ribosome, part 1 Chairs: Günter Kramer and Martine Collart		
09:00 – 09:30	Rachel Green	Quality control at the ribosome
09:30 – 10:00	Toshi Inada	Protein quality control at the ribosome
10:00 – 10:30	Claudio Joazeiro	Ribosome-associated quality control and neurodegeneration
10:30 – 10:50	Kim Schneider (ST)	The benefit of eIF2a phosphorylation, a central hub in stress responses **Note: ST = Short Talk**
10:50 – 11:10		**COFFEE BREAK**
Quality control at the ribosome, part 2 Chairs: Günter Kramer and Martine Collart		
11:10 – 11:40	Susan Ackerman	Translation Fidelity and Neurodegeneration
11:40 – 12:10	Zoya Ignatova	Controlling translation under stress
12:10 – 12:30	Irmgard Sinning (ST)	RAC/Ssb - a unique chaperone triad on the ribosome
12:30 – 13:00	Ulrich Hartl	Ribosome quality control for nuclear encoded mitochondrial proteins
13:00 – 14:00		**LUNCH**
Chaperones, Part 1 Chairs: Jaroslaw Marszalek and Patricija van Oosten-Hawle		
15:45 – 16:15	Elke Deuerling	Chaperones acting at central hubs of protein biogenesis
16:15 – 16:35	Claes Andréasson (ST)	Releasing misfolded proteins from Hsp70
16:35 – 16:55	Tania Morán Luengo (ST)	The function of Hsp90 in protein folding
16:55 – 17:30		**COFFEE BREAK**
Chaperones, Part 2 Chairs: Jaroslaw Marszalek and Patricija van Oosten-Hawle		
17:30 – 18:00	Bernd Bukau	Co-translational protein folding and assembly
18:00 – 18:20	Daniel Southworth (ST)	Substrate translocation by the Hsp104 disaggregase visualized by cryo-EM
18:20 – 18:40	Marta Carroni (ST)	Different mechanisms but conserved functions: coiled-coil domains control AAA+ protein activity
18:40 – 19:00	Jorge Cuéllar (ST)	The role of CCT-PhLP1 system in the folding of mLST8, a key component of mTOR complex
19:00 – 20:30		**DINNER**
20:30 – 22:30		**POSTER SESSION 1: POSTER NUMBERS 1-40**

TUESDAY 16TH MAY

Chaperone mechanisms, Part 1 Chairs: Didier Picard and Jason Young		
09:00 – 09:30	Johannes Buchner	Chaperone dynamics
09:30 – 10:00	Sander Tans	Probing chaperone action at the single molecule level
10:00 – 10:20	Jaroslaw Marszalek (ST)	Probing molecular mechanisms of J-protein-Hsp70 interaction with chaperones dedicated for iron-sulfur cluster biogenesis.
10:20 – 10:40	Matthias Mayer (ST)	Molecular mechanism of the regulation of Hsp70s by J-domain cochaperones.
10:40 – 11:10		**COFFEE BREAK**
Chaperone mechanisms, Part 2 Chairs: Didier Picard and Jason Young		
11:10 – 11:40	Rachel Klevit	Rescuing the rescuer: small heat shock proteins in disease
11:40 – 12:10	Jason Gestwicki	Targeting chaperones for therapeutics
12:10 – 12:40	Manajit Hayer-Hartl	Rubisco, a paradigm for chaperone-assisted assembly and conformational remodeling
12:40 – 13:00	Krzysztof Liberek (ST)	Two component sHsp bacterial system in counteracting irreversible protein aggregation and promoting Hsp70/Hsp100-dependent efficient refolding
13:00 – 14:00		**LUNCH**
Protein Degradation, Part 1 Chairs: Thibault Mayor & Eri Sakata		
15:15 – 15:45	Shigeo Murata	Activation of Nrf1 compensates for proteasome dysfunction
15:45 – 16:15	Michael Knop	A systematic approach to dissect proteasome degradation in yeast
16:15 – 16:45	Jeff Brodsky	ER associated degradation
16:45 – 17:05	Adrien Rousseau (ST)	An evolutionarily conserved pathway controls proteasome homeostasis
17:05 – 17:30		**COFFEE BREAK**
Protein Degradation, Part 2 Chairs: Thibault Mayor & Eri Sakata		
17:30 – 18:00	Eilika Weber-Ban	Substrate recruitment strategies to the bacterial proteasome
18:00 – 18:30	Ron Kopito	Functional genomic analysis of protein quality control networks in the mammalian endoplasmic reticulum and cytosol
18:30 – 19:00	Pedro Carvalho	Mechanisms of ER-associated protein degradation
19:00 – 20:30		**DINNER**
20:30 – 22:30		**POSTER SESSION 2: POSTER NUMBERS 40-80**

WEDNESDAY 17TH MAY

Cellular strategies against misfolded proteins Chairs: Dr. Gian Gaetano Tartaglia and Anat Ben-Zvi		
09:00 – 09:30	Judith Frydman	Role of molecular chaperones in protein quality control
09:30 – 10:00	Lea Sistonen	Transcriptional memory and re-programming of gene expression in cell stress
10:00 – 10:20	Martin Vabulas (ST)	CHIP as a Sensor of Proteostasis Stress
10:20 – 10:40	Thibault Mayor (ST)	Should I stay or should I go - clearance of cytosolic misfolded proteins
10:40 – 11:00		**COFFEE BREAK**
The Sue Lindquist Memorial session Chair: Yuri Chernoff		
11:00 – 11:10	Jeff Kelly	Remembering Sue Lindquist
11:10 – 11:40	Rick Morimoto	Resetting Organismal proteostasis by chromatin remodelling and transcellular signalling
11:40 – 12:10	Simon Alberti	RNP granules: how they form, age and cause disease

12:10 – 12:30	Ritwick Sawarkar (ST)	Molecular chaperones regulate transcriptional response to stress
12:30 – 13:00	Jeff Kelly	The role of the proteostasis network in changing protein structure relative to spontaneous folding
13:00 – 19:00		**AFTERNOON EXCURSION – WITH PACKED LUNCH**
19:00 – 20:30		**DINNER**
20:30 – 22:30		**POSTER SESSION 3: POSTER NUMBERS 80-119**

THURSDAY 18TH MAY

Protein quality control in organelles, Part 1 Chairs: Rebecca Taylor and Christian Münch		
09:00 – 09:30	Kazu Mori	The unfolded protein response through evolution
09:30 – 09:50	Avi Ashkenazi (ST)	Coordination between two branches of the unfolded protein response determines cell fate
09:50 – 10:20	Peter Walter	The unfolded protein response in health and disease
10:20 – 10:40	Niko Amin-Wetzel (ST)	An ER-localized J protein recruits BiP to IRE1α and represses the Unfolded Protein Response
10:40 – 11:00	Mike Cheetham (ST)	The role of the unfolded protein response in retinal degeneration
11:00 – 11:20		**COFFEE BREAK**
Protein quality control in organelles, Part 2 Chairs: Rebecca Taylor and Christian Münch		
11:20 – 11:50	Agnieszka Chacińska	Crosstalk between mitochondria and cellular protein quality control mechanisms
11:50 – 12:20	Cole Haynes	Adaptation to mitochondrial stress
12:20 – 12:50	Jean-Francois Collet	Revisiting lipoprotein sorting in Gram-negative bacteria: disorder matters
12:50 – 13:10	Hemmo Meyer (ST)	Orchestration of the Endo-Lysosomal Damage Response (ELDR) by VCP/p97
13:10 – 16:00		**AFTERNOON EXCURSION – WITH PACKED LUNCH DEPENDING ON INTEREST**
Protein aggregation in disease, Part 1 Chairs: Konstanze Winklhofer and David Vilchez		
16:00 – 16:30	Sheena Radford	Mechanism of amyloids formation
16:30 – 16:50	Janine Kirstein (ST)	Complete Suppression of HTT Fibrilization and Disaggregation of HTT Fibrils by a Trimeric Chaperone Complex
16:50 – 17:10	Anthony Fitzpatrick (ST)	The atomic structures of Tau filaments from Alzheimer's disease brain
17:10 – 17:30		**COFFEE BREAK**
Protein aggregation in disease, Part 2 Chairs: Konstanze Winklhofer and David Vilchez		
17:30 – 18:00	Harm Kampinga	Heat shock proteins and protein aggregation diseases
18:00 – 18:20	Serena Carra (ST)	Aberrant compartment formation by HSPB2 mislocalizes lamin A and compromises nuclear integrity and function
18:20 – 18:40	Georgios Karras (ST)	A role for protein folding in driving the age-related genome instability of cancers
18:40 – 18:55	Anne Bertolotti & Bernd Bukau	End of Meeting Summary
19:30 – 22:00		**GALA DINNER & PARTY**

FRIDAY 19TH MAY

DEPARTURE

Abstracts List

Membrane protein insertion and folding by the bacterial holo-translocon
Sara Alvira[1], Joanna Komar[1], Mathieu Botte[3], Imre Berger[1, 3], Dafydd Jones[2], Christiane Schaffiztel[1, 3], Ian Collinson[1]

[1]*School of Biochemistry. University of Bristol. UK*
[2]*School of Biosciences. University of Cardiff. UK*
[3]*EMBL-Grenoble. France*

In all organisms, many proteins must be transported across and into membranes before arriving at their functional destination. In bacteria, the SecYEG translocon is a protein-conducting membrane channel that can function promoting the transport of secretory proteins or assisting the insertion of integral membrane proteins. In this last case, SecYEG associates with YidC and SecDF-YajC to form a functional complex called the holo-translocon (HTL) able to insert and fold membrane proteins in a co-translational translocation mechanism universally conserved. In this work, we use classical biochemistry combined with synthetic biology approaches and structural techniques, mainly electron microscopy, to unravel the interactions of a membrane protein with the HTL, from its synthesis at the ribosome to the passage through the HTL, and to address how the HTL folds and promotes the assembling of membrane proteins within the lipid bilayer.

An ER-localized J protein directs BiP to mammalian IRE1α to repress the Unfolded Protein Response
Niko Amin-Wetzel, Reuben Saunders, Maarten Kamphius, Claudia Rato, Steffen Preissler, David Ron

Cambridge Institute for Medical Research

A BiP-IRE1 complex correlates with UPR repression but the mechanisms of its formation are unknown. Like other Hsp70 chaperones, BiP binding to substrates is catalysed by J-domain co-chaperones that stimulate ATP hydrolysis and guide the chaperone to its targets. Here we report the discovery of an ER-localised J-protein that associates with the human IRE1α luminal domain and promotes formation of a complex between BiP and IRE1α. Complex formation observed in vitro with purified components requires a functional J domain and ATP hydrolysis by BiP. Inactivation of the gene encoding this ER-localised J-domain protein selectively de-represses the IRE1α branch of the UPR. Reintroduction of the J-protein into the mutant cells attenuates IRE1α signalling and enhances the recovery of a BiP-IRE1α complex. These findings suggest a role for an ER localised J-protein in repressing the IRE1α branch of the UPR by accelerating the formation of a repressive BiP-IRE1α complex.

Protein quality control: Releasing misfolded proteins from Hsp70
Naveen NK Gowda, Jayasankar M Kaimal, Claes Andréasson

Stockholm University, Department of Molecular Biosciences, The Wenner-Gren Institute, 106 91 Stockholm, Sweden

The chaperone Hsp70 participates in protein quality control by associating with proteins that occupy non-native conformations. Nucleotide-exchange factors accelerate the release of proteins from Hsp70 and give them the opportunity to fold and interact with quality control factors. We are seeking to understand the functions of the conserved nucleotide-exchange factors of the cytoplasm and endoplasmic reticulum, Hsp110/Grp170 and Fes1/Sil1. We have found that Fes1 plays a dedicated role to release proteins from Hsp70 and therefor to target misfolded proteins for ubiquitin-dependent degradation. Functional analysis shows that Fes1 and its human homologue HspBP1 depend on a flexible substrate-release domain (SRD) for function. Fes1 associates with Hsp70, triggers nucleotide exchange and concomitantly hinders substrate rebinding by SRD interactions. The findings provide mechanistic insight into protein quality control active at the interface of chaperone and ubiquitin-proteasome systems.

The J-Protein Family of C. elegans – Expression analyses during Aging and Stress
Kristin Arnsburg, Janine Kirstein

Leibniz Institute for Molecular Pharmacology (FMP Berlin)

J-proteins are co-chaperones, which bind client proteins and stimulate the ATP hydrolysis activity of their Hsp70. Caenorhabditis elegans encodes for 9 different HSP-70 yet 31 J-proteins. This high diversity within the J-protein family suggests that these co-chaperones play an important role in the specificity of the Hsp70 machinery. So far, only little is known about their specific expression pattern. We identified dnj-13 as the most heat-inducible J-protein. Interestingly, overexpression of dnj-13 was beneficial during acute heat shock, but decreased the median life expectancy. In addition, we identified different J-protein expression patterns in various neurodegenerative disease models, which points to specific cellular responses. For example, only the JB6/8 homolog, dnj-24, was induced in the Huntington's disease model. Our results indicate a J-protein signature during various proteotoxic challenges and give insight into the organismal J-protein network in aging and disease.

Coordination between the PERK and IRE1 branches of the unfolded protein response
Avi Ashkenazi, Tsun-Kai Chang, Min Lu, Scot Marsters, David Lawrence, Jenille Tan, Scott Martin

Genentech Inc.

The mammalian UPR branches—PERK, IRE1 and ATF6—are well studied, but their coordination is incompletely understood. PERK and IRE1 have an ER-lumenal domain that senses misfolded proteins and in turn activates a cytoplasmic kinase module. PERK phosphorylates eIF2α to inhibit mRNA translation. IRE1 further harbors a cytoplasmic RNAse moiety, which activates the transcription factor XBP1s to expand ER capacity, and performs regulated IRE1-dependent mRNA decay (RIDD) to decrease translational load. Whereas PERK stimulation persists, IRE1 activation is transient. We discovered that the PERK-eIF2α pathway reverses IRE1 phosphorylation under ER stress in a phosphatase-dependent manner. SiRNA library screening revealed a specific phosphatase that mediates IRE1 dephosphorylation. Its disruption prolonged IRE1 phosphorylation and oligomerization and augmented XBP1s generation and RIDD under ER stress. Thus, PERK controls the duration of IRE1's activity to ensure temporal coordination of the UPR.

CHOP deletion reduces hepatic Z alpha-1 antitrypsin and p62 accumulation in PiZ mice

Sergio Attanasio[1], Nunzia Pastore[1], Raffaele Castello[1], Pasquale Piccolo[1], Nicola Brunetti-Pierri[1, 2]

[1]*Telethon Institute of Genetics and Medicine, 80078, Pozzuoli, Naples, Italy.*
[2]*Department of Translational Medicine, Federico II University, 80131, Naples, Italy.*

Liver disease in alpha-1 antitrypsin deficiency is due to accumulation of the mutant Z-alpha-1 antitrypsin (ATZ) in the endoplasmic reticulum (ER) of hepatocytes that leads to liver injury. Although ATZ accumulation causes ER stress, the role of unfolded protein response (UPR) has been controversial in the last years. We found that livers of PiZ transgenic mice, expressing the human ATZ, have activation of the UPR and sustained upregulation of CHOP. In PiZ livers, CHOP induces the gene expression and protein accumulation of p62, an autophagy receptor involved in liver protein aggregates. p62 was found to bind the mutant Z-alpha-1 antitrypsin and was found to form protein aggregates in PiZ livers. Finally, genetic ablation of Chop in PiZ mice resulted in reduction of hepatic ATZ and p62 accumulation. In summary, our data suggest that CHOP activation leads to p62-positive aggregates formation in PiZ mouse livers aggravating the proteotoxicity of ATZ.

Three is a crowd: protein disaggregation at the single-molecule level

Mario Avellaneda[1], Nadinath Nillegoda[2], Axel Mogk[2], Bernd Bukau[2], Sander Tans[1]

[1]*AMOLF*
[2]*ZMBH Heidelberg*

The proper functioning of proteins relies on their capability to fold to their native structure. Under stress, however, the folding process can be perturbed, resulting in the generation of misfolded and aggregated proteins. Aggregation can be critical for cells and, in humans, give rise to many diseases and disorders, such as Parkinson or Alzheimer. Fortunately, organisms are provided provided with sophisticated protein machineries that disrupt aggregates: chaperones.
However, the molecular mechanism by which chaperones interact with aggregates, leading to its eventual disruption, remains largely unknown. By looking at single aggregates and measuring how they respond to different conditions, it will possible to unravel the steps involved in chaperone-mediated protein disaggregation. We use optical tweezers to study in real time what is the folding state of a single aggregate and how it is affected by each of the components of the bacterial and eukaryotic disaggregation machineries.

Mechanism of actin folding by the eukaryotic chaperonin TRiC/CCT

David Balchin, Goran Milicic, Manajit Hayer-Hartl, F. Ulrich Hartl

Max Planck Institute of Biochemistry, Department of Cellular Biochemistry

TRiC, the essential heterooligomeric chaperonin in eukarya, folds 10% of the proteome. The most abundant TRiC substrate is the cytoskeletal protein actin, which cannot be folded by simpler chaperonin systems such as GroEL/ES in bacteria. We used various spectroscopic and structural techniques to determine the mechanism by which TRiC promotes the conformational progression of actin to the native state. We find that actin does not fold spontaneously, but reaches a dynamic misfolded state in the absence of aggregation. Binding of actin to specific TRiC subunits avoids misfolding and promotes the early acquisition of native-like secondary structure. ATP binding to TRiC effects an asymmetric conformational change in the chaperonin ring. This step causes partial release of actin, priming it for folding upon complete release into the chaperonin cavity, mediated by ATP hydrolysis. Our results reveal, for the first time, how TRiC directs the folding pathway of an obligate eukaryotic substrate.

Relocalization of the Hsp40 chaperone Sis1 into the nucleus differentially affects yeast prions

Yury Barbitoff[4], Andrew Matveenko[1, 2, 3], Svetlana Moskalenko[1, 3], Olga Zemlyanko[1, 2], Yury Chernoff[4, 2, 4], Galina Zhouravleva[1]

[1]*Department of Genetics and Biotechnology, St. Petersburg State University, St. Petersburg, Russia*
[2]*Laboratory of Amyloid Biology, St. Petersburg State University, St. Petersburg, Russia*
[3]*St. Petersburg Branch, Vavilov Institute of General Genetics, Russian Academy of Sciences, St. Petersburg, Russia*
[4]*School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia, USA*

Yeast self-perpetuating protein aggregates (prions) provide a convenient model for studying the protein quality control (PQC) system. The major components of the yeast PQC system are molecular chaperones and protein sorting factors. Here, we show that the chaperone sorting factor Cur1 oppositely affects yeast prions, curing [URE3] while enhancing [PSI+]. We link these effects to the ability of Cur1 to relocalize the Hsp40-Sis1 from cytoplasm into the nucleus. We demonstrate that both curing of [URE3] and enhancement of [PSI+] in the presence of excess Cur1 are counteracted by Sis1 in a dosage-dependent manner. We also show that the magnitude of both pro- and anti-prion effects of Cur1 correlates with the degree of Sis1 relocalization. Finally, we demonstrate that the effect of Cur1 on prions parallels effects of the attachment of nuclear localization signal to Sis1. These findings highlight the differential specificities of different prions to the Hsp40 chaperone balance.

Modeling Hsp70/Hsp40 interaction by multi-scale molecular simulations and co-evolutionary sequence analysis

Duccio Malinverni[1], Alfredo Jost Lopez[2], Paolo De Los Rios[1, 3], Gerhard Hummer[2, 4], Alessandro Barducci[5, 6]

[1]*Laboratoire de Biophysique Statistique, Faculte de Sciences de Base, Ecole Polytechnique Federale de Lausanne - EPFL, CH-1015 Lausanne, Switzerland*
[2]*Max Planck Institute of Biophysics, Max-von-Laue-Str. 3, D-60438 Frankfurt am Main, Germany*
[3]*Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Federale de Lausanne - EPFL, CH-1015 Lausanne, Switzerland*
[4]*Institut fur Biophysik, Johann Wolfgang Goethe Universitat Frankfurt, 60438 Frankfurt am Main, Germany*
[5]*Inserm, U1054, Montpellier, France*
[6]*Universite de Montpellier, CNRS, UMR 5048, Centre de Biochimie Structurale, Montpellier, France*

The interaction between the Heat Shock Proteins 70 and 40 is at the core of the ATPase regulation of the chaperone machinery that maintains protein homeostasis. However, the structural details into the interaction are still elusive and contrasting models have been proposed for the transient Hsp70/Hsp40 complexes. Here we combine molecular simulations based on both coarse-grained and atomistic models with co-evolutionary sequence analysis to shed light on this problem by focusing on the bacterial DnaK/DnaJ system. The integration of these

complementary approaches resulted in a novel structural model that rationalizes previous experimental observations. We identify an evolutionarily conserved interaction surface formed by helix II of the DnaJ J-domain and a groove on lobe IIA of the DnaK nucleotide binding domain, involving the inter-domain linker.

(bioRxiv, 067421 https://doi.org/10.1101/067421, currently under review in eLife)

BMC Biology editor - Protein Quality Control

Graham Bell

BMC Biology, BioMed Central, Springer Nature

BMC Biology is the flagship biology journal of the online open access publisher BioMed Central: we publish across the entire spectrum of biology, aiming for research papers of particular interest or importance, under the editorship of Miranda Robertson.

We are very interested in the topic of this meeting and will be launching a thematic series focussing on this area in the near future. The broad aim of the series will be to focus on causes and consequences of protein misfolding at membranes in particular, and will include research articles as well as reviews, Q+A articles etc, under the guest editorship of Peter Walter, Jonathan Weissman, Manu Hegde, and Anne Bertolotti.

Graham Bell, Senior Editor at BMC Biology, and who handles submissions on this topic, will hope to attend the meeting to hear about, and discuss with the excellent list of speakers, the latest work in this field.

Regulation of the Anaphase Promoting Complex/Cyclosome (APC/C) by the Proteasome Mediated Degradation of its Catalytic Subunit, Apc11

Shay Ben-Aroya[1], Marina Volpe[1], Gali Prag[2], Netanel Rosenstein[1], Nelly Levinton[1]

[1]*Bar-Ilan University*
[2]*Faculty of life sciences, Tel-Aviv University*

One of the challenges encountered by the protein quality control (PQC) is the need to ensure that members of multiprotein complexes are available in the correct proportions, otherwise, unassembled members may engage in promiscuous activity when not regulated in the context of their complex. To elucidate this issue, we identified the ubiquitin proteasome system (UPS) mediated degradation of Apc11, the catalytic subunit of the anaphase promoting complex/cyclosome (APC/C), primarily the excess subunits that do not incorporate into the fully assembled complex. Further work has shown that Apc11 accumulation is detrimental to cellular survival. This is at least partially explained by the resulting mistimed entry to the metaphase stage of the mitotic cycle.
We thus provide evidence that the overlooked UPS-mediated degradation of Apc11 is an essential aspect of APC/C regulation, which adds to the multiple layers of controls that have been well described in numerous studies.

Differentiation can determine cellular proteostasis

Yael Bar-Lavan[1], Netta Shemesh[1], Shiran Dror[1], Rivka Ofir[2], Esti Yeger-Lotem[3], Anat Ben-Zvi[1]

[1]*Department of Life Sciences and The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel*
[2]*Regenerative Medicine and Stem Cell Research Center, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel*
[3]*Department of Clinical Biochemistry and Pharmacology and The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel*

In multicellular organisms, the composition of the proteome, and by extension, protein-folding requirements, varies between cells. In agreement, chaperone network composition differs between tissues. Here, we ask how cellular differentiation affects chaperone expression. Our bioinformatics analyses show that the myogenic transcription factor HLH-1 can bind to the promoters of muscle-expressed chaperones. When we employed HLH-1 myogenic potential to genetically modulate cellular differentiation of C. elegans embryonic cells, we found that HLH-1-dependent myogenic conversion induced the expression of HLH-1-regulated chaperones in differentiating muscle cells. Disrupting HLH-1 function in muscle cells reduced the expression of HLH-1-regulated chaperones and compromised muscle proteostasis, disrupting, in turn, the folding of muscle proteins and thus, myogenesis. We propose that cellular differentiation could establish a proteostasis network dedicated to the folding of the muscle proteome.

The plasticity of the Hsp90 co-chaperone system

Maximilian Biebl, Priyanka Sahasrabudhe, Julia Rohrberg, Daniel Rutz, Johannes Buchner
Technische Universität München

The Hsp90 chaperone system is required for the maturation of a very diverse set of clients, including steroid hormone receptors, kinases and E3 ubiquitin ligases. The conformational cycle of Hsp90 which is closely connected to client maturation is regulated by a plethora of co-chaperones. Despite many studies that investigated the mechanisms of interactions between Hsp90 and co-chaperones, the question how the cohort of co-chaperones influences the processing of client proteins in vivo has remained elusive. Here we comprehensively studied the effect of Hsp90 co-chaperones on the activity of different Hsp90 client proteins in S. cerevisiae. Our results highlight the complexity of the Hsp90 co-chaperone network and define client-specific co-chaperone requirements as well as co-chaperones that are universally important for client maturation. Thus, co-chaperones adapt the Hsp90 cycle to the requirements of the client protein and ensure optimal client activation by the Hsp90 machinery.

Ameliorating ageing through protein synthesis improvements

Victoria Martinez Miguel[1], Celia Lujan[1], Tobias von der Haar[2], Ivana Bjedov[1]

[1]*University College London; UCL Cancer Institute; Paul O'Gorman Building; London, UK*
[2]*University of Kent; Kent Fungal Group; School of Biosciences; Canterbury; UK*

A remarkable discovery in the field of ageing research is that a single gene mutation in any of the major nutrient signalling pathways leads to a pronounced lifespan extension and improved health in model organisms from yeast to mammals. The most well-studied anti-ageing alterations are down-regulation of insulin signalling, target-of-rapamycin signalling or dietary restriction. Interestingly, all these interventions decrease protein synthesis, but the mechanism why lessened translation increases lifespan is largely elusive. We performed a focused RNAi screen to uncover novel anti-ageing genes that are implicated in protein synthesis. We are currently examining various hypotheses to explain longevity of translation mutants, such as improved proteostasis and quality of synthesised proteins. Given the importance of protein translation in ageing, as evidenced by several longevity screens and ageing studies, elucidating the underlying mechanism is timely and critical for better understanding of the ageing process and the discovery of novel drug targets which could lead to healthier ageing.

Molecular chaperones control the physical state of membrane-less compartments

Edgar Boczek[1], Daniel Mateju[1], Marcus Jahnel[1], Louise Jawerth[1], Ina Poser[1], Andrii Kopach[1], Serena Carra[2], Stephan Grill[1], Franz-Ulrich Hartl[3], Anthony Hyman[1], Simon Alberti[1]

[1]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*
[2]*University of Modena and Reggio Emilia, Department of Biomedical, Metabolic and Neural Sciences, Modena, Italy*
[3]*Max Planck Institute of Biochemistry, Department of Cellular Biochemistry, Martinsried, Germany*

Stress granules (SGs) are membrane-less organelles that assemble through phase separation. In vitro, SG proteins like Fused In Sarcoma (FUS) form liquid droplets that undergo a transition into solid fibrils. This process of molecular aging is accelerated by mutations in FUS that have been linked to amyotrophic lateral sclerosis (ALS). Here, we show that diverse chaperone systems maintain the liquid-like properties of SGs. A special small heat shock protein stops the molecular aging process and a disease-associated mutation abrogates this housekeeper ability. In contrast, more general chaperones preferentially target misfolded proteins that accumulate in the liquid droplet phase of SGs. We propose that aberrant phase transitions within liquid-like compartments lie at the heart of age-related diseases such as ALS and that the cellular chaperone machinery specifically targets and controls these processes.

A novel Hsp90 co-chaperone, CHP-1, in brain of patients with Parkinson's disease and Lewy Bodies Dementia

Anastasiia Bohush[1], Anna Filipek[1], Serge Weis[2]

[1]*Anastasiia Bohush, Anna Filipek*
Nencki Institute of Experimental Biology, Warsaw, Poland
[2]*Serge Weis*
Neuropathology, Neuromed Campus, Kepler University Hospital, Johannes Kepler University, Linz, Austria

In both Parkinson's disease (PD) and Dementia with Lewy bodies (DLB) inclusions of misfolded alpha-synuclein are present. It has been suggested that formation of these inclusions might be inhibited by chaperones and co-chaperones.
In this work we analyzed the localization and estimated the level of a Hsp90 co-chaperone, CHP-1, in the brain of patients with PD and DLB. Applying immunohistochemistry, we have found that CHP-1 is localized in Lewy bodies and by means of qRT-PCR and Western blot we have determined that its level is different in PD and DLB brains compared to control. Also, the level of the CHP-1 mRNA/protein is up-regulated in the cortex (frontal and temporal) and in the substantia nigra of patients with PD and DLB. Using mouse neuroblastoma NB2a cells and applying the proximity ligation assay we have shown that CHP-1 interacts with alpha-synuclein. Thus, our results suggest that a Hsp90 co-chaperone, CHP-1, might be involved in the pathogenesis of PD and DLB.

Genetic misregulation of the stability of the transcription factor Pap1 alters the tolerance of fission yeast to oxidative stress

Luis Marte, Susanna Boronat, Elena Hidalgo

Oxidative Stress and Cell Cycle Group, Universitat Pompeu Fabra, C/ Dr. Aiguader 88, 08003 Barcelona, Spain

One of the toxicity hallmarks associated to oxidative stress is protein carbonylation. In a search for S. pombe genes involved in carbonylated protein degradation, we identified some protein quality control (PQC) deletion mutants with lower carbonylated protein levels upon oxidative stress than wild-type cells. Some of these mutants had enhanced levels of the transcription factor Pap1. Upon mild oxidative stress, Pap1 activates H2O2-detoxifying genes transcription what may lead to decreased protein damage in our mutants. To assess the role of our newly identified genes in Pap1 degradation, we studied the stability of nuclear or cytosolic forms of Pap1 expressed in our PQC mutant strains. We observed that some of these PQC mutants had a cell localization specific effect in Pap1 degradation. We also observed that Pap1 levels in the mutants were not sufficient to explain decreased protein carbonyls, suggesting a direct role of the PQC system in the degradation of carbonylated proteins.

Genetic mediators of Alpha 1-Antitrypsin Deficiency –mediated liver toxicity

Philippe Joly[1], Hélène Vignaud[2], Julie di Martino[2], Roman Garin[1], Lioara Restier[1], Christophe Cullin[1], Christelle Marchal[2], Aaron D. Gitler[3], Alain Lachaux[1], Julien Couthouis[3], Marion Bouchecareilh[2]

[1]*Service d'hépatologie-gastroentérologie et nutrition pédiatriques, Service d'hépatologie-gastroentérologie et nutrition pédiatriques, Hôpital Femme Mère Enfant, CHU de Lyon*
[2]*CNRS IBGC UMR5095, Bordeaux, France*
[3]*Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA*

Alpha 1-antitrypsin deficiency (α1ATD) is a rare genetic disorder that manifests as emphysema and cirrhosis. The main cause of α1ATD is the retention of mutant α1AT proteins in the endoplasmic reticulum (ER). The most common and severe disease causing alleles is Z-α1AT. In

homozygous patients, the protein is not only inefficiently exported but also accumulates in the ER which is the main cause of liver disease. The mechanisms by which Z-α1AT aggregates cause ER dysfunctions remain to be elucidated. Based on recent evidences and on the frequency of liver disease occurrence in Z-α1AT patients, it seems likely that likely that liver damages are linked to unknown genetic factors yet to be elucidated. To this end, we have developed an innovative approach combining next generation exome sequencing with genomics screens. So far, the results obtained suggest that slightly impaired Endoplasmic Reticulum Associated Degradation (ERAD) machinery could cause the liver damages associated with α1ATD.

Designing proteasome adaptors to degrade specific targets

Kimberly Bowen, Grace Kago, Shameika Wilmington, Mai Dao, Andreas Matouschek

University of Texas at Austin

Here we present a system to deplete specific proteins from cells using adaptors, called degradons, which shuttle target proteins to the proteasome for destruction. The degradons interact with the proteasome through a ubiquitin-like domain and recognize the target through an affinity domain derived from the tenth domain of fibronectin 3, called a monobody. The degradons recognize the Src homology 2 (SH2) domains of Abl kinase or Shp2 phosphatase. We have shown that, in vitro, degradons acted catalytically to shuttle fluorescent model proteins containing SH2 domains to the proteasome. In cultured cells, degradons delivered by transient transfection were able to deplete stably expressed model proteins by two- to four-fold. Finally, we found that degradons led to nearly 50% reduction in the levels of endogenous Shp2 in HEK293T cells. Currently, we are optimizing the degradons to increase the degradation efficiency of their targets.

Chaperone-mediated substrate selection during endoplasmic reticulum associated degradation (ERAD)

Jeffrey Brodsky

University of Pittsburgh

Once selected by chaperones, proteins in the ER are removed via a process termed ER associated degradation (ERAD). Many of the critical players in the ERAD pathway have been identified and their functions discerned. However, a growing number of substrate-specific modifiers of ERAD have been identified. Because the ERAD pathway has been associated with ~70 diseases, we propose that ERAD modifiers might be disease modifiers. We also propose that the correction of misfolded, disease-linked secreted proteins will require ERAD-targeted drugs. To this end, we have used yeast-based expression systems to dissect the ERAD pathway for proteins linked to human diseases and identify uncharacterized factors and pathways that modulate ERAD. Candidates from these efforts are then tested in mammalian models.

Structural Studies Of The CCT-Gelsolin Complex

María Teresa Bueno[1], Jorge Cuéllar[1], Andreas Svanström[2], Julie Grantham[2], José M. Valpuesta[1]

[1]*Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, 28049, Madrid, España*
[2]*Department of Chemistry and Molecular Biology, University of Gothenburg, Medicinaregatan 9C, 40530 Gothenburg, Sweden*

The chaperonin CCT is involved in assisting the folding of 10% of cytosolic proteins but some of them do not require interactions with CCT to get its proper folding. Gelsolin is an actin filament severing protein that increases actin dynamics by generating filament ends for further actin polymerization. The binding to CCT is slow and gelsolin is accumulated over time suggesting that this protein is not a real folding substrate of the chaperonin. Therefore, CCT could have a regulatory effect on gelsolin, acting indirectly in actin filament dynamics.

The aim of this project is the structural characterization of the CCT-Gelsolin complex by electron-microscopy to elucidate the binding mechanism that mediates such interaction and whether CCT has an actual role on actin regulation. To face this, we performed binding assays between CCT and Gelsolin adding a crosslinker to stabilize the complex, which was purified by gel filtration and now is being used for further structural characterization.

The role of untranslated protein response in the elimination of B cells lacking the B cell receptor.

Marieta Caganova, Claudia Grosse, Klaus Rajewsky
The Max Delbrück Center for Molecular Medicine (MDC)

Acute ablation of the BCR in resting mature naïve B cells in mice leads to a fast disappearance of BCR- B cells from peripheral lymphoid system. This phenomenon has been mainly explained by a "tonic" signal provided by the BCR, essential for B cell survival. However, other possible mechanisms may exist. It has been proposed that ablation of BCR from surface of a B cell activates a "danger" signal leading to apoptosis of BCR- B cells. Indeed, ex vivo purified BCR- B cells show signs of endoplasmatic reticulum (ER) stress and UPR. A causal relationship between UPR induction and BCR- B cell death remains to be established. In my research project I will address the role of UPR in elimination of BCR- B cells in vivo. By addressing this point my work will give the first-time evidence for the existence of "a danger signal" in a B cell lacking functional BCR.

Granulostasis: protein quality control of stress granules

Simon Alberti[1], Daniel Mateju[1], Laura Mediani[2], Serena Carra[2]

[1]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, 01307, Germany*
[2]*University of Modena and Reggio Emilia, Department of Biomedical, Metabolic and Neural Sciences and Center for Neuroscience and Nanotechnologies, Via Campi 287, 41125 Modena, Italia*

Stress granules (SGs) are ribonucleoprotein particles that assemble when translation is inhibited. In amyotrophic lateral sclerosis and inclusion body myopathy, the SG components TDP-43, FUS, hnRNPA1 accumulate in the form of protein aggregates. We therefore investigated the mechanisms that drive the conversion of physiological SGs into aggregates. We find that liquid-like SGs can sequester misfolded proteins, which promote the aberrant conversion of SGs into solid aggregates. We identify a specific protein quality control process that prevents the accumulation of misfolding-prone proteins in SGs and, by doing so, preserves SG dynamics. This quality control process is referred to as

granulostasis, and relies on the action of HSPB8-BAG3-HSP70. BAG1 and BAG6 cannot functionally replace BAG3. Finally, we show that aberrant SGs are preferentially disassembled in an HSP70-dependent manner in the stress recovery phase and only a minor proportion of SGs is targeted to autophagy.

Aberrant compartment formation by HSPB2 mislocalizes lamin A and compromises nuclear integrity and function

Federica Morelli[1], Jessika Bertacchini[1], Milena Nasi[2], Simon Alberti[3], Serena Carra[1]

[1]*University of Modena and Reggio Emilia, Department of Biomedical, Metabolic and Neural Sciences and Center for Neuroscience and Nanotechnologies, Via Campi 287, 41125 Modena, Italia*
[2]*Department of Surgery, Medicine, Dentistry and Morphology, University of Modena and Reggio Emilia, Modena, Italy*
[3]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, 01307, Germany*

HSPB2 is a small heat shock protein that contains intrinsically disordered regions (IDRs). In cells, IDR-containing proteins drive the assembly of liquid droplets via a process known as liquid-liquid demixing. Evidence that HSPBs can phase separate into liquid droplets is lacking. We report that, in cells, HSPB2 phase separates to form nuclear assemblies that behave as liquid droplets. We find that the intrinsically disordered C-terminal domain of HSPB2 is required for phase separation. We also show that HSPB2 nuclear droplets sequester lamin A and displace chromatin, with detrimental consequences for nuclear function and integrity. Importantly, aberrant phase separation of HSPB2 is negatively regulated by its partner HSPB3, but not by two HSPB3 mutations associated with myopathy. Finally, we report that HSPB2 nuclear foci colocalize with lamin A in differentiating myoblasts. These results suggest that HSPB2 might participate in cellular reorganization during myoblast differentiation.

Structural analysis of ClpC activity regulation

Marta Carroni[1, 3], Kamila Franke[2], Michael Maurer[2], Jasmin Jäger[2], Bernd Bukau[2], Axel Mogk[2]

[1]*Cryo-EM centre, Science for Life Laboratory, Tomtebodavägen 23, Solna, Stockholm, Sweden*
[2]*Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Deutsches Krebsforschungszentrum (DKFZ), DKFZ-ZMBH Alliance, Im Neuenheimer Feld 282, Heidelberg D-69120, Germany*
[3]*Department of Crystallography/Biological Sciences, Institute of Structural and Molecular Biology, Birkbeck College, London WC1E 7HX, UK.*

The AAA+ unfoldase ClpC forms together with the peptidase ClpP an ATP-dependent proteasome-like complex in bacteria. ClpC/ClpP control regulatory and developmental processes but also play crucial role in proteostasis. ClpC alone is inactive and requires cooperating adaptor proteins (e.g. MecA), which target substrates and concurrently stimulate ClpC ATPase activity. Here, we dissected the molecular basis of ClpC activity control by determining its structure using cryo-EM single particle analysis. We show that ClpC in absence of adaptors forms a unique, pseudohelical assembly formed by two spirals that interact via coiled-coil M-domains (MDs) in a head-to-head manner. This resting state is inactive and does not allow for binding of substrate and ClpP. Binding of MecA to MDs or mutating key MD residues convert the resting state into functional ClpC hexamers. Our data identify MDs as a molecular switch, repressing ClpC in the ground state and allowing for activation by adaptors.

Coordination between the PERK and IRE1 branches of the unfolded protein response

Tsun-Kai Chang, Min Lu, Scot Marsters, David Lawrence, Jenille Tan, Scott Martin, and Avi Ashkenazi

Cancer Immunology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080 USA

The mammalian UPR branches—PERK, IRE1 and ATF6—are well studied, but their coordination is incompletely understood. PERK and IRE1 have an ER-lumenal domain that senses misfolded proteins and in turn activates a cytoplasmic kinase module. PERK phosphorylates eIF2α to inhibit mRNA translation. IRE1 further harbors a cytoplasmic RNAse moiety, which activates the transcription factor XBP1s to expand ER capacity, and performs regulated IRE1-dependent mRNA decay (RIDD) to decrease translational load. Whereas PERK stimulation persists, IRE1 activation is transient. We discovered that the PERK-eIF2α pathway reverses IRE1 phosphorylation under ER stress in a phosphatase-dependent manner. SiRNA library screening revealed a specific phosphatase that mediates IRE1 dephosphorylation. Its disruption prolonged IRE1 phosphorylation and oligomerization and augmented XBP1s generation and RIDD under ER stress. Thus, PERK controls the duration of IRE1's activity to ensure temporal coordination of the UPR.

Altered organisation of the intermediate filament cytoskeleton and relocalisation of proteostasis modulators in cells lacking the chaperone domain protein sacsin

Emma Duncan, Teisha Bradshaw, Lisa Romano, Paul Chapple

Queen Mary University of London

The neurodegeneration protein sacsin contains multiple proteostasis linked domains. Sacsin's role is unknown, but consequences of loss of function include reduced mitochondrial health and neurofilament abnormalities. Here, we demonstrate that loss of sacsin also causes perinuclear accumulation of vimentin, including in cells from patients with the disease ARSACS where sacsin is mutated. Reorganization of the vimentin cytoskeleton is known to occur when misfolded proteins are targeted to aggresomes. In sacsin deficient cells Hsp70, ubiquitin and the autophagy-lysosome pathway proteins Lamp2 and P62 relocalised to the area of the vimentin accumulation. There was no overall increase in ubiquitinated proteins, but autophagic flux was increased under starvation conditions. Data indicate that loss of sacsin impacts on organisation of the intermediate filament cytoskeleton, leading to changes in cellular distribution of the proteostasis machinery and functional adaptation.

The role of the unfolded protein response in retinal degeneration

Michael Cheetham[1], Dimitra Athanasiou[1], Monica Aguila[1], James Bellingham[1], Naheed Kanuga[1], Peter Adamson[2]

[1]*UCL Institute of Ophthalmology, 11-43 Bath Street, London, UK*
[2]*Ophthiris Discovery Performance Unit, GlaxoSmithKline Ophthalmology, Stevenage, UK*

Several forms of inherited retinal degeneration have been associated with the unfolded protein response (UPR). In particular, mutations in rhodopsin, such as P23H, cause rhodopsin misfolding and induction of the UPR. Nevertheless, the role of the UPR in retinal degeneration is unclear. We investigated the effect of PERK inhibition on retinal degeneration in the P23H-1 transgenic rat. PERK inhibition led to reduced ERG responses and decreased photoreceptor survival. Additionally, PERK inhibition increased inclusion formation in cultured cells overexpressing P23H rod opsin, and increased rhodopsin aggregation in the P23H-1 rat retina, suggesting enhanced P23H misfolding and aggregation. In contrast, treatment of P23H-1 rats with an inhibitor of eIF2α phosphatase, salubrinal, led to improved photoreceptor survival. Collectively, these data suggest the activation of PERK is part of a protective response to mutant rhodopsin that protects against photoreceptor cell death.

The yeast vigilin has widespread impact on the polyQ-mediated aggregation landscape of the cell

Matthew Cheng[1, 2], Patrick Hoffmann[3], Ralf-Peter Jansen[1]

[1]*International Max Planck Research School "From Molecules to Organisms", Spemannstraße 35, 72076 Tübingen, Germany*
[2]*Interfaculty Institute of Biochemistry, Eberhard-Karls-Universität Tübingen, Hoppe-Seyler-Straße 4, 72076 Tübingen, Germany*
[3]*MRC Laboratory of Molecular Biology, Francis Crick Avenue, CB2 0QH, Cambridge, United Kingdom*

Expansion of polyglutamine (polyQ) repeats underlie a number of neurodegenerative diseases, caused in part by polyQ-mediated protein aggregation. However, polyQ regions are common in eukaryotic proteomes and believed to mediate protein-protein interactions. Moreover, aggregation of certain polyQ-containing proteins is important for their biological functions.
The RNA-binding protein Scp160p is the yeast homolog of the vigilin protein family, characterized by 14 hnRNP K homology (KH) domains. Scp160p has been implicated in ploidy maintenance, mRNA localization, and translation. I present data which show that Scp160p plays a role in the aggregation of exogenous polyQ reporters. Furthermore, a combination of filter trap binding and comparative mass spectrometry show that Scp160p facilitates aggregation of many endogenous polyQ-containing proteins. Scp160p's influence on the aggregation of Cyc8p, a polyQ-containing transcription factor identified by our comparative mass spectrometry was validated. Together, our data demonstrate a role for the RNA-binding protein Scp160p in modulating polyQ-mediated protein-protein interactions.

Prion-based cellular memories of stress

Yury Chernoff[1, 2], Tatiana Chernova[3], Rebecca Howie[1], Lina Jay Garcia[1], Keith Wilkinson[3]

[1]*School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia, USA*
[2]*St. Petersburg State University, St. Petersburg, Russia*
[3]*Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia, USA*

Ordered protein aggregates (amyloids) and their infectious or heritable variety (prions) are associated with human diseases and yeast heritable traits. We show that chaperones of the ribosome associated complex (RAC), not inducible by heat, are aggregated and/or relocated during heat stress, thus modulating the effect of stress on a yeast prion. We also demonstrate that the heat-inducible actin assembly protein Lsb2 forms a metastable prion in response to heat stress. This prion can promote prion formation by other proteins and persists in a fraction of yeast cells for a number of cell generations after stress, thus generating a cellular memory of stress. The ability to form a prion is traced to a single amino acid substitution in Lsb2. Evolutionary acquisition of this substitution coincides with the acquisition of thermotolerance in the Saccharomyces phylogenetic lineage, pointing to the potential link between prion-forming properties and adaptation to higher growth temperatures.

A Novel Yeast Model for Interactions between T-synthase and its Molecular Chaperone Cosmc

Tatiana A. Chernova, Qian Wang, Tongzhong Ju

Emory University School of Medicine, Atlanta, GA 30322, USA

O-glycosylation is a common protein posttranslational modification, which play pivotal roles in many biological processes. The aberrant O-glycans contribute to the development of cancer. Core 1 beta3 Galactosyltransferase (T-synthase) is the key enzyme in the O-glycosylation pathway. Biosynthesis of active T-synthase requires specific molecular chaperone Cosmc. Cosmc assists the folding of T-synthase by preventing its aggregation and proteasomal degradation. Alterations in Cosmc are associated with cancers and Tn-syndrome. The molecular interactions of T-synthase and Cosmc are not well understood. We have developed a yeast model for investigating these interactions. In this model, we can inducibly express T-synthase and Cosmc, confirm their correct cellular localization and measure the enzymatic activity. We discovered that T-synthase forms amyloid-like aggregates in yeast. Our model will aid in understanding how dysfunction of Cosmc causes human diseases such as colon cancer.

Cellular response to ribosome-stalled polypeptides

Young-Jun Choe[1], Timm Hassemer[1], Roman Koerner[1], Barbara Hummel[2], Ritwick Sawarkar[2], Manajit Hayer-Hartl[1], F. Ulrich Hartl[1]

[1]*Max Planck Institute of Biochemistry*
[2]*Max Planck Institute of Immunobiology and Epigenetics*

Translating ribosomes can stall for various reasons. The resulting protein products are cleared by the ribosome-associated quality control complex (RQC). Stalled polypeptide chains that are not degraded by this pathway are C-terminally extended with Ala/Thr residues (CAT tailed) (1). Recent reports have shown that these CAT tails drive the aggregation of stalled chains (2,3,4). It remained unclear, however, whether aggregates containing CAT-tail proteins are toxic or beneficial. In this study, we investigate the effects of stalled chain aggregates on cell growth under different conditions. We also analyze cellular responses to CAT-tail protein aggregates. Finally, we try to identify conditions in which the RQC pathway is expected to play a crucial role in maintaining protein homeostasis.

1) Shen et al. (2015) Sciecne
2) Choe et al. (2016) Nature
3) Yonashiro et al. (2016) eLife
4) Defenouillère et al. (2016) J Biol Chem

Hsp83 loss suppresses proteasomal activity resulting in an upregulation of caspase-dependent compensatory autophagy.

Courtney Choutka[1, 2], Lindsay Devorkin[1, 2], Nancy Erro Go[1, 2], Ying-Chen Claire Hou[1], Annie Moradian[1, 3], Gregg Morin[1, 4], Sharon Gorski[1, 2, 5]

[1]*Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, 675 West 10th Avenue, Vancouver, BC, Canada, V5Z 1L3*
[2]*Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6*
[3]*Beckman Institute, California Institute of Technology, Pasadena, CA, USA 91125*
[4]*Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada, V6H 3N1*
[5]*Centre for Cell Biology, Development, and Disease, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6*

The molecular coordination of the two main degradative pathways, the ubiquitin-proteasome system and autophagy, has limited understanding. Here, we demonstrate an essential role for a caspase in the activation of compensatory autophagy when proteasomal activity is compromised. Functional loss of Hsp83, the Drosophila homolog of human heat-shock protein 90, resulted in reduced proteasomal activity and elevated levels of the effector caspase Dcp-1. Surprisingly, genetic analyses showed that the caspase was not required for cell death in this context, but instead was essential for the ensuing compensatory autophagy, female fertility, and organism viability. The zymogen pro-Dcp-1 was found to interact with Hsp83 and undergo proteasomal regulation in an Hsp83-dependent manner. Our work reveals unappreciated roles for Hsp83 in proteasomal activity and regulation of Dcp-1 as well as identifies an effector caspase as a key regulatory factor for sustaining adaptation to cell stress in vivo.

Not4 and Not5 are essential for co-translational assembly of the SAGA histone acetyltransferase and for global protein acetylation

Martine Collart, Sari Kassem, Zoltan Villanyi

University of Geneva, Faculty of Medicine, Department of Microbiology and Molecular Medicine

Acetylation of histones regulates gene expression in eukaryotes. In the yeast S.cerevisiae it depends mainly upon the ADA and SAGA histone acetyltransferase (HAT) complexes. Previous screens have determined that global acetylation is reduced in cells lacking subunits of the Ccr4-Not complex, a global regulator of eukaryotic gene expression. In our study we have characterized the functional connection between the Ccr4-Not complex and SAGA. We show that SAGA mRNAs encoding a core set of SAGA subunits are tethered together for co-translational assembly of the encoded proteins. The Ccr4-Not complex is present at this site to promote the co-translational assembly of these subunits, and this is needed for integrity of SAGA. In addition, we determine that a glycolytic enzyme, the glyceraldehyde-3-phosphate dehydrogenase Tdh3, a prototypical moonlighting protein, is tethered at this site of Ccr4-Not-dependent co-translational SAGA assembly and functions as a chaperone.

Structure and function of the ribosome associated chaperone Ssb

Charlotte Conz[1, 2], Genis Valentin Gese[3], Andrea Gumiero[3], Ying Zhang[3], Felix Weyer[3], Karine Lapouge[3], Tamas Fischer[3], Sabine Rospert[1, 2], Irmgard Sinning[3]

[1]*Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, University of Freiburg, D-79104 Freiburg, Germany*
[2]*BIOSS Centre for Biological Signaling Studies, University of Freiburg, D-79104 Freiburg, Germany.*
[3]*Heidelberg University Biochemistry Center (BZH), INF 328, D-69120 Heidelberg, Germany.*

The ribosome-associated complex (RAC) and Ssb are co-translational chaperones that assist in folding of newly synthesized proteins. RAC is a stable complex composed of the Hsp40 Zuotin (Zuo) and the atypical Hsp70 Ssz. RAC acts as a co-chaperone of the Hsp70 Ssb, that binds to the nascent polypeptide. We determined the X-ray structure of Ssb in the ATP-bound state at 2.6 Å resolution. Based on the structure, sequence alignments and ribosome binding studies, we identified a positively charged region at the C-terminus, that is involved in ribosome binding. Crosslinking experiments showed that Ssb binds close to the tunnel exit via contacts with ribosomal proteins and rRNA. Using Ssb and Zuo1 variants allowed us to correlate specific contacts at the ribosome with Ssb switching between the open and closed conformation. These data allowed us to derive a molecular model of how Ssb and RAC cooperate in the interaction with nascent chains.
Reference:
Gumiero et al. (2016) Nat. Comms. 7: 13563.

The role of CCT-PhLP1 system in the folding of mLST8, a key component of mTOR complex

Jorge Cuéllar[1], Takuma Aoba[2], Madhura Dhuvale[2], Grant Ludlam[2], Rebecca Plimpton[2], Barry M. Willardson[2], José M. Valpuesta[1]

[1]*Centro Nacional de Biotecnología (CNB-CSIC). Madrid. Spain*
[2]*Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT. USA*

The mechanistic target of rapamycin (mTOR) forms two multi-protein complexes (mTORC1 and mTORC2) that are master regulators of cell growth, survival and autophagy and they are involved in many pathological processes. mLST8 is a 37 kDa B-propeller protein which is a core component of both mTOR complexes.
We have characterized the folding pathway of another B-propeller protein, the G protein B-subunit, a process assisted by CCT and its co-chaperone PhLP1. A extension of our work on G was to ask if B-propeller proteins involved in other signaling complexes such as mLST8 share the same folding mechanism. We tested the binding of mLST8 to CCT and PhLP1 by IP and found strong evidence of the formation of a ternary complex.
Recently we have started the structural characterization of the purified CCT-mLST8 complex by combining CryoEM and XL-MS. A preliminary 3D reconstruction indicates that mLST8 binds deep within the CCT folding cavity in close association with the CCTa and CCTg subunits.

HSF2 protects cells against proteotoxic insults through cell-cell adhesion

Jenny Joutsen[1, 2], Alejandro J Da Silva[1, 2], Jens Luoto[1, 2], Eva Henriksson[1, 2], Valerie Mezger[3], Lea Sistonen[1, 2]

[1]*Faculty of Science and Engineering, Cell Biology, Åbo Akademi University, 20520 Turku, Finland*
[2]*Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, 20520 Turku, Finland*
[3]*UMR 7216 Epigenetics and Cell Fate, University Paris Diderot, 75013 Paris, France*

Every human cell is dependent on the correct function of its proteins. During proteotoxicity, cells respond to stress by initiating transcriptional programs counteracting the damage. One such program is called the heat shock response (HSR) and it is mediated by heat shock factors HSFs. From human HSFs (HSF1-4), HSF1 is essential for HSR, whereas HSF2 is dispensable for it and hence the exact role of HSF2 in proteotoxic stresses is unknown. Here, we establish HSF2 as an important factor in chronic proteotoxic stress, such as prolonged proteasome inhibition. We reveal, that lack of HSF2 impairs cell adhesion and sensitizes cells to sustained proteotoxicity. Also, we identify cadherin superfamily proteins as the main adhesion molecules depleted in HSF2 KO cells and show that cadherin inhibition promotes lethality of proteasome inhibition. Altogether we show that HSF2 is a pro-survival factor during chronic stress and describe a novel regime in the HSF-mediated cell protection.

IRE1 Activation - is it as Simple as 1, 2, 3?

Sam Dawes[1, 2], Lukasz Wieteska[2], Beining Chen[1], Anastasia Zhuravleva[2]

[1]*University of Sheffield*
[2]*University of Leeds/Astbury Centre*

Folding and maturation of the majority of secretory proteins occurs in the endoplasmic reticulum. To cope with these varying expression levels, the unfolded protein response (UPR), controlled by three transmembrane stress sensors, upregulates chaperones and halts translation via downstream effectors. Misregulation of this complex system has been linked to neurodegenerative diseases, various cancers and other diseases. We have characterised the initial stages of activation of the most conserved stress sensor, inositol requiring enzyme 1 (IRE1), namely dimerization and disulphide bond formation, and the role of the Hsp70 chaperone, BiP in these processes using, SDS-PAGE, microscale thermophoresis, nuclear magnetic resonance, mass spectrometry and molecular dynamics simulations. Our results highlight that IRE1 and BiP interactions are competitive, that oligomerisation is a time dependent process and provides further insight in to disulphide bond formation of IRE1.

A spiral track of allosteric communication in the bacterial disaggregase ClpB

Célia Deville[1], Marta Carroni[2], Kamila Franke[3], Axel Mogk[3], Bernd Bukau[3], Helen Saibil[1]

[1]*ISMB, Birkbeck, University of London, United Kingdom*
[2]*SciLifeLab, Stockholm, Sweden*
[3]*ZMBH, Heidelberg, Germany*

Solubilisation of protein aggregates, i.e. disaggregation, is essential to prevent accumulation of aggregates, which can cause toxicity and cell death. In prokaryotes and non-metazoan eukaryotes, disaggregation involves cooperation between Hsp100 and Hsp70 proteins. Hsp100 disaggregases (ClpB in bacteria) extract polypeptides from aggregates and thread them through a central channel. Because of the inherent plasticity, flexibility and asymmetry of these hexameric proteins, the underlying mechanism of translocation remains elusive. We have determined cryo-EM structures of a ClpB variant in the absence and presence of an unfolded substrate. Density can be assigned to the substrate in the central channel and shows specific interactions with ClpB. Comparison of the two structures reveals allosteric communication spiraling around the hexamer, likely mediated by conformational changes of the middle domains which play an important role in regulation of the complex by Hsp70.

Profiling of Ssb interactions with nascent proteins reveals principles of Hsp70 assisted co-translational folding

Kristina Döring[1, 2], Nabeel Ahmed[3], Trine Riemer[1, 2], Harsha Garadi Suresh[1, 2], Yevhen Vainshtein[1], Markus Habich[5], Jan Riemer[5], Matthias Mayer[1], Edward O'Brien[3, 4], Günter Kramer[1, 2], Bernd Bukau[1, 2]

[1]*Center for Molecular Biology of Heidelberg University (ZMBH), DKFZ-ZMBH Alliance, Heidelberg, Germany*
[2]*German Cancer Research Center (DKFZ), Heidelberg, Germany*
[3]*Bioinformatics and Genomics Graduate Program, The Huck Institutes of the Life Sciences, Pennsylvania State University, Pennsylvania, USA*
[4]*Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania 16802, USA*
[5]*Institute of Biochemistry, University of Cologne, Zuelpicher Str. 47, 50674, Cologne, Germany*

The yeast Hsp70 Ssb interacts with ribosomes and nascent chains to assist protein folding. To reveal its working principle, we determined the nascent chain-binding pattern of Ssb at near-residue resolution by in vivo selective ribosome profiling. Ssb associates broadly with cytosolic, nuclear and hitherto unknown substrate classes of mitochondrial and ER proteins. Ssb engages most substrates by multiple binding-release cycles to a degenerate sequence enriched in positively charged and aromatic amino acids. Timely association with this motif upon emergence at the ribosomal tunnel exit requires ribosome-associated complex RAC. Ribosome footprint densities along orfs reveal faster translation at times of Ssb binding, mainly imposed by biases in mRNA secondary structure and codon usage. Ssb thus employs substrate-tailored dynamic nascent chain associations to coordinate co-translational protein folding, facilitate accelerated translation and support targeting of organellar proteins.

In situ architecture and cellular interactions of polyQ inclusions

Felix Bäuerlein[1], Itika Saha[2], Archana Mishra[3], Maria Kalemanov[1, 4], Rüdiger Klein[3, 5], Irina Dudanova[3], Mark Hipp[2, 5], Ulrich Hartl[2, 5], Wolfgang Baumeister[1], Ruben Fernandez-Busnadiego[1]

[1]*Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Germany.*
[2]*Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Germany.*
[3]*Department of Molecules, Signaling, Development, Max Planck Institute of Neurobiology, Germany.*

[4]*Graduate School of Quantitative Biosciences Munich, 81337 Munich, Germany.*
[5]*Munich Cluster for Systems Neurology (SyNergy), D-80336 Munich, Germany.*

In many neurodegenerative disorders protein aggregation results in cytotoxicity and the formation of intracellular inclusion bodies (IBs). However, the role of IBs in pathology is not well understood, and the native structure of protein aggregates inside cells has not been determined. Here we employ advanced cryo-electron tomography (cryo-ET) methods to analyze the structure of IBs formed by polyglutamine (polyQ)-expanded huntingtin exon 1 within their intact cellular context and to an unprecedented level of detail. We find that in primary mouse neurons and immortalized human cells, polyQ IBs consist of amyloid-like fibrils that interact with cellular endomembranes, particularly those of the ER. These interactions lead to membrane deformation, the local impairment of ER organization and profound alterations in ER membrane dynamics. These results suggest that aberrant interactions between IB fibrils and endomembranes contribute to the deleterious cellular effects of protein aggregation.

The atomic structures of Tau filaments from Alzheimer's disease brain

Anthony Fitzpatrick[1], Benjamin Falcon[1], Shaoda He[1], Alexey Murzin[1], Garib Murshudov[1], Holly Garringer[2], Anthony Crowther[1], Bernardino Ghetti[2], Michel Goedert[1], Sjors Scheres[1]

[1]*MRC Laboratory of Molecular Biology, Francis Crick Avenue, CB2 0QH, Cambridge, UK*
[2]*Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA*

Alzheimer's disease (AD) is the most common neurodegenerative disease, and there are no mechanism-based therapies. Abundant neurofibrillary lesions and neuritic plaques are the defining pathological characteristics of AD. Paired helical and straight Tau filaments (PHFs and SFs) with the tinctorial and biophysical characteristics of amyloid are the major components of the neurofibrillary lesions. Here, we present the atomic structures of PHFs and SFs extracted from AD brain, as determined by single-particle cryo-electron microscopy. We show that PHFs and SFs form ultrastructural polymorphs, where the C-shaped subunit of each protofilament of Tau is arranged in a base-to-base or back-to-back manner, respectively. Details of the atomic structures of PHFs and SFs will be presented, yielding insights into the self-assembly, polymorphisms and propagation of tau aggregates in AD and other Tauopathies. Strategies for structure-based drug design will also be discussed.

The prion domain of Sup35 senses stress and promotes cell survival by pH-dependent protein phase separation

Titus Franzmann[1], Marcus Jahnel[2], Julia Mahamid[3], Andrei Pouniakovsky[1], Wolfgang Baumeister[3], Stefan Grill[2], Anthony Hyman[1], Simon Alberti[1]

[1]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*
[2]*TU Dresden, BIOTEC, Dresden, Germany*
[3]*Max Planck Institute, Molecular Structural Biology, Martinsried, Germany*

Yeast respond to stresses with an intracellular acidification, which promotes a fluid to solid transition of the cytoplasm. How yeast mount adaptive responses to environmental changes is still unclear. Proteins with intrinsically disordered regions (IDRs) are sensitive to physicochemical changes and can adopt different material states, including liquids, gels and amyloids. We hypothesized that IDRs function as stress sensors. We demonstrate that the prion protein Sup35 forms reversible particles in vivo in a pH-dependent manner in response to stresses. In vitro, acidic pH induces Sup35 phase separation into reversible gels. pH-sensing is encoded in the IDR and links its phase behavior to the cytoplasmic pH. Removing the IDR makes Sup35 aggregate irreversibly, which impairs cell fitness. This suggests that IDRs may re-route the phase behavior of proteins toward reversible assemblies. We propose that the ancestral function of IDRs is mounting adaptive responses through phase separation.

Characterization of the RAC-ribosome interaction by EPR

Sandra Fries[1, 2], Theresa Braun[2, 3], Christoph Globisch[3], Christine Peter[3], Malte Drescher[3], Elke Deuerling[1]

[1]*Department of Biology, University of Konstanz, 78457 Konstanz (Germany)*
[2]*Konstanz Research School Chemical Biology, University of Konstanz, 78457 Konstanz (Germany)*
[3]*Department of Chemistry, University of Konstanz, 78457 Konstanz (Germany)*

Correct folding of newly synthesized polypeptides is mediated by ribosome-associated chaperones. In eukaryotes, one of these chaperones systems includes the ribosome-associated complex (RAC), which acts as co-chaperone of an Hsp70-family protein interacting directly with nascent polypeptides. RAC is tethered to the ribosomes via three contacts, two to the large ribosomal subunit (60S) and one to the small ribosomal subunit (40S). In this study electron paramagnetic resonance (EPR) spectroscopy coupled with site-directed spin labeling (SDSL) is used to elucidate RAC's direct interaction to the 40S ribosomal subunit. The small size of a nitroxide spin label enables measurements directly in the binding interface without interfering binding ability. Here we demonstrate - as proof of principle - that EPR spectroscopy is a suitable method to characterize binding interfaces of the ribosome-RAC interaction but also rule out the pitfall of high viscosity caused by the ribosomes.

De novo design of a biologically active amyloid

Rodrigo Gallardo[1, 2], Meine Ramakers[1, 2], Filip Claes[1, 2], Sofie Nyström[3], Laurence J. Young[4], Lydia Young[5, 6], Bart De Strooper[1, 7], Per Hammarström[3], Louise Serpell[8], Joost Schymkowitz[1, 2], Frederic Rousseau[1, 2], et al.

[1]*VIB Center for Brain & Disease Research, Switch Laboratory, Leuven, Belgium.*
[2]*KU Leuven, Department of Cellular and Molecular Medicine, Switch Laboratory, Leuven, Belgium.*
[3]*IFM Department of Chemistry, Linköping University, Linköping, Sweden.*
[4]*Department of Chemical Engineering and Biotechnology, University of Cambridge, UK.*
[5]*Astbury Centre for Structural Molecular Biology, University of Leeds, UK.*
[6]*School of Molecular and Cellular Biology, University of Leeds, UK*
[7]*KU Leuven, Department of Neurosciences, Leuven Institute for Neurodegenerative Diseases (LIND), Leuven, Belgium.*
[8]*School of Life Sciences, University of Sussex, UK.*

Most human proteins possess amyloidogenic segments, but only about 30 are associated with amyloid-associated pathologies, and it remains unclear what determines amyloid toxicity. We designed vascin, a synthetic amyloid peptide, based on an amyloidogenic fragment of vascular endothelial growth factor receptor 2 (VEGFR2), a protein that is not associated to amyloidosis. Vascin recapitulates key biophysical and biochemical characteristics of natural amyloids, penetrates cells, and seeds the aggregation of VEGFR2 through direct interaction. We found that amyloid toxicity is observed only in cells that both express VEGFR2 and are dependent on VEGFR2 activity for survival. Thus, amyloid toxicity here appears to be both protein-specific and conditional. It is determined by VEGFR2 loss of function in a biological context in which this protein's function is essential.

Two factors in the eukaryotic cell that sense slow ribosomes: eIF5A and Dhh1

Anthony Schuller, Colin Wu, Allen Buskirk, Karole D'Orazio, Rachel Green

HHMI, Johns Hopkins University School of Medicine

While the rate of elongation by the ribosome is thought to be relatively even across all coding sequences, there are examples where ribosomes pause at specific mRNA and nascent peptide motifs. Recent studies have established that synthesis of poly-proline stretches is particularly challenging, inducing pauses that can be resolved in bacteria and eukaryotes by a specialized elongation factor known as EFP and eIF5A, respectively. Importantly, these factors function through binding to the ribosome E site which typically becomes accessible on slow peptidyl transferase substrates. Using ribosome profiling and biochemistry, we have extended these initial observations in eukaryotes, finding that eIF5A is critical for elongation on hundreds of tripeptide motifs, including many with no prolines. In addition, eIF5A acts generally to promote rapid peptidyl hydrolysis during termination. These data rationalize the high cellular concentration and essential nature of eIF5A in all eukaryotes.

A potential mechanism for integrating ADP-ribosylation, ubiquitination and proteasome regulation

Yetis Gultekin, Hermann Steller

The Strang Laboratory of Apoptosis and Cancer Biology The Rockefeller University

The proteasome is responsible for the selective breakdown of intracellular proteins.Interestingly, the substrates of a poly-ADP-ribose polymerase TNKS have only halflives of minutes after they are tagged for destruction. Our hypothesis is that posttranslational modification by ADP-ribosylation provides a molecular scaffold to promote fast and efficient degradation of labile proteins. Iduna is evolutionarily conserved and functional ubiquitin ligase on the degradation of ADP-ribosylated proteins. Up to date, there is no loss-of-function mutant of Iduna in any model organisms. In order to study the physiological roles of Iduna in vivo, we generate Iduna-Null mutants of Drosophila by CRISPR-Cas9 genome editing. Hence, Iduna inactivation promotes Axin accumulation that leads to over proliferation of intestinal progenitors in Drosophila midgut. We show that regulation of Axin proteolysis by Iduna is necessary to maintain intestinal homeostasis by buffering Axin in the Wingless pathway.

Somatic increase of CCT8 mimics proteostasis of human pluripotent stem cells and extends C. elegans lifespan

Ricardo Gutierrez-Garcia[1], Alireza Noormohammadi[1], Amirabbas Khodakarami[1], Hyun Ju Lee[1], Seda Koyuncu[1], Tim König[1], Christina Schindler[1], Isabel Saez[1], Azra Fatima[1], Christoph Dieterich[2], David Vilchez[1]

[1]*Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Joseph Stelzmann Strasse 26, Cologne 50931, Germany*
[2]*Section of Bioinformatics and Systems Cardiology, Department of Internal Medicine III and Klaus Tschira Institute for Computational Cardiology, Neuenheimer Feld 669, University Hospital, Heidelberg 69120, Germany*

Human embryonic stem cells (hESCs) do not undergo senescence and, therefore, can replicate continuously. This capacity may demand avoidance of any imbalance in protein homeostasis that would otherwise compromise hESC identity. We show that hESCs exhibit enhanced assembly of the TRiC/CCT complex. Moreover, increased expression of CCT subunits is required for the remarkable ability of stem cells to avoid mutant Huntingtin aggregation. Thus, mimicking proteostasis of hESCs in somatic tissues could protect from damaged proteins. By using C. elegans as a model organism, we found that somatic increased expression of a single subunit (CCT8) extends lifespan in a TRiC/CCT-dependent manner. Furthermore, ectopic expression of CCT8 is sufficient to ameliorate the age-associated demise of proteostasis and correct proteostatic deficiencies in HD models. Our results establish a novel regulation of proteostasis that links organismal longevity with hESC immortality.

A small heat shock protein involved in the protein quality control system of Bacillus subtilis

Ingo Hantke, Kürsad Turgay

Institut für Mikrobiologie,
Leibniz Universität Hannover

Small heat shock proteins (sHsp) occur in all cells and fulfil their protective role by preventing the aggregation of proteins. It was suggested that sHsp can actively sequester misfolded proteins and thereby facilitate the subsequent refolding by ATP-dependent chaperones. Recent experiments suggested that sHsp could also display aggregase activity, important for such a sequestration activity. We identified a sHsp in the Gram positive model organism B. subtilis, which is involved in thermo and salt tolerance development. Our experiments revealed a salt and heat stress induced synthesis and localization of YocM to intracellular protein aggregates. Furthermore, we could successfully utilize YocM fused to mCherry as an aggregate marker in vivo. Interestingly, purified YocM appears to accelerate protein aggregation of different model substrates in vitro. This suggests a potentially new and interesting role of a sHsp with an aggregase activity in cellular protein quality control systems.

Small heat shock proteins from cyanophages are necessary to stabilize the host photosystems
Martin Haslbeck, Sabine Rittinger, Marina Daake, Annika Strauch, Johannes Buchner, et al.

Center for Integrated Protein Science Munich (CIPSM) and Department Chemie.
Technische Universität München. D-85748 Garching. Germany

Small heat shock proteins (sHsps) represent a first line of defense against proteotoxic stress and prevent the aggregation of unfolding proteins. Their chaperone activity correlates with their propensity to form dynamic ensembles of higher oligomers. Here we studied sHsps encoded in marine cyanophages which infect cyanobacteria of the Synechococcus genera. A virus commonly uses the protein folding machinery of the infected host, thus, it remains elusive why a cyanophage would need especially a sHsp for its reproductive cycle. Because the host spectrum of a cyanophages is commonly narrow, we investigated two Synechococcus strains and their specific infecting phages. Interestingly, the analysis of the structural and functional properties of the respectively encoded sHsps showed that the host and the phage sHsps co-operate in synergistic manner in stabilizing the components of the photosystem II complex of the cyanobacteria.

Failure of RQC machinery causes protein aggregation and proteotoxic stress Timm Hassemer, Young-Jun Choe, Roman Koerner, Franz-Ulrich Hartl

Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried/Germany

The translation of aberrant mRNAs leads to ribosomal stalling at the 3' end of the mRNA. In case of mRNAs lacking an in-frame stop codon, translation of the 3'-poly(A) tail results in the addition of a C-terminal polylysine tract to the nascent polypeptide chain, causing ribosomal stalling due to electrostatic interactions with the ribosomal exit channel. The translation products of erroneous mRNAs are rapidly recognized and targeted for proteasomal degradation by the ribosomal quality control (RQC) machinery. We could show in a yeast model that deletion of the E3 ligase Ltn1p, a key component of the RQC complex, causes stalled polypeptides to form detergent-insoluble aggregates. The formation of these aggregates is dependent on another RQC component, Rqc2p, which catalyzes the addition of a C-terminal alanine/threonine (CAT) tail to the stalled polypeptide1. The aggregates interfere with general cellular protein quality control through sequestration of multiple cytosolic chaperones, including the essential Hsp40 chaperone Sis1p. Our results emphasize the crucial role of the RQC machinery in proteostasis maintenance and provide a possible explanation for the neurodegenerative phenotype observed in RQC-deficient mice.

1. Shen, P. S. *et al.* Protein synthesis. Rqc2p and 60S ribosomal subunits mediate mRNA-independent elongation of nascent chains. *Science* **347**, 75-78, (2015)

HSF2 protects cells against proteotoxic insults through cell-cell adhesion
Jenny Joutsen, Alejandro J. Da Silva, Jens Luoto, Eva Henriksson, Valerie Mezger, Lea Sistonen

Åbo Akademi University, Finland

Every human cell is dependent on the correct function of its proteins. During proteotoxicity, cells respond to stress by initiating transcriptional programs counteracting the damage. One such program is called the heat shock response (HSR) and it is mediated by heat shock factors HSFs. From human HSFs (HSF1-4), HSF1 is essential for HSR, whereas HSF2 is dispensable for it and hence the exact role of HSF2 in proteotoxic stresses is unknown. Here, we establish HSF2 as an important factor in chronic proteotoxic stress, such as prolonged proteasome inhibition. We reveal, that lack of HSF2 impairs cell adhesion and sensitizes cells to sustained proteotoxicity. Also, we identify cadherin superfamily proteins as the main adhesion molecules depleted in HSF2 KO cells and show that cadherin inhibition promotes lethality of proteasome inhibition. Altogether we show that HSF2 is a pro-survival factor during chronic stress and describe a novel regime in the HSF-mediated cell protection.

A comprehensive analysis of modulators regulating tau aggregation
Shoshiro Hirayama, Yasuyuki Sakurai, Kazuki Murata, Shigeo Murata

Graduate School of Pharmaceutical Sciences, the University of Tokyo

The aggregation of the microtubule binding protein tau is a key pathological feature of neurodegenerative diseases. Using a tau repeat domain (tau RD) aggregation model cell line that propagates tau RD aggregates, we performed an unbiased, genome-wide siRNA screen for genes suppressing aggregation of tau RD. We identified 14 aggregation suppressor genes, of which two were from the SUMO modification pathway. Of the three SUMO isoforms, we found that only knockdown of SUMO2 strongly enhanced aggregation. SUMO2 modification was observed in soluble tau RD but not in aggregated tau RD. We also found that knockdown of SUMO2 suppressed degradation of soluble tau RD in aggregation negative cells. SUMO2 modification was diminished in K340R mutant of tau RD and it was observed that delayed degradation of tau RD (K340R). These results suggested that SUMO2 modification on K340 leads to rapid degradation of soluble tau RD thereby preventing its subsequent aggregation.

The influence of cell non-autonomous UPR signaling on the toxicity of misfolded proteins in nematode models
Soudabeh Imanikia, Rebecca Taylor

Medical Research Council, Laboratory of Molecular Biology

Activation of the unfolded protein response in the endoplasmic reticulum extends lifespan of C. elegans. When activated in neurons, it results in non-autonomous signals in intestine. This may be due to an improved proteostasis. Rescuing age-onset loss of ER proteostasis via XBP-1s, a constitutively active spliced form of the UPR transcription factor XBP-1, we address whether the UPR benefits protein folding capacity, cell autonomously or non-autonomously. This can be achieved by expressing tissue-specific XBP-1s with misfolded proteins to determine how UPR activation in different tissues affects these toxic species. Our findings suggest that UPR activation in neurons or intestine can reduce the toxicity throughout the organism. Protection against these proteotoxic species varies depending on their type and subcellular localization, although these mechanisms are yet to be elucidated. Our results suggest that UPRER activation may increase lifespan, in part, through improved proteostasis.

A Novel Complex Targets Ubiquitinated Ribosome to Ribosome Quality Control

Yoshitaka Matsuo[1], Ken Ikeuchi[1], Yasushi Saeki2[2], Shintaro Iwasaki[3], Christian Schmidt[4], Tsuyoshi Udagawa[1], Thomas Becker[4], Keiji Tanaka[2], Nicholas Ingolia[3], Roland Beckmann4[4], Toshifumi Inada[1]

[1]*Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan.*
[2]*Laboratory of Protein Metabolism, Tokyo Metropolitan Institute of Medical Science, Setagaya-ku, Tokyo 156-8506, Japan.*
[3]*Department of Molecular and Cell Biology, University of California, Berkeley, CA94720, United States.*
[4]*Gene Center and Center for integrated Protein Science Munich, Department of Biochemistry, Feodor-Lynen-Str. 25, University of Munich, 81377 Munich, Germany.*

Translation arrest by polybasic sequences induces ribosome stalling, and the arrest product is degraded by the RQC system. Here, we report that ubiquitination of the 40S ribosomal protein uS10 by the E3 ubiquitin ligase Hel2 (or RQT1) was required for RQC. We identified a novel RQT complex composed of the RNA helicase-family protein Slh1/Rqt2, the ubiquitin binding protein Cue3/Rqt3, and yKR023W/Rqt4 that is required for RQC. The defects in RQC of the RQT mutants correlated with sensitivity to anisomycin, which stalls ribosome at the rotated form. Cro-EM analysis revealed that Hel2 bound-ribosome were dominantly the rotated form with hybrid tRNAs. Ribosome profiling revealed that ribosomes stalled at the rotated state with specific pairs of codons at P-A sites serve as RQC substrates. Rqt1 specifically ubiquitinates these arrested ribosomes to target them to the RQT complex, allowing subsequent RQC reactions including dissociation of the stalled ribosome into subunits.

KSHV Modulates the IRE1-XBP1 Axis of the Unfolded Protein Response during Lytic Replication

Benjamin Johnston[1, 2], Craig McCormick[1, 2]

[1]*Department of Microbiology & Immunology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4R2*
[2]*Beatrice Hunter Cancer Research Institute*

Kaposi's sarcoma-associated herpesvirus is a DNA tumour virus that hijacks multiple cellular stress responses during infection, which is thought to aid tumor initiation. One stress response linked to KSHV infection is the unfolded protein response (UPR), which is activated by ER stress. The ER stress sensor IRE1α splices out a 26-nucleotide intron of xbp1 mRNA, shifting the reading frame to translate the active transcription factor XBP1s. In addition to transactivating UPR genes, XBP1s drives the expression of the KSHV latent-lytic switch gene, K-RTA, which results in initiation of lytic replication. We found that K-RTA-mediated lytic replication also activates IRE1α to induce xbp1 splicing; however, XBP1s protein failed to accumulate and cellular XBP1s-target genes were not upregulated. Strikingly, ectopic expression of XBP1s inhibited viral progeny. Our findings suggest that XBP1s may be downregulated by products of the lytic replication program to permit efficient viral replication.

A link between metabolite homeostasis and Alzheimer's disease: two metabolites downregulated in AD rescue an Aβ C elegans model

Priyanka Joshi, Ryan Limbocker, Michele Perni, Sean Chia, Johnny Habchi, Christopher M Dobson, Michele Vendruscolo

Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, UK

Cellular homeostasis, including protein homeostasis and metabolite homeostasis, refers to all molecular processes occurring at steady-state inside the cell. Recent studies indicate that cellular homeostasis, in particular protein homeostasis, becomes disrupted in neurodegenerative diseases. Although protein homeostasis is regulated by several mechanisms including molecular chaperones, detoxifying enzymes and protein clearance, the effect of endogenous metabolites as small molecule proteostasis regulators has remained relatively unexplored. We have identified two metabolites that are downregulated in Alzheimer's disease, and when administered, rescue a C elegans model of AD by inducing an HSF-1 dependent molecular chaperone pathway. We propose that metabolite homeostasis is a crucial link to the maintenance of protein homeostasis, thus representing an alternative therapeutic approach towards the treatment of AD.

HSF2 protects cells against proteotoxic insults through cell-cell adhesion

Jenny Joutsen[1, 2], Alejandro Da Silva[1, 2], Jens Luoto[1, 2], Eva Henriksson[1, 2], Valerie Mezger[3], Lea Sistonen[1, 2]

[1]*Faculty of Science and Engineering, Cell Biology, Åbo Akademi University, 20520 Turku, Finland*
[2]*Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, 20520 Turku, Finland*
[3]*UMR 7216 Epigenetics and Cell Fate, University Paris Diderot, 75013 Paris, France*

Every human cell is dependent on the correct function of its proteins. During proteotoxicity, cells respond to stress by initiating transcriptional programs counteracting the damage. One such program is called the heat shock response (HSR) and it is mediated by heat shock factors HSFs. From human HSFs (HSF1-4), HSF1 is essential for HSR, whereas HSF2 is dispensable for it and hence the exact role of HSF2 in proteotoxic stresses is unknown. Here, we establish HSF2 as an important factor in chronic proteotoxic stress, such as prolonged proteasome inhibition. We reveal, that lack of HSF2 impairs cell adhesion and sensitizes cells to sustained proteotoxicity. Also, we identify cadherin superfamily proteins as the main adhesion molecules depleted in HSF2 KO cells and show that cadherin inhibition promotes lethality of proteasome inhibition. Altogether we show that HSF2 is a pro-survival factor during chronic stress and describe a novel regime in the HSF-mediated cell protection.

The structure of human αA-crystallin, a redox-sensitive chaperone

Christoph Kaiser[1], Carsten Peters[1], Maria Stavropoulou[1, 4], Philipp Schmid[1], Evgeny Mymrikov[1], Juri Rappsilber[2], Martin Zacharias[3], Martin Haslbeck[1], Bernd Reif[1, 4], Johannes Buchner[1], Sevil Weinkauf[1], et al.

[1]*Department Chemie and Center for Integrated Protein Science Munich (CIPSM), Technische Universität München, Lichtenbergstr. 4, 85748 Garching, Germany*
[2]*Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh EH9 3JR, UK*
[3]*Physics Department and Center for Integrated Protein Science Munich (CIPSM), Technische Universität München, James-Franck-Str. 1, 85748 Garching, Germany*
[4]*Helmholtz-Zentrum München (HMGU), Ingolstädter Landstr. 1, 85764 Neuherberg, Germany*

The small heat shock protein αA-crystallin (αA) is of critical importance in maintaining eye lens transparency. We present the structures of various human αA assemblies obtained by cryo-electron microscopy (cryo-EM) and single particle 3D-reconstruction. The oligomers are hollow, barrel-shaped structures consisting of tetrameric units. Higher-order oligomer formation is mediated mainly via N-terminal interactions, while the C-terminal tails exist in different conformations as supported by cryo-EM and solution-state nuclear magnetic resonance spectroscopy. The formation of an intra-molecular disulfide bond in human αA, as it occurs in vivo , seems to be coupled to significant conformational changes and leads to larger yet more dynamic oligomers with a higher chaperone activity towards certain substrates.

Chaperone mediated delivery of ubiquitylated misfolded proteins to the proteasome.
Ganapathi Kandasamy and Claes Andreasson.

Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University

In the ubiquitin mediated protein quality control pathway, misfolded proteins are recognized by molecular chaperones, ubiquitylated by dedicated quality-control ligases and delivered to 26S proteasome by general shuttle factor proteins for degradation. Hsp70 is a chaperone plays role at the ubiquitylation step; Hsp70 binds to misfolded proteins and facilitates their ubiquitylation. We have investigated the requirement of Hsp70 and its chaperone partner Hsp110 in the proteasomal degradation of ubiquitin modified proteins using yeast model. We find that Hsp70 and Hsp110 function at the post-ubiquitylation step to deliver aggregation prone ubiquitylated misfolded proteins to the 26S proteasome. Hsp70 binds to the 26S proteasome suggesting a mechanism for the delivery of its interacting proteins for proteolysis. In contrast Hsp110 forms complexes with Hsp70 and thereby releases the chaperone from its binding site at the proteasome. Our data suggests that aggregation prone ubiquitylated misfolded proteins are shuttled to proteasome by chaperone-mediated mechanisms

---

An unfolded protein-induced conformational switch activates mammalian IRE1
G Elif Karagöz[1], Vivian Chen[2], Jasmin Schäffer[3], Peter Walter[1]

[1]*Department of Biochemistry and Biophysics, Howard Hughes Medical Institute, University of California, San Francisco, CA 94143, USA*
[2]*Department of Plant Biology, Carnegie Institution for Science, Stanford, CA 94305, USA*
[3]*Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Heidelberg, Germany*

To adjust the folding capacity of the endoplasmic reticulum (ER), cells rely on the unfolded protein response (UPR), a signaling network that relays the ER folding-status to the nucleus via ER-resident sensors. IRE1 is the most conserved sensor, found from yeast to human. Here, we demonstrate that, similarly to yeast, IRE1 activation in mammals relies on direct and selective binding of ~12 amino-acid long hydrophobic stretches exposed on unfolded proteins. Nuclear magnetic resonance spectroscopy experiments revealed that unfolded proteins bind to the MHC-like-groove in human IRE1 ER-lumenal domain (LD) and induce conformational changes promoting its oligomerization. Binding of ER-resident chaperone BiP to IRE1 LD does not prevent IRE1 LD's interaction with peptides, or break preformed IRE1 LD dimers, instead it allosterically modulates IRE1 LD dimerization. Our results support the hypothesis that unfolded protein binding activates IRE1 directly and BiP binding buffers IRE1 activity.

RAPP, a Ribosome-Associated Protein Quality Control, in Health and Disease
Elena Tikhonova[1], Zemfira Karamysheva[2], Andrey Karamyshev[1]

[1]*Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX, 79430, USA*
[2]*Department of Biological Sciences, Texas Tech University, Lubbock, TX, 79409, USA*

Regulation of Aberrant Protein Production (RAPP) is a ribosome-associated quality control. It monitors proteins during their synthesis, senses defective proteins and degrades their mRNA templates. Normally, nascent polypeptides emerged from the ribosome interact with targeting or folding factors. When a mutation prevents these interactions, the Ago2 protein, a sensor in RAPP, detects the interaction loss and triggers mRNA degradation. RAPP activation leads to inhibition of protein expression that precedes mRNA degradation suggesting that Ago2 induces translational repression. We found that disease-causing mutations in the signal sequences of secretory proteins lead to significant reductions in their mRNA levels demonstrating that the molecular mechanisms of some cases of pycnodystosis, Crigler-Najjar syndrome, Norrie disease, aspartylglucosaminuria, and autoimmune disease are associated with RAPP.

Proteasome regulation by the yeast protein quality control machinery
Ofri Karmon, Lee Zeev Peters, Shay Ben-Aroya

Bar Ilan University, Ramat Gan, Israel

Proteasome storage granules (PSGs) are created in yeast as part of an extensive and programmed reorganization of proteins into reversible assemblies upon carbon source depletion. Here, we demonstrate that cells distinguish dysfunctional proteasomes from PSGs on the cytosolic insoluble protein deposit (IPOD). Furthermore, we provide evidence that this is a general mechanism for the reorganization of additional proteins into reversible assemblies. Our study expands the roles of the IPOD, which might serve not only as the specific depository for amyloidogenic and misfolded proteins, but also as a potential hub from which proteins are directed to distinct cellular compartments. These findings therefore provide a framework for understanding how cells discriminate between intact and abnormal proteins under stress conditions to ensure that only structurally 'correct' proteins are deployed.

A role for protein folding chaperones in driving the age-related genome instability of cancers
Georgios Karras

Whitehead Institute for Biomedical Research

Age-related loss of physiological homeostasis drives the characteristic genomic and proteomic instabilities of cancers. Examining hundreds of genome maintenance factors, we uncovered direct links between genome maintenance processes and the protein folding machinery in human cells. Cancer mutations in critical genome maintenance proteins alter their functional dependence on protein folding chaperones. The protein-folding chaperone HSP90 can rescue the function of mutants that retain most physical interactions with their functional partners. In contrast, HSP70 engages with mutants that have reduced binding to functional partners with critical roles in genome maintenance. These results uncover

a delicate relationship between genome maintenance and the protein folding machinery in normal cells that is readily perturbed in cancer cells. This work provides an approach for identifying mutations that enable the coupling of genome instability to age-related proteotoxic stresses in cancer.

Identification of distinct chaperone complexes that suppress Htt fibrilization and disaggregate Htt amyloid fibrils
Janine Kirstein

Leibniz Institute for Molecular Pharmacology, Berlin, Germany

We can demonstrate that a trimeric chaperone complex composed of Hsp70, Hsp110 and type II J-protein family completely suppresses the amyloid fibril formation of HttExon1Q48 using a novel FRET-based in vitro assay. We also demonstrate for the first time a disaggregation of HttExon1Q48 fibrils by this chaperone complex. The combination of different Hsp70 and J-protein chaperones together with Hsp110 forms distinct chaperone complexes that exhibit different suppression and disaggregation activities. Depletion of these chaperones in HD patient-derived neuronal progenitor cells lead to a pronounced increase of aggregation of the endogenous Htt protein. We could confirm the importance of these chaperones to maintain the solubility of HttQn and related polyQ proteins on an organismal level in C. elegans. Consequently, overexpression of the rate-limiting J-protein DNAJB1 in HEK cells expressing robustly aggregating HttExon1Q97 lead to a pronounced decrease of HttExon1Q97 aggregation.

The role of BAG6 in protein quality control
Yee Hui Koay, et al.

Division of molecular and cellular function, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Oxford Road, Manchester, M13 9PT, UK

Failure of protein quality control pathways can lead to harmful accumulation of aggregation-prone protein, as occurs in many human diseases. Defining the range of quality control systems that cells use to handle such aggregation-prone protein is vital for understanding the molecular basis of these diseases and the development of new treatments. BAG6 has been implicated in a wide range of functions, but recent work has focused on its role in cellular protein quality control systems. BAG6 has the ability to bind exposed hydrophobic stretches and this is likely to underlie its ability to contribute to the quality control of different types of unfolded protein in the cells. BAG6 contributes to at least two distinct quality control pathways: ERAD which removes misfolded protein from the ER, and a cytoplasmic quality control system that degrades mislocalised membrane protein. In this study, we show that BAG6, RNF126 and UBR4 promote ubiquitination and degradation of a model ERAD substrate.

Single molecule experiments on the human Glucocorticoid Receptor (GR)
Eline Koers[1], Mario Avellaneda[1], David-Paul Minde[2], Sander Tans[1]

[1]*AMOLF*
[2]*current affiliation: Cambridge Centre for Proteomics*

The Glucocorticoid Receptor (GR) resides in the cytosol when it is in the unliganded state. Both Hsp70/Hsp40 and Hsp90 play an essential role in the ability of the receptor to accept its ligand cortisol. In particular, Hsp90 is thought - according to biochemical studies - to open up the ligand binding site.
We placed the GR Ligand Binding Domain (LBD) in an optical tweezer setup to study these interaction on a single-molecule level. For this, the protein needed to be tethered to polystyrene beads which are held or moved by a laser beam. Traditional bioconjugation strategies utilize cysteine modifications for tethering, but these are undesirable due to the presence of surface exposed native cysteines in GR. We present a alternative method involving small tags which can be used to study the effect of chaperones on the GR-LBD, but could also be used for other proteins with exposed cysteines opening up this method for a variety of substrates.

CHIP as a Sensor of Proteostasis Stress
Yannick Kopp[1, 2], Tobias B. Schuster[1, 2], Wei-Han Lang[1, 2], Adrían Martínez-Límon[1, 2], Harald F. Hofbauer[1, 3], Robert Ernst[1, 3, 4], Giulia Calloni[1, 2], R. Martin Vabulas[1, 2]

[1]*Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany*
[2]*Institute of Biophysical Chemistry, Goethe University Frankfurt, Frankfurt am Main, Germany*
[3]*Institute of Biochemistry, Goethe University Frankfurt, Frankfurt am Main, Germany*
[4]*Institute of Biochemistry, Medical Faculty, University of Saarland, Homburg, Germany*

The mechanisms underlying the early adaptation of cellular compartments to cytosolic protein aggregation are not clear. We show that the HSP70- and HSP90-interacting ubiquitin ligase CHIP, when freed from chaperones during acute stress, can dock on cellular membranes thus performing a proteostasis sensor function. We reconstituted this process in vitro and found that mainly phosphatidic acid and phosphatidylinositol-4-phosphate enhance association of chaperone-free CHIP with liposomes. HSP70 and membranes compete for mutually exclusive binding to the TPR domain of CHIP. At new cellular locations, access to compartment-specific substrates enables CHIP to participate in the reorganization of the respective organelles, as exemplified by the fragmentation of the Golgi apparatus (effector function). We propose that the unmasking of chaperone-associated determinants of cellular localization may be a general mechanism to rapidly adjust cellular architecture to proteostasis stress.

CAT-tailing as a fail-safe mechanism for efficient degradation of stalled nascent polypeptides
Kamena Kostova[1, 2], Kelsey Hickey[1], Jonathan Weissman[1, 2]

[1]*University of California, San Francisco*
[2]*Howard Hughes Medical Institute*

Ribosome stalling leads to subunit dissociation and recruitment of the Ribosome Quality control Complex (RQC), which targets the partially synthesized polypeptide for proteasomal degradation through the action of the Ltn1p ubiquitin ligase. A second core RQC component, Rqc2p,

modifies the nascent polypeptide with the addition of a Carboxy-terminal Alanine and Threonine (CAT) tail though a non-canonical translation reaction. Here we explore the function of CAT tails in vivo. We show that Ltn1p is able to access only nascent chain lysine residues immediately proximal to the ribosome exit tunnel. For substrates that have no Ltn1p-accessible lysines, CAT tail addition enables degradation by exposing lysines sequestered in the ribosome exit tunnel or the ER lumen. Thus, CAT tails do not serve as a degron, but rather provide a fail-safe mechanism that greatly expands the range of RQC-degradable substrates.

The E3 ubiquitin ligase UBR5 maintains proteostasis of huntingtin in immortal pluripotent stem cells

Seda Koyuncu, Isabel Sáez Martínez, Giuseppe Calculli, et al.

CECAD-– Cluster of Excellence

Defects in proteostasis lead to accumulation of misfolded and aggregated proteins that is associated to onset of age-related diseases such as Huntington's disease(HD).HD is caused by abnormal expansion of CAG-encoded polyQ repeats in HTT gene, which causes formation of protein aggregates in neurons. Interestingly, length of pathological polyQ does not influence survival and pluripotency of iPSCs derived from HD patients(HD-iPSCs). These suggest that iPSCs might have enhanced mechanisms to maintain proteostasis of mutant HTT.Here, we examined mechanisms by which iPSCs maintain proteostasis of HTT. We have uncovered that iPSC have increased levels of UBR5.We show that UBR5 interacts with HTT and regulates both mutant & wild-type HTT.Loss of UBR5 results in accumulation of HTT protein and polyQ aggregates in HD-iPSCs.Besides, in C.elegans model of HD, orthologue of UBR5 regulates levels of polyQ aggregates.Thus, we define UBR5 as a novel modulator of HTT by studying proteostasis of iPSCs.

Investigating the flux of newly synthesized proteins through the network of co-translationally acting chaperones

Carla Galmozzi, Kristina Döring, Josef Auburger, Yu-Wei Shieh, Lena Grave, Bernd Bukau, Günter Kramer

Center for Molecular Biology of Heidelberg University (ZMBH) and German Cancer Research Center (DKFZ), DKFZ-ZMBH Alliance

Biogenesis of proteins integrates the action of multiple chaperones that engage the nascent polypeptide to support folding. We used selective ribosome profiling to explore the nascent substrate pool of the Hsp70 chaperone DnaK in E. coli and its functional interplay with Trigger Factor (TF) in protein folding. Analyzing the pattern of nascent chain engagement in wild-type and chaperone mutants, we find that TF binding in most cases precedes the engagement by DnaK. The rhythm of TF binding and release is coordinated with the emergence of domains, suggesting TF coordinates co-translational domain-wise folding. DnaK binds most proteins late during translation, but TF absence shifts binding to earlier time points, implying both chaperones compete for overlapping binding sites and providing a rationale for their redundant function. We finally show that TF and DnaK also guide quaternary structure formation, by binding nascent subunits to facilitate co-translational protein complex assembly.

TOR-mediated sensing of chaperone activity alters glucose metabolism and extends lifespan

Matea Peric[1], Ana Saric[2], Marina Musa[1], Peter Bou Dib[3], Marina Rudan[1], Sandra Sobocanec[2], Kristian Vlahovicek[4], Nuno Raimundo[3], Anita Krisko[1]

[1]*Mediterranean Institute for Life Sciences, Mestrovicevo setaliste 45, 21000 Split, Croatia*
[2]*Division of Molecular Medicine, Rudjer Boskovic Institute, Bijenicka 54, 10000 Zagreb, Croatia*
[3]*Universitätsmedizin Göttingen, Institut für Zellbiochemie, Humboldtallee 23, D-37073 Göttingen*
[4]*Bioinformatics Group, Department of Molecular Biology, Division of Biology, Faculty of Science, University of Zagreb, Croatia*

Despite the indisputable importance of cellular proteostasis on multiple levels, the relationships between proteostasis and other cellular pathways remain poorly understood. Here we show that enhanced chaperone activity is sensed by the TOR pathway via the activity of Hsp82. Chaperone enrichment decreases the level of Hsp82, deactivating TOR pathway and consequently activating Snf1/AMPK, regardless of glucose availability. This mechanism culminates in the extension of yeast replicative lifespan (RLS) that is fully reliant on both TOR deactivation and Snf1/AMPK activation. Specifically, we identify oxygen consumption increase as the downstream effect of Snf1 activation responsible for the entire RLS extension. Our results set a novel paradigm for the role of proteostasis in aging: modulation of the misfolded protein level can affect cellular metabolic features, and consequently, modify lifespan.

Repression of the antioxidant NRF2 pathway in premature aging

Nard Kubben[1], T. Voss[1], W. Zhang[2, 3], L. Wang[2, 4], J. Yang[2], J. Qu[4], G.H. Liu[2, 3, 5], T. Misteli[1]

[1]*National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA*
[2]*National Laboratory of Biomacromolecules, Chinese Academy of Sciences, Beijing 100101, China*
[3]*FSU-CAS Innovation Institute, Foshan University, Foshan, Guangdong 528000, China*
[4]*Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China*
[5]*Beijing Institute for Brain Disorders, Beijing 100069, China*

Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare fatal premature aging disorder. HGPS is caused by production of progerin, a mutant form of the nuclear architectural protein lamin A, causing extensive morphological, (epi)genetic damage and mesenchymal stem cell (MSC) attrition through unknown mechanisms.

Using a high-throughput high-content imaging-based siRNA screen we identify the antioxidative and proteostatic NRF2 pathway as a driver mechanism in HGPS. Progerin sequesters and impairs NRF2 transcriptional activation of antioxidants. Suppressed NRF2 activity or consequential chronic oxidative stress recapitulate HGPS aging defects and re-activation of NRF2 activity in HGPS patient cells reverses aging defects and restores in vivo MSC viability (Kubben, Cell, 2016).

These findings establish the NRF2 antioxidative response as a key contributor to aging, and suggest NRF2/proteostasis-promoting compounds as a promising novel therapeutic strategy in HGPS and aging diseases.

Protein homeostasis of a metastable subproteome associated with Alzheimer's disease

Rishika Kundra[1], Richard Morimoto[2], Christopher Dobson[1], Michele vendruscolo[1]

[1]*Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, UK*
[2]*Department of Molecular Biosciences, Northwestern University, Evanston IL 60208, USA*

Alzheimer's disease is the most common cause of dementia. A hallmark of this disease is the presence of aberrant deposits formed primarily by the Aβ peptide (amyloid plaques) and the tau protein (neurofibrillary tangles) in the brains of affected individuals. Increasing evidence suggests that the formation of these deposits is associated with the age-related dysregulation of a set of highly expressed and aggregation prone proteins that make up a metastable subproteome. To better understand the origins of such dysregulation, we identify components of the protein homeostasis system associated with these metastable proteins by using a co-expression analysis. Our results specifically characterize the importance of the protein clearance and trafficking mechanisms, in particular the endo-lysosomal system and ubiquitin-proteasome system, in maintaining the cellular homeostasis of the metastable subproteome associated with Alzheimer's disease.

Mimicking the heterozygous condition of alpha-1-antitrypsin deficiency in cellular models

Mattia Laffranchi, Romina Berardelli, Riccardo Ronzoni, Annamaria Fra

Dept. of Molecular and Translational Medicine, University of Brescia.

The common genotype associated with alpha-1-antitrypsin deficiency is homozygosity for the polymerogenic Z mutant, but a relevant number of AATD patients carry Z AAT in association with the S AAT or with rarer mutants. To investigate the formation in vivo of mixed polymers between Z and S AAT, we set up an experimental strategy that mimics in cellular models the heterozygous condition.
We demonstrated by pull-down assays that Z is able to form hetero-complexes with other AAT variants which are recognised by the polymer specific conformational mAb 2C1.
In immunofluorescence analyses, we observed that S and Z AAT co-localise within dilated cisternae of the ER in close proximity, as assessed by the Proximity Ligation Assay. Interestingly, Z interacts also with the wt M AAT, although to a much lesser extent. Taken together, these results provide the first evidence of intracellular co-localization and co-polymerization of AAT mutants expressed in heterozygosity.

RAC, a unique Hsp70-Hsp40 pair at the ribosome.

Karine Lapouge, Felix A. Weyer, Andrea Gumiero, Genís V. Gesé, Irmgard Sinning

Heidelberg University Biochemistry Center (BZH), INF 328, D-69120 Heidelberg, Germany.

Co-translational chaperones assist in the de novo folding of nascent polypeptides. In eukaryotes, the co-translational chaperone RAC (ribosome-associated complex) and the Hsp70 Ssb are functionally linked. RAC is composed of the Hsp40 Zuo1 and the atypical Hsp70 Ssz1. While the interaction between Ssz1 and Zuo1 had been analysed, high resolution structural information of the complex was missing. We determined the structure of the RAC core comprising full length Ssz1 and the Zuo1 N terminus. The structure shows how Zuo1 binds to the Ssz1 linker region, interacts with the NBD and complements the SBDβ by β-augmentation. Thereby, this Hsp70-Hsp40 pair is molded into a functional unit, in which Zuo1 stabilizes Ssz1 in trans through interactions that in canonical Hsp70s occur in cis. We provide molecular insights into the coupling of a special Hsp70-Hsp40 pair, which in concert with Ssb, forms a unique chaperone triad at the ribosomal tunnel exit.
Weyer et al. (2017) NSMB 24:144-151

Membrane remodeling by chaperones and presynaptic protein quality control

Elsa Lauwers[1, 2], Yu-Chun Wang[1, 2], Patrik Verstreken[1, 2]

[1]*KU Leuven, Department of Human Genetics*
[2]*VIB Center for Brain & Disease Research*

Chaperones safeguard proteome integrity by balancing protein (un)folding with proteolysis. We recently identified several chaperones in a screen for brain-enriched proteins that remodel membranes, revealing an intriguing link bewteen membrane dynamics and protein quality control. We focused on a presynaptically enriched chaperone, Hsc70, and demonstrated that it deforms the endosomal membrane to drive intraluminal vesicles formation into multivesicular bodies (MVBs). This Hsc70 activity is responsible for turning over a particular set of presynaptic proteins, leaving a rejuvenated protein pool and promoting neurotransmission. We are now pursuing how another membrane deforming chaperone, Hsp90, affects synaptic organelles, protein levels and function. Our data suggests that Hsp90 is involved in the fusion of MVBs with the presynaptic plasma membrane to release exosomes. This work reveals unique strategies used by chaperones to coordinate synaptic proteostasis and membrane dynamics.

An Evolutionarily Conserved Ribosome-associated Quality Control Mechanism Maintains Epidermal Stem Cell Homeostasis

Kif Liakath-Ali[1], Eric Mills[2], Inês Sequeira[1], Beate Lichtenberger[1, 4], Ajay Mishra[1], Angela Oliveira Pisco[1], Ibrahim Adham[3], Rachel Green[2], Fiona Watt[1]

[1]*1. Centre for Stem Cells and Regenerative Medicine, King's College London, 28th floor, Guy's Tower Wing, London SE1 9RT, UK.*
[2]*2. Howard Hughes Medical Institute, Johns Hopkins School of Medicine, Department of Molecular Biology and Genetics, Baltimore, MD 21205, USA.*
[3]*3. Institute of Human Genetics, University Medical Centre of Göttingen, D-37073, Göttingen, Germany.*
[4]*4. Skin & Endothelium Research Division, Department of Dermatology, Medical University of Vienna, Lazarettgasse 14, 1090 Vienna, Austria.*

mRNA quality control is one of a major processes involved in protein quality control mechanism which ensure fidelity of protein translation. An evolutionarily conserved component of the quality control machinery, Dom34/Pelota (Pelo), rescues stalled ribosomes. Here we show that Pelo is required for mammalian epidermal homeostasis, surprisingly not dermal homeostasis. Conditional deletion in murine epidermal stem cells expressing Lrig1 results in hyperproliferation and abnormal differentiation, whereas deletion in other stem cells does not. Loss of Pelo results in truncated ribosome footprints and global upregulation of transcription and translation. Translational inhibition by rapamycin-

mediated down regulation of mTOR rescues the epidermal phenotype. This study reveals a novel role for the ribosome-rescue machinery in mammalian tissue homeostasis and an unanticipated specificity in its impact on different stem cell populations.

Two-component sHsps bacterial system cooperation in counteracting irreversible aggregation and Hsp70/Hsp100-dependent efficient protein refolding

Igor Obuchowski, Artur Piróg, Szymon Żwirowski, Stijn De Moor, Krzysztof Liberek

Intercollegiate Faculty of Biotechnology, University of Gdansk, Gdansk, Poland

Small heat-shock proteins (sHsps) are an evolutionary conserved class of ATP independent chaperones that protect cells against proteotoxic stress. sHsps form assemblies with aggregation-prone misfolded proteins, which facilitates subsequent substrate solubilization and refolding by ATP dependent Hsp70 and Hsp100 chaperones. Upon heat shock, sHsps rapidly bind misfolded proteins, preventing their irreversible aggregation. This interaction has to be broken for efficient Hsp70/Hsp100-mediated refolding. Bacteria usually harbor one or two sHsp-coding genes, whose products, when two variants are present, interact with each other. However, their activities and mechanism of action was investigated mostly separately. Here we analyze functional cooperation within a two-component sHsp bacterial system (E. coli IbpA and IbpB) in comparison to a single sHsp (E. amylovora HspB) in preventing protein irreversible aggregation and providing efficient Hsp70/Hsp100-mediated misfolded protein recovery.

Investigating altered proteostasis as underlying mechanism of autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)

Fabiana Longo[1, 2], Peter De Jonghe[3], Paola Podini[4], Angelo Quattrini[1, 5, 6], Francesca Maltecca[1, 6]

[1]*San Raffaele Scientific Institute, Division of Genetics and Cell biology, Neurogenomics Unit, Milan, Italy*
[2]*University of Insubria, Varese, Italy*
[3]*VIB-Department of Molecular Genetics, University of Antwerpen, Belgium*
[4]*San Raffaele Scientific Institute, Division of Neuroscience - INSPE - Institute of Experimental Neurology, Milan, Italy*
[5]*Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy*
[6]*Vita-Salute San Raffaele University, Milan, Italy*

ARSACS is caused by mutations in the 520 kDa protein sacsin. Sacsin is composed of a ubiquitin-like domain binding the proteasome, three repeating regions with Hsp90 homology and a J-domain homologous to Hsp40. Although these domains suggest a chaperone-like function, sacsin cellular role is unknown. In ARSACS patient cells in which sacsin is almost absent, we observed that the intermediate filament vimentin forms a perinuclear 'cage' void of organelles. This peculiar feature was confirmed by ultrastructural analysis, also showing increased number of autophagosomes and autolysosomes, some of them containing electron-dense material suggestive of proteinaceous content. Increased levels of LC3II upon inhibition of lysosomal degradation confirmed an autophagy boost in patient cells. These phenotypes may be consistent with formation of aggresomes, induced by misfolding and accumulation of putative sacsin substrates, indicating that altered proteostasis could underlie ARSACS pathogenesis.

A Concentration-Dependent Liquid Phase Separation Can Cause Toxicity upon Increased Protein Expression

Benedetta Bolognesi[1, 2, 3], Nieves Lorenzo Gotor[1, 2], Riddhiman Dhar[1, 3], Davide Cirillo[1, 2], Gian Gaetano Tartaglia[1, 2, 4], Ben Lehner[1, 3, 4]

[1]*Universitat Pompeu Fabra (UPF), 08003 Barcelona, Spain.*
[2]*Bioinformatics and Genomics Programme, Centre for Genomic Regulation, The Barcelona Institute of Science and Technology, Barcelona, Spain;*
[3]*EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation, The Barcelona Institute of Science and Technology, Barcelona, Spain;*
[4]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain.*

Multiple human diseases are associated with a liquid-to-solid phase transition resulting in the formation of protein aggregates. We present an alternative mechanism for cellular toxicity based on a concentration-dependent liquid-liquid demixing. Analyzing dosage sensitive proteins in yeast reveals that they share physicochemical properties with proteins that participate in physiological liquid-liquid demixing. Increasing the concentration of one of these proteins indeed results in the formation of cytoplasmic foci with liquid properties. Demixing occurs at the onset of toxicity and titrates proteins and mRNAs from the cytoplasm. Focus formation is reversible, and resumption of growth occurs as the foci dissolve as protein concentration falls. Preventing demixing abolishes the dosage sensitivity of the protein. We propose that triggering inappropriate liquid phase separation may be an important cause of dosage sensitivity and a determinant of human disease.

Probing molecular mechanisms of J-protein-Hsp70 interaction with chaperones dedicated for iron-sulfur cluster biogenesis.

Bartłomiej Tomiczek[1], Wojciech Delewski[1], Brenda Schilke[2], Rafal Dutkiewicz[1], Szymon J. Ciesielski[2], Lukasz Nierzwicki[3], Igor Grochowina[1], Jacek Czub[3], Elizabeth A. Craig[2], Jaroslaw Marszalek[1]

[1]*Intercollegiate Faculty of Biotechnology, University of Gdansk, Poland*
[2]*Department of Biochemistry, University of Wisconsin-Madison, USA*
[3]*Department of Physical Chemistry, Gdansk University of Technology, Gdansk, Poland*

Hsp70 chaperones are critical for protein biogenesis and homeostasis in all cellular compartments. Obligate J-protein co-chaperones drive the specificity of Hsp70 function by controlling its ATPase activity and thus its interaction with substrate. But, much remains to be learned about their mechanisms of action, mostly because typical multifunctional Hsp70s interact with several J-proteins. Yet, in the specialized system for the biogenesis of iron-sulfur (FeS) cluster in S. cerevisiae a dedicated J-protein (Jac1) and dedicated Hsp70 (Ssq1) interact with a single substrate, the protein scaffold on which clusters are built. Hsp70 interaction with the cluster bound scaffold is critical for cluster transfer to recipient proteins. This system enables us to combine high-resolution mechanistic studies with in vivo verification of their results. By combining computational, and experimental approaches we identified critical residues for interaction between the J-domain of Jac1 and Ssq1.

Genetic misregulation of the stability of the transcription factor Pap1 alters the tolerance of fission yeast to oxidative stress
Luis Marte, Susanna Boronat, Elena Hidalgo

Oxidative Stress and Cell Cycle Group, Universitat Pompeu Fabra

One of the toxicity hallmarks associated to oxidative stress is protein carbonylation. In a search for S. pombe genes involved in carbonylated protein degradation, we identified some protein quality control (PQC) deletion mutants with lower carbonylated protein levels upon oxidative stress than wild-type cells. Some of these mutants had enhanced levels of the transcription factor Pap1. Upon mild oxidative stress, Pap1 activates H2O2-detoxifying genes transcription what may lead to decreased protein damage in our mutants. To assess the role of our newly identified genes in Pap1 degradation, we studied the stability of nuclear or cytosolic forms of Pap1 expressed in our PQC mutant strains. We observed that some of these PQC mutants had a cell localization specific effect in Pap1 degradation. We also observed that Pap1 levels in the mutants were not sufficient to explain decreased protein carbonyls, suggesting a direct role of the PQC system in the degradation of carbonylated proteins.

Potential link of mitochondrial biogenesis and quality control
Christoph U. Mårtensson[1, 2], Lars Ellenrieder[1, 2], Lena-Sophie Wenz[1, 2], Nikolaus Pfanner[1, 3], Thomas Becker[1, 3]

[1]*Institute for Biochemistry and Molecular Biology, University of Freiburg, Germany*
[2]*Faculty of Biology, University of Freiburg, Germany*
[3]*BIOSS Centre for Biological Signaling Studies*

The mitochondrial proteome consists of up to 1000 (yeast)/ 1500 (human) proteins. 99% of mitochondrial proteins are nuclear encoded, synthesized as precursors on cytosolic ribosomes and imported into mitochondria. The translocase of the outer membrane (TOM) complex forms a general entry pore for these precursors. Upon translocation via the TOM complex intramitochondrial import machineries sort the precursor proteins into the submitochondrial compartments like inner membrane, intermembrane space and matrix. During mitochondrial biogenesis unassembled and misfolded proteins might impair mitochondrial function and need to be identified and degraded. However, it is not yet known how mitochondrial biogenesis is connected to quality control. Here, we report of a potential link between mitochondrial biogenesis and quality control.

Effect of protein translation in ageing using Drosophila melanogaster.
Victoria Martinex Miguel

Cancer Institute, University College London

A remarkable discovery in the field of aging is that a single gene mutation can lead to the reduction of major nutrient signalling pathways. This change results in pronounced lifespan extension and improved health in model organisms from yeast to mammals. The most well studied anti-aging alterations are down-regulation of insulin signalling, target-of-rapamycin signalling or dietary restriction. Recently, my group showed that a pharmacological approach to inhibit TOR signalling extends lifespan and down-regulation of translation and increased autophagy is essential for its benefits. Guided by our recent unpublished data, we examined various translation-related genes for their effect on aging. We also tested major hypothesis to understand how reduced translation can contribute to healthier aging. In conclusion, using our established molecular and analytical methods and a multi-disciplinary approach we aim to gain an in-depth insight into the translation mediated longevity.

An aberrant phase transition of stress granules triggered by misfolded proteins and prevented by chaperone function
Daniel Mateju[1], Titus Franzmann[1], Avinash Patel[1], Andrii Kopach[1], Edgar Boczek[1], Shovamayee Maharana[1], Hyun Lee[1], Serena Carra[2], Anthony Hyman[1], Simon Alberti[1]

[1]*Max Planck Institute of Molecular Cell Biology and Genetics*
[2]*University of Modena and Reggio Emilia*

Stress granules (SG) are membraneless compartments involved in regulating mRNAs during stress. Aberrant forms of SGs have been implicated in age-related diseases, such as amyotrophic lateral sclerosis (ALS), but the molecular events triggering their formation are unknown. Here, we find that misfolded proteins, such as ALS-linked variants of SOD1, tend to aggregate in SGs in human cells. This promotes a conversion of SGs into an aberrant state with reduced dynamics and solid-like material properties. With the accumulation of misfolded proteins, SGs also recruit chaperones, which prevent excessive co-aggregation with misfolded proteins and promote SG disassembly. Moreover, we identify a backup system for SG clearance, which involves transport of aberrant SGs to the aggresome for degradation by autophagy. We propose that SGs are vulnerable to co-aggregation with misfolded proteins and that cells use a system of protein quality control to prevent a conversion of SGs into an aberrant state.

Genotoxin-induced transcriptional dynamics regulate selective aggregation of a splicing factor
Veena Mathew[1], Annie Tam[1], Karissa Milbury[1], Analise Hofmann[2], Christopher Hughes[3], Gregg Morin[3, 4], Christopher Loewen[2], Peter Stirling[1, 4], et al.

[1]*Terry Fox Laboratory, BC Cancer Agency*
[2]*Department of Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia, Vancouver, Canada*
[3]*Michael Smith Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, Canada*
[4]*Department of Medical Genetics, University of British Columbia, Vancouver, Canada*

Upon genotoxic stress, dynamic relocalization events control DNA repair, and alterations of the transcriptome and proteome enabling stress recovery. How these events may influence one another is only partly known. Beginning with a cytological screen for genome maintenance proteins, we find that, upon alkylation stress, the splicing factor Hsh155 localizes to both nuclear and cytoplasmic protein quality control aggregates. Under stress, an ordered sequestration of Hsh155 occurs at nuclear and then cytoplasmic aggregates regulated by molecular chaperones. Hsh155 aggregation is cell cycle dependent and responsive to dynamic changes in ribosomal protein gene expression. Indeed, loss of ribosomal protein gene transcription factor Sfp1 allows general aggregate formation but prevents Hsh155 recruitment. Together, our

analyses suggest a model in which eviction of proteins from chromatin undergoing transcriptional remodeling during stress can be coupled to protein sequestration in aggregates.

Molecular mechanism of the regulation of Hsp70s by J-domain cochaperones

Roman Kityk[1], Jürgen Kopp[2], Matthias Mayer[1]

[1]*Center for Molecular Biology Heidelberg University (ZMBH)*
[2]*Biochemistry Center Heidelberg University (BZH)*

The versatility of Hsp70 chaperones depends on the targeting function of J-domain cochaperones. J-domain proteins either bind to Hsp70 substrates themselves or are located where Hsp70 substrates appear and stimulate Hsp70's ATPase activity in synergism with the substrate itself. Thereby J-domain proteins convert Hsp70s from the ATP bound state with high substrate association rates into the ADP bound state with low substrate dissociation rates, allowing Hsp70s to efficiently trap substrates. Stimulation of the ATPase activity of Hsp70s by J-domain proteins is essential for Hsp70 functions, however, the molecular mechanism of this process is largely unknown.
We solved the crystal structure of the J-domain of E. coli DnaJ in complex with the E. coli Hsp70 DnaK in the ATP bound conformation. Our structure is consistent with a large body of biochemical and biophysical evidence and reveals the molecular mechanism of J-domain protein-mediated stimulation of the ATPase activity of Hsp70s.

Deubiquitinase activity is required for the proteasomal degradation of misfolded cytosolic proteins upon heat-stress

Thibault Mayor, Mang Zhu, Amalia Rose, Sophie Comyn, Nancy Fang

Department of Biochemistry and Molecular Biology, Michael Smith Laboratories, University of British Columbia

Protein quality control pathways can either assist refolding, target terminally misfolded proteins for degradation or retained misfolded proteins in specific compartments; a major challenge is to decipher how these triage decisions are made at the molecular level. We have characterized several pathways that rely on E3 ubiquitin ligases to target cytosolic misfolded proteins for proteasome degradation. Our latest work shows how the Rsp5 E3 ligases is "reprogrammed" under heat stress conditions with the help of two deubiquitinases in order to target misfolded proteins for proteasome degradation. Notably, these deubiquitinases ensure that K48-linked ubiquitin chains are assembled on misfolded substrates. Using proteomics, we also deciphered which proteins evade the ubiquitin proteasome system and assembled into heat stress granules. Our work provides a better understanding of the targeting of misfolded polypeptides by quality control pathways that play a major role in protein homeostasis.

Regulation of sHsps by hetero-oligomer formation

Mareike Mentzel[1], Evgeny Mymrikov[1], Carsten Peters[2], Sevil Weinkauf[2], Martin Haslbeck[1], Johannes Buchner[1]

[1]*Chair of Biotechnology/Department of Chemistry/Technical University Munich*
[2]*Institute of Electron Microscopy/Department of Chemistry/Technical University Munich*

Small heat shock proteins play a major role in protein quality control due to their holdase function. While their interaction is well established, the effects of the hetero-oligomer formation are still enigmatic. In our study, we focused on three human sHsp (Hsp27, αB-crystallin and Hsp20) as these co-expressed in several human cell lines. Size and activity of these three sHsps hetero-oligomers were analyzed in vitro and in human cell lines. We investigated changes in size distribution of hetero-oligomers by SEC-MALS and by EM. The potential regulation of sHsps activity was characterized by chaperone assays using model substrates and cell lysates. Taken together, our data provide a comprehensive picture on the effect of hetero-oligomerization on the structure and function of human sHsps.

VCP/p97 maintains cellular homeostasis by driving autophagy of ruptured lysosomes

Hemmo Meyer

Molecular Biology I, Faculty of Biology, Centre for Medical Biotechnology, University of Duisburg-Essen, Germany

The AAA-ATPase VCP/p97 is best known for its role in promoting proteasomal degradation. We now demonstrate that p97 governs a pathway, which we term ELDR (for EndoLysosomal Damage Response) that drives the clearance of damaged lysosomes by autophagy and thus maintains lysosomal homeostasis. Upon damage, p97 is recruited to lysosomes and cooperates with a specific of cofactors to turn over K48-linked ubiquitin conjugates and facilitate engulfment of the damaged organelle by autophagosomal membranes. Of note, lysosomal clearance is compromised by mutations in p97 associated with a multisystem proteinopathy (MSP-1), and damaged lysosomes accumulate in affected patient tissue carrying the mutations. Moreover, we show that p97 helps clear late endosomes/lysosomes ruptured by endocytosed tau fibrillar tangles. Thus, our data reveal an important mechanism of how p97 maintains lysosomal and protein homeostasis, and implicate the pathway as a modulator of degenerative diseases.

Single particle cryo-EM reconstruction of chaperonin:actin complexes

Goran Milicic, David Balchin, Ulrich Hartl, Manajit Hayer-Hartl

Max Planck Institute of Biochemistry

Actin, the most abundant protein in eukaryotic cells, depends on the group II chaperonin TRiC in order to reach its native state. Actin cannot be folded by the group I chaperonin GroEL, although unfolded actin binds GroEL and is encapsulated in the GroEL-GroES folding cage. To gain insight into the mechanism by which these two chaperonins differ, we used single particle cryo-EM to solve the structures of GroEL:actin and TRiC:actin complexes in various states. Comparison of the structures showed that the specificity of binding to the heterooligomeric TRiC is critical for successful folding, a requirement that the homooligomeric GroEL is unable to support. Moreover, The TRiC cycle includes an additional conformational step preceding substrate release, which allows actin to adopt an intermediate state that folds successfully upon release into the folding cage.

Cellular aggregases maintain basal and balanced proteostasis activities

Axel Mogk, Chiting Ho, Tomas Grousl, Bernd Bukau

Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Deutsches Krebsforschungszentrum (DKFZ), DKFZ-ZMBH Alliance, Im Neuenheimer Feld 282, Heidelberg D-69120, Germany

Protein homeostasis is governed by a protein quality control network preventing the accumulation of misfolded proteins through refolding and degradation pathways. The organized sequestration of misfolded proteins by cellular aggregases represents a third proteostasis strategy, however, its physiological relevance and interdigitation with refolding and degrading activities remains poorly understood.
Misfolded protein sequestration in S. cerevisiae is executed by Btn2 and Hsp42 aggregases. Here we report on a systematic genetic analysis revealing that Btn2 and Hsp42 functions are specifically linked to Hsp70-dependent activities. Btn2 and Hsp42 act as crucial proteostasis backup systems and become essential for cell growth at physiological growth temperatures upon limiting Hsp70 capacity. Promoting the aggregation of misfolded proteins reduces Hsp70 overload and thereby is maintaining a basal level of Hsp70 capacity and re-adjusting the balance between degrading and refolding pathways.

Hsp90 releases the break on the Hsp70 folding path

Tania Morán Luengo[1], Roman Kityk[2], Matthias P. Mayer[2], Stefan G. D. Rüdiger[1]
[1]*Cellular Protein Chemistry, Bijvoet Center for Biomolecular Research, Utrecht University, The Netherlands*
[2]*Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Heidelberg, Germany*

Protein folding in the cell relies on the orchestrated action of conserved families of molecular chaperones, the Hsp70 and Hsp90 systems. Hsp70 acts in earlier intermediates and exhibits refolding activity whilst Hsp90 acts downstream of Hsp70 and does not show refolding function on its own. The overall process has always remained unclear, since Hsp90 function has not been established.
We observed that under relevant physiological conditions, the protein folding capacity of Hsp70 is dependent on Hsp90. We show that Hsp90, acting in an ATP dependent manner, releases the substrate from the Hsp70 folding trap. The refolding rate is independent from chaperone concentrations implying early action of the chaperones on the folding path followed by a chaperone-free slow reaction leading to functional protein. Together, our data suggest that this is a general pathway for chaperone-assisted protein folding.

Functional CRISPR screening identifies the ufmylation pathway as a regulator of SQSTM1/p62.

Francesca Moretti[1], Rowena De Jesus[2], Gregory McAllister[2], Zuncai Wang[2], Phil Bergman[2], Shanming Liu[2], Elizabeth Frias[2], John Alford[2], John S Reece-Hoyes[2], Alicia Lindeman[2], Jennifer Kelliher[2], et al.

[1]*Novartis Institutes for BioMedical Research, Switzerland*
[2]*Novartis Institutes for BioMedical Research, United States*
[3]*Massachusetts General Hospital, Harvard Medical School, United States*

SQSTM1 is an adaptor protein that integrates multiple cellular signaling pathways and whose expression is tightly regulated at the transcriptional and post-translational level. We describe a forward genetic screening paradigm exploiting CRISPR-mediated genome editing coupled to a cell selection step by FACS to identify regulators of SQSTM1. Through systematic comparison of pooled libraries, we show that CRISPR is superior to RNAi in identifying known SQSTM1 modulators. A genome-wide CRISPR screen exposed MTOR signalling and the entire macroautophagy machinery as key regulators of SQSTM1 and identified several novel modulators, including the ufmylation cascade. We show that ufmylation regulates SQSTM1 by eliciting a cell type-specific ER stress response which induces SQSTM1 expression and results in its accumulation. This study validates pooled CRISPR screening as a powerful method to map the repertoire of cellular pathways that regulate the fate of an individual target protein.

Hsp70 modulates proteasome activity and undergoes ubiquitin-independent degradation by the 20S proteasome.

Alexey Morozov[1], Tatiana Astakhova[2], David Garbuz[1], Marina Serebryakova[3], Olga Zatsepina[1], Vadim Karpov[1], Michail Evgen'ev[1]

[1]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str.32, 119991, Moscow, Russia*
[2]*Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Vavilov str. 26, 119334, Moscow, Russia.*
[3]*Belozersky Institute of Physico-Chemical Biology MSU, Leninskie Gory 1 building 40, 119992, Moscow, Russia.*

In frames of protein quality control stress-inducible heat shock protein 70 (Hsp70) cooperates with ubiquitin-proteasome system (UPS). Hsp70 together with its co-chaperones determines the fate of damaged proteins. If the client-protein can not be repaired, it is ubiquitinated and escorted for degradation by the UPS. Soon after stress relief Hsp70 itself undergoes ubiquitination and is rapidly hydrolyzed by the 26S proteasome. Although interplay between the UPS and Hsp70 is of special interest the effect of Hsp70 on the functional state of different forms of proteasomes was not sufficiently studied.
We have shown that incubation with recombinant Hsp70 conversely affects the activity of purified 20S and 26S proteasomes: 20S proteasomes were inhibited while 26S proteasomes were activated. Furthermore, we demonstrated that Hsp70 can be cleaved by the 20S proteasome independent of ubiquitination. The obtained results reveal new aspects of the interplay between Hsp70 and the UPS.

Cellular consequences of mitochondrial proteotoxic stress

Anne Kathrin Müller-Rischart, Nadja Lebedeva, Barbara Conradt

Department Biology II, Cell and Developmental Biology, Center for integrated protein science – CIPSM, Ludwig-Maximilian-University Munich, Germany

Upon accumulation of excessive or misfolded mitochondrial proteins, the mitochondrial unfolded protein response (UPRmt) is activated in a compartment specific manner in mammalian cells. We are specifically interested in whether activation of the intermembrane space (IMS) UPRmt affects mitochondrial morphology, function and, eventually, leads to the degradation of entire organelles.
As a starting point, we analyzed mitochondrial morphology in HeLa cells upon activation of IMS UPRmt. Strikingly, we observed massive mitochondrial clustering around the nucleus. We are currently examining different parameters of mitochondrial function.

Furthermore, we hypothesize that activation of IMS UPRmt triggers mitophagy. However, we failed to observe accumulation of PINK1 protein or translocation of parkin to mitochondria. Therefore, we are analyzing parkin/PINK1 independent autophagy pathways in order to determine the fate of mitochondria harboring terminally misfolded proteins.

The mammalian mitochondrial unfolded protein response

Christian Münch

Goethe University Frankfurt

The correct implementation of mitochondrial function in cells plays a crucial role in cellular survival. Thus, the mitochondrial proteome needs to be tightly controlled and perturbations have been implicated in a wide range of human pathologies. Upon misbalanced mitochondrial proteostasis, the mitochondrial unfolded protein response (UPRmt) activates in an attempt to restore accurate protein folding. The mechanisms controlling the mammalian UPRmt remain poorly understood, despite our extensive knowledge gained in C. elegans. Through RNA-sequencing and assessment of cellular stress signaling pathways, we've gained insight into the complex UPRmt signaling network and its coordination with other stress responses. Furthermore, we uncovered a locally acting new branch of the UPRmt that controls mitochondrial pre-RNA processing and translation. These effects are acute and reversible and constitute a temporally controlled rapid response to mitochondrial proteostasis defects.

The mechanism for Nrf1 activation in response to proteasome inhibition

Shun Koizumi, Shigeo Murata

Graduate School of Pharmaceutical Sciences, the University of Tokyo

The 26S proteasome is a large protease complex comprised of 33 different subunits. The proteasome degrades ubiquitinated proteins to regulate cellular homeostasis, and thus its activity needs to be properly regulated. In response to proteasome dysfunction, cells upregulate expression of all the proteasome genes by activating Nrf1. Nrf1 is an ER-resident transcription factor that is continually retrotranslocated and degraded by the proteasome. Upon proteasome inhibition, Nrf1 escapes degradation and is cleaved to become active. We recently identified the aspartyl protease DNA-damage inducible 1 homolog 2 (DDI2) as a responsible processing enzyme for Nrf1 through a genome-wide siRNA screen. Deletion of DDI2 reduced the cleaved form of Nrf1 and increased the full-length cytosolic form of Nrf1, resulting in poor upregulation of proteasomes in response to proteasome inhibition. Our results provide a new therapeutic target for cancer treatment in combination with proteasome inhibitors.

Mechanistic insights into aggregate solubilization by the human Hsp70-based protein disaggregase

Nadinath Nillegoda[1], Franziska Kundel[2], David Klenerman[2], Bernd Bukau[1]

[1]*Center for Molecular Biology of Heidelberg University (ZMBH), German Cancer Research Center (DKFZ), DKFZ-ZMBH Alliance, Heidelberg, Germany.*
[2]*Department of Chemistry, University of Cambridge, Cambridge, UK.*

Protein aggregates form when the generation of misfolded proteins exceeds the refolding and degradative capacity of cells. Protein aggregation is a reversible process, which is mediated by Hsp70-based disaggregases in humans. However, the mechanism by which this is achieved is unclear. We recently showed that interclass J-protein complexing power Hsp70 machinery to synergize protein disaggregation. Mechanistically, this implies the formation of transient higher order complexes of J-proteins that could efficiently recruit multiple Hsp70s onto aggregates. In support of this hypothesis, we provide biochemical evidence showing that both J-protein types, in complex, are required to simultaneously recruit Hsp70s to form an active disaggregase. We also provide complementary microscopic evidence showing the formation of distinct Hsp70 assemblies on aggregates. Taken together, our findings suggest a transient chaperone assembly containing multiple Hsp70s drives protein disaggregation in humans.

Two-component sHsps bacterial system cooperation in counteracting irreversible aggregation and Hsp70/Hsp100-dependent efficient protein refolding

Igor Obuchowski, Artur Piróg, Szymon Żwirowski, Stijn De Moor, Krzysztof Liberek

Intercollegiate Faculty of Biotechnology, University of Gdansk, Gdansk, Poland

Small heat-shock proteins (sHsps) are an evolutionary conserved class of ATP independent chaperones that protect cells against proteotoxic stress. sHsps form assemblies with aggregation-prone misfolded proteins, which facilitates subsequent substrate solubilization and refolding by ATP dependent Hsp70 and Hsp100 chaperones. Upon heat shock, sHsps rapidly bind misfolded proteins, preventing their irreversible aggregation. This interaction has to be broken for efficient Hsp70/Hsp100-mediated refolding. Bacteria usually harbor one or two sHsp-coding genes, whose products, when two variants are present, interact with each other. However, their activities and mechanism of action was investigated mostly separately. Here we analyze functional cooperation within a two-component sHsp bacterial system (E. coli IbpA and IbpB) in comparison to a single sHsp (E. amylovora HspB) in preventing protein irreversible aggregation and providing efficient Hsp70/Hsp100-mediated misfolded protein recovery.

Molecular mechanism of XBP1u translational pausing

Miku Ohfurudono[1], Ash-way Sogawa[2], Yukio Kimata[1], Kenji Kohno[1]

[1]*Nara Institute of Science and Technology*
[2]*Osaka Medical Center for Cancer and Cardiovascular Diseases*

It has not been well highlighted that peptide elongation on eukaryotic ribosomes is physiologically regulated. However, our previous study revealed that a functional translational pausing occurs on the XBP1u mRNA. This phenomenon leads to stabilization of ribosome-nascent chain (RNC) complex that contains the XBP1u mRNA and its translation product, which is carried to the endoplasmic-reticulum (ER) membrane. Then the XBP1u mRNA is spliced and matured by the ER-located endoribonuclease IRE1α. We also found that the C-terminal

sequence of the XBP1u peptide (236th~261st) is important for translational pausing. However, the molecular mechanism is unclear, and I am thus addressing this issue.
I thus performed photocrosslinking assay, which indicated that multiple ribosomal proteins (RPL3, RPL4 and RPL7) physically interact with the XBP1u nascent chain. Actually, gene knockdown of these ribosomal proteins affected the strength of translational pausing of the XBP1u mRNA.

An ortho-Iminoquinone Compound Reacts with Lysine Inhibiting Aggregation while Remodeling Mature Amyloid Fibrils.
Luiza Fernandes[1], Nathalia Moraes[1], Fernanda Sagrillo[3], Augusto Magalhães[1], Marcela Moraes[3], Jeffery Kelly[2], Debora Foguel[1], Neil Grimster[2], Fernando Palhano[1]

[1]*Universidade Federal do Rio de Janeiro.*
[2]*The Scripps Research Institute.*
[3]*Fluminense Federal University.*

Protein aggregation is a hallmark of several neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. It has been shown that lysine residues play a key role in the formation of these aggregates. Thus the ability to disrupt aggregate formation by covalently modifying lysine residues could lead to the discovery of therapeutically relevant anti-amyloidogenesis compounds. Herein, we demonstrate that an ortho-iminoquinone (IQ) can be utilized to inhibit amyloid aggregation. Using synuclein, Aβ1-40 and IAPP8-24 as model systems, we observed that IQ was able to react with lysine residues and reduce amyloid aggregation. We also observed that IQ reacts with free amines within the amyloid fibril cross-linking them, and thus preventing their dissociation and their seeding capacity.

Regulation of the neuroprotective chaperone DNAJB2 in motor neurons
David Parfitt[1], Daniele Ottaviani[2], Oriano Marin[2, 3], Michaela Auer-Grumbach[4], Burkhard Gess[5], Maria Ruzzene[2], Michael Cheetham[1], et al.

[1]*UCL Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, UK*
[2]*Department of Biomedical Sciences, University of Padova, Via U. Bassi 58/b 35131, Padova, Italy*
[3]*Proteomics Center, University of Padova and Azienda Ospedaliera di Padova, Via G. Orus 2/B, 35129, Padova, Italy*
[4]*Department of Orthopaedics, Medical University Vienna, Austria*
[5]*Department of Sleep Medicine and Neuromuscular Disorders, University of Muenster, Germany*

Neurons are particularly vulnerable to disorders to imbalances of proteostasis. Mutations in the DNAJ protein DNAJB2 (HSJ1) lead to inherited neuropathies. DNAJB2 binds ubiquitylated proteins through its ubiquitin interacting motifs (UIMs) and functions with HSP70 proteins to facilitate their delivery to the proteasome for degradation. DNAJB2 overexpression can reduce aggregation of neurodegeneration-associated proteins in vitro and in vivo; however, the regulation of DNAJB2 function in motor neurons is little understood. Here we show that CK2 phosphorylates DNAJB2 within its second UIM, and that phosphorylation is accompanied by a reduced ability to bind ubiquitylated clients and stimulate proteasome degradation. Furthermore, motor neurons generated from motor neuropathy patient-derived iPS cells and DnajB2 knockout mice serve as a model for investigating the cellular function of DNAJB2 and build our understanding of the specific proteostasis requirements of motor neurons.

Glutathionylation of DnaK provides a link between oxidative stress and the heat shock response
Sarah Perrett, et al.

Institute of Biophysics, Chinese Academy of Sciences

Cys modification has been detected in different Hsp70 family members in vivo. However, the physiological significance of Cys modification of Hsp70 remains to be elucidated. Oxidative stress results in temporary inactivation of DnaK due to depletion of cellular ATP and thiol modifications such as glutathionylation, until normal cellular ATP levels and a reducing environment are restored. We observed glutathionylation of DnaK in lysates of E. coli cells that had been subjected to oxidative stress. In vitro, we found that glutathionylation of DnaK reversibly changes the secondary structure and tertiary conformation, leading to reduced nucleotide and peptide binding ability. Glutathionylation of DnaK also reversibly weakens interaction of DnaK with DnaJ, GrpE or Sigma32, which may facilitate release of Sigma32 from its interaction with DnaK, thus triggering the heat shock response. Such a mechanism provides a possible link between oxidative stress and the heat shock response in bacteria.

Posttranslational modifications of tyrosine residues modulate α-synuclein cytoxicity and protein turnover in a yeast model of Parkinson´s Disease
Blagovesta Popova[1, 2], Alexandra Kleinknecht[1, 2], Diana Lázaro[2, 3], Oliver Valerius[1], Tiago Outeiro[2, 3], Gerhard Braus[1, 2]

[1]*Institute of Microbiology and Genetics, Department of Molecular Microbiology and Genetics, University of Göttingen, Göttingen, Germany*
[2]*Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany*
[3]*University of Göttingen Medical School, Department of NeuroDegeneration and Restorative Research, Göttingen, Germany*

Parkinson's disease is characterized by loss of dopaminergic neurons and presence of α-synuclein (αSyn) protein inclusions. Expression of human αSyn in Saccharomyces cerevisiae resembles the pathology of the disease resulting in cytotoxicity and aggregate formation. αSyn is abundantly phosphorylated at serine S129 and possesses four tyrosines (Y39, Y125, Y133, and Y136) that can be posttranslationally modified by nitration or phosphorylation. We addressed the molecular mechanism how tyrosine posttranslational modifications affect αSyn cytotoxicity. Tyrosine nitration can contribute to αSyn toxicity or can be part of a cellular salvage pathway when di-tyrosine-crosslinked dimers are formed. The Y133 residue, which can be either phosphorylated or nitrated, determines whether S129 is protectively phosphorylated and αSyn inclusions are cleared. This complex interplay with S129 phosphorylation demonstrates a dual role for C-terminal tyrosine residues that control αSyn protein turnover.

Chloroplast protein quality control mediated by DNAJ-like proteins
Pablo Pulido, Dario Leister

Ludwig-Maximilians-Universität (LMU) Munich, Germany

Plant chloroplastic HSP70s are involved in processes such as the folding of imported proteins synthetized in the cytosol and refolding after exposure to stress. It is known that the specificity of HSP70 is determined by its DNAJ partners that deliver the substrates. However, several DNAJ isoforms have lost the motifs required for the interaction with HSP70, displaying HSP70-independent chaperone activity. Recently, we have reported that the DNAJ-like protein SCO2 is essential for the assembly of LHC to PSII complexes. Furthermore, we have unveiled a large number of DNAJ-like proteins in the model plant Arabidopsis thaliana. Besides the three classical types of DNAJ proteins (DNAJA, DNAJB and DNAJC), we propose to classify DNAJ-like proteins into three different groups. The DNAJD type comprises proteins with a J-like domain, DNAJE contains a zinc finger domain and DNAJF displays a C-terminal domain. Interestingly, other eukaryotes also display similar DNAJ-like proteins.

Structural insights into the Hsp70-mediated, Ubiquitin Proteasome degradation pathway
Lucia Quintana-Gallardo, Jaime Martín-Benito, Jose Maria Valpuesta

National Center for Biotechnology CSIC, Madrid

The maintenance of protein homeostasis in the cell includes parallel and interconnected strategies that focus on the folding, degradation or accumulation of dangerous potential species in different compartments. Molecular chaperones are involved in these tasks, either protecting the substrates against misfolding interactions and directing them to functional native states or driving them towards degradation. This latter process is achieved thanks to the presence of the co-chaperone CHIP, which can act as E3 ligase directing the Hsp70:substrate complex towards substrate degradation by interacting with different E2 ligases. We have purified the chaperone Hsp70 and the co-chaperone CHIP, and formed a complex with the a version of the tumour suppressor protein 53. We have generated a 3D reconstruction by Cryo-EM that allow us to locate the different components of the complex and suggest a mechanism for the substrate ubiquitination.

Understanding the molecular mechanism of adaptive thermotolerance in E.coli
Manish Rai[1, 2], Rohan Dandge[1, 2], Kanika Verma[1, 2], Rajashekhar Donaka[1], Kanika Saxena[1, 4], Gopal Gunanathan Jayaraj[1, 2], Souvik Maiti[1, 2, 3, 4], Kausik Chakraborty[1, 2, 4], et al.

[1]*Chemical and systems Biology Unit, CSIR-Institute of Genomics and Integrative Biology, CSIR, Mathura Road, Delhi 110 025, India.*
[2]*Academy of Scientific and Innovative Research (AcSIR), Anusandhan Bhawan, 2 Rafi Marg, New Delhi-110001, India.*
[3]*CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, CSIR, Pune 411008, India.*
[4]*Savitribai Phule Pune University, Ganeshkhind, Pune, Maharashtra 411007*

Bacteria are able to survive under wide range of temperature but how it manages to fold its proteome in this wide range is unknown. Mutations that are selected during adaptive evolution for growth at high temperature are known in E.coli. However the mechanisms of adaptive thermotolerance have not been delineated, nor has been its link to intracellular protein folding. To this extent, we evolved E.coli at 47^{0}C for 800 generations. The growth rate and competitive fitness of evolved strains is better than the unevolved strain at 47^{0}C but interestingly without any growth disadvantage at 37^{0}C. To understand the link between thermotolerance and folding capacity of evolved E.coli, we used slow folding mutants of proteins as a folding sensor, and find that evolved strains are able to buffer mutations better. This clearly shows that evolved bacteria have better folding capacity. In this work we describe the different mechanisms that we identify as crucial regulators of proteostasis in E. coli.

Thio-modification of tRNA at the wobble position as regulator of the kinetics of decoding and translocation on the ribosome
Namit Ranjan, Marina Rodnina

Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Uridine 34 (U34) at the wobble position of the tRNA anticodon is post-transcriptionally modified, usually to mcm5s2, mcm5, or mnm5. The lack of the mcm5 or s2 modification at U34 of tRNALys, tRNAGlu, and tRNAGln causes ribosome pausing, protein misfolding and aggregation in yeast. The pauses occur during the elongation step, but the mechanism is not known. Using real-time kinetic analysis we show that tRNALys lacking the s2 group has a lower affinity of binding to the cognate codon and is more efficiently rejected during initial selection and proofreading phases. The lack of s2 modification also slows down the rearrangements in the ribosome–EF-Tu–GDP–Pi–Lys-tRNALys complex following GTP hydrolysis by EF-Tu. Finally, tRNA–mRNA translocation is slower with the s2-deficient tRNALys. These observations explain the observed ribosome pausing during translation and demonstrate how the s2 modification helps to ensure the optimal translation rates that maintain proteome homeostasis of the cell.

Silent mutations affectings CFTRs translational landscape rescue folding mutations
Robert Rauscher, Marta Guevara-Ferrer, Zoya Ignatova

University of Hamburg/Institute for biochemistry and molecular biology

Proteins biosynthesis rates can modulate their folding efficiency. Transcript sequences evolved to coordinate translational velocity and cotranslational folding. To learn how local alterations affect CFTR biogenesis and cotranslational folding, we combined ribosome profiling with biochemical assessment of disease relevant mutations combined with a silent polymorphism, T2562G, which locally decreases ribosomal speed.
We found defined regions of slow translation. Thus, the CFTR transcript does not only encode vectorial information of amino acid sequence, but also coordinates ribosomal velocity to optimally fine tune cotranslational folding. Next, when combining the sSNP T2562G with disease relevant mutations, we found increased CFTR steady state levels and altered protease susceptibility. In conclusion, CFTR folding is coordinated by regions of slow translation along the transcript and alterations in the velocity add to the effect of altered amino acid in disease.

Identification of Molecular chaperones having crucial neuronal functions; the potential therapeutic targets for neurodegenerative diseases

Sandeep Raut[1], Bhagaban Mallik[2], Chandan Sahi[1], Vimlesh Kumar[2]

[1]1. Chaperone Biology Lab, Department of Biological Sciences, Indian Institute of Science Education and Research (IISER) Bhopal, Bhauri, Bhopal, Madhya Pradesh, India.
[2]2. Laboratory of Neurogenetics, Department of Biological Sciences, Indian Institute of Science Education and Research (IISER) Bhopal, Bhauri, Bhopal, Madhya Pradesh, India.

Most of the chaperones are constitutively expressed in neurons and several are repeatedly linked to various neurodegenerative disorders (NDDs). Hence, they are considered as potential therapeutic targets. Drosophila melanogaster is a predominant model system for ongoing research on NDDs. However identification of Drosophila chaperones having neuron specific function is missing. We performed RNAi mediated reverse genetic screen to identify such potential candidates. We identified and classified all Drosophila chaperones. Ubiquitous knockdown of these proteins revealed number of essential candidates. Pan-neuronal and eye-specific knockdown of these essential chaperones along with robust behavioural assays evince the candidates having crucial neuronal functions and as potential therapeutic targets for (NDDs). Interestingly, most of these chaperones are linked to cellular protein quality control, mitochondrial function and cytoskeletal organization.

FKBP51 links to a novel pathway to autophagy by regulating Beclin1

Nils Gassen[1], Jens Stepan[1], Jakob Hartmann[2], Andreas Genewsky[2], Mathias Schmidt[2], Matthias Eder[2], Theo Rein[1]

[1]Max Planck Institute of Psychiatry, Department of Translational Research in Psychiatry, Kraepelinstr. 10, Munich 80804, Germany
[2]Max Planck Institute of Psychiatry, Department of stress neurobiology and neurogenetics, Kraepelinstr. 10, Munich 80804, Germany

FK506 binding protein 51 (FKBP51) is established as Hsp90 co-chaperone and regulator of the glucocorticoid receptor, and consequently of stress physiology. It is engaged in adaptive processes through several molecular feedback loops.
Recently we discovered an autophagy-inducing effect of FKBP51, concomitant with elevated levels of the autophagy regulator Beclin1. Here we elucidate the underlying mechanism: FKBP51 scaffolds a heterocomplex controlling the phosphorylation and thus activity of a novel Beclin1 E3 ligase. Several different assays reveal that targeting this E3 ligase by small molecules enhances Beclin1 protein stability and drives autophagy. Furthermore, these novel autophagy inducers elicit antidepressant-like effects in mice and enhance synaptic function in mice and as well as in brain slices. We are currently testing potentially beneficial effects of these compounds in neurodegenerative diseases as well as in viral infection.

Modulation of translation efficiency: a new player in dendritic cell function

Marisa Reverendo[1], Rafael Argüello[1], Evelina Gatti[1, 2], Philippe Pierre[1, 2]

[1]CNRS, INSERM, CIML, Aix Marseille University, Marseille, France
[2]Institute for Research in Biomedicine - iBiMED and Aveiro Health Sciences Program, University of Aveiro, Aveiro, Portugal

Dendritic cells are immune cells that change their gene expression pattern rapidly after activation by microbes and are key immune regulators. The control of protein synthesis during DC activation is a major determinant and tRNAs are fundamental for this process. However, stress conditions can alter their pool and modifications dynamics, which can skew protein synthesis accuracy. To understand how DCs couple activation, protein synthesis and proteome stability we are studying the mechanisms that allow DCs to coordinate their translational program upon activation. Using transcriptomic analysis, whole genome codon usage, and tRNAs expression, we observed a connection between variations in the tRNA species and DC function. Indeed, protein synthesis and tRNA levels are regulated by toll like receptors and this is vital for DC activation and subsequent T cell priming. Therefore, we believe that the regulation of protein synthesis in DCs can affect their immune functions.

Identification of new human amyloidogenic proteins

Andrey Zelinsky[1], Nina Romanova[1], Andrey Kajava[2], Alexandr Rubel[1], Pavithra Chandramowlishwaran[3], Yury Chernoff[1, 3]
[1]Laboratory of Amyloid biology, St. Petersburg State University, St. Petersburg, Russia
[2]Centre de Recherche en Biologie cellulaire de Montpellier, Montpellier, France
[3]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

Amyloids are self-assembled fibrous cross-beta protein aggregates, associated with a variety of human diseases and implicated in some positive biological functions. We have developed a new yeast-based experimental assay for identification of amyloidogenic proteins, and applied this assay to human proteins, that contain cross-beta structures as predicted by in silico algorithm ArchCandy. Our data confirm that at least some of these proteins indeed form amyloids. Specifically, some isoforms of the PHC3 protein, a member of chromatin-modifying complex PRC1, possess amyloidogenic properties. We propose that amyloid formation modulates the function of PRC1 in epigenetic regulation of chromatin. Our data provide a new strategy for identifying biologically important amyloids in human proteome.

Authors acknowledge support from RSF (14-50-00069) and from SPbSU Resource Centers “Development of Molecular and Cellular Technologies” and “Biobank”.

A small molecular compound modulating Heat Shock Factor activity

Sally Svartsjö, Jenny Öster, Viktor Bäck, Pia Roos-Mattjus

Faculty of Science and Engineering - Biochemistry, Åbo Akademi University, Åbo, Finland

Cellular stress responses enable cells to sense and respond to changes in the cellular environment. The heat shock response to various proteotoxic stresses is regulated by a family of heat shock transcription factors (HSF1-4), with HSF1 being the master regulator. HSFs are also implicated in development, aging and in many pathophysiological conditions including cancer and neurodegenerative diseases. Therefore, there is significant interest in the discovery and development of small molecules that modulate the activity of HSFs. We have studied a natural product used in Chinese medicine. This compound induces a stress response in a HSF1-dependent manner. Pretreatment of the compound with excess free thiols inhibits the response, suggesting that the compound is thiol-responsive. We will present our work in progress at the meeting.

An evolutionarily conserved pathway controls proteasome homeostasis
Adrien Rousseau, Anne Bertolotti

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, CB2 0QH, UK

The proteasome is essential for the selective degradation of most cellular proteins, but how cells maintain adequate amounts of proteasome is unclear. We recently identified an evolutionarily conserved signaling pathway controlling proteasome homeostasis. Central to this pathway is TORC1, the inhibition of which induced all known yeast 19S regulatory particle assembly-chaperones (RACs), as well as proteasome subunits. Downstream of TORC1 inhibition, the yeast mitogen-activated protein kinase, Mpk1, acts to increase the supply of RACs and proteasome subunits under challenging conditions in order to maintain proteasomal degradation and cell viability. This adaptive pathway was evolutionarily conserved, with mTOR and ERK5 controlling the levels of the four mammalian RACs and proteasome abundance. Thus, the central growth and stress controllers, TORC1 and Mpk1/ERK5, endow cells with a rapid and vital adaptive response to adjust proteasome abundance in response to the rising needs of cells.

Structural insights into the ATPase cycle of the 26S proteasome
Marc Wehmer[1], Till Rudack[2, 4], Florian Beck[1], Antje Aufderheide[1], Friedrich Förster[1, 3], Klaus Schulten[4], Wolfgang Baumeister[1], Eri Sakata[1]

[1]*Max Planck Institute of Biochemistry, Germany*
[2]*Ruhr University Bochum, Germany*
[3]*Utrecht University, Netherlands*
[4]*University of Illinois, Urbana–Champaign, USA*

The 26S proteasome mediates selective protein degradation in eukaryotic cells to control protein turnover. To understand how the proteasome functions, a detailed understanding of the conformational states that the proteasome adopts during the ATP hydrolysis cycle is required. Applying single-particle cryo electron microscopy (cryo-EM) and image classification to samples, we showed the high-resolution EM maps of four distinct conformational states identified in the presence of different nucleotides and nucleotide analogs. The resolution of the four conformers allowed for the construction of atomic models of the AAA+ ATPase module as it progresses through the functional cycle. Strikingly, in an unobserved conformational state (s4), the gate controlling access to the 20S CP is open. Our structures suggest that gate opening of 20S CP is coupled with the ATP hydrolysis cycle.

Characterising the molecular chaperone systems involved in the defective trafficking of KCNQ1 channel complexes in the Long QT Syndrome
Grace Salsbury, Andrew Tinker, J. Paul Chapple, Stephen C. Harmer

William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, U.K.

KCNQ1 and KCNH2 form the alpha subunits of two cardiac potassium ion channels. Mutations in either channel underlie the Long QT Syndrome (LQTS) which can lead to sudden cardiac death. Mutations in KCNQ1 or KCNH2 can result in defective trafficking and prevent these channels from reaching the plasma membrane (PM). Previous work has identified that the molecular chaperones Hsp70 and Hsp90 assist wild-type and mutant KCNH2 channel trafficking but whether they play a role in KCNQ1 channel trafficking has not been determined. Interestingly, the inhibition of Hsp90 (using geldanamycin) or the overexpression of an Hsp70 dominant negative mutant did not significantly affect the PM expression of wild-type or mutant KCNQ1 (KCNQ1-G325R). In contrast these interventions did act to significantly reduce the PM expression of KCNH2. In summary, these results highlight that KCNQ1 utilises different chaperone systems from KCNH2 to fold and traffic to the cell surface.

An ER-localized J protein directs BiP to mammalian IRE1α to repress the Unfolded Protein Response
Niko Amin-Wetzel, Reuben Saunders, Maarten Kamphius, Claudia Rato, Steffen Preissler, David Ron

University of Cambridge Institute for Medical Research

A BiP-IRE1 complex correlates with UPR repression but the mechanisms of its formation are unknown. Like other Hsp70 chaperones, BiP binding to substrates is catalysed by J-domain co-chaperones that stimulate ATP hydrolysis and guide the chaperone to its targets. Here we report the discovery of an ER-localised J-protein that associates with the human IRE1α luminal domain and promotes formation of a complex between BiP and IRE1α. Complex formation observed in vitro with purified components requires a functional J-domain and ATP hydrolysis by BiP. Inactivation of the gene encoding this ER-localised J-domain protein selectively de-represses the IRE1α branch of the UPR. Reintroduction of the J-protein into the mutant cells attenuates IRE1α signalling and enhances the recovery of a BiP-IRE1α complex. These findings suggest a role for an ER localised J-protein in repressing the IRE1α branch of the UPR by accelerating the formation of a repressive BiP-IRE1α complex.

Molecular chaperones at chromatin regulate stress-response and buffer genetic variation
Ritwick Sawarkar, Barbara Hummel, Erik Hansen, Fernando Aprile-Garcia, Parul Tomar

Max Planck Institute of Immunobiology and Epigenetics

Understanding how genotypes are linked to phenotypes is important in biomedical and evolutionary studies. The chaperone heat-shock protein 90 (HSP90) buffers genetic variation by stabilizing proteins with variant sequences, thereby uncoupling phenotypes from genotypes. Here we report an unexpected role of HSP90 in buffering cis-regulatory variation affecting gene expression in different mouse strains. By employing the KAP1-mediated epigenetic pathway, HSP90 represses the regulatory influence of endogenous retroviruses (ERVs) on neighboring genes in the mouse genome. In addition to buffering genetic variation, chromatin-associated chaperones also control the response of mammalian cells to environmental stress. Our data link protein-misfolding to recruitment of RNA pol II pausing proteins to chromatin dependent on p38-kinase activity. Thus our findings add a new regulatory layer through which HSP90 uncouples phenotypic outcomes from individual genotypes.

The benefit of eIF2α phosphorylation, a central hub in stress responses
Kim Schneider, Geoff Nelson, Laura Luh, Anne Bertolotti

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, CB2 0QH, United Kingdom

Phosphorylation of the eukaryotic translation initiation factor eIF2α to reduce global protein synthesis is a first line of defense, essential for cells to survive multiple forms of stress. However, despite the utmost importance of the eIF2α phospho-signaling pathway, the global consequences of the eIF2α-dependent translational attenuation are still poorly characterized. Here, using high throughput sequencing of total and polysomal RNAs, we provide an unbiased and comprehensive analysis of the stress-induced translational reprogramming in vivo. We also provide a global analysis of the protective mechanisms underlying a prolonged eIF2α phosphorylation using Sephin1, a selective inhibitor of the stress-induced eIF2α phosphatase. This study provides the molecular rationale explaining why eIF2α phosphorylation has evolved as a powerful defense system against many forms of stresses.

The combination of Hsc70, type II J-protein and Hsp110 is effective in prevention of Htt aggregation and disaggregation
Annika Scior, Janine Kirstein

FMP Berlin, Robert-Rössle-Str. 10, 13125 Berlin

Protein aggregation represents a hallmark of neurodegenerative diseases. To circumvent the deleterious effects of protein aggregation a sophisticated chaperone network copes with misfolded or aggregated proteins. We utilized various C. elegans chaperones to study their interaction with aggregation-prone proteins. We provide biochemical assays that analyze the effectivity of different Hsp70/J-protein combinations to prevent Huntingtin aggregation and also provide evidence for disaggregation of existing fibrils. We could show that the constitutively expressed Hsp70 in combination with a type II J-protein was most effective in prevention of aggregation as well as disaggregation. Also the different inducible Hsp70 could suppress aggregation, however they where less effective compared to the constitutively expressed Hsp70. These data provide insight into how inducible as well as constitutive chaperones cooperate or might substitute for the loss of each other to combat protein aggregation.

RAC/Ssb - a unique chaperone triad on the ribosome
Irmgard Sinning

Heidelberg University Biochemistry Center, INF 328, D-69120 Heidelberg, Germany

During protein synthesis at the ribosome numerous factors act early on the nascent polypeptide chain. These can be grouped into three major classes – chaperones that assist in folding, enzymes that modify the nascent chain and targeting factors that assist in protein localization. As all of them need access to the nascent polypeptide chain, they utilize partially overlapping binding sites at the ribosomal tunnel exit, but their interplay is poorly understood. Our data provide the structural framework for interactions of co-translational factors at the ribosomal tunnel exit. In yeast, the canonical Hsp70 protein Ssb acts together with the ribosome associated complex (RAC), which consists of the inactive Hsp70 protein Ssz and the Hsp40 protein Zuotin. Together, they form a unique chaperone triad at the ribosome. Structure determination of Ssb and RAC together with ribosome binding studies provide detailed insights into the interplay of this chaperone system, which evolved to link translation and protein folding.

Transcriptional memory and re-programming of gene expression in cell stress
Lea Sistonen

Faculty of Science and Engineering, Turku Centre for Biotechnology, Åbo Akademi University

Cellular stress responses are fundamental for cells and organisms to survive and adapt to hostile conditions. As a manifestation of the critical role for stress responses, cancer cells enhance their survival and metastatic properties by activating heat shock factor 1 (HSF1), the master inducer of transcription upon proteotoxicity. The mechanisms by which stress influences long-term transcriptional processes and whether cells encode a memory to the harmful conditions, have remained unclear. Here, we examined how human K562 erythroleukemia cells adjust to repeated exposures to proteotoxic stimuli, by profiling nascent transcription, chromatin environment, mRNA and protein expression prior to, during and after the exposures. The dynamic regulation of transcriptionally engaged RNA polymerase and the simultaneous re-programming of heat-responsive gene promoters, revealed that the transcriptional memory to stress is epigenetically determined and inherited through mitosis.

A reporter assay to monitor protein aggregation in vivo in mammalian cells
Marisa Pereira[1, 2], Diogo Tomé[1, 2], Ana Sofia Domingues[1], Ana Sofia Varanda[1], Manuel Santos[1], Ana Soares[1, 3]

[1]1 Institute of Biomedicine-IBIMED, Department of Medical Sciences, University of Aveiro, Aveiro, Portugal
[2]These authors contributed equality to this work
[3]This research was supported by the Portuguese Foundation for Science &Technology through the POCH program and COMPETE2020 by the grants SFRH/BPD/77528/2011, PTDC/BIM-MEC/1719/2014 and UID/BIM/04501/2013.

Protein conformational diseases (PCD) are characterized by accumulation of aggregated proteins. However, the genes and pathways involved in loss of protein conformation are not fully known. Hspb1, an oligomeric small heat shock protein that binds and keeps unfolded proteins in a folding competent state, can be used to monitor protein aggregation in vivo by following the cellular re-localization of Hspb1-GFP fluorescence to foci. We constructed and validated a HeLa stable cell line expressing the HSPB1-GFP reporter protein. This cell line was exposed to different stressors: tunicamycin, arsenite and MG132. Proteasome inhibition by MG132 led to re-localization of HSPB1-GFP fluorescence to foci, confirming that our reporter system is functional. This reporter is now being used to set up high throughput screens to identify genes and pathways involved in protein aggregation. We hope to identify novel therapeutic targets that can be used for the development of PCD therapies.

Inorganic Polyphosphate (Polyp) as a Mitochondrial Chaperone in Aging
Maria de la Encarnación Solesio Torregrosa, Evgeny V Pavlov

NYU-Department of Basic Science

Mitochondria, where ROS are physically generated, are most likely to show dysregulated protein aggregation, as ROS are main contributors to this process. Classical chaperones/proteases are the main defense mechanisms against increased protein aggregation, but they are insufficient to explain explain the robust mitochondrial mechanism against increased protein aggregation. PolyP is a well-conserved and ubiquous polymer, composed of multiple subunits of phosphate, showing a preferential mitochondrial location. It has been proposed as a chaperone in different organisms. We tested the hypothesis that polyP is a mitochondrial chaperone. Our data show that cells lacking polyP have increased levels of mitochondrial misfolded proteins. The depletion of polyP increased cell death in response to ROS and heat shock. Intracellular distribution of α-synuclein was affected by the absence of polyP. Electron microscopy images showed clear differences in the mitochondrial structure in absence of polyP.

The structural basis for polypeptide translocation by the Hsp104 disaggregase.
Daniel Southworth[1], Stephanie Gates[1], Adam Yokom[1], JiaBei Lin[2], Meredith Jackrel[2], James Shorter[2]

[1]*Department of Biological Chemistry*
Life Sciences Institute
University of Michigan, Ann Arbor, MI 48109 U.S.A.
[2]*Department of Biochemistry and Biophysics*
Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, U.S.A

Hsp100 polypeptide translocases are hexameric AAA+ machines that maintain the proteome by unfolding aberrant and toxic proteins for refolding or proteolytic degradation. The mechanisms of substrate interaction and ATP hydrolysis-driven translocation remain major questions. The Hsp104 disaggregase solubilizes amorphous aggregates and structured amyloids. We have determined cryo-EM structures of Hsp104 bound to AMPPNP and ADP to subnanometer resolution that reveal a remarkable open spiral architecture of the hexamer. Nucleotide-specific conformations of an essential middle domain are identified that explain its allosteric control mechanism. Recently, we have characterized a novel substrate-bound structure of Hsp104 in a closed conformation that identifies the structural basis for substrate recognition. Together, this work reveals a model for processive substrate translocation by AAA+ enzymes and identifies a remarkable structural plasticity of Hsp104-catalyzed disaggregation.

Mitochondrial membrane protein misfolding and aggregation in S. cerevisiae
Karen Stroobants[1], Piotr Chroscicki[2], Rishika Kundra[1], Agnieszka Chacinska[2], Christopher Dobson[1], Michele Vendruscolo[1]

[1]*University of Cambridge*
[2]*International Institute of Molecular and Cell Biology in Warsaw*

The amyloid hypothesis is one of the most influential theories of Alzheimer's disease, and is based on the observation that proteins aggregate into amyloid fibrils as a hallmark of the disease. In this context, we have demonstrated that membrane proteins can exhibit aggregation behaviour similar to that typically seen for cytosolic proteins. In parallel, by carrying out transcriptome-wide studies we have identified a set of proteins that are metastable against aggregation and downregulated in Alzheimer's disease. Many of these proteins are closely involved in the oxidative phosphorylation (OP) pathway, and mitochondrial in nature. To investigate the aggregation behaviour and the role in neurodegenerative disorders of these proteins, we exploit the S. cerevisiae model system. We use strains with defective mitochondrial import and overexpressing specific OP proteins to investigate the structural properties of their aggregates, and to determine the cellular responses to their aggregation.

Hyperaccurate mitochondrial translation increases cellular proteostatic capacity
Tamara Suhm, Martin Ott, et al.

Department of Biochemistry and Biophysics, Stockholm University, Sweden

Mitochondria are the power plant of the cell as they provide most of its ATP. Because the respiratory chain complexes are composed of proteins encoded by the nuclear as well as the mitochondrial genome, mitochondrial translation plays an important role in maintaining normal cellular physiology. To establish how changes in mitochondrial translation accuracy impact cell physiology, we generated and analyzed mutants with altered translation accuracy. We thereby reveal a novel mitochondria-to-nucleus signaling pathway that profoundly reprograms cellular stress signaling. Hyperaccurate mitochondrial translation activates this retrograde signaling pathway, thereby increasing cytosolic proteostatic capacity. This protects against oxidative stress and increases the cell´s probability to escape from senescence. Contrary, decreased translation accuracy provokes hypersensitivity to stress. Our results highlight that cellular stress response directly depends on mitochondrial translation accuracy.

Increase in protein abundance causes ribonucleoprotein granule formation
Nieves de Lorenzo[1, 2], Benedetta Bolognesi[1, 2], Gian Gaetano Tartaglia[1, 2, 3]

[1]*Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr Aiguader 88, 08003 Barcelona, Spain*
[2]*Universitat Pompeu Fabra (UPF), 08003 Barcelona, Spain*
[3]*Institució Catalana de Recerca i Estudis Avançats (ICREA), 23 Passeig Llu?s Companys, 08010 Barcelona, Spain*

Mutations in RNA binding proteins (RBPs) such as Tar DNA binding protein-43 TDP-43, Fused in sarcoma FUS, survival of motor neuron SMN1, Ataxin-2 ATX2, Optineurin OPT and Angiogenenin ANG promote formation of granules and cause motor neuron diseases. We found that structural disorder, nucleic acid binding propensity and amino acid patterns such as arginine-glycine and phenylalanine-glycine are key features of granule-forming proteins. We developed a computational approach, catGRANULE, to identify RBPs assembling into granules and we validated our predictions using advanced microscopy and Fluorescence Recovery After Photo-bleaching FRAP. One important result of our studies is that over-expression of granule-forming RBPs results in formation of foci that are toxic to the cell. Indeed, the RNAs trapped in the

granules cannot be translated, which decreases cell viability. Moreover, RBPs localized to foci are not available for other interactions, causing impairment of homeostasis.

Neurotoxic mutants of the prion protein are subjected to distinct intracellular quality control pathways

Monique Puschkarow[1], Sebastian Jung[1], Konstanze F. Winklhofer[2] and Jörg Tatzelt[1]

[1]*Department Biochemistry of Neurodegenerative Disease, Institute of Biochemistry and Pathobiochemistry, Ruhr University Bochum, Germany*
[2]*Department Molecular Cell Biology, Institute of Biochemistry and Pathobochemistry, Ruhr University Bochum, Germany*

Prion diseases in humans and animals are characterized by progressive neurodegeneration and the formation of infectious particles. Both features are linked to aberrant conformers of the prion protein (PrP), a GPI-anchored neuronal glycoprotein. To specifically investigate signaling pathways that are activated by neurotoxic PrP conformers in the absence of infectious prion replication we have established novel cell culture models for PrP mutants linked to inherited prion disease in humans. We have chosen three different PrP mutants that are characterized by C-terminal deletion of increasing size. The largest mutant forms both infectious and neurotoxic conformers, while the two shorter ones are not converted into infectious prions. Interestingly, we could show that the three mutants were subjected to different quality control pathways in the cytosol and secretory pathway that interfered with trafficking and maturation of PrP at various steps. As a consequence, the neurotoxic mutants accumulated in different intracellular compartments. We are now focusing on the compartment-specific signaling pathways activated by the pathogenic conformers.

Identification of Selective Vulnerabilities in the Prostate Cancer Proteostasis Network

Isabelle Taylor[1], Hao Shao[1], Michael Moses[2], Len Neckers[2], Jane Trepel[3], Xavier Salvatella[4], Martin Kampmann[1], Jason Gestwicki[1]

[1]*Institute for Neurodegenerative Disease, University of California at San Francisco, San Francisco, USA*
[2]*Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA*
[3]*Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA*
[4]*Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Spain*

Castrate-resistant prostate cancer (CRPC) is the 2nd leading cause of cancer death among men. It has recently been appreciated that these cells remain highly dependent on the activity of androgen receptor (AR). The goal of this project is to uncover how the proteostasis network is re-wired in CRPC to allow for the amplification and truncation of AR that drives these cancers. Using a custom shRNA library targeting ~140 genes in the proteostasis network ("the proteostasis library"), we have identified specific chaperones essential for the survival of prostate cancer cells. Moreover, we have used inhibitors of specific chaperones, including Hsp70 and Hsp90, in combination with the shRNA collection, to confirm known interactions and identify unexpected vulnerabilities. This work suggests new drug targets for the treatment of CRPC, while also providing a possible template for studying the roles of the proteostasis network in other systems.

How does UPR signaling influence ageing?

Ming Sheng, Rebecca Taylor

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, UK

Activation of cellular stress responses can extend longevity in model organisms. One of these stress responses, the endoplasmic reticulum unfolded protein response (UPR), can increase lifespan when activated specifically within the nervous system of C. elegans, through an inter-tissue signaling pathway that communicates UPR activation between neurons and the intestine. We aim to determine the components of this signaling pathway, as well as the downstream mechanisms that mediate the effects of neuronal UPR activation on ageing. To this end, we have carried out genetic screens in order to determine genes acting in neurons to control the release of signals that activate the UPR in other tissues. We have also examined the metabolic changes that occur downstream of inter-tissue UPR signaling, and, surprisingly, have identified changes in lipid and carbohydrate metabolism that may underlie the effects of cell non-autonomous UPR activation on lifespan.

Two-component sHsps bacterial system cooperation in counteracting irreversible aggregation and Hsp70/Hsp100-dependent efficient protein refolding

Igor Obuchowski, Artur Piróg, Szymon Żwirowski, Bartłomiej Tomiczek, Krzysztof Liberek

Intercollegiate Faculty of Biotechnology, University of Gda?sk, Gda?sk, Poland

Small heat-shock proteins (sHsps) are an evolutionary conserved class of ATP independent chaperones that protect cells against proteotoxic stress. sHsps form assemblies with aggregation-prone misfolded proteins, which facilitates subsequent substrate solubilization and refolding by ATP dependent Hsp70 and Hsp100 chaperones. Upon heat shock, sHsps rapidly bind misfolded proteins, preventing their irreversible aggregation. This interaction has to be broken for efficient Hsp70/Hsp100-mediated refolding. Bacteria usually harbor one or two sHsp-coding genes, whose products, when two variants are present, interact with each other. However, their activities and mechanism of action was investigated mostly separately. Here we analyze functional cooperation within a two-component sHsp bacterial system (E. coli IbpA and IbpB) in comparison to a single sHsp (E. amylovora HspB) in preventing protein irreversible aggregation and providing efficient Hsp70/Hsp100-mediated misfolded protein recovery.

Probing molecular mechanisms of J-protein-Hsp70 interaction with chaperones dedicated for iron-sulfur cluster biogenesis.

Bartlomiej Tomiczek[1], Wojciech Delewski[1], Brenda Schilke[2], Rafal Dutkiewicz[1], Szymon Ciesielski[2], Lukasz Nierzwicki[3], Igor Grochowina[1], Milena Stolarska[1], Jacek Czub[3], Elizabeth Craig[2], Jaroslaw Marszalek[1]

[1]*Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland*
[2]*Department of Biochemistry, University of Wisconsin-Madison*
[3]*Department of Physical Chemistry, Gdansk University of Technology, Gdansk, Poland*

Hsp70 chaperones are critical for protein biogenesis and homeostasis in all cellular compartments. Obligate J-protein co-chaperones drive the specificity of Hsp70 function by controlling its ATPase activity and thus its interaction with substrate. But, much remains to be learned about

their mechanisms of action, mostly because typical multifunctional Hsp70s interact with several J-proteins. Yet, in the specialized system for the biogenesis of iron-sulfur (FeS) cluster in S. cerevisiae a dedicated J-protein (Jac1) and dedicated Hsp70 (Ssq1) interact with a single substrate, the protein scaffold on which clusters are built. Hsp70 interaction with the cluster bound scaffold is critical for cluster transfer to recipient proteins. This system enables us to combine high-resolution mechanistic studies with in vivo verification of their results. By combining computational, and experimental approaches we identified critical residues for interaction between the J-domain of Jac1 and Ssq1.

CHIP as a Sensor of Proteostasis Stress

Yannick Kopp[1, 2], Tobias B. Schuster[1, 2], Wei-Han Lang[1, 2], Adrian Martinez-Limon[1, 2], Harald F. Hofbauer[1, 3], Robert Ernst[1, 2, 3, 4], Giulia Calloni[1, 2], R. Martin Vabulas[1, 2]

[1]*Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany*
[2]*Institute of Biophysical Chemistry, Goethe University Frankfurt, Frankfurt am Main, Germany*
[3]*Institute of Biochemistry, Goethe University Frankfurt, Frankfurt am Main, Germany*
[4]*Institute of Biochemistry, Medical Faculty, University of Saarland, Homburg, Germany*

The mechanisms underlying the early adaptation of cellular compartments to cytosolic protein aggregation are not clear. We show that the HSP70- and HSP90-interacting ubiquitin ligase CHIP, when freed from chaperones during acute stress, can dock on cellular membranes thus performing a proteostasis sensor function. We reconstituted this process in vitro and found that mainly phosphatidic acid and phosphatidylinositol-4-phosphate enhance association of chaperone-free CHIP with liposomes. HSP70 and membranes compete for mutually exclusive binding to the TPR domain of CHIP. At new cellular locations, access to compartment-specific substrates enables CHIP to participate in the reorganization of the respective organelles, as exemplified by the fragmentation of the Golgi apparatus (effector function). We propose that the unmasking of chaperone-associated determinants of cellular localization may be a general mechanism to rapidly adjust cellular architecture to proteostasis stress.

Mapping the mechanisms of retinal degeneration caused by mutations in the co-chaperone AIPL1

Jacqueline van der Spuy, Almudena Sacristan-Reviriego

Institute of Ophthalmology, University College London

Mutations in the photoreceptor/pineal-expressed gene AIPL1 cause Leber congenital amaurosis (LCA), the most severe form of childhood inherited retinopathy. AIPL1 is a photoreceptor-specific co-chaperone that interacts with HSP90 via a C-terminal tetratricopeptide repeat (TPR) domain to facilitate the correct assembly and activity of retinal cGMP phosphodiesterase (PDE6). The AIPL1 N-terminal FKBP-like domain interacts directly with the isoprenyl moiety of the PDE6 catalytic subunits. We investigated the functional impact of novel LCA-associated AIPL1 variants. Our data reveal that the relative domain organization and integrity of AIPL1 is important for PDE6-mediated catalysis, with variants mapping to one domain also affecting the activity of the other independently folded domain. The functional assessment and confirmation of likely pathogenic AIPL1 variants is moreover important for the accurate diagnosis and effective triage of patients for AIPL1-targeted gene replacement therapy.

The transcription factor PQM-1 is a novel regulator of proteostasis and mediator of transcellular chaperone signalling in C. elegans.

Daniel O'Brien, Rebecca Aston, Vijay Shanmugiah, David Westhead, Patricija van Oosten-Hawle

Faculty of Biological Sciences, School of Molecular and Cell Biology and Astbury Centre for Structural Molecular Biology, University of Leeds

The significance of cell protective stress response mechanisms is now widely appreciated to not just act at the level of single cells, but at the "multicellular" level. Evolutionary conserved stress responses initiate "transcellular chaperone signalling" (TCS) that allows protective chaperone expression to be signalled from one tissue to another. How TCS functions at the molecular level however remains an open question to date. Using a systems-wide approach and further genetic analysis, we have identified the GATA transcription factor PQM-1 as a mediator of TCS in C. elegans. We demonstrate that PQM-1 is required for proteostasis maintenance and show how depletion of pqm-1 suppresses induction of TCS-mediated hsp90 expression in several target tissues. Transcriptional activity of PQM-1 in the C. elegans intestine is increased during TCS-activating conditions, suggesting the requirement of the intestine as a key organ to transduce TCS across different tissues.

Human cells cope with tRNA misexpression by activating protein quality control mechanisms

Ana Sofia Varanda[1, 2, 4, 5], Mafalda Santos[1, 2], Ana Raquel Soares[1, 5], Carla Oliveira[2, 3], Manuel A.S. Santos[1]

[1]*iBiMED&Health Sciences, University of Aveiro, 3810-193 Aveiro, Portugal (UID/BIM/04501/2013)*
[2]*Institute of Molecular Pathology and Immunology, University of Porto (IPATIMUP), 4200-465 Porto, Portugal*
[3]*Faculty of Medicine, University of Porto, 4200-465 Porto, Portugal*
[4]*SFRH/BD/76417/2011*
[5]*PTDC/BIM-MEC/1719/2014*

Alterations in protein synthesis components, namely tRNAs, increase the level of protein synthesis errors (PSE) and are related with diseases, from cancer to neurodegeneration. Still, the cause-effect mechanisms remain to be elucidated. To understand how human cells cope with PSE, we modified the anticodon of a human tRNASer, to incorporate serine at various non-cognate sites. Stable HEK293 cell lines expressing these tRNAs were analyzed at different cell passages. tRNAs misexpression led to accumulation of misfolded proteins, but activation of ubiquitin-proteasome system and the unfolded protein response (UPR) protected these cells from proteotoxic stress, maintaining viability in all passages. In some cases, adaptation was mainly due to increased protein turnover, while in other cases, UPR activation with consequent protein synthesis inhibition was the main adaptation mechanism. Our data provide new insights on how mammalian cells cope and adapt to proteotoxic stress induced by PSE.

ER Stress in a Premature Aging Disease

Sandra Vidak, Tom Misteli

National Cancer Institute, NIH, Bethesda, MD 20892, United States

The rare premature-aging disease Hutchinson-Gilford Progeria Syndrome (HGPS) is caused by constitutive production of progerin, a mutant form of the nuclear architectural protein lamin A. Cells from HGPS patients exhibit numerous cellular defects, but the exact mechanisms are poorly understood. We hypothesize that extensive nuclear aggregation of progerin leads to the accumulation of misfolded proteins, affecting proteostasis in the rest of the cell/nucleus. Our preliminary observations using high-throughput imaging suggest accumulation of Hsp110 chaperone in the ER of progerin-expressing cells, accompanied by a significant increase in ER chaperones (GRP94,GRP78,calnexin), transcriptional activation of several UPR genes and increased IRE1α phosphorylation. Since prolonged UPR activation can promote apoptosis of vascular smooth muscle cells, a phenotype readily observed in HGPS, our results allow for a possible contribution of ER stress to the HGPS pathology.

Investigation on structural features of Hsp60, amateur chaperone in amyloid-beta fibrillogenesis

Silvia Vilasi[1], Maria Rosalia Mangione[1], Claudia Marino[1, 2], Rita Carrotta[1], Fabio Librizzi[1], Claudio Canale[4], Maria Grazia Ortore[5], Caterina Ricci[5], Francesco Cappello[3], Donatella Bulone[1], Pier Luigi San Biagio[1]

[1]*Institute of Biophysics, National Research Council, Palermo, Italy*
[2]*Department of Neurology, University of Texas Medical Branch, Galveston, (TX) USA*
[3]*Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, Italy*
[4]*Nanophysics Department, Istituto Italiano di Tecnologia*
[5]*Università Politecnica delle Marche*

There are several mechanisms by which chaperones exert their protective action in assisting protein folding, targeting, transport, as well as, in fighting the consequences of protein misfolding and aggregation. However, in many cases, the scarcity of structural data has impeded an understanding of the recognition and antiaggregation mechanisms, that result crucially important when considering how to screen for and characterize potential inhibitors of amyloidosis. Here we show how a human chaperonin, Hsp60, can specifically influence fibrillogenesis of Aβ40 peptide involved in Alzheimer Disease (doi:10.1016/j.bbagen.2016.07.019). Inhibition studies are correlated with structural, self organization and stability properties of the chaperonin under study (doi:10.1016/j.bpc.2015.07.006; doi:10.1371/journal.pone.0097657), analyzed by a battery of biophysical methods and aimed to understand what are molecular determinants that are responsible for this inhibitory action.

Somatic increase of CCT8 mimics proteostasis of human pluripotent stem cells and extends C. elegans lifespan

David Vilchez[1], Alireza Noormohammadi[1], Amirabas Khodakarami[1], Ricardo Gutiérrez-Garcia[1], Hyun Ju Lee[1], Seda Koyuncu[1], Tim König[1], Christina Schindler[1], Isabel Saez[1], Azra Fatima[1], Christoph Dieterich2[2]

[1]*CECAD-University of Cologne*
[2]*Klaus Tschira Institute for Computational Cardiology*

Human pluripotent stem cells (hESCs) can replicate indefinitely while maintaining their undifferentiated state and, therefore, are immortal in culture. This capacity may demand avoidance of any imbalance in proteostasis that would otherwise compromise hESC identity. Here we show that hESCs exhibit enhanced assembly of the TRiC/CCT complex, a chaperonin that facilitates the folding of 10% of the proteome. We find that ectopic expression of a single subunit (CCT8) is sufficient to increase TRiC/CCT assembly. Moreover, increased TRiC/CCT complex is required to avoid aggregation of mutant Huntingtin protein. We further show that increased expression of CCT8 in somatic tissues extends C. elegans lifespan in a TRiC/CCT-dependent manner. Moreover, CCT8 ameliorates the age-associated demise of proteostasis and corrects proteostatic deficiencies in models of Huntington's disease. Our results suggest proteostasis is a common principle that links organismal longevity with hESC immortality.

Oligomerization contributes to HSPB5 R120G and D109H aggregation and dysfunction

Jan Vonk[1], Maarten Hoppenbrouwers[1], Bianca Brundel[2], Harm Kampinga[1]

[1]*Department of Cell Biology, University Medical Center Groningen, Groningen, The Netherlands*
[2]*Department of Physiology, Institute for Cardiovascular Research, VU University Medical Center, Amsterdam, The Netherlands*

The HSPB5 R120G and D109H mutations lead to HSPB5 aggregation and dilated cardiomyopathy (DCM). The mechanism underlying this is not known. We hypothesized that rather than being intrinsically misfolded and aggregation-prone, the oligomerization dynamics of HSPB5 R120G and D109H may contribute to their dysfunction and lead to aggregation.
When expressing the disease-related HSPB5 R120G and D109H mutants in mouse HL-1 cardiomyocytes, we found aggregation which was associated with contractile dysfunction. One of the interactions within HSPB5 oligomers is between the C-terminal IPI domain and a hydrophobic groove of the α-crystallin domain. Disruption of this interaction in the HSPB5 R120G and D109H background partially prevented the aggregation and partially reversed contractile dysfunction.
The data show that HSPB5 R120G and D109H are not misfolded proteins per se, however that their altered oligomerization dynamics contribute to their aggregation and dysfunction.

The L-isoaspartate modification in a cytotoxic amylin peptide delays fibrillization and alters fibril structure

Rebeccah Warmack, Steven Clarke

Department of Chemistry and Biochemistry
University of California, Los Angeles

Little is known about the modifications that may drive proteins into toxic aggregates. L-isoaspartate (L-isoD) formation is a damaging age-related modification whose role in aggregation remains unclear. To investigate the role of L-isoD in aggregation, an L-isoD-containing fibril-forming segment of the human islet amyloid polypeptide (hIAPP) was generated. Aggregation assays of the modified peptide showed delayed

aggregate formation compared to native. Fibril formation was confirmed by electron microscopy. X-ray diffraction patterns for both fibrils displayed reflections at 4.6 Å, indicating the presence of stacked β-sheets as shown previously. Variable reflections at 8.7 Å and 9.0 Å might correlate to increased distances between the stacked β-sheets within the fibril. These and other shifts in diffraction were integrated into a model of the L-isoD fibril. The results suggest that slight changes in the fibril structure may be sufficient to hinder the rate of fibril formation.

Molecular basis for chaperoning of inner mitochondrial membrane proteins across the intermembrane space

Katharina Weinhäupl[1], Audrey Hessel[1], Tobias Jores[2], Doron Rapaport[2], Martha Brennich[3], Paul Schanda[1]

[1]*Institut de Biologie Structurale, CEA-CNRS-Université Grenoble Alpes, 38044 Grenoble, France*
[2]*Interfaculty Institute of Biochemistry, Hoppe-Seyler-Str. 4, University of Tübingen, 72076 Tübingen, Germany*
[3]*EMBL Grenoble, 71 avenue des Martyrs, 38042 Grenoble, France*

TIM9/10 is the main chaperone in the mitochondrial intermembrane space and responsible for the import of nuclear encoded membrane proteins. The primary substrate of TIM9/10 are mitochondrial carriers, a large family of proteins crucial for metabolite transport in mitochondria.
Although the X-ray structure of TIM9/10 has been solved more than 10 years ago, structural data of substrate bound TIM9/10 is still lacking. A reason for this might be the dynamics such systems often exhibit. While dynamics pose a serious obstacle for most structural biology techniques NMR is ideally suited to tackle this problem.
By combining NMR, to get atomic resolution insight on the binding site, with small angle X-ray scattering, to obtain an overall view of the ensemble, we report here the first structural model of TIM9/10 bound to a mitochondrial carrier. We found that substrates are bound on the outside of TIM9/10 and that the stoichiometry of the complex is dependent on the length of the client protein.

Linear ubiquitination reduces protein aggregate toxicity

Verian Bader[1], Eva van Well[1], Jens Meschede[1], Maria Patra[2], Cathrin Schnack[1], Petra Goldmann[1], Dominik Sehr[1], Andreas C. Woerner[3], Elisabeth Petrasch-Parwez[4], Alina Blusch[5], Gisa Ellrichmann[5], Ralf Gold[5], Thomas Arzberger[6], Mark S. Hipp[3], F. Ulrich Hartl[3], Jörg Tatzelt[7], and Konstanze F. Winklhofer[1,2]*

[1]*Department of Molecular Cell Biology, Institute of Biochemistry and Pathobiochemistry, Ruhr University Bochum, Germany,*
[2]*Neurobiochemistry, Adolf Butenandt Institute, Ludwig-Maximilians-University Munich, Germany.*
[3]*Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Martinsried, Germany,* [4]*Neuroanatomy, Ruhr University Bochum, Germany,*
[5]*Department of Neurology, St Josef Hospital, Ruhr University Bochum, Germany,*
[6]*Centre for Neuropathology and Prion Research, Ludwig-Maximilians-University Munich, Germany,*
[7]*Department of Biochemistry of Neurodegenerative Diseases, Institute of Biochemistry and Pathobiochemistry, Ruhr University Bochum, Germany*

Neurodegenerative diseases are characterized by the accumulation of misfolded protein species in the brain. How protein aggregation and cellular handling of misfolded proteins is linked to neuronal dysfunction and cell death has become a central question that is crucial to develop causal therapies. Here we report that components of the linear ubiquitin assembly complex (LUBAC) are recruited to mutant huntingtin (Htt-polyQ) to promote its modification by linear ubiquitin chains. As a consequence, toxicity of misfolded Htt-polyQ is markedly reduced and its subcellular localization is altered. Silencing of HOIP, the catalytic E3 ubiquitin ligase of LUBAC, increases Htt-polyQ-induced toxicity and decreases nuclear localization of Htt-polyQ, whereas silencing of OTULIN, a deubiquitinase with unique specificity for linear polyubiquitin, has the opposite effect. These findings identify linear ubiquitination as a potential target for disease-modifying strategies in Huntington's disease.

Cotranslational chaperone action at the single-molecule level

Florian Wruck[1], Alexandros Katranidis[2], Martin Hegner[3], Günter Kramer[4, 5], Beate Zachmann-Brand[4, 5], Matthias P. Mayer[4, 5], Bernd Bukau[4, 5], Sander J. Tans[1]

[1]*AMOLF institute, 1098 XG Amsterdam, The Netherlands*
[2]*Forschungszentrum Jülich, Institute of Complex Systems ICS-5, Jülich, Germany*
[3]*CRANN-The Naughton Institute, School of Physics, Trinity College Dublin, Ireland*
[4]*Center for Molecular Biology of Heidelberg University (ZMBH), DKFZ-ZMBH Alliance, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany*
[5]*German Cancer Research Center (DKFZ), Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany*

Although translation and the role of chaperones in protein folding are well studied subjects, cotranslational chaperone action has been proven difficult to observe. How proteins adopt their native structure with efficient fidelity while being synthesized by the ribosome remains largely unexplored. Several crucial mechanistic questions concerning the effects of chaperones on cotranslational folding remain unanswered: For instance, how does trigger factor (TF) and the major bacterial heat shock protein 70 (DnaK) affect cotranslational protein folding? Do they affect the translation rate? When and how often do they (un)bind? How do these chaperones assure reliable and fast native folding during protein synthesis? Here, we introduce a combined optical tweezers and confocal laser scanning microscopy approach to investigate the action of chaperones on co- and posttranslational folding in real-time.

Development of PERK/CHOP Branch Activators as an Anti-Cancer Therapy

Min Wu, Mohmmad Hafiz, Jennifer Curran, Ben Munoz

Proteostasis Therapeutics, Inc., 200 Technology Sq., 4th Floor, Cambridge, MA 02139

Hyperactiva¬tion of oncogenes often leads to an increase in protein synthesis, which results in an increased protein-folding load in the endoplasmic reticulum (ER). In addition, stresses such as hypoxia, nutrient deprivation and acidification in the tumor microenvironment disturb protein folding in the ER. In response to the accumulation of unfolded or misfolded proteins within the ER, three branches of Unfolded Protein Response (UPR) are activated. Adaptive responses are activated through the IRE1 and ATF6 branches to reduce ER workload and prevent further accumulation of unfolded proteins. Under sustained ER stress, the PERK/eIF2a/ATF4/CHOP branch is activated to induce

apoptosis. We have identified UPR activators that preferentially activate the PERK/CHOP branch. The PERK/CHOP activators induce apoptosis in cancer cells, and have demonstrated different selectivity profiles against cancer cell vs. non-transformed cells, and among various cancer cell lines profiled. Activation of the PERK/CHOP branch is a promising therapeutic approach to target cancer.

Characterizing a Novel Intermediate in the Reaction Cycle of the GroEL/ES Chaperonin

Xiao Yan[1], Qiaoyun Shi[1, 2], Goran Miličić[1], Andreas Bracher[1], F. Ulrich Hartl[1], Manajit Hayer-Hartl[1]

[1]*Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany*
[2]*iHuman Institute, ShanghaiTech University, Shanghai 201210, China*

The bacterial chaperonin GroEL and its cofactor GroES form a nano-cage that functions as a molecular machine for protein folding. GroEL and GroES undergo an ATP-regulated interaction cycle that serves to transiently encapsulate substrate protein by opening and closing the folding cage. The GroEL oligomer consists of two identical heptameric rings stacked back-to-back. How the two rings are functionally coordinated during the conformational cycle is not completely understood. We used a range of biophysical approaches, including dual-color fluorescence cross-correlation spectroscopy, x-ray crystallography, electron microscopy and FRET, to probe the conformational cycle of GroEL/ES. We identify a previously uncharacterized, functionally important transient intermediate in the GroEL/ES folding cycle. Our results establish a link between inter-ring allostery, relative population of asymmetric and symmetric GroEL:GroES complexes, and substrate release.

High resolution structural studies and direct visual observation of Hsp104 from *Chaetomium thermophilum* suggest the role of the spiral architecture

Yosuke Inoue[1], Yuya Hanazono[2], Kentaro Noi[3], Akihiro Kawamoto[4], Kazuki Takeda[2], Keiichi Noguchi[1], Keiichi Namba[4], Teru Ogura[3], Kunio Miki[2], Kyosuke Shinohara[1], Masafumi Yohda[1]

[1]*Department of Bioengineering and Life Sciences, Tokyo University of Agriculture and Technology, Koganei*
[2]*Department of Chemistry, Graduate School of Science, Kyoto University*
[3]*Department of Molecular Cell Biology, Institute of Molecular Embryology and Genetics, Kumamoto University*
[4]*Graduate School of Frontier Biosciences, Osaka University*

We have been studying protein disaggregation mechanism by Hsp104 using that of Chaetomium thermophilum (CtHsp104). We determined the crystal structure of the N-terminal domain deficient mutant (CtHsp104□N) in complex with ADP at 2.7 Å resolution. CtHsp104□N assembles into an oligomer of infinite-bound spiral form. Then, we carry out cryo-EM observation of the wild type CtHsp104 in the presence of ADP. We observed spiral form morphology of Hsp104 hexamer. Unexpectedly, however, the closed ring morphology was also observed under the same condition, suggesting that Hsp104 takes two distinct conformations of closed ring and spiral form in the ADP bound state. Finally, we carry out AFM observation of CtHsp104. The major population (~80 %) of CtHsp104□N was the spiral form whereas the closed ring was observed as a minor population. Our data suggests that Hsp104 changes conformation between the closed ring and the spiral form during the reaction cycle.

Quality control pathways of the hERG potassium channel

Jason Young[1, 3], Christine Hantouche[2, 3], Brittany Williamson[1, 3], Alvin Shrier[2, 3]

[1]*Dept. of Biochemistry, McGill University*
[2]*Dept. of Physiology, McGill University*
[3]*Groupe de Recherche Axé sur la Structure des Protéines*

Mutations in the hERG/Kv11.1 potassium channel which cause its misfolding and loss of function, are responsible for cardiac long QT syndrome. The mechanisms of hERG degradation are still being explored, but are different from other models of ER-associated degradation. Hsc70/Hsp70 acts in both the folding and degradation of hERG at the ER. The co-chaperones DNAJA1 and DNAJA2 regulate degradation by Hsc70/Hsp70 and CHIP E3 ligase. In contrast, Bag1 promotes chaperone-independent degradation by the ER-associated E3 ligase TRC8. The transmembrane region of TRC8 recognizes hERG, and selects misfolded wild-type and mutant hERG for degradation. A small molecule that stabilizes the structure of misfolded hERG prevents TRC8-mediated degradation. Our findings reveal a previously unknown quality control function of TRC8.

List of Participants

Prof. Susan Ackerman
sackerman@ucsd.edu
University of California, San Diego, La Jolla, CA, United States

Prof. Simon Alberti
alberti@mpi-cbg.de
Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, Dresden, Germany

Dr. Sara Alvira
sara.alvira@bristol.ac.uk
University of Bristol, University Walk, Clifton, BS8 1TD, Bristol UK

Mr. Niko Amin-Wetzel
na395@cam.ac.uk
Cambridge Institute for Medical Research, Hills Road, CB2 0XY, Cambridge, UK

Dr. Claes Andréasson
claes.andreasson@su.se
Stockholm University, Svante Arrhenius väg 20B, 10691, Stockholm, Sweden

Ms. Kristin Arnsburg
arnsburg@fmp-berlin.de
Leibniz Institute for Molecular Pharmacology (FMP Berlin), Robert-Roessle-Str. 10, 13125, Berlin, Germany

Dr. Avi Ashkenazi
aa@gene.com
Genentech Inc., 1 DNA Way, 94080, South San Francisco, CA, United States

Mr. Sergio Attanasio
s.attanasio@tigem.it
Telethon Institute of Genetics and Medicine (TIGEM), Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy

Mr. Mario Avellaneda
m.avellaneda@amolf.nl
AMOLF, Science Park 104, 1098XG, Amsterdam, Netherlands

Dr. David Balchin
dbalchin@gmail.com
Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152, Munich, Bayern, Germany

Mr. Yury Barbitoff
barbitoff@bk.ru
St. Petersburg State University, Universitetskaya nab. 7/9, 199034, St. Petersburg, Russia

Dr. Alessandro Barducci
alessandro.barducci@cbs.cnrs.fr
Centre de Biochimie Structurale CNRS UMR 5048 INSERM U1054, 29, rue de Navacelles, 34000, Montpellier, France

Dr. Kim Baumann
k.baumann@nature.com
Springer Nature, 4 Crinan Street, N1 9XW, London, United Kingdom

Dr. Graham Bell
graham.bell@biomedcentral.com
BioMed Central, Springer Nature, Floor 6, 236 Gray's Inn Road, London, WC1X 8HB, London, UK

Prof. Shay Ben-Aroya
benaroyashay@gmail.com
Bar-Ilan University, The Nano Center, Building 206 room B-840, 52900, Ramat-Gan, Israel

Dr. Anat Ben-ZVi
anatbz@bgu.ac.il
Ben-Gurion University of the Negev, P.O.Box 653, 84105, Beer-Sheva, Israel

Dr. Anne Bertolotti
aberto@mrc-lmb.cam.ac.uk
MRC Laboratory of Molecular Biology, Francis Crick Avenue, CB2 0QH, Cambridge, UK

Mr. Maximilian Biebl
maxi.biebl@tum.de
Technical University of Munich, Lichtenbergstraße 4, 85748, Garching, Germany

Dr. Ivana Bjedov
i.bjedov@ucl.ac.uk
University College London, UCL Cancer Institute, Paul O Gorman Building, 72 Huntley Street, WC1E 6DD, London, UK

Dr. Edgar Boczek
boczek@mpi-cbg.de
Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, Dresden, Germany

Mrs. Anastasiia Bohush
a.bohush@nencki.gov.pl
Nencki Institute of Experimental Biology PAS, Pasteur St. 3, 02-093, Warsaw, Poland

Dr. Susanna Boronat
susanna.boronat@upf.edu
Universitat Pompeu Fabra, C/ Dr. Aiguader, 88, 08003, Barcelona, Spain

Dr. Marion Bouchecareilh
marionb@ibgc.cnrs.fr
CNRS, IBGC UMR5095, 1 rue Camille Saint Saens, Bordeaux, France

Ms. Kimberly Bowen
kimberly.bowen@utexas.edu
University of Texas at Austin, 2506 Speedway, NMS 4.242, 78712, Austin, TX, United States

Prof. Jeffrey Brodsky
jbrodsky@pitt.edu
University of Pittsburgh, A320 Langley Hall, Department of Biological Sciences, 1526, Pittsburgh, PA, United States

Dr. Mirella Bucci
m.bucci@us.nature.com
Nature Chemical Biology, 22 Bush Street, 94402, San Francisco, California, United States

Prof. Johannes Buchner
johannes.buchner@tum.de
Technische Universität München, Lichtenbergstrasse 4, 85747, Garching, Germany

Ms. Maria Teresa Bueno-Carrasco
mtbueno@cnb.csic.es
Centro Nacional Biotecnología – CSIC, Calle Darwin nº 3 - Campus Cantoblanco, 28049, Madrid, Spain

Prof. Bernd Bukau
bukau@zmbh.uni-heidelberg.de
Center for Molecular Biology Heidelberg (ZMBH) Im Neuenheimer Feld 282, 69120, Heidelberg, Germany

Dr. Marieta Caganova
m.2891.c@gmail.com
The Max Delbrück Center for Molecular Medicine (MDC), Robert-Rössle-Str. 10, 13125 Berlin-Buch, Berlin, Germany

Prof. Serena Carra
serena.carra@unimore.it
University of Modena and Reggio Emilia, Department of Biomedical, Metabolic and Neural Sciences and Center for Neuroscience and Nanotechnologies, Giuseppe Campi 287, 41125, Modena, Italy

Dr. Marta Carroni
marta.carroni@scilifelab.se
SciLife Laboratory Sweden, Tomtebodavägen 23A, 17165 Stolna, Stockholm, Sweden, 17165, Solna, Stockholm, Sweden

Mr. Pedro Carvalho
pedro.carvalho@path.ox.ac.uk
University of Oxford, Sir William Dunn School Pathology South parks road, OX1 3RE, Oxford, UK

Prof. Agnieszka Chacinska
achacinska@iimcb.gov.pl
International Institute of Molecular and Cell Biology in Warsaw, 4 Ks. Trojdena Street, 02-109, Warsaw, Poland

Dr. Chiung-Wen (Mary) Chang
Chiung-Wen.Chang@bcm.edu
Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Texas 77030, Houston, Texas, United States

Dr. Tsun-Kai Chang
chang.tsunkai@gene.com
Genentech Inc. USA, 1 DNA way, 94080, South San Francisco, California, United States

Prof. Paul Chapple
j.p.chapple@qmul.ac.uk
Queen Mary University of London, Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine, Charterhouse Square, London, UK

Prof. Mike Cheetham
michael.cheetham@ucl.ac.uk
UCL Institute of Ophthalmology, 11-43 Bath Street, EC1V 9EL, London, UK

Mr. Matthew Cheng
matthew.cheng@ifib.uni-tuebingen.de
International Max Planck Research School, Interfaculty Institute of Biochemistry, Universität Tübingen, Hoppe-Seyler Straße 4, 72076, Tübingen, Germany

Prof. Yury Chernoff
yury.chernoff@biology.gatech.edu
Georgia Institute of Technology, 950 Atlantic Drive, Engineered Biosystems Building (EBB), M/C 2000, 30332-2000, Atlanta, GA, United States

Dr. Tatiana Chernova
tanyaachernova@gmail.com
Emory University, 1510 Clifton Rd RRC rm, 4070, 30322, Atlanta, GA, United States

Dr. Young-Jun Choe
choe@biochem.mpg.de
Max Planck Institute of Biochemistry, Am Klopferspitz 18, Martinsried, Germany

Ms. Courtney Choutka
cchoutka@bcgsc.ca
BC Cancer Research Centre, 675 West 10th Avenue, V5Z 1L3, Vancouver, BC, Canada

Prof. Martine Collart
martine.collart@unige.ch
Faculty of Medicine, University of Geneva, CMU, 1 rue Michel Servet, Dpt MiMol, 1211, Geneva 4, Switzerland

Prof. Jean-Francois Collet
jfcollet@uclouvain.be
de Duve Institute, Universite de Louvain, Avenue Hippocrate 75, 1200, Brussels, Belgium

Dr. Charlotte Conz
charlotte.conz@biochemie.uni-freiburg.de
Institute of Biochemistry and Molecular Biology, Stefan-Meier-str. 17, D-79104, Freiburg, Germany

Dr. Jorge Cuéllar
jcuellar@cnb.csic.es
National Centre for Biotechnology (CSIC), C/ Darwin, 3 Campus UAM, 28049, Madrid, Spain

Mr. Alejandro Da Silva
adasilva@abo.fi
Åbo Akademi, Department of Biosciences, Åbo Akademi University, Tykistökatu 6, 20520, Turku, Finland

Mr. Sam Dawes
sjdawes1@sheffield.ac.uk
University of Sheffield, Department of Chemistry, Western Bank, S3 7HF, Sheffield, United Kingdom

Prof. Elke Deuerling
elke.deuerling@uni-konstanz.de
University of Konstanz, Dept. Biology, Universitaetsstr, 10, 78464, Konstanz, Germany

Dr. Celia Deville
c.deville@mail.cryst.bbk.ac.uk
Birkbeck, university of London, Malet Street, WC1E7HK, London, UK

Dr. Kristina Döring
k.doering@zmbh.uni-heidelberg.de
ZMBH, Heidelberg University, Im Neuenheimer Feld 282, 69120, Heidelberg, Germany

Dr. Ruben Fernandez-Busnadiego
ruben@biochem.mpg.de
Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152, Martinsried, Germany

Dr. Anthony Fitzpatrick
awpfitzpatrick@gmail.com
MRC Laboratory of Molecular Biology, Francis Crick Avenue, CB2 0QH, Cambridge, UK

Dr. Titus Franzmann
titus.franzmann@mpi-cbg.de
Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr 108, 01307, Dresden, Germany

Ms. Sandra Fries
sandra.fries@uni-konstanz.de
University of Konstanz, Universitätsstraße 10, Konstanz, Germany

Prof. Judith Frydman
jfrydman@stanford.edu
Stanford University, E200 Clark Center, Stanford University, 94305, Stanford, CA, United States

Dr. Rodrigo Gallardo
rodrigo.gallardo@switch.vib-kuleuven.be
Switch Laboratory, Herestraat 49, Box 802, Room 08.683, 3000, Leuven, Belgium

Prof. Jason Gestwicki
jason.gestwicki@ucsf.edu
UCSF, 675 Nelson Rising Lane, 94158, San Francisco, California, United States

Prof. Rachel Green
ragreen@jhmi.edu
JHU – SOM, 725 N. Wolfe Street, 21210, Baltimore, MD, United States

Mr. Yetis Gultekin
ygultekin@rockefeller.edu
The Rockefeller University, 1230 York Av The Rockefeller Univ Box 210, 10065, NYC, NY, United States

Mr. Ricardo Gutierrez
rgutier2@smail.uni-koeln.de
CECAD Research Center - University of Cologne, Joseph-Stelzmann-Str. 26, 50931, Cologne, Germany

Mr. Ingo Hantke
ingo.hantke@ifmb.uni-hannover.de
Institut für Mikrobiologie, Leibniz Universität Hannover, Herrenhäuser Straße 2, 30419, Hannover, Germany

Prof. Ulrich Hartl
uhartl@biochem.mpg.de
Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152, Martinsried, Germany

Dr. Martin Haslbeck
martin.haslbeck@tum.de
Technische Universität München /Department Chemie /Lehrstuhl für Biotechnologie, Lichtenbergstrasse 4, 85748, Garching, Germany

Mr. Timm Hassemer
hassemer@biochem.mpg.de
Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152, Planegg, Germany

Dr. Manajit Hayer-Hartl
mhartl@biochem.mpg.de
Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152, Martinsried, Germany

Dr. Cole Haynes
cole.haynes@umassmed.edu
University of Massachusetts Medical School, 347 Plantation Street, LRB 425, 01605, Worcester, Massachusetts, United States

Dr. Eva Henriksson
eva.henriksson@abo.fi
Åbo Akademi University, Finland, Tykistökatu 6A, 20520, Turku, Finland

Dr. Shoshiro Hirayama
s.hirayama@mol.f.u-tokyo.ac.jp
The University of Tokyo, Hongo, 113-0033, Bunkyo-ku, Tokyo, Japan

Prof. Zoya Ignatova
zoya.ignatova@uni-hamburg.de
University of Hamburg, Martin-Luther-King-Pl. 6, 20146, Hamburg, Germany

Dr. Soudabeh Imanikia
imanikia@mrc-lmb.cam.ac.uk
MRC, Laboratory of Molecular Biology, Francis Crick Avenue, CB2 0QH, Cambridge, UK

Prof. Toshifumi Inada
tinada@m.tohoku.ac.jp
Tohoku University, Aobaku 6-3, 980-8578, Sendai, Japan

Prof. Claudio Joazeiro
c.joazeiro@zmbh.uni-heidelberg.de
Heidelberg University (ZMBH), Im Neuenheimer Feld 282, 69120, Heidelberg, Germany

Mr. Ben Johnston
ben.johnston@dal.ca
Dalhousie University, 5850 College Street, Room 7F, PO BOX 15000, B3H 4R2, Halifax, Nova Scotia, Canada

Dr. Priyanka Joshi
pj291@cam.ac.uk
Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, UK

Mrs. Jenny Joutsen
jenny.joutsen@abo.fi
Åbo Akademi University, Tykistökatu 6, 20520, Turku, Finland

Dr. Christoph Kaiser
christoph.kaiser@tum.de
Department Chemie, Technische Universität München, Lichtenbergstr, 4, 8574, Garching, Germany

Prof. Harm Kampinga
h.h.kampinga@umcg.nl
UMCG, Ant. Deusinglaan 1, 9791 AV, Groningen, Netherlands

Dr. Ganapathi Kandasamy
ganapathi.kandasamy@su.se
Stockholm University, Svante Arrhenius väg 20C, Stockholm, Sweden

Dr. Gulsun Elif Karagoz
elif@walterlab.ucsf.edu
UCSF, 600, 16th street, MC2200, UCSF, 94158, San Francisco, CA, United States

Dr. Andrey Karamyshev
andrey.karamyshev@ttuhsc.edu
Texas Tech University Health Sciences Center, 3601 4th Street, Mail Stop 6540, Lubbock, Texas, United States

Mrs. Ofri Karmon
ofri_yogev@hotmail.com
Bar Ilan University, Ramat Gan, Israel

Dr. Georgios Karras
gkarras@wi.mit.edu
Whitehead Institute for Biomedical Research, 455 Main Street, Nine Cambridge Center, 02142, Cambridge, MA, United States

Prof. Jeffery Kelly
jwk@scripps.edu
The Scripps Research Institute, 10550 N. Torrey Pines Rd., BCC 265, 92037, La Jolla, CA, United States

Dr. Janine Kirstein
kirstein@fmp-berlin.de
Leibniz Institute for Molecular Pharmacology (FMP), Robert Rössle-Strasse 10, 13125 Berlin, Germany

Prof. Rachel Klevit
klevit@uw.edu
University of Washington, Dept. of Biochemistry, Box 357350, 98195, Seattle, Washington, United States

Mr. Michael Knop
m.knop@zmbh.uni-heidelberg.de
ZMBH/University ofHeidelberg, Im Neuenheimer Feld 282, 69120, Heidelberg, Germany

Ms. Yee Hui Koay
yeehui.koay@manchester.ac.uk
University of Manchester, Oxford Road, M13 9PT, Manchester, UK

Dr. Eline Koers
eline_koers@hotmail.com
AMOLF Science Park 102, 1098 XG, Amsterdam, Netherlands

Prof. Ron Kopito
kopito@stanford.edu
Stanford University, 164 Camellia Ave, 94061, Redwood City, CA, United States

Mr. Yannick Kopp
Y.Kopp@em.uni-frankfurt.de
Buchmann Institute for Melecular Life Science, Max-von-Laue-Str. 15, 60438, Frankfurt am Main, Germany

Ms. Kamena Kostova
kamena.kostova@ucsf.edu
University of California, San Francisco, 1700 4th Street, Byers Hall Room 404, 94158, San Francisco, CA, United States

Ms. Seda Koyuncu
sedakyncu@gmail.com
CECAD-– Cluster of Excellence, Joseph-Stelzmann-Strasse, 26, 50931, Cologne, Germany

Dr. Günter Kramer
g.kramer@zmbh.uni-heidelberg.de
Center for Molecular Biology of Heidelberg University (ZMBH) and German Cancer Research Center (DKFZ), DKFZ-ZMBH Alliance Im Neuenheimer Feld 282, 69120, Heidelberg, Germany

Dr. Stefan Kreft
stefan.kreft@uni-konstanz.de
Dept. of Biology, Molecular Microbiology, AG Deuerling, University of Konstanz, Universitaetsstr. 10, 78457, Konstanz, Germany

Dr. Anita Krisko
anita.krisko@medils.hr
Mediterranean Institute for Life Sciences, Mestrovicevo setaliste 45, 21000, Split, Croatia

Dr. Nard Kubben
kubbenlp@mail.nih.gov
National Institutes of Health, 41 Library Drive, Building 41, Room B513, 20892, Bethesda, Maryland, United States

Dr. Karin Kuehnel
karin.kuehnel@nature.com
Nature Communications, 4 Crinan Street, N1 9XW, London, UK

Ms. Rishika Kundra
rk489@cam.ac.uk
University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge, UK

Dr. Mattia Laffranchi
m.laffranchi005@unibs.it
Dept. of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, 25123, Brescia, Italy

Dr. Karine Lapouge
karine.lapouge@bzh.uni-heidelberg.de
Biochemistry Center (BZH) /University of Heidelberg, Im Neuenheimer Feld 328, 69120, Heidelberg, Germany

Dr. Elsa Lauwers
Elsa.Lauwers@cme.vib-kuleuven.be
VIB / KU Leuven, 49 Herestraat, 3000, Leuven, Belgium

Dr. Kif Liakath-Ali
kif.liakath-ali@kcl.ac.uk
Centre for Stem Cells & Regenerative Medicine, King's College London, 28th Floor, Guy's Hospital, King's College London School of Medicine, Great Maze Pond, SE1 9RT, UK

Prof. KRZYSZTOF Liberek
liberek@biotech.ug.edu.pl
University of Gdansk, Bażyńskiego 8, 80-309, Gdansk, Poland

Ms. Fabiana Longo
longo.fabiana@hsr.it
Ospedale San Raffaele, Via Olgettina 60, 20132, Milano, Italy

Ms. Nieves Lorenzo Gotor
nieves.lorenzo@crg.eu
CRG, dr. aiguader, 88, 08003, Barcelona, Spain

Prof. Jaroslaw Marszalek
jaroslaw.marszalek@biotech.ug.edu.pl
Intercollegiate Faculty of Biotechnology, University of Gdansk, Abrahama 58, 80-307, Gdansk, Poland

Mr. Luis Marte
luis.marte@upf.edu
Universitat Pompeu Fabra, Dr. Aiguader 88, 08003, Barcelona, Spain

Mr. Christoph U. Mårtensson
christoph.martensson@biochemie.uni-freiburg.de
Institute for Biochemistry and Molecular Biology, University of Freiburg, Stefan-Meier-Straße 17, Freiburg, Germany

Ms. Victoria Martinez Miguel
victoria.martinez.miguel.14@ucl.ac.uk
University College London, 72 Huntley Street, WC1E 6DD, London, UK

Mr. Daniel Mateju
mateju@mpi-cbg.de
Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307, Dresden, Germany

Dr. Veena Mathew
vmathew@bccrc.ca
Terry Fox Laboratory, BC Cancer Agency, 675 West 10th Ave, V5Z1L3, Vancouver, British Columbia, Canada

Prof. Matthias Mayer
m.mayer@zmbh.uni-heidelberg.de
University of Heidelberg, Im Neuenheimer Feld 282, D-69120, Heidelberg, Germany

Dr. Thibault Mayor
mayor@mail.ubc.ca
University of British Columbia, 2125 East Mall, NCE306, V6T1Z4, Vancouver, BC, Canada

Ms. Mareike Mentzel
mareike.mentzel@tum.de
Chair of Biotechnology/Department of Chemistry/Technical University Munich, Lichtenbergstraße 4, 85748, Garching, Bavaria, Germany

Prof. Hemmo Meyer
hemmo.meyer@uni-due.de
Centre for Medical Biotechnology, Faculty of Biology, University of Duisburg-Essen, Universitaetsstr. 5, 45117, Essen, Germany

Mr. Goran Milicic
milicic@biochem.mpg.de
Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152, Martinsried, Germany

Dr. Axel Mogk
a.mogk@zmbh.uni-heidelberg.de
ZMBH, University of Heidelberg, Im Neuenheimer Feld 282, 69120, Heidelberg, Germany

Ms. Tania Morán Luengo
T.MoranLuengo@uu.nl
Utrecht University, Jansdam 1E, 3512HA, Utrecht, Netherlands

Dr. Francesca Moretti
morettifrancesca83@gmail.com
Novartis Institutes for BioMedical Research, Fabrikstrasse 2, 4056, Basel, Switzerland

Prof. Kazutoshi Mori
mori@upr.biophys.kyoto-u.ac.jp
Department of Biophysics, Graduate School of Science, Kyoto University, Kitashirakawa-oiwake, Sakyo-ku, 606-8502, Kyoto, Japan

Prof. Richard Morimoto
r-morimoto@northwestern.edu
Northwestern University, 2205 Tech Dr., Hogan 2-100, 60208, Evanston, IL, United States

Dr. Alexey Morozov
Runkel@inbox.ru
Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str. 32, 119991, Moscow, Russia

Dr. Anne Kathrin Müller-Rischart
mueller-rischart@bio.lmu.de
Cell- and Developmental Biology, Ludwig-Maximilian-University Munich, Großhaderner Str. 2, 82152, Planegg-Martinsried, Germany

Dr. Christian Münch
ch.muench@em.uni-frankfurt.de
Goethe University Frankfurt, Institute for Biochemistry II, Building 75, Theodor-Stern-Kai 7, 60590, Frankfurt, Germany

Prof. Shigeo Murata
smurata@mol.f.u-tokyo.ac.jp
The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-0033, Tokyo, Japan

Dr. Anne Nielsen
nielsen@embo.org
The EMBO Journal, Meyerhofstrasse 1, 69117, Heidelberg, Germany

Dr. Nadinath Nillegoda
n.nillegoda@zmbh.uni-heidelberg.de
Center for Molecular Biology, University of Heidelberg, Im Neuenheimer Feld 282, 69120, Heidelberg, Germany

Mr. Igor Obuchowski
igor.obuchowski@gmail.com
Intercollegiate Faculty of Biotechnology, University of Gdansk, Gdansk, Poland, Jana Bażyńskiego 8, 80-309, Gdansk, Pomorskie, Poland

Ms. Miku Ohfurudono
ohfurudono.miqu@gmail.com
Nara Institute of Science and Technology, #1-507 (NASIT Dormitory) 8916-5, 630-0101, Takayama-cho Ikoma-shi, Nara-ken, Japan

Prof. Fernando Palhano
palhano@bioqmed.ufrj.br
Universidade Federal do Rio de Janeiro, Av Bauhinia 400, N/A, Rio de Janeiro, Brazil

Dr. David Parfitt,
d.parfitt@ucl.ac.uk
UCL Institute of Ophthalmology, 11-43 Bath Street, EC1V 9EL, London, UK

Prof. Sarah Perrett
sarah.perrett@cantab.net
Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing, China

Prof. Didier Picard
Didier.Picard@unige.ch
Department of Cell Biology, University of Geneva, Sciences III, 1211, Geneva, Switzerland

Dr. Blagovesta Popova
bpopova@gwdg.de
University of Göttingen, Grisebachstr. 8, 37077, Göttingen, Germany
Dr. Pablo Pulido
pablo.pulido@lmu.de
Ludwig Maximilian University (LMU) of Munich, Germany, Großhaderner Str. 2-4, D-82152, Planegg-Martinsried, Bayern, Germany

Ms. Lucia Quintana
lquintana@cnb.csic.es
National Center for Biotechnology CSIC, C/Darwin 3, 28049, Madrid, Spain

Prof. Sheena Radford
s.e.radford@leeds.ac.uk
The University of Leeds, Woodhouse Lane, LS2 9JT, Leeds, West Yorkshire, UK

Mr. Manish Rai
manish.rai@igib.in
CSIR-IGIB, Sukhdev Vihar, 110025, New Delhi, Delhi, India

Dr. Namit Ranjan
namit.ranjan@mpibpc.mpg.de
Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077, Göttingen, Germany

Mr. Robert Rauscher
robert.rauscher@chemie.uni-hamburg.de
University of Hamburg/Institute For Biochemistry And Molecular Biology, Martin-Luther-King-Platz 6, 20146, Hamburg, Germany

Mr. Sandeep Raut
sraut@iiserb.ac.in
Indian Institute Of Science Education And Research, Bhopal, Bhopal Bypass Road, 462066, Bhauri, Madhya-Pradesh, India

Dr. Brinda Ravikumar
brinda.ravikumar@abbvie.com
Foundational Neuroscience Center, Abbvie, 200 Sidney Street, Cambridge, MA, United States

Dr. Theo Rein
theorein@psych.mpg.de
Max Planck Institute of Psychiatry, Kraepelinstr. 10, 80804, München, Germany

Dr. Marisa Reverendo
marisa.reverendo@gmail.com
CIML - Centre d'Immunologie de Marseille-Luminy, Parc Scientifique & Technologique de Luminy, Case 906, 13288 Marseille cedex 09, Marseille, France

Dr. Nina Romanova
n.romanova@spbu.ru
Saint-Petersburg State University, 7/9 Universitetskaya nab, 199034, Saint-Petersburg, Russia

Dr. Pia Roos-Mattjus
proos@abo.fi
Abo Akademi University, Artillerigatan 6 A 3rd floor, Biocity, 20520, Åbo, Finland

Mr. Adrien Rousseau
rousseau@mrc-lmb.cam.ac.uk
MRC Laboratory of Molecular Biology, Francis Crick Avenue, CB2 0QH, Cambridge, UK

Prof. Helen Saibil
h.saibil@mail.cryst.bbk.ac.uk
Birkbeck College, Malet St, WC1E 7HX, London, UK

Dr. Eri Sakata
sakata@biochem.mpg.de
Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152, Martinsried, Germany

Ms. Grace Salsbury
g.a.salsbury@qmul.ac.uk
William Harvey Research Institute, Charterhouse Square, EC1M 6BQ, London, UK
Mr. Reuben Saunders

raps3@cam.ac.uk
University of Cambridge Institute for Medical Research, Cambridge Biomedical Campus, Wellcome Trust /MRC Building, Hills Road, CB2 0XY, Cambridge, UK

Dr. Ritwick Sawarkar
sawarkar@ie-freiburg.mpg.de
Max Planck Institute of Immunobiology and Epigenetics, Stübeweg 51, 79108, Freiburg, Germany
Dr. Kim Schneider
kschneid@mrc-lmb.cam.ac.uk
MRC Laboratory of Molecular Biology, Francis Crick Avenue, CB2 0QH, Cambridge, UK

Dr. Annika Scior
scior@fmp-berlin.de
FMP Berlin, Robert-Rössle-Str. 10, 10119, Berlin, Germany

Dr. Irmgard Sinning
irmi.sinning@bzh.uni-heidelberg.de
Heidelberg University Biochemistry Center, INF 328, D-69120, Heidelberg, Germany

Prof. Lea Sistonen
lea.sistonen@btk.fi
Åbo Akademi University, BioCity, Tykistokatu 6, 20520, Turku, Finland

Dr. Ana Soares
ana.r.soares@ua.pt
Institute for biomedicine - University of Aveiro, 3810-193, Aveiro, Portugal

Dr. Maria de la Encarnación Solesio Torregrosa
ms8790@nyu.edu
NYU, Department of Basic Science, 345 East, 24th Street, Room#1030S, 10010, NYC, NY, United States

Dr. Daniel Southworth
dsouth@umich.edu
University of Michigan, Life Sciences Institute, 210 Washtenaw Ave, 48109, Ann Arbor, MI, United States

Dr. Karen Stroobants
stroobantskaren@gmail.com
University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge, UK

Ms. Tamara Suhm
tamara.suhm@dbb.su.se
Stockholm University, Svante Arrhenius väg 16C, Stockholm, Sweden

Prof. Sander Tans
tans@amolf.nl
FOM Institute AMOLF, Science Park 104, 1098 XG, Amsterdam, APO/FPO EUROPE/ATLANTIC, Netherlands

Dr. Gian Gaetano Tartaglia
gian@tartaglialab.com
Centre for Genomic Regulation (CRG), Doctor Aiguader, 88, E08003, Barcelona, Spain

Prof. Jorg Tatzelt
joerg.tatzelt@rub.de
Ruhr University Bochum, Universitaetsstrasse 150, Bochum, Germany

Ms. Isabelle Taylor
isabelle.taylor@ucsf.edu
University of California, San Francisco, 675 Nelson Rising Lane, 94158, San Francisco, CA, United States

Dr. Rebecca Taylor
rtaylor@mrc-lmb.cam.ac.uk
MRC Laboratory of Molecular Biology, Francis Crick Avenue, CB2 0QH, Cambridge, UK

Mr. Bartlomiej Tomiczek
bartlomiej.tomiczek@ug.edu.pl
University of Gdansk, Abrahama 58, 80-307 PL, Gdansk, Poland

Dr. R. Martin Vabulas
vabulas@em.uni-frankfurt.de
Goethe University Frankfurt, Max-von-Laue-Str.15, D-60438, Frankfurt am Main, Germany

Dr. Jacqueline van der Spuy
j.spuy@ucl.ac.uk
Institute of Ophthalmology, University College London, 11 - 43 Bath Street, EC1V 9EL, London, United Kingdom

Dr. Patricija van Oosten-Hawle
p.vanoosten-hawle@leeds.ac.uk
University of Leeds, Faculty of Biological Sciences, School of Molecular and Cell Biology, LS2 9JT, Leeds, UK

Mrs. Ana Sofia Varanda
sofiavaranda@ua.pt
University of Aveiro, Institute of Biomedicine – iBiMED, University of Aveiro, 3810-193, Aveiro, Portugal

Dr. Sandra Vidak
sandra.vidak@nih.gov
National Cancer Institute, NIH, 9000 Rockville Pike, Building 41, 20892, Bethesda, Maryland (MD), United States

Dr. Silvia Vilasi
silvia.vilasi@pa.ibf.cnr.it
CNR, Via Ugo La Malfa 153, Palermo, Italy

Dr. David Vilchez
dvilchez@uni-koeln.de
CECAD -University of Cologne, Joseph Stelzmann Strasse, 26 50931 Cologne, Germany, 50931, Cologne, Germany

Mr. Jan Vonk
j.j.vonk@umcg.nl
UMCG Antonius Deusinglaan 1, Groningen, Netherlands

Dr. Peter Walter
Peter@walterlab.ucsf.edu
UCSF/HHMI, 800 Kirkham Street, 94122, San Francisco, California, United States

Ms. Rebeccah Warmack
rawarmack@ucla.edu
University of California, Los Angeles, UCLA Department of Chemistry & Biochemistry, Paul Boyer Hall 640, Box 951569, 90095, Los Angeles, CA, United States

Prof. Eilika Weber-Ban
eilika@mol.biol.ethz.ch
ETH Zurich, Institute for Molecular Biology & Biophysics Otto-Stern-Weg 5, 8093, Zurich, Switzerland

Ms. Katharina Weinhäupl
kathi.weinhaeupl@gmail.com
Institut de Biologie Structurale, 71 avenue des Martyrs, CS 10090, 38044, Grenoble Cedex 9, France

Prof. Jonathan Weissman
joan.kanter@ucsf.edu
UCSF, 1700 4th ST, Room 403b, Mail code 2542, 94158, San Francisco, CA, United States

Prof. Konstanze Winklhofer
konstanze.winklhofer@rub.de
Ruhr University Bochum, Universitaetsstrasse 150, 44801, Bochum, Germany

Dr. Florian Wruck
f.wruck@amolf.nl
AMOLF Science Park 104, 1098 XG, Amsterdam, Noord-Holland, Netherlands

Dr. Min Wu
min.wu@proteostasis.com
Proteostasis Therapeutics, Inc., 200 Technology Sq. | 4th Floor, 02139, Cambridge, MA, United States

Mr. Xiao Yan
xiaoyan@biochem.mpg.de
Max-Planck Institute of Biochemistry, Am Klopferspitz 18, 82152, Martinsried, Munich, Germany

Dr. Masafumi Yohda
yohda@cc.tuat.ac.jp
Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Kogane, 184-8588, Tokyo, Japan

Dr. Jason Young
jason.young2@mcgill.ca
McGill University, 3649 Promenade Sir William Osler, H3G 0B1, Montreal, Quebec, Canada

POSTER NUMBERS AND TITLES

	Name	Title of Poster
1	Dr. Sara Alvira	Membrane protein insertion and folding by the bacterial holo-translocon.
2	Ms. Kristin Arnsburg	The J-protein Family of C. elegans - Expression Analyses during Aging and Stress.
3	Mr. Sergio Attanasio	Chop deletion reduces hepatic Z alpha1-antitrypsin and SQSTM1/p62 accumulation in PiZ mice
4	Mr. Mario Avellaneda	Three is a crowd: protein disaggregation at the single-molecule level
5	Dr. David Balchin	Mechanism of actin folding by the eukaryotic chaperonin TRiC/CCT
6	Mr. Yury Barbitoff	Relocalization of the Hsp40 chaperone Sis1 into the nucleus differentially affects yeast prions
7	Dr. Alessandro Barducci	Modeling Hsp70/Hsp40 interaction by multi-scale molecular simulations and co-evolutionary sequence analysis
8	Prof. Shay Ben-Aroya	Regulation of the Anaphase Promoting Complex/Cyclosome (APC/C) by the Proteasome Mediated Degradation
9	Dr. Anat Ben-Zvi	Differentiation can determine cellular proteostasis
10	Mr. Maximilian Biebl	The plasticity of the Hsp90 co-chaperone system
11	Dr. Ivana Bjedov & Ms. Victoria Martinez Miguel	Decreased protein synthesis for healthy ageing
12	Dr. Edgar Boczek	Molecular chaperones control the physical state of membrane-less compartments
13	Mrs. Anastasiia Bohush	A novel Hsp90 co-chaperone, CHP-1, in brain of patients with Parkinson's disease and Lewy Bodies Dementia
14	Dr. Susanna Boronat & Mr. Luis Marte	Genetic misregulation of the stability of the transcription factor Pap1 alters the tolerance of fission yeast to oxidative stress
15	Dr. Marion Bouchecareilh	Genetic mediators of Alpha 1-Antitrypsin Deficiency–mediated liver toxicity
16	Ms. Kimberly Bowen	Designing Proteasome Adaptors to Degrade Specific Targets
17	Ms. Maria Teresa Bueno-Carrasco	Structural studies of the CCT-gelsolin complex
18	Dr. Marieta Caganova	The role of Untranslated Protein Response in the elimination of B cells lacking the B cell receptor
19	Dr. Tsun-Kai Chang	PERK coordinates IRE1 attenuation to control cell fate
20	Prof. Paul Chapple	Altered organisation of the intermediate filament cytoskeleton and relocalisation of proteostasis modulators in cells lacking the ataxia protein sacsin
21	Mr. Matthew Cheng	The yeast vigilin has widespread impact on the polyQ-mediated aggregation landscape of the cell
22	Prof. Yury Chernoff	Prion-based cellular memories of stress
23	Dr. Tatiana Chernova	A Novel Yeast Model for Interactions between T-synthase and its Molecular Chaperone Cosmc
24	Dr. Young-Jun Choe	Toxic aggregation of stalled nascent polypeptides
25	Ms. Courtney Choutka	Heat-shock proteins modulate autophagy by regulating effector caspases
26	Prof. Martine Collart	Not5-dependent co-translational assembly of Ada2 and Spt20 is essential for functional integrity of SAGA
27	Dr. Charlotte Conz	Interaction of the cotranslational Hsp70 Ssb with ribosomal proteins and rRNA depends on its lid domain
28	Dr. Jorge Cuéllar	The role of CCT-PhLP1 system in the folding of mLST8, a key component of mTOR complex
29	Mrs. Jenny Joutsen & Mr. Alejandro Da Silva	HSF2 protects cells against protein aggregates through cadherin superfamily proteins
30	Mr. Sam Dawes	Strategies for tackling Alzheimer's disease: The unfolded protein response and small molecule inhibitors
31	Dr. Celia Deville	Substrate transfer and threading through the ClpB disaggregase
32	Dr. Kristina Döring	Profiling of Ssb interactions with nascent proteins reveals principles of Hsp70 assisted co-translational folding
33	Dr. Ruben Fernandez-Busnadiego	Cellular interactions and structural organization of mutant huntingtin inclusions studied in situ
34	Dr. Titus Franzmann	A prion domain that senses stress to promote cell survival by pH-driven protein phase separation
35	Ms. Sandra Fries	Characterization of the RAC-ribosome interaction by EPR
36	Dr. Rodrigo Gallardo	De novo design of a biologically active amyloid
37	Mr. Yetis Gultekin	Regulation of Axin proteolysis by Iduna is required for the intestinal homeostasis of *Drosophila*
38	Mr. Ricardo Gutierrez	Somatic increase of CCT8 mimics proteostasis of human pluripotent stem cells and extends C. elegans lifespan
39	Mr. Ingo Hantke	A small heat shock protein involved in the stress response of *Bacillus subtilis*

40	Dr. Martin Haslbeck	Small heat shock proteins from cyanophages are necessary to stabilize the host photosystems
41	Mr. Timm Hassemer	RNA and protein quality control pathways cooperate to protect cells against stalled nascent polypeptides
42	Dr. Shoshiro Hirayama	A comprehensive analysis of modulators regulating tau aggregation
43	Dr. Soudabeh Imanikia	The influence of cell non-autonomous UPRER (ER in superscript) signaling on the toxicity of misfiled proteins in the nematode *C. elegans* models
44	Mr. Ben Johnston	KSHV Modulates the IRE1-XBP1 Branch of the Unfolded Protein Response during Lytic Replication
45	Dr. Priyanka Joshi	A link between metabolite homeostasis and Alzheimer's disease: two metabolites downregulated in AD rescue an Aβ C elegans model
46	Dr. Christoph Kaiser	The structure of human αA-crystallin, a redox-sensitive chaperone
47	Dr. Ganapathi Kandasamy	Chaperone mediated delivery of ubiquitylated misfolded protein to the Proteasome
48	Dr. Gulsun Elif Karagoz	An unfolded protein-induced conformational switch activates mammalian IRE1
49	Dr. Andrey Karamyshev	RAPP, a Ribosome-Associated Protein Quality Control, in Health and Disease
50	Mrs. Ofri Karmon	How the protein quality control machinery distinguishes between functional and dysfunctional proteasomes
51	Ms. Yee Hui Koay	The role of BAG6 and UBR4 in ERAD
52	Dr. Eline Koers	Single molecule experiments on the human Glucocorticoid Receptor (GR)
53	Mr. Yannick Kopp	CHIP as a Sensor of Proteostasis Stress
54	Ms. Kamena Kostova	Role of C-terminal Alanine and Threonine Extensions (CAT tails) in Nascent Polypeptide Degradation
55	Ms. Seda Koyuncu	The E3 ubiquitin ligase UBR5 maintains proteostasis of huntingtin in immortal pluripotent stem cells'
56	Dr. Günter Kramer	Analysis of the co-translational interplay of chaperones by Selective Ribosome Profiling
57	Dr. Anita Krisko	TORC1-mediated sensing of chaperone activity alters glucose metabolism and extends lifespan
58	Dr. Nard Kubben	Repression of the antioxidant NRF2 pathway in premature aging
59	Ms. Rishika Kundra	Protein homeostasis of a metastable subproteome associated with Alzheimer's disease
60	Dr. Mattia Laffranchi	Mimicking the heterozygous condition of alpha-1-antitrypsin deficiency in cellular models
61	Dr. Karine Lapouge	RAC, a unique Hsp70-Hsp40 pair at the ribosome.
62	Dr. Elsa Lauwers	Membrane remodeling by chaperones and presynaptic protein quality control.
63	Dr. Kif Liakath-Ali	An Evolutionarily Conserved Ribosome-associated Quality Control Mechanism Maintains Epidermal Stem Cell Homeostasis
64	Ms. Fabiana Longo	Investigating altered proteostasis as underlying mechanism of autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)
65	Ms. Nieves Lorenzo Gotor	Liquid de-mixing as a mechanism of dosage sensitivity
66	Prof. Jaroslaw Marszalek & Mr. Bartlomiej Tomiczek	Probing molecular mechanisms of J-protein-Hsp70 interaction with chaperones dedicated for iron-sulfur cluster biogenesis.
67	Mr. Christoph U. Mårtensson	A potential link between mitochondrial biogenesis and quality control.
68	Mr. Daniel Mateju	Aberrant phase transition of stress granules triggered by misfolded proteins and prevented by chaperone function
69	Dr. Veena Mathew	Genotoxin-induced transcriptional repression regulates selective protein aggregation
70	Dr. Thibault Mayor	Deubiquitinase activity is required for the proteasomal degradation of misfolded cytosolic proteins upon heat-stress
71	Ms. Mareike Mentzel	Regulation of sHsp size distribution and activity by hetero-oligomerization
72	Mr. Goran Milicic	Single particle cryo-EM analysis of chaperonin:actin complex
73	Dr. Axel Mogk	Cellular aggregases maintain basal Hsp70 capacity ensuring balanced proteostasis
74	Dr. Francesca Moretti	Functional CRISPR screening identifies the ufmylation pathway as a regulator of SQSTM1/p62
75	Dr. Alexey Morozov	Hsp70 modulates proteasome activity and undergoes ubiquitin-independent degradation by the 20S proteasome.
76	Dr. Anne Kathrin Müller-Rischart	Cellular consequences of mitochondrial proteotoxic stress
77	Dr. Christian Münch	The mitochondrial unfolded protein response controls matrix translation
78	Dr. Nadinath Nillegoda	Mechanistic insights into aggregate solubilization by the human Hsp70-based protein disaggregase
79	Mr. Igor Obuchowski	Two-component sHsps bacterial system cooperation in counteracting irreversible aggregation and Hsp70/Hsp100-dependent efficient protein refolding
80	Ms. Miku Ohfurudono	Molecular mechanism of XBP1u translational pausing
81	Prof. Fernando Palhano	An ortho-Iminoquinone Compound Reacts with Lysine Inhibiting Aggregation while Remodeling Mature Amyloid Fibrils

82	Dr. David Parfitt	Regulation of the neuroprotective chaperone DNAJB2 in motor neurons
83	Prof. Sarah Perrett	Glutathionylation of DnaK provides a link between oxidative stress and the heat shock response
84	Dr. Blagovesta Popova	Posttranslational modifications of tyrosine residues modulate α-synuclein cytotoxicity and protein turnover in a yeast model of Parkinson´s Disease
85	Dr. Pablo Pulido	Chloroplast protein quality control mediated by DNAJ-like proteins
86	Ms. Lucia Quintana	Structural characterisation of the Hsp70:Hsp40:GR complex
87	Mr. Manish Rai	Understanding the molecular mechanism of adaptive thermotolerancy in *E.coli*
88	Dr. Namit Ranjan	Thio-Modification of tRNA at the Wobble Position as Regulator of the Kinetics of Decoding and Translocation on the Ribosome
89	Mr. Robert Rauscher	Silent mutations affect translational landscape and rescue CFTR folding mutations
90	Mr. Sandeep Raut	Identification of *Drosophila* chaperones regulating eye and neuromuscular junction morphology: the potential therapeutic targets for neurodegenerative diseases
91	Dr. Theo Rein	FKBP51 links to a novel pathway to autophagy by regulating Beclin1
92	Dr. Marisa Reverendo	Modulation of translation: a new player in dendritic cell function
93	Dr. Pia Roos-Mattjus	A small molecular compound modulating Heat Shock Factor activity
94	Dr. Eri Sakata	Structural insights into the ATPase cycle of the 26S proteasome
95	Ms. Grace Salsbury	Characterising the molecular chaperone systems involved in the defective trafficking of KCNQ1 channel complexes in the Long QT Syndrome
96	Mr. Reuben Saunders	An ER-localized J protein recruits BiP to repress Ire1 dimerization and the Unfolded Protein Response
97	Dr. Annika Scior	A chaperone complex consisting of Hsp70, a J-protein and Hsp110 prevents and reverses the formation of Htt amyloid fibrils
98	Dr. Ana Soares	A reporter assay to monitor protein aggregation *in vivo* in mammalian cells
99	Dr. Maria de la Encarnación Solesio Torregrosa	PolyP promotes protein aggregation to protect mitochondria against stress
100	Dr. Karen Stroobants	Mitochondrial membrane protein misfolding studied in S. cerevisiae as model system
101	Ms. Tamara Suhm	Mitochondrial translation governs cytoplasmic protein homeostasis
102	Dr. Gian Gaetano Tartaglia	Increase in protein abundance causes ribonucleoprotein granule formation
103	Ms. Isabelle Taylor	Identification of Selective Vulnerabilities in the Prostate Cancer Proteostasis Network
104	Dr. Patricija van Oosten-Hawle	The transcription factor PQM-1 is a novel regulator of proteostasis and mediator of transcellular chaperone signalling in *C. elegans*
105	Dr. Jacqueline van der Spuy	Mapping the mechanisms of retinal degeneration caused by mutations in the co-chaperone AIPL1
106	Mrs. Ana Sofia Varanda	Human cells cope with tRNA misexpression by activating Protein Quality Control mechanisms
107	Dr. Sandra Vidak	The Contribution of ER Stress to Premature Aging
108	Dr. Silvia Vilasi	Investigation on structural features of Hsp60, amateur chaperone in amyloid-beta fibrillogenesis
109	Mr. Jan Vonk	Oligomerization contributes to HSPB5 R120G and D109H aggregation and dysfunction
110	Ms. Rebeccah Warmack	The L-isoaspartate modification in a cytotoxic amylin peptide delays fibril formation and alters fibril structure
111	Ms. Katharina Weinhäupl	Molecular basis for chaperoning of inner mitochondrial membrane proteins across the intermembrane space
112	Prof. Konstanze Winklhofer	Linear ubiquitination protects from protein aggregate toxicity
113	Dr. Florian Wruck	Cotranslational chaperone action at the single-molecule level
114	Dr. Min Wu	Identification of PERK Branch (CHOP) Activators As Anti-Cancer Therapeutics
115	Mr. Xiao Yan	Characterizing a Novel Intermediate in the Reaction Cycle of the GroEL/ES Chaperonin
116	Dr. Masafumi Yohda	High resolution structural studies and direct visual observation of Hsp104 from Chaetomium thermophilum suggest the role of the spiral architecture
117	Dr. Jason Young	Quality Control Pathways of the hERG Potassium Channel
118	Dr. Nina Romanova	Identification of new human amyloidogenic proteins
119	Dr. Chiung-Wen (Mary) Chang	Structural Elements Regulating AAA+ Protein Quality Control Machines