The first Autumn School on Proteostasis: from molecular mechanisms to organismal consequences

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Accepted: 15 April 2019 / Published online: 9 May 2019
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Abstract

The first Autumn School on Proteostasis was held at the Mediterranean Institute for Life Sciences (MedILS) in Split, Croatia, from November 12th–16th, 2018, bringing together 44 graduate students and postdoctoral fellows and 22 principal investigators from around the world. This meeting was geared towards providing students with an in-depth understanding of the field of proteostasis, with the aim of broadening their perspectives of the field. Session topics covered multiple aspects of cellular and organismal proteostasis, including fundamental principles, responses to heat shock, aging and disease, and protein folding, misfolding, and degradation. The structure of the meeting and the restricted number of participants afforded the students and postdocs the opportunity to interact with principal investigators to discuss not only their latest research, but also their career prospects and progression in a close, supportive environment.

Keywords Proteostasis · Heat shock · Aging · Disease · Protein folding · Protein degradation

Introduction

In November 2017, a year before the first Autumn School on Proteostasis was held, a small group of young investigators from the Genie Network (Group of European New Investigators) came together for a meeting at MedILS to discuss proteostasis in aging and disease. It was here that

PROGENIE was created—PROteostasis Group of European New Investigators—and the idea for a school on proteostasis was born. The school was organized by Matthias J. Feige (Technical University of Munich), Ritwick Sawarkar (Max-Planck Institute of Immunology and Epigenetics, Freiburg), and Ruth Scherz-Shouval (Weizmann Institute of Science, Israel). The aim of this school was to provide students with access to experts in the field of proteostasis in an intimate setting where they would not only learn about the history of the field through review lectures, but also be introduced to the latest breakthroughs from the principal investigators themselves, all within a framework for networking and career development. The organizers received many more applications than anticipated. To keep the participant number optimal for informal discussions, the organizers had to select participants from among the applicants based on the letters of intent. Participants arrived from the USA, Canada, China, India, and almost all European countries. The main themes of the sessions included fundamental principles in proteostasis, proteostasis in aging and human disease, chaperone mechanisms, and protein degradation, with each session including review seminars focused on particular areas of proteostasis, and research seminars in which the principal investigators presented their unique area of interest. Beyond the main seminar sessions, there were also short talks from selected graduate students and postdoctoral fellows, a poster session, a career

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development panel, and round-table discussion groups covering specific topics of interest to the next generation of young scientists. Many of the sessions and discussions among participants and speakers were held in front of the impressive scenery of the Croatian coast side (Fig. 1). In addition, the participants experienced Uri Alon's (Weizmann Institute of Science, Israel) famous guitar talk "How to choose a good research project", which provided much inspiration and amusement. This report will provide an overview of the topics covered in both the review and research seminars across all sessions at the meeting.

Fundamental principles in proteostasis

The term "proteostasis" was first coined in a review published in *Science* in 2008, to describe the phenomenon of protein homeostasis (Balch et al. 2008). As defined in the opening sentence of this publication, "proteostasis refers to controlling the concentration, conformation, binding interactions...and location of individual proteins making up the proteome by readapting the innate biology of the cell...". Rick Morimoto (Northwestern University, Evanston, USA), one of the authors of this seminal review, opened the first session of the meeting

with a review seminar titled "The path to proteostasis", in which he expounded on the many aspects of the proteostasis field and the historical achievements that have given us the understanding we have today. Basic concepts such as protein folding landscapes, protein kinetics and stability, and the proteostasis boundary were covered, as well as proteostasis networks, chaperones, and the role of proteostasis in disease and aging (Hutt et al. 2009; Labbadia and Morimoto 2015b). The challenges faced by the cell to deal with off-pathway protein intermediates caused by mutation or damage, which are prone to misfolding and aggregation, are great, and there is much we still need to learn about how these pathways are regulated and the effects of stress and protein misfolding. While we are making headway in our understanding of these pathways in cells, for example in identifying regulators of the proteostasis network (Silva et al. 2011), organismal wide proteostasis through tissue communication is still largely unexplored and warrants future study.

Next, Miroslav Radman, director of the MedILS and host of the school, provided a historical and philosophical perspective on how cellular stress responses interact, impacting lifespan and aging. Dr. Radman's seminal discovery and characterization of the SOS response in *E. coli* in 1970 was one of

Fig. 1 Participants and speakers during the Autumn School on Proteostasis that took place at the spectacular coast of Split, Croatia. Photograph courtesy of Aleksandar Topalovic

the first examples of how genome integrity is influenced by epigenetic effects (what he referred to as the “smell of Lamarckism in a Darwinian world”). More recently, it is becoming increasingly clear that the interplay between the rate of protein synthesis, protein homeostasis, and oxidation modulate the cellular and organismal predisposition to disease and aging. For instance, levels of protein oxidation are increased by lowering transcriptional or translational fidelity (Dukan et al. 2000) as well as overall protein synthesis rates. In turn, the level of irreversible protein oxidation (carbonylation) dictates whether a bacterium develops a mutator or anti-mutation phenotype (Krisko and Radman 2013) and a functional degeneration similar to aging. Dr. Radman hypothesized that protein folding and protein oxidation are antagonistically competitive systems that mark and even determine the rate of aging.

The third speaker of this session was Uri Alon (Weizmann Institute of Science, Israel), who described the fascinating role of senescent cells in aging and disease (McHugh and Gil 2018). Stemming from the observation that genetically identical animals raised in the same conditions will die at different times, it was hypothesized that there are additional factors that contribute to age-related decline. One of these factors is the accumulation of senescent cells, which are normally cleared by the immune system, keeping their numbers low in young animals. However, as organisms age and more damage occurs, the production of senescent cells increases while their rate of removal is reduced due to overwhelming of the removal mechanisms, leading to accumulation in the body. To help the audience visualize this process, Dr. Alon described an organism as a city, constantly producing garbage, which, in a young organism, is cleared away by 100 garbage trucks—the cell’s proteostasis systems. However, with age, the city accumulates more and more trash, yet still has only 100 garbage trucks, leading to overwhelming of the system, cellular dysfunction, and death. Senescent cells are known to secrete pro-inflammatory signals, creating conditions of chronic low-grade inflammation, thus exacerbating age-related diseases such as cancer (Coppé et al. 2010). A major challenge in this field is to further understand the interplay between the immune system and levels of senescent cells, as the potential to direct the immune system may allow increased clearance during aging.

Proteostasis networks

Numerous cellular pathways function in an integrated manner to maintain the integrity of the proteome, thus forming the dynamic proteostasis network. This network functions to ensure that protein synthesis, folding, modification, targeting, and turnover are coordinated to ensure that proteins reach their cellular destinations both correctly folded and functional. As the internal or external environment of the cell changes in

response to a variety of stresses, the proteostasis network adapts, fine-tuning the protein quality control (PQC) response to prevent protein misfolding and aggregation. Loss of this balanced response can occur due to disease, genetic mutation, environmental exposures, and aging, allowing toxic misfolded protein or aggregates to accumulate.

One way to examine how the proteostasis network is rewired or imbalanced in stressed states is to use a systems approach, allowing a comprehensive view of this complex, dynamic network. Esti Yeger-Lotem (Ben-Gurion University of the Negev, Israel) described her lab’s approach aimed at elucidating the landscape of molecular chaperones across human tissues. Previously, using molecular interaction networks constructed by correlating tissue expression datasets for 16 different tissues and physical protein interactions, they examined how genes causal for hereditary diseases lead to phenotypes in a tissue-specific manner (Barshir et al. 2014). Though hereditary diseases generally manifest clinically in specific tissues (e.g., BRCA1 is associated with breast and ovarian cancer), most genes and interactions are globally expressed, leading to the question: if causal genes are expressed across most tissues, why do they only cause damage at specific sites? Dr. Yeger-Lotem’s group found that in merely 6% of disease, the causal gene is expressed only in diseased tissue, while in 30% of disease, the causal gene is upregulated, and in another 25% of disease, the causal gene has a disease-related tissue-specific partner. The genotype tissue-expression (GTEx) project analyzed genomic sequences and performed RNA-seq on over 50 tissues sampled from 900 donors to create an atlas of healthy tissues (Aguet et al. 2017; Basha et al. 2018). Using these data, Yeger-Lotem’s group pointed to the role of paralogs of causal genes in determining the diseased tissue (Barshir et al. 2018). This leads to the second question that was addressed, which is how chaperones are organized across human tissues. By knocking out individual chaperones and measuring cell growth, it becomes obvious that chaperones have a higher impact on growth than other protein-coding genes. “Core” chaperones, those whose expression is relatively stable across tissues, are less associated with disease, while “variable” chaperones, whose expression levels and tissue specificity are distinct, are more often related to disease (Netta Shemesh et al., unpublished data). For more resources allowing examination of quantitative relationships in chaperone networks, see ProteinNet, TissueNet, and ChaperoneNet (<http://netbio.bgu.ac.il/chapnet/>) via the Yeger-Lotem website (<http://netbio.bgu.ac.il/labwebsite/>).

Uri Alon (Weizmann Institute of Science, Israel) redirected focus to the molecular level. After bringing meeting participants outside to enjoy the Croatian coastline, he began an informal review of molecular recognition, challenging the audience to consider the varied historical models describing how enzyme and substrate specificity is achieved within a noisy biochemical environment. Early models described a rigid

lock-and-key relationship (Fischer 1894), but molecular recognition usually involves conformational changes; this is likely due to a gain in specificity in which enzymes utilize a hindrance barrier (Koshland Jr. 1958), meaning they sacrifice affinity for their correct ligand in order to increase the barrier for binding of the wrong ligand. For example, the DNA-binding protein, RecA, stretches DNA as it scans it to find an appropriate binding site; while not optimal for RecA binding, this induced-fit in which the protein changes conformation upon binding requires the protein to bend, consuming energy, thus decreasing binding (Savir and Tlusty 2007, 2010). False substrates are excluded from enzyme binding as it requires too much free energy. Kinetic proofreading, described by Hopfield in the 1970s (Hopfield 1974), achieves high fidelity binding beyond the level expected from the free-energy difference between correct and incorrect interactions. For example, tRNA binding to complementary mRNA occurs correctly 100 times more than predicted. This increased accuracy is due to a unidirectional intermediate step in which GTP must be converted to GDP before an amino acid can be incorporated into the peptide chain. Once bound, there is a race to complete GDP phosphorylation before falling off, creating a filter in which only those tRNAs which stay bound long enough to complete the intermediate step ultimately contribute to protein synthesis. Another form of specificity described in the 1970s by Savageau (Savageau 1974) addresses why certain sites use activators versus repressors to regulate genes. While both approaches prevent non-specific binding, high-demand genes are generally bound by activators which need only to fall off to cause repression while low-demand genes are bound by repressors, thus requiring loss of binding for activation. In essence, each type of regulation uses the least-demanding mechanism to achieve the desired goal.

Rick Morimoto (Northwestern University, Evanston, USA) presented an overview of a vital part of the proteostasis network: the heat shock response (HSR), originally observed in *Drosophila* in the 1960s (Ritossa 1962). Description of the induction of heat shock proteins in *Drosophila*, such as Hsp70, by Lindquist, Henikoff, and Meselson (Lindquist McKenzie et al. 1975) in the 1970s was later shown to be conserved in humans by Morimoto (Hunt and Morimoto 1985; Wu et al. 1985) who went on to describe heat shock elements (HSEs) which are targeted by the HSF transcription factor family upon heat shock or stress (Abravaya et al. 1991). Much of Morimoto's recent research has used *C. elegans* to study the HSR, applying genetics on an organismal level. He showed that neuronal signaling via AFD thermosensory neurons coordinates an organismal heat shock response in a non-cell-autonomous manner in multiple somatic tissues in animals exposed to environmental stress, while downregulation of neuronal AFD signaling restored the cell autonomous response to chronic proteotoxic stress (Gidalevitz et al. 2006; Prahlad et al. 2008). This finding prompted the question, does

a misfolded protein in a specific tissue evoke a systemic response? Using temperature-sensitive (ts) myosin, Morimoto was able to induce an organismal heat shock response, thus identifying transcellular chaperone signaling, which coordinates feedback between somatic tissues to regulate organismal proteostasis. Overexpression of Hsp90 in distant tissues suppresses ts-myosin misfolding in muscle, showing that information is being sent to damaged tissues from intact tissues (van Oosten-Hawle et al. 2013). Another question Morimoto addressed is whether the decline of proteostasis in aging is gradual or sudden. Protein misfolding occurring in early adulthood in *C. elegans* causes induction of heat shock genes; however, upon reproductive maturity, there is an abrupt decrease in the HSR, apparently due to decreased chromatin accessibility of HSF and RNA polymerase. Increased expression of the demethylase, JMJD3.1, or ablation of the germline rescues this effect. In fact, JMJD3.1 overexpression can rescue age-associated declines in the UPR, UPR^{mito}, and damage caused by oxidative stress. Induction of mild mitochondrial stress, either through knockdown of OXPHOS proteins or pathogen exposure can repress this age-related decline in HSR, presumably through hormesis (Labbadia et al. 2017). Thus, proteostasis collapse appears to be controlled by a precise genetic switch activated upon reproductive maturity (Labbadia and Morimoto 2015a).

Proteostasis in aging

The next session was opened with a review of the collapse of proteostasis during aging, by Rebecca Taylor (MRC Laboratory of Molecular Biology, UK). As organisms age, various facets of the proteostasis network fail, including protein folding, aggregation clearance, and protein degradation, while the cellular stress responses concurrently fail to activate, leading to loss of homeostasis and cellular dysfunction (Taylor and Dillin 2011). However, research has shown that the aging process can be modulated by various means, such as caloric intake restriction (Donati et al. 2008), alterations in the insulin signaling pathway (Bartke 2008), and via changes in the metabolic and respiratory rate (Dillin et al. 2002). In addition, the activation of the unfolded protein response in the endoplasmic reticulum (UPR^{ER}) in response to stress, particularly in neurons, can influence other cells and tissues within an organism, rescuing the loss of ER stress resistance that is associated with aging and leading to increased lifespan (Imanikia et al. 2017).

Miroslav Radman approached the question on the role of proteostasis in aging from the perspective of maintenance of genomic integrity, which deteriorates as organisms age. The bacteria *Deinococcus radiodurans* remarkably survives complete desiccation and high exposures to ionizing radiation. In both conditions, the bacteria are able to reassemble their genome from thousands of pieces with extreme fidelity (Zahradka et al. 2006). The high level of protection is not

due to decreased susceptibility of the DNA to break or to a novel or more efficient ability to repair DNA. Instead, the unique survival ability is due to an extremely efficient anti-oxidation system, able to prevent carbonylation, i.e., irreversible proteome oxidation (Krisko and Radman 2010). Carbonylation increases rapidly within an hour of ionizing radiation, staying at a high level for roughly 8 h. After this, a new wave of carbonylation ensues at which point massive bacteria death is observed. Dr. Radman left the audience to discuss the potential mechanism behind of this second wave of cellular death.

The session was closed by Anita Krisko (MedILS) working in the Molecular Basis of Aging Lab. Her work focuses on the crosstalk between metabolism and proteostasis, trying to elucidate the molecular basis for the phenomenon of heat-induced hormesis. While it is commonly assumed that the favorable effects of mild heat shock in the replicative lifespan in yeast are due to the increased expression of chaperones, Dr. Krisko showed compelling evidence that in fact, metabolic changes are necessary for these beneficial outcomes. Work in her lab shows that the phenotype cannot be recapitulated by single genetic interventions impacting either the respiratory chain, protein synthesis, or glutathione reduction, nor by mutants that show constitutive induction of the heat-shock response. Moreover, proteostasis and metabolism are known to interact through pleiotropic mechanisms mediated by TORC1 and Hsp90 (Gopinath and Leu 2016; Perić et al. 2017). Taken together, Dr. Krisko's work suggests that global metabolic reprogramming is required to achieve lifespan extension in yeast (Musa et al. 2018).

Proteostasis in human disease

Tumors are notoriously stressful environments, with the cancer cells as well as the cells of the surrounding microenvironment experiencing oxidative stress (Sosa et al. 2013), proteotoxic stress (Martin et al. 2015), and nutrient deprivation (Reid and Kong 2013). As described by Ruth Scherz-Shouval (Weizmann Institute of Science) in her review seminar titled “The Chaperoning of Cancer”, the stress response activated in the cells of the microenvironment, specifically cancer-associated fibroblasts (CAFs), is coordinated by HSF1, leading to the expression of various chaperones which promote tumor survival, evolution, and malignancy (Scherz-Shouval et al. 2014). One of the downstream outcomes of activated HSF1 is the promotion of extracellular matrix (ECM) remodeling, which precedes tumor growth and promotes an anti-inflammatory wound healing response. Loss of HSF1 by genetic manipulation leads to CAFs that are impaired in their ability to secrete ECM, thus attenuating tumor formation and development.

Paul Workman's team (The Institute of Cancer Research, London, UK) discovered a small-molecule bisamide

compound that potently inhibits the HSF1-pathway, and further medicinal chemistry optimization yielded a chemical probe (CCT251236) with improved solubility and oral availability. CCT251236 also limited the growth of a human ovarian carcinoma xenograft in mice (Cheeseman et al. 2017). In the same study, pirin was identified as a specific target and was crystallized in complex with CCT251236. Expression of HSF1 and its target genes was shown to be associated with poorer myeloma patient survival. In addition, shRNA-mediated knockdown of HSF1 or pharmacologic inhibition of the HSF1-pathway reduced viability and induced apoptosis in human myeloma cells, and CCT251236 inhibited the growth of a human myeloma xenograft (Fok et al. 2018). A pharmaceutically enhanced derivative CCT251236 is scheduled for clinical trial during 2020, targeting ovarian cancer and myeloma as the primary disease indications. It will be exciting to follow further studies of the Workman lab which will focus on the molecular mechanism of action and likely add to our general understanding of the HSF1-pathway.

The day ended with an atypical “guitar talk” by Uri Alon (Weizmann Institute of Science, Rehovot, Israel) focused on the human aspect of research and publication. The talk's aim was to fuel an open conversation around the effects that research has on scientists as human beings. A number of self-imposed stereotypes and idealizations around the way we perform our research were analyzed and broken down through a dialogue lead by Uri but centered on the audience members and their contributions. Topics like being scooped, the peer-review process, and the importance of mentorship were each first addressed with a song by Uri then discussed with the audience (Levy-Tzedek et al. 2018). The musical introduction serves two important purposes: to loosen the inhibitions around discussing these difficult topics with akin-minded strangers, and to move the focus away from the strictly objective realm of scientific endeavor towards the subjective experience of the scientist's life (more topics can be found here (Alon 2010; Lahav 2010)). The guitar talk was a great way to end the first day of the first Autumn School on Proteostasis, as it set a positive, open tone to the scientific dialogue for the rest of the conference.

Organelle proteostasis

Proper organelle function is vital for cellular homeostasis. This relies upon maintenance of precise protein folding, maturation, and degradation in all distinct cellular compartments, such as the endoplasmic reticulum (ER) and mitochondria.

Anne Bertolotti (MRC Laboratory of Molecular Biology, UK) began with a broad molecular and historical overview of the ER stress response in her first lecture before moving on to specifics of her current research. The ER unfolded protein response (UPR^{ER}) is a highly conserved process activated by the presence of unfolded

or misfolded proteins in the lumen of the ER which acts to ensure that these proteins are not released into the cell or its environment. Upon activation, translation is slowed, giving the protein-folding machinery extra time to focus on refolding proteins or to target them for degradation. A complex signaling pathway is engaged and molecular chaperone expression is increased. Together, these components act to increase the ER protein quality control (PQC) capacity, restore proteostasis, and avoid death. When it was discovered that folding mutants, but not loss-of-function mutants of hemagglutinin (HA) were retained in the ER (Gething et al. 1986), one of the first founding principles of UPR^{ER} was established—that mechanisms exist to identify and retain misfolded proteins in the ER. The next fundamental discovery in understanding the UPR^{ER} was that misfolded proteins retained in the ER are bound by BiP (Bole et al. 1986). BiP/GRP78 was found to be a HSP-like protein (Munro and Pelham 1986) acting in concert with other newly discovered ER-resident HS proteins, such as GRP94/HSP90 (Sorger and Pelham 1987). Forced expression of misfolded HA in the ER was sufficient to induce expression of BiP (Kozutsumi et al. 1988), proving that a mechanism to monitor events in the lumen of ER was able to transduce signals across the lipid bilayer of ER to the nucleus. Studies in yeast further fleshed out the UPR^{ER}, identifying ER chaperone homolog to BiP (Normington et al. 1989), the UPR DNA-binding element (UPRE) (Mori et al. 1992; Kohno et al. 1993), and ER-to-nucleus signaling components, the kinase, Ern1/Ire1 (Kohno et al. 1993; Cox et al. 1993), later shown to be conserved in mammals (Tirasophon et al. 1998; Wang et al. 1998), as well as the transcription factor, HAC1 (Cox and Walter 1996; Mori et al. 1996). UPR sensing occurs when BiP, bound to Ire1 or PERK, is released causing dimerization of Ire1 and PERK (Bertolotti et al. 2000; Carrara et al. 2015). BiP also regulates via ATF6 (Haze et al. 1999) and XBP1 (Calton et al. 2002). The result of this signaling, increased ER chaperone expression and translation attenuation via phosphorylation of eIF2 α , decreases the biosynthetic load in a process Dr. Bertolotti describes as a coordinated translation and transcriptional reprogramming response.

Building upon the foundation she provided, Anne Bertolotti then went on to describe the identification of factors that help cells survive PQC failure that occurs during protein misfolding in neurodegenerative disease. Phosphorylation of eIF2 α slows translation, giving quality control systems extra time to respond to misfolded proteins, thus increasing the cell's repair capacity (Tsaytler et al. 2011). Identification of Guanabenz (GBZ), an α -2 adrenergic agonist which reversibly inhibits eIF2 α dephosphorylation by binding the PP1C phosphatase regulatory subunit, R15A, allowed fine-tuning of the rate of protein

synthesis, attenuating translation and increasing PQC capacity. A more specific R15A-binding compound, Sephin1, similarly increases PQC capacity and protects mice suffering from ER-associated misfolding diseases (Das et al. 2015). Dr. Bertolotti argued for the potential of targeting phosphatases as previously underexploited drug targets, via selectivity that can be achieved by targeting their regulatory subunits. A drug discovery effort identified Raphin1, which selectively targets the more ubiquitously expressed PP1C regulatory subunit, R15B, rendering it susceptible to degradation and causing a reversible pause in translation because of an R15A-dependent feedback loop. Raphin1 was able to increase PQC capacity to target cytosolic aggregates, such as huntingtin polyQ proteins (Krzyzosiak et al. 2018). Mechanistically, R15 inhibition provides the same benefit with R15A being useful for proteostatic defects in the ER and R15B in other cellular compartments. By prolonging eIF2 α phosphorylation and pausing translation, R15B inhibition increases chaperone capacity to repair misfolded proteins, enhancing proteostasis and overcoming protein misfolding stress in cell culture and in mice. Dr. Bertolotti has since founded CAMPHOS Therapeutics, aimed at identifying selective phosphatase inhibitors for use in treating human disease.

Christian Münch (Goethe University Frankfurt, Germany) provided an overview of the mitochondrial unfolded protein response (UPR^{mt}), a conserved pathway activated by mitochondrial protein misfolding and regulated by mitochondrial-to-nuclear signaling (Münch 2018). Early studies showed that either dysregulation of the mitochondrial genome or accumulation of misfolded protein in the matrix resulted in nuclear upregulation of mitochondrial chaperonins (Martinus et al. 1996; Zhao et al. 2002). General protein quality control in the mitochondria is achieved by numerous mitochondrial targeted chaperones (Hsp60, Hsp70, Hsp90) which attempt to refold proteins, and matrix proteases (ClipXP, Lon) which can degrade proteins that fail to fold properly. Activation of the UPR^{mt} triggers metabolic adaptations and mitochondrial biogenesis to promote cellular survival and has been shown to increase lifespan in *C. elegans* (Durieux et al. 2011; Shpilka and Haynes 2018). Dr. Münch stressed the need and difficulty in differentiating between the integrated stress response (ISR), the UPR^{ER}, and UPR^{mt} and further described multiple axes of the UPR^{mt}; these include the canonical UPR^{mt} axis, the UPR^{mt} sirtuin axis, the UPR^{IMS}/ER α axis, and the UPR^{mt} translation axis. This multi-axis response is likely mediated by multiple pathways with complex and incompletely defined molecular mechanisms. Together, these integrated pathways lead to distinct effects including chaperone induction, antioxidant response, translation inhibition, and enhanced protease activity with the ultimate goal of increasing the PQC capacity and promoting cell survival (Münch; Münch and Harper 2016).

Cellular principles of folding and degradation

The next session was opened by a review seminar on the role of chaperones during development by Anat Ben-Zvi (Ben Gurion University on the Negev, Israel). Dr. Ben-Zvi first compared the chaperoning systems between prokaryotes and eukaryotes, highlighting the divergent roles of two sets of chaperones in higher organisms, one set dedicated to stress-induced proteotoxicity and a second set of constitutively active cytosolic chaperones called CLIPS (Albanèse et al. 2006). The divergence of chaperone systems continued through evolution, with complex organisms developing overlapping but distinct chaperone sets that act independent of stress and whose pattern of expression is tissue-specific and dynamic in time. The family of the mammalian heat shock factor (HSF) exemplifies the complex interaction between chaperone programs. Different HSFs are required for organismal development, albeit at different stages, but the requirement for the HSF in development does not correlate with the stress-induced heat shock response. Moreover, it is clear that chaperone expression is controlled multi-factorially as it cannot be recapitulated fully by the expression levels or activity of the HSF family members (Åkerfelt et al. 2010). Finally, the cell differentiation process during development includes the regulation of chaperone levels, illustrated by the work from Dr. Ben-Zvi and her lab on the key role of HLH1/MyoD in muscle development in *C. elegans* (Bar-Lavan et al. 2016).

Stress granules (SGs) are phase-separated liquid condensates in the cell cytoplasm induced by stress. SG components include prion-like proteins that are inherently prone to aggregate and genetically linked to neurodegenerative diseases (Patel et al. 2015). In her seminar, Serena Carra (Università degli Studi di Modena e Reggio Emilia, Italy) showed that misfolded proteins and defective ribosomal products (DRiPs) are sequestered into the SG phase and drive the formation of aberrant solid-like SGs (Mateju et al. 2017). Dr. Carra's lab further found that the HSPB8-BAG3-HSP70 chaperone complex ensures the proteostasis of the granules (thus termed "granulostasis") and targets DRiPs for degradation (Ganassi et al. 2016). In a parallel attempt, the group focused on small heat shock proteins and found that HspB2 is able to undergo aberrant phase separation in the nucleus of myoblasts thereby displacing chromatin and sequestering Lamin A leading to nuclear disintegration (Morelli et al. 2017). This process was triggered by overexpression of HspB2 but prevented by binding of HspB3 to HspB2 in the cytoplasm. Intriguingly, two mutants of HspB3 that are associated with myopathy limit the interaction with HspB2 and drive HspB2 condensate formation.

The session was closed by Anton Khmelinskii (IMB Mainz, Germany) who provided a comprehensive overview of methods used to study protein degradation in vivo. The development of these methods began as early as the 1930s,

with the discovery of deuterium and ^{15}N paving the way for the first metabolic labelling techniques (pulse-chase) using deuterated fatty acids and ^{15}N -labeled amino acids. Since then, sophisticated methods have been developed permitting the analysis of protein dynamics in cells, such as pulse-chase SILAC (Fierro-Monti et al. 2013; Simon and Kornitzer 2014; Terzi and Cambridge 2017), global protein stability profiling (Yen et al. 2008) and the application of fluorescent protein timers (Khmelinskii and Knop 2014) just to name a few. The remarkable array of techniques currently available has undoubtedly facilitated many groundbreaking discoveries and will continue to be indispensable in the future.

Chaperone mechanisms

Johannes Buchner (Technische Universität München, Germany) gave two remarkably comprehensive seminars about the discovery and characteristics of molecular chaperones. The heat shock response first reported by Ritossa in *Drosophila melanogaster* (Ritossa 1962) leads to upregulation of heat shock proteins (Tissières et al. 1974). Later on, the term "molecular chaperone" was coined by Laskey for the role of nucleoplasm in histone assembly (Laskey et al. 1993). Buchner reminded the audience of the intrinsic instability of proteins that leads to dynamics and flexibility, which are crucial for protein function. This comes with the cost of an aggregation risk that is minimized by chaperones, which may also explain the great variety of chaperone systems that developed many times during evolution and may have co-evolved with their clients. Buchner went on to illustrate the main groups of molecular chaperones in the cell ranging from Hsp70s, Hsp90s, small heat shock proteins, chaperonins, and Hsp100s (Richter et al. 2010). The broad chaperone spectrum makes a strict definition of the term difficult. For example, Hsp12 stabilizes the cell membrane without binding to proteins, thus ruling out proteins as the sole clients of chaperones (Welker et al. 2010). Polyphosphate can act as a primordial chaperone, being an example of a non-proteinaceous chaperone molecule (Gray et al. 2014). Also, some clients are bound to chaperones most of their lifetime, like kinases to Hsp90 (Boczek et al. 2015). This leads to the rather general conclusion that a chaperone is a cellular factor that stabilizes an unfolded or pre-folded client while not being part of the final and functional complex. Buchner went on to introduce the Hsp90 machinery and small heat shock proteins in more detail as these systems are extensively studied in his lab. Hsp90 is a ubiquitous chaperone ensuring the folding of numerous kinases, E3 ligases, and transcription factors (Schopf et al. 2017), which positions it at a central hub of cellular control. This way, Hsp90 adds an additional layer of complexity to the regulation of its clients by tuning their conformations and enabling them to be responsive to stress. Hsp90 undergoes a remarkable ATP-dependent conformational cycle

which is influenced by the coordinated binding and release of a cohort of dedicated co-chaperones (Sahasrabudhe et al. 2017). Another area of Buchner's research interest is the field of small heat shock proteins (Haslbeck et al. 2018). These ATP-independent chaperones form dynamic oligomeric assemblies able to stabilize their substrates in a refolding-competent state, ready to be refolded by the Hsp70 system following stress release. The two elaborate seminars given by Dr. Buchner left the audience well prepared for diving deep into chaperone research.

The last talk of the session was given by Matthias Feige (Technical University of Munich, Munich, Germany). The talk reviewed the current knowledge and the latest advances in the understanding of the regulation of secretory pathway proteins. Protein trafficking through the ER has dedicated sets of chaperones (e.g., BiP) and co-chaperones, as well as specialized quality control systems and stress responses. The fascinating question is how are all of these activities coordinated within the time frame between translation and secretion? The question is especially important in the context of the immune system, as three major classes of proteins are all trafficking through the ER, (1) antibodies, (2) immune receptors, and (3) interleukins. The heavy chain of antibodies helped unveil fundamental paradigms of how folding is used to control complex assembly within the ER. Unlike other heavy chain protein domains, the C_H1 domain of the antibodies remains in a partially unfolded state. The latter state is bound by the chaperone BiP blocking its secretion and slowing down its ERAD-mediated degradation. The C_H1 domain only folds when BiP binding is outcompeted by the light chain C domain, after which the antibody is secreted. The coupling between binding and folding events ensures that only complete antibody complexes are secreted (Feige et al. 2009). Moreover, chaperones within the ER fall into two classes, one class that binds sites dispersed through the antibody domains and another class that evolved to bind specific sequences. Interestingly, these sequences are both pro-degradation and prone to aggregation, but in the fully folded state, the sequences are buried inside the protein (Behnke et al. 2016). Hence, the chaperones co-evolved with their binding sequence, optimizing the quality control for these molecules.

Despite extensive research, it remains a mystery how the cell manages to prevent misfolding and aggregation in this crowded environment while ensuring the productive assembly of protein complexes. In his illuminating seminar, **Bernd Bukau** (Universität Heidelberg, Germany) explained how cells cope with this intricate challenge. His group found that in prokaryotes the efficiency of complex assembly strongly decreases when the subunits are synthesized from separate mRNAs, showing that the process seems directly coupled to translation (Shieh et al. 2015). These findings add another

layer of complexity to the operon originally found to coordinate transcriptional regulation. Using ribosome profiling, Dr. Bukau's group could further show that while being released from the exit tunnel, the nascent chain is bound by ribosome-associated chaperones like trigger factor (TF) and DnaK, preventing unproductive interactions and aberrant folding. Interestingly, while TF binds early to the nascent chain, DnaK comes in later and stays until full folding is achieved, revealing a hierarchy of chaperone factors in the process. In eukaryotes, where polycistronic mRNAs are rare, the question arises whether there is a similar link between complex assembly and translation. Using ribosome profiling, Bukau's laboratory found that nine out of 12 protein complexes in *S. cerevisiae* assembled co-translationally with the three exceptions being substrates of specialized, dedicated chaperones (Shiber et al. 2018). The primary synthesized subunit stabilizes the subsequent one, thereby preventing aggregation. Like TF and DnaK in prokaryotes, the eukaryotic chaperone Ssb transiently interacts with the newly synthesized subunit and dissociates upon establishment of productive subunit interactions to prevent aberrant subunit association. These results suggest that eukaryotic translation, protein folding, and complex assembly are strictly connected (Kramer et al. 2019). Dr. Bukau went on to explain how yeast reacts to an overload upon enduring unfolding stress. Hsp42 uses its prion-like domain to bind and sequester unfolded substrates in dedicated organelles termed CytoQ's (Boczek and Alberti 2018; Grousl et al. 2018). These molecular "garbage dumps" can subsequently be recycled upon stress release by the Hsp100 machinery, which is an ATP-dependent pore that is able to lift back unfolded proteins in a refolding competent state via a threading mechanism (Mogk et al. 2018).

Analyzing global proteostasis

The final session of the meeting, on the topic of using proteomics to study proteostasis, was dedicated to an overview of mass spectrometry theory and methods that are a cornerstone of many protein analysis techniques today. This seminar, given by Christian Münch (Goethe University, Frankfurt, Germany), covered the basics of how mass spectrometers work, with a focus on the Orbitrap technology (Eliuk and Makarov 2015). Quantitative mass spectrometry techniques are now the gold standard for large-scale proteomic analysis, with tools and applications readily available for many aspects of proteostasis, for example ubiquitin signaling (Ordureau et al. 2015) and other protein degradation mechanisms (Savitski et al. 2018).

Conclusions and perspectives

The first Autumn School on Proteostasis provided seminal insights into the field for all participants. However, even more

importantly, it enabled everyone to integrate their own research into the work of the community of scientists that all strive together to solve big questions. This very special and warm meeting strongly facilitated exchange and friendship between the participants and the speakers and is likely to become a cornerstone for the proteostasis community. Indeed, this meeting has already inspired a pre-workshop for junior scientists at the upcoming EMBO workshop on Protein Quality Control in April 2019, which will introduce PhD students and post-docs to various aspects of proteostasis with the aim of encouraging dialogue about the future of this field.

Funding information Funding for the first Autumn School on Proteostasis was provided by Leica, Zeiss, the German Society for Biochemistry and Molecular Biology (GBM), and the Cell Stress Society International.

References

- Abravaya K, Phillips B, Morimoto RI (1991) Heat shock-induced interactions of heat shock transcription factor and the human hsp70 promoter examined by in vivo footprinting. Downloaded from. *Mol Cell Biol* 11:586–592
- Aguet F, Brown AA, Castel SE et al (2017) Genetic effects on gene expression across human tissues. *Nature* 550:204–213. <https://doi.org/10.1038/nature24277>
- Åkerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. *Nat Rev Mol Cell Biol* 11:545–555. <https://doi.org/10.1038/nrm2938>
- Albanèse V, Yam AY-W, Baughman J, Parnot C, Frydman J (2006) Systems analyses reveal two chaperone networks with distinct functions in eukaryotic cells. *Cell* 124:75–88. <https://doi.org/10.1016/j.cell.2005.11.039>
- Alon U (2010) How to build a motivated research group. *Mol Cell* 37:151–152. <https://doi.org/10.1016/j.molcel.2010.01.011>
- Balch WE, Morimoto RI, Dillin A, Kelly JW (2008) Adapting proteostasis for disease intervention. *Science* 319:916–919. <https://doi.org/10.1126/science.1141448>
- Bar-Lavan Y, Shemesh N, Dror S, Ofir R, Yeger-Lotem E, Ben-Zvi A (2016) A differentiation transcription factor establishes muscle-specific proteostasis in *Caenorhabditis elegans*. *PLoS Genet* 12:e1006531. <https://doi.org/10.1371/journal.pgen.1006531>
- Barshir R, Shwartz O, Smoly IY, Yeger-Lotem E (2014) Comparative analysis of human tissue interactomes reveals factors leading to tissue-specific manifestation of hereditary diseases. *PLoS Comput Biol* 10:e1003632. <https://doi.org/10.1371/journal.pcbi.1003632>
- Barshir R, Hekselman I, Shemesh N, Sharon M, Novack L, Yeger-Lotem E (2018) Role of duplicate genes in determining the tissue-selectivity of hereditary diseases. *PLoS Genet* 14:e1007327. <https://doi.org/10.1371/journal.pgen.1007327>
- Bartke A (2008) Insulin and aging. *Cell Cycle* 7:3338–3343. <https://doi.org/10.4161/cc.7.21.7012>
- Basha O, Shpringer R, Argov CM, Yeger-Lotem E (2018) The DifferentialNet database of differential protein-protein interactions in human tissues. *Nucleic Acids Res* 46:D522–D526. <https://doi.org/10.1093/nar/gkx981>
- Behnke J, Mann MJ, Scruggs F-L, Feige MJ, Hendershot LM (2016) Members of the Hsp70 family recognize distinct types of sequences to execute ER quality control. *Mol Cell* 63:739–752. <https://doi.org/10.1016/j.molcel.2016.07.012>
- Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D (2000) Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat Cell Biol* 2:326–332. <https://doi.org/10.1038/35014014>
- Boczek EE, Alberti S (2018) One domain fits all: using disordered regions to sequester misfolded proteins. *J Cell Biol* 217:1173–1175. <https://doi.org/10.1083/jcb.201803015>
- Boczek EE, Reefschläger LG, Dehling M, Struller TJ, Häusler E, Seidl A, Kaila VRI, Buchner J (2015) Conformational processing of oncogenic v-Src kinase by the molecular chaperone Hsp90. *Proc Natl Acad Sci* 112:E3189–E319. <https://doi.org/10.1073/pnas.1424342112>
- Bole DG, Hendershot LM, Kearney JF (1986) Posttranslational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. *J Cell Biol* 102:1558–1566
- Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, Clark SG, Ron D (2002) IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 415:92–96. <https://doi.org/10.1038/415092a>
- Carrara M, Prisch F, Nowak PR, Kopp MC, Ali MMU (2015) Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein CH1 to initiate ER stress signaling. *Elife* 4:e03522. <https://doi.org/10.7554/eLife.03522.001>
- Cheeseman MD, Chessum NEA, Rye CS, Pasqua AE, Tucker MJ, Wilding B, Evans LE, Lepri S, Richards M, Sharp SY, Ali S, Rowlands M, O'Fee L, Miah A, Hayes A, Henley AT, Powers M, te Poele R, de Billy E, Pellegrino L, Raynaud F, Burke R, van Montfort RLM, Eccles SA, Workman P, Jones K (2017) Discovery of a chemical probe bisamide (CCT251236): an orally bioavailable efficacious pirin ligand from a heat shock transcription factor 1 (HSF1) phenotypic screen. *J Med Chem* 60:180–201. <https://doi.org/10.1021/acs.jmedchem.6b01055>
- Coppé J-P, Desprez P-Y, Krtolica A, Campisi J (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol Mech Dis* 5:99–118. <https://doi.org/10.1146/annurev-pathol-121808-102144>
- Cox JS, Walter P (1996) A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* 87:391–404
- Cox JS, Shamu CE, Walter P (1993) Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* 73:1197–1206. [https://doi.org/10.1016/0092-8674\(93\)90648-A](https://doi.org/10.1016/0092-8674(93)90648-A)
- Das I, Krzyzosiak A, Schneider K, Wrabetz L, D'Antonio M, Barry N, Sigurdardottir A, Bertolotti A (2015) Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science* 348:239–242. <https://doi.org/10.1126/science.aaa4484>
- Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C (2002) Rates of behavior and aging specified by mitochondrial function during development. *Science* 298:2398–2401. <https://doi.org/10.1126/science.1077780>
- Donati A, Recchia G, Cavallini G, Bergamini E (2008) Effect of aging and anti-aging caloric restriction on the endocrine regulation of rat liver autophagy. *J Gerontol - Ser A Biol Sci Med Sci* 63:550–555. <https://doi.org/10.1093/gerona/63.6.550>
- Dukan S, Farewell A, Ballesteros M, Taddei F, Radman M, Nystrom T (2000) Protein oxidation in response to increased transcriptional or translational errors. *Proc Natl Acad Sci* 97:5746–5749. <https://doi.org/10.1073/pnas.100422497>
- Durieux J, Wolff S, Dillin A (2011) The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* 144:79–91. <https://doi.org/10.1016/j.cell.2010.12.016>
- Eliuk S, Makarov A (2015) Evolution of Orbitrap mass spectrometry instrumentation. *Annu Rev Anal Chem* 8:61–80. <https://doi.org/10.1146/annurev-anchem-071114-040325>

- Feige MJ, Groscurth S, Marcinowski M, Shimizu Y, Kessler H, Hendershot LM, Buchner J (2009) An unfolded CH1 domain controls the assembly and secretion of IgG antibodies. *Mol Cell* 34: 569–579. <https://doi.org/10.1016/j.molcel.2009.04.028>
- Fierro-Monti I, Racle J, Hernandez C, Waridel P, Hatzimanikatis V, Quadroni M (2013) A novel pulse-chase SILAC strategy measures changes in protein decay and synthesis rates induced by perturbation of proteostasis with an Hsp90 inhibitor. *PLoS One* 8:e80423. <https://doi.org/10.1371/journal.pone.0080423>
- Fischer E (1894) Einfluss der Configuration auf die Wirkung der Enzyme. *Ber Dtsch Chem Ges* 27:2985–2993. <https://doi.org/10.1002/cber.18940270364>
- Fok JHL, Hedayat S, Zhang L, Aronson LI, Mirabella F, Pawlyn C, Bright MD, Wardell CP, Keats JJ, De Billy E, Rye CS, Chessum NEA, Jones K, Morgan GJ, Eccles SA, Workman P, Davies FE (2018) HSF1 is essential for myeloma cell survival and a promising therapeutic target. *Clin Cancer Res* 24(10):2395–2407. <https://doi.org/10.1158/1078-0432.CCR-17-1594>
- Ganassi M, Mateju D, Bigi I, Mediani L, Poser I, Lee HO, Seguin SJ, Morelli FF, Vinet J, Leo G, Pansarasa O, Cereda C, Poletti A, Alberti S, Carra S (2016) A surveillance function of the HSPB8-BAG3-HSP70 chaperone complex ensures stress granule integrity and dynamism. *Mol Cell* 63:796–810. <https://doi.org/10.1016/j.molcel.2016.07.021>
- Gething M-J, McCammon K, Sambrook J (1986) Expression of wild-type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. *Cell* 46:939–950. [https://doi.org/10.1016/0092-8674\(86\)90076-0](https://doi.org/10.1016/0092-8674(86)90076-0)
- Gidalevitz T, Ben-Zvi A, Ho KH, Brignull HR, Morimoto RI (2006) Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science* 311:1471–1474. <https://doi.org/10.1126/science.1124514>
- Gopinath RK, Leu J-Y (2016) Hsp90 maintains proteostasis of the galactose utilization pathway to prevent cell lethality. *Mol Cell Biol* 36: 1412–1424. <https://doi.org/10.1128/MCB.01064-15>
- Gray MJ, Wholey W-Y, Wagner NO, Cremers CM, Mueller-Schickert A, Hock NT, Krieger AG, Smith EM, Bender RA, Bardwell JCA, Jakob U (2014) Polyphosphate is a primordial chaperone. *Mol Cell* 53:689–699. <https://doi.org/10.1016/j.molcel.2014.01.012>
- Grousl T, Ungelenk S, Miller S, Ho CT, Khokhrina M, Mayer MP, Bukau B, Mogk A (2018) A prion-like domain in Hsp42 drives chaperone-facilitated aggregation of misfolded proteins. *J Cell Biol* 217:1269–1285. <https://doi.org/10.1083/jcb.201708116>
- Haslbeck M, Weinkauff S, Buchner J (2018) Small heat shock proteins: simplicity meets complexity. *J Biol Chem* 294:2121–2132. <https://doi.org/10.1074/jbc.REV118.002809>
- Haze K, Yoshida H, Yanagi H, Yura T, Mori K (1999) Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol Biol Cell* 10:3787–3799. <https://doi.org/10.1091/mbc.10.11.3787>
- Hopfield JJ (1974) Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. *Proc Natl Acad Sci U S A* 71:4135–4139. <https://doi.org/10.1073/PNAS.71.10.4135>
- Hunt C, Morimoto RI (1985) Conserved features of eukaryotic hsp70 genes revealed by comparison with the nucleotide sequence of human hsp70 (gene structure/heat shock/evolution). *Proc Natl Acad Sci USA* 82:6455–6459
- Hutt DM, Powers ET, Balch WE (2009) The proteostasis boundary in misfolding diseases of membrane traffic. *FEBS Lett* 583:2639–2646. <https://doi.org/10.1016/j.febslet.2009.07.014>
- Imanikia S, Sheng M, Taylor RC (2017) Cell non-autonomous UPRER signaling. Springer, Cham, pp 27–43
- Khmelniskii A, Knop M (2014) Analysis of protein dynamics with tandem fluorescent protein timers. *Methods Mol Biol* 1174:195–210. https://doi.org/10.1007/978-1-4939-0944-5_13
- Kohno K, Normington K, Sambrook J, Gething MJ, Mori K (1993) The promoter region of the yeast K4R2 (BiP) gene contains a regulatory domain that responds to the presence of unfolded proteins in the endoplasmic reticulum. *Mol Cell Biol* 13:877–890
- Koshland DE Jr (1958) Application of a theory of enzyme specificity to protein synthesis. *Proc Natl Acad Sci U S A* 44:98–104
- Kozutsumi Y, Segal M, Normington K, Gething MJ, Sambrook J (1988) The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. *Nature* 332:462–464. <https://doi.org/10.1038/332462a0>
- Kramer G, Shiber A, Bukau B (2019) Mechanisms of cotranslational maturation of newly synthesized proteins. *Annu Rev Biochem* 88: annurev-biochem-013118-111717. <https://doi.org/10.1146/annurev-biochem-013118-111717>
- Krisko A, Radman M (2010) Protein damage and death by radiation in *Escherichia coli* and *Deinococcus radiodurans*. *Proc Natl Acad Sci U S A* 107:14373–14377. <https://doi.org/10.1073/pnas.1009312107>
- Krisko A, Radman M (2013) Phenotypic and genetic consequences of protein damage. *PLoS Genet* 9:e1003810. <https://doi.org/10.1371/journal.pgen.1003810>
- Krzyzosiak A, Sigurdardottir A, Luh L et al (2018) Target-based discovery of an inhibitor of the regulatory phosphatase PPP1R15B. *Cell* 174:1216–1228.e19. <https://doi.org/10.1016/j.cell.2018.06.030>
- Labbadia J, Morimoto RI (2015a) Repression of the heat shock response is a programmed event at the onset of reproduction. *Mol Cell* 59: 639–650. <https://doi.org/10.1016/j.molcel.2015.06.027>
- Labbadia J, Morimoto RI (2015b) The biology of proteostasis in aging and disease. *Annu Rev Biochem* 84:435–464. <https://doi.org/10.1146/annurev-biochem-060614-033955>
- Labbadia J, Brielmann RM, Neto MF et al (2017) Mitochondrial stress restores the heat shock response and prevents proteostasis collapse during aging. *Cell Rep* 21:1481–1494. <https://doi.org/10.1016/j.celrep.2017.10.038>
- Lahav G (2010) How to survive and thrive in the mother-mentor marathon. *Mol Cell* 38:477–480. <https://doi.org/10.1016/j.molcel.2010.05.012>
- Laskey RA, Mills AD, Philpott A et al (1993) The role of nucleoplasmin in chromatin assembly and disassembly [and discussion]. *Philos Trans R Soc B Biol Sci* 339:263–269; discussion 268–9. <https://doi.org/10.1098/rstb.1993.0024>
- Levy-Tzedek S, Moran GS, Alon U, Sal-Man N (2018) Peer power. *EMBO Rep* 19:e47246. <https://doi.org/10.15252/embr.201847246>
- Lindquist McKenzie S, Henikoff S, Meselson M (1975) Localization of RNA from heat-induced polysomes at puff sites in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 72:1117–1121
- Martin DDO, Ladha S, Ehrnhoefer DE, Hayden MR (2015) Autophagy in Huntington disease and huntingtin in autophagy. *Trends Neurosci* 38:26–35. <https://doi.org/10.1016/j.tins.2014.09.003>
- Martinus RD, Garth GP, Webster TL, Cartwright P, Naylor DJ, Høj PB, Hoogenraad NJ (1996) Selective induction of mitochondrial chaperones in response to loss of the mitochondrial genome. *Eur J Biochem* 240:98–103. <https://doi.org/10.1111/j.1432-1033.1996.0098h.x>
- Mateju D, Franzmann TM, Patel A et al (2017) An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. *EMBO J* 36:1669–1687. <https://doi.org/10.15252/embr.201695957>
- McHugh D, Gil J (2018) Senescence and aging: causes, consequences, and therapeutic avenues. *J Cell Biol* 217:65–77. <https://doi.org/10.1083/jcb.201708092>
- Mogk A, Bukau B, Kampina HH (2018) Cellular handling of protein aggregates by disaggregation machines. *Mol Cell* 69:214–226. <https://doi.org/10.1016/j.molcel.2018.01.004>

- Morelli FF, Verbeek DS, Bertacchini J, Vinet J, Mediani L, Marmioli S, Cenacchi G, Nasi M, de Biasi S, Brunsting JF, Lammerding J, Pegoraro E, Angelini C, Tupler R, Alberti S, Carra S (2017) Aberrant compartment formation by HSPB2 mislocalizes Lamin A and compromises nuclear integrity and function. *Cell Rep* 20:2100–2115. <https://doi.org/10.1016/j.celrep.2017.08.018>
- Mori K, Sant A, Kohnol K et al (1992) A 22 bp cis-acting element is necessary and sufficient for the induction of the yeast KAR2 (BiP) gene by unfolded proteins. *EMBO J* 11:2583–2593
- Mori K, Kawahara T, Yoshida H et al (1996) Signalling from endoplasmic reticulum to nucleus: transcription factor with a basic-leucine zipper motif is required for the unfolded protein-response pathway. *Genes Cells* 9:803–817
- Münch C (2018) The different axes of the mammalian mitochondrial unfolded protein response. *BMC Biol* 16:81. <https://doi.org/10.1186/s12915-018-0548-x>
- Münch C, Harper JW (2016) Mitochondrial unfolded protein response controls matrix pre-RNA processing and translation HHS public access. *Nature* 534:710–713. <https://doi.org/10.1038/nature18302>
- Munro S, Pelham HRB (1986) An Hsp70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. *Cell* 46:291–300
- Musa M, Perić M, Bou Dib P et al (2018) Heat-induced longevity in budding yeast requires respiratory metabolism and glutathione recycling. *Aging (Albany NY)* 10:2407–2427. <https://doi.org/10.18632/aging.101560>
- Normington K, Kohno K, Kozutsumi Y, Gething MJ, Sambrook J (1989) *S. cerevisiae* encodes an essential protein homologous in sequence and function to mammalian BiP. *Cell* 57:1223–1236. [https://doi.org/10.1016/0092-8674\(89\)90059-7](https://doi.org/10.1016/0092-8674(89)90059-7)
- Ordureau A, Münch C, Harper JW (2015) Quantifying ubiquitin signaling. *Mol Cell* 58:660–676
- Patel A, Lee H, Jawerth L et al (2015) A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell* 162:1066–1077. <https://doi.org/10.1016/j.cell.2015.07.047>
- Perić M, Lovrić A, Šarić A, Musa M, Bou Dib P, Rudan M, Nikolić A, Sobočanec S, Mikecin AM, Dennerlein S, Milošević I, Vlahoviček K, Raimundo N, Kriško A (2017) TORC1-mediated sensing of chaperone activity alters glucose metabolism and extends lifespan. *Aging Cell* 16:994–1005. <https://doi.org/10.1111/ace.12623>
- Prahlad V, Cornelius T, Morimoto RI (2008) Regulation of the cellular heat shock response in *Caenorhabditis elegans* by thermosensory neurons. *Science* 320:811–814. <https://doi.org/10.1126/science.1156093>
- Reid MA, Kong M (2013) Dealing with hunger: metabolic stress responses in tumors. *J Carcinog* 12:1477–3163. <https://doi.org/10.4103/1477-3163.119111>
- Richter K, Haslbeck M, Buchner J (2010) The heat shock response: life on the verge of death. *Mol Cell* 40:253–266. <https://doi.org/10.1016/j.molcel.2010.10.006>
- Ritossa F (1962) A new puffing pattern induced by temperature shock and DNP in *drosophila*. *Experientia* 18:571–573. <https://doi.org/10.1007/BF02172188>
- Sahasrabudhe P, Rohrberg J, Biebl MM, Rutz DA, Buchner J (2017) The plasticity of the Hsp90 co-chaperone system. *Mol Cell* 67:947–961. <https://doi.org/10.1016/j.molcel.2017.08.004>
- Savageau MA (1974) Comparison of classical and autogenous systems of regulation in inducible operons. *Nature* 252:546–549. <https://doi.org/10.1038/252546a0>
- Savir Y, Tlusty T (2007) Conformational proofreading: the impact of conformational changes on the specificity of molecular recognition. *PLoS One* 2:e468. <https://doi.org/10.1371/journal.pone.0000468>
- Savir Y, Tlusty T (2010) RecA-mediated homology search as a nearly optimal signal detection system. *Mol Cell* 40:388–396. <https://doi.org/10.1016/j.molcel.2010.10.020>
- Savitski MM, Zinn N, Faelth-Savitski M, PoECKel D, Gade S, Becher I, Muelbaier M, Wagner AJ, Strohmer K, Werner T, Melchert S, Petretich M, Rutkowska A, Vappiani J, Franken H, Steidel M, Sweetman GM, Gilan O, Lam EYN, Dawson MA, Prinjha RK, Grandi P, Bergamini G, Bantscheff M (2018) Multiplexed proteome dynamics profiling reveals mechanisms controlling protein homeostasis. *Cell* 173:260–274. <https://doi.org/10.1016/j.cell.2018.02.030>
- Scherz-Shouval R, Santagata S, Mendillo ML, Sholl LM, Ben-Aharon I, Beck AH, Dias-Santagata D, Koeva M, Stemmer SM, Whitesell L, Lindquist S (2014) The reprogramming of tumor stroma by HSF1 is a potent enabler of malignancy. *Cell* 158:564–578. <https://doi.org/10.1016/j.cell.2014.05.045>
- Schopf FH, Biebl MM, Buchner J (2017) The HSP90 chaperone machinery. *Nat Rev Mol Cell Biol* 18:345–360. <https://doi.org/10.1038/nrm.2017.20>
- Shiber A, Döring K, Friedrich U, Klann K, Merker D, Zedan M, Tippmann F, Kramer G, Bukau B (2018) Cotranslational assembly of protein complexes in eukaryotes revealed by ribosome profiling. *Nature* 561:268–272. <https://doi.org/10.1038/s41586-018-0462-y>
- Shieh YW, Minguez P, Bork P, Auburger JJ, Guilbride DL, Kramer G, Bukau B (2015) Operon structure and cotranslational subunit association direct protein assembly in bacteria. *Science* 350:678–680. <https://doi.org/10.1126/science.aac8171>
- Shpilka T, Haynes CM (2018) The mitochondrial UPR: mechanisms, physiological functions and implications in ageing. *Nat Rev Mol Cell Biol* 19:109–120. <https://doi.org/10.1038/nrm.2017.110>
- Silva MC, Fox S, Beam M, Thakkar H, Amaral MD, Morimoto RI (2011) A genetic screening strategy identifies novel regulators of the proteostasis network. *PLoS Genet* 7:e1002438. <https://doi.org/10.1371/journal.pgen.1002438>
- Simon E, Kornitzer D (2014) Pulse-chase analysis to measure protein degradation. *Methods Enzymol*. <https://doi.org/10.1016/B978-0-12-420070-8.00006-4>
- Sorger PK, Pelham HRB (1987) The glucose-regulated protein grp94 is related to heat shock protein hsp90. *J Mol Biol* 194:341–344. [https://doi.org/10.1016/0022-2836\(87\)90380-9](https://doi.org/10.1016/0022-2836(87)90380-9)
- Sosa V, Moliné T, Somoza R, Paciucci R, Kondoh H, LLeonart ME (2013) Oxidative stress and cancer: an overview. *Ageing Res Rev* 12:376–390. <https://doi.org/10.1016/j.arr.2012.10.004>
- Taylor RC, Dillin A (2011) Aging as an event of proteostasis collapse. *Cold Spring Harb Perspect Biol* 3:a004440. <https://doi.org/10.1101/cshperspect.a004440>
- Terzi F, Cambridge S (2017) An overview of advanced SILAC-labeling strategies for quantitative proteomics. *Methods Enzymol* 585:29–47. <https://doi.org/10.1016/bs.mie.2016.09.014>
- Tirasophon W, Welihinda AA, Kaufman RJ (1998) A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes Dev* 12:1812–1824
- Tissières A, Mitchell HK, Tracy UM (1974) Protein synthesis in salivary glands of *Drosophila melanogaster*: relation to chromosome puffs. *J Mol Biol* 84:389–392. [https://doi.org/10.1016/0022-2836\(74\)90447-1](https://doi.org/10.1016/0022-2836(74)90447-1)
- Tsaytler P, Harding HP, Ron D, Bertolotti A (2011) Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. *Science* 332:91–94. <https://doi.org/10.1126/science.1201396>
- van Oosten-Hawle P, Porter RS, Morimoto RI (2013) Regulation of organismal proteostasis by transcellular chaperone signaling. *Cell* 153:1366–1378. <https://doi.org/10.1016/j.cell.2013.05.015>
- Wang XZ, Harding HP, Zhang Y, Jolicoeur EM, Kuroda M, Ron D (1998) Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *EMBO J* 17:5708–5717. <https://doi.org/10.1093/emboj/17.19.5708>
- Welker S, Rudolph B, Frenzel E, Hagn F, Liebisch G, Schmitz G, Scheuring J, Kerth A, Blume A, Weinkauff S, Haslbeck M, Kessler H, Buchner J (2010) Hsp12 is an intrinsically unstructured stress

- protein that folds upon membrane association and modulates membrane function. *Mol Cell* 39:507–520. <https://doi.org/10.1016/j.molcel.2010.08.001>
- Wu B, Hunt C, Morimoto R (1985) Structure and expression of the human gene encoding major heat shock protein HSP70. *Mol Cell Biol* 5:330–341
- Yen HCS, Xu Q, Chou DM, Zhao Z, Elledge SJ (2008) Global protein stability profiling in mammalian cells. *Science* 322:918–923. <https://doi.org/10.1126/science.1160489>
- Zahradka K, Slade D, Bailone A, Sommer S, Aeverbeck D, Petranovic M, Lindner AB, Radman M (2006) Reassembly of shattered chromosomes in *Deinococcus radiodurans*. *Nature* 443:569–573. <https://doi.org/10.1038/nature05160>
- Zhao Q, Wang J, Levichkin IV et al (2002) A mitochondrial specific stress response in mammalian cells. *EMBO J* 21:4411–4419. <https://doi.org/10.1093/emboj/cdf445>

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