

Communication

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From recordings of disulfide isomerases in action to reversal of maladaptive endoplasmic reticulum stress responses: *proceedings on the ER & Redox Club Meeting held in Venice, April 2015*

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Abstract: The endoplasmic reticulum (ER) interacts and cooperates with other organelles as a central hub in cellular homeostasis. In particular, the ER is the first station along the secretory pathway, where client proteins fold and assemble before they travel to their final destination elsewhere in the endomembrane system or outside the cell. Protein folding and disulfide bond formation go hand in hand in the ER, a task that is achieved with the help of ER-resident chaperones and other folding factors, including oxidoreductases that catalyze disulfide bond formation. Yet, when their combined effort is in vain, client proteins that fail to fold are disposed of through ER-associated degradation (ERAD). The ER folding and ERAD machineries can be boosted through the unfolded protein response (UPR) if required. Still, protein folding in the ER may consistently fail when proteins are mutated due to a genetic defect, which, ultimately, can lead to disease. Novel developments in all these fields of study and how new insights ultimately can be exploited for clinical or biotechnological purposes were highlighted in a rich variety of presentations at the ER & Redox Club Meeting that was held in Venice from 15 to 17 April 2015. As such, the meeting provided the participants an excellent opportunity to mingle and discuss key advancements and outstanding questions on ER function in health and disease.

Keywords: autophagy; BiP; chaperone; endoplasmic reticulum; ER-associated degradation; ER-stress; mitochondria; protein disulfide isomerase; protein folding; redox regulation; unfolded protein response

Introduction

The ER & Redox Club Meeting 2015 held at Vento di Venezia on Certosa Island in Venice was the follow-up of a series of meetings that have been organized over the years by European scientists who have an interest in the ER as a protein folding compartment. Three aspects within the scope of that interest traditionally have stood central: disulfide bond formation – which explains the “redox” in the name – homeostasis of the ER (in health & disease), and – with a keen eye on biotechnology – how we may exploit knowledge of ER function, for instance for production of antibodies. The latter aspect is also reflected in the fact that earlier installments of the meeting were called “European Colloquia on Cells as Protein Factories”.

Since the inaugural meeting in Heidelberg in 1998, the meeting has been held first on an annual and later on a biannual basis in various places across Europe. Many of the scientists who were invited to come to earlier installments of the meeting enjoyed its format to the extent that since then they have become ‘regulars’. This year’s installment in Venice reunited several familiar groups and welcomed a few newly recruited ones. While the scope of the meeting has not changed fundamentally over the years, the program of this year’s meeting was more ambitious than ever before with 31 oral and 31 poster presentations featuring research from 33 different groups hailing from 14 different countries.

The field of (oxidative) protein folding in the ER always has provided a rich ground for establishing ways to improve protein production strategies for clinical and biotechnological purposes, but in recent years the field is undergoing a further rise in popularity, having been “phagocytosed” by the proteostasis field [1, 2]. Indeed, with an aging population, the realization that disorders may stem from folding deficiencies (e.g. Alzheimer and

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Parkinson disease) has become wide-spread. No wonder there is a growing research focus also on ER-homeostasis in health and disease. In that respect it is of note that Peter Walter and Kazutoshi Mori received the Lasker Award last year for their discovery and delineation of the UPR [3].

Diseases related to ER function may ensue as a loss-of-function disorder when a mutated ER-client protein is amiss at a destination it cannot reach due to misfolding and retention in the ER or disposal through ERAD. Gain-of-function disorders may arise when homeostatic re-adjustments that are invoked in response to an ER-client's failure to fold turn maladaptive. The challenge in the first case is to find ways to let the client protein – despite its defect – reach the proper destination in a functional state, while in the second case the challenge is to let the cell's homeostatic circuitry be rewired to preempt or counteract the maladaptive response. Moreover, homeostatic pathways, including ER-stress pathways, may be exploited by cancer cells for survival and ER-stress pathways seem to play a role in inflammation as well.

The journal „Endoplasmic Reticulum Stress in Diseases“ aims to be in step with the proteostatic trend by covering research on how ER function may become compromised, causing disease. At the end of the meeting it was therefore decided to let proceedings on the scientific developments that were discussed in Venice appear in this startup journal, a feat not wholly unprecedented as Adam Benham reported in the FEBS journal on the same meeting held six years earlier in Elsinore, Denmark [4].

Connections of the ER with mitochondria

In recent years it has become evident that the ER leads a busy social life, making connections with most if not all other organelles [5]. Particularly well studied are the so-called mitochondria-ER associated membranes (MAMs), which turn out to be a platform for the regulation of Ca^{2+} signaling, lipid biogenesis, mitochondrial dynamics, and autophagy [6]. Luca Scorrano and his group from the Università di Padova, Italy, already had identified mitofusin 2 as an important tether between ER and mitochondrial membranes [7]. At the meeting, Déborah Naón from the Scorrano group presented a talk on whether a splice variant of mitofusin 2 may serve a role distinct from the 'conventional' mitofusin 2, while Emilie Schrepfer from the same group sketched on a poster her approaches to find new mitofusion 2 interactors.

Entry of clients into the ER

It has long been established that proteins destined to enter the secretory pathway are translocated across the ER membrane through translocons, but surprising new insights on this topic have been emerging lately or seem to be on the horizon [8, 9]. Tslil Ast from the laboratory of Maya Schuldiner at the Weizman Institute in Rehovot, Israel, presented a talk about a new mechanism for releasing clogged translocation pores. She demonstrated that proteins translocating through the translocon can, at some rate, block the pore if they start folding at both termini before translocation is complete. Using a whole genome screen, a protease was identified that directly cleaves the jammed proteins thus releasing the translocation apparatus. Stefan Schorr from Richard Zimmermann's laboratory at the Universitätsklinikum des Saarlandes in Homburg, Germany, addressed how Ca^{2+} leakage from the ER lumen through translocation pores is prevented. They had already shown that the ER-resident chaperone BiP interacting with the pore component Sec61 α is key for this process [10], and at the meeting Stefan presented a talk on which Hsp40 co-chaperones help BiP with this task.

ER exit and retrograde entry into the ER

The ER to Golgi trafficking step is the first in the vesicular traffic network of the secretory pathway. During this step mature, correctly folded, processed and assembled proteins (cargo) are distinguished from immature proteins and ER-resident proteins by sorting into COPII coated vesicles that will allow their progress to the later compartments of the secretory pathway. Binding to the COPII coat can occur directly or via adaptors called cargo receptors [11]. Sefi Geva, from the Maya Schuldiner laboratory, reported on a poster the identification of a new cargo receptor dedicated to allow exit of a subset of ER-clients to travel to the Golgi in yeast.

Matteo Fossati presented in a talk his work carried out in the laboratory of Nica Borgese at the Consiglio Nazionale delle Ricerche in Milan, Italy, on secretory membrane cargo recycling from the Golgi back to the ER [12]. He showed that secretory membrane proteins endowed with an ER export signal accumulated in the Golgi upon a Golgi-to-plasma membrane block in transport, but in the absence of an export signal underwent Rab6-dependent recycling to the ER. While so far recruitment to ER exit sites was considered to be the main rate-limiting step for export from the early secretory pathway, Matteo's work

showed that cargo membrane proteins in addition must escape from this apparently futile recycling pathway to proceed through the Golgi.

There is a special class of proteins that hijack retrograde trafficking to reach the ER and that exploit the retro-translocation machinery of ERAD as “a backdoor” through which to get into the cytosol. These are bacterial toxins: Shiga toxins, ricin and cholera toxin [13]. Ludger Johannes from the Institut Curie in Paris, France, and colleagues earlier reported on the identification of compounds that selectively block the toxin retrograde trafficking [14]. At the meeting, Ludger presented a talk on how one of these compounds, Retro-2, targets an ER-resident protein. Surprisingly, Retro-2 binding to or ablation of this target protein make that its partner protein will commit to endocytosis in a manner that precludes the toxin entry.

ER-resident chaperones

Upon entry of client proteins into the ER and prior to their exit, they fold, obtain N-linked glycans and/or disulfide bonds, and undergo assembly steps. All these maturation events are under tight scrutiny to ensure that only products of the highest quality emerge from the ER [15]. Key quality control decisions are made by ER-resident chaperones, which assist client maturation. Matthias Feige, until recently working in the laboratory of Linda Hendershot at St. Jude Children’s Research Hospital in Memphis, Tennessee, but now at the Technische Universität in Munich, Germany, presented a talk on how BiP, its Hsp40 co-chaperones and the ER-resident large Hsp70, GRP170 [16, 17] differ in their substrate-binding specificity, recognizing peptide motifs in their clients that differ in amino-acid composition and physicochemical characteristics. These findings in part explain how (co-) chaperones vary to the extent of which clientele they cater to.

Along these lines it was of interest that Naomi Lodder from the laboratory of Ineke Braakman at the Universiteit Utrecht, Holland, presented in a talk that pErp1, a potential GRP94 co-chaperone that assists the maturation of antibodies in plasma cells [18-20], seems to be superfluous for the maturation of any client protein tested so far apart from antibodies. The intricacies of client binding also depend on the interplay between chaperones, co-chaperones and in the case of BiP, the nucleotide exchange factors, BAP (also known as Sil1) and the aforementioned GRP170. Mathias Rosam from the laboratory of Johannes Buchner at the Technische

Universität in Munich presented a talk on how conformational changes in BAP and BiP, as assessed by single molecule Förster resonance energy transfer (FRET) and validated by biochemistry, lie at the basis of how BAP influences BiP function.

Disulfide bond formation & the ERO-PDI relay

Protein folding and disulfide bond formation go hand in hand in the ER [21]. Thus, as soon as a nascent ER-client emerges through the translocon into the lumen and exposes free sulfhydryl groups on cysteines, it may start to undergo oxidative folding. Philip Robinson from the laboratory of Neil Bulleid at the University of Glasgow, Scotland, presented a talk about how he addresses these early oxidative events, making use of nascent chains of various lengths that are stalled on ribosomes. As such, he demonstrated how to precisely pinpoint when disulfide bond formation starts depending on how far the nascent chain has reeled into the ER lumen yet.

To sustain disulfide bond formation, the ER hosts an impressive array of protein relays that provide on the one hand the oxidizing equivalents necessary for insertion of disulfide bonds into client proteins, and on the other hand facilitate the shuttling of free electrons that are generated during the process in a manner that minimizes formation of harmful radical oxygen species [22]. Protein disulfide isomerase (PDI) is the archetypal disulfide donor in the ER. Upon oxidation of a client protein, PDI gets to become re-oxidized by ER oxidoreductase (ERO) proteins, of which the most prominent in mammals is Ero1 α . The ERO-PDI relay indeed has been well established as a primary mechanism for the provision of disulfide bonds to clients in the ER [23]. H₂O₂ generated from the ERO-PDI system can be recycled by ER-resident peroxiredoxin IV and glutathione peroxidases to support further disulfide bond formation [22, 24]. Ero1 α oxidizes PDI with reduction of O₂ to H₂O₂ on a bound FAD molecule. Previous studies indicated that different pairings of four cysteines, contained in an electron shuttle loop, tightly regulate the Ero1 α activity to avoid ER hyperoxidation [25]. Shingo Kanemura from the laboratory of Kenji Inaba at Tohoku University in Sendai, Japan, presented a poster on which he sketched the identification of yet another regulatory disulfide bond in Ero1 α .

Adam Benham from Durham University, England, presented a talk in which he discussed how Ero1 α can be partially reduced by homocysteine [26], an α -amino acid associated with inflammatory disease, and how

homocysteine influences the conformation of Ero1 α , thereby modulating its interactions with PDI. The interplay between Ero1 α and PDI is well documented [27], but Lei Wang from the laboratory of Chih-chen Wang at the Institute of Biophysics of the Chinese Academy of Sciences in Beijing, China, presented a talk in which he shed new light on how Ero1 α and PDI cross-influence each other's function [28]. He moreover revealed that in yeast the interplay between Ero1p and Pdi1p seems to be controlled in a different way than in mammals.

PDI and PDI-like proteins in action

The study of PDI function was already initiated in the 60's in the laboratory of C. Anfinsen [29], but despite a wealth of investigations since then, the precise molecular mechanisms underlying the PDI-catalyzed disulfide introduction and isomerization of a wide variety of substrate proteins still remain unclear. Masaki Okumura from the laboratory of Kenji Inaba demonstrated on a poster how using high-speed atomic force microscopy (AFM) allows direct visualization of individual PDI and PDI-like protein dynamics during catalysis of oxidative protein folding. Masaki's spectacular results justly earned him the Armenise-Harvard Foundation-sponsored poster award, which was handed to him at the closing session by Ineke Braakman. Catalysis of disulfide bond formation in the ER is a responsibility PDI shares with numerous family members [30, 31]. In accordance, Anna Chatsisvili from Ineke Braakman's laboratory presented a poster that highlighted that ablation of PDI has a limited effect on oxidative folding of various client proteins, as presumably other PDI-like proteins step in to do the job.

PDI-like proteins differ in their domain organization and redox properties, which likely accounts for their diverse and specialized roles [30, 31]. From Aldo Ceriotti's laboratory at the Consiglio Nazionale delle Ricerche in Milan, William Remelli (in a talk) and Anna Paola Casazza (on a poster) showed the preliminary characterization of AtPDIL5-1, a PDI-like protein from *A. thaliana* with a single redox-active domain. Helena Safavi-Hemami from the laboratory of Lars Ellgaard at Københavns Universitet in Copenhagen, Denmark, highlighted in a talk that through analysis of protein folding in cone snails it appears that disulfide bond formation of cone snail toxins (conotoxins) may be the dedicated job of a subset of specialized PDIs. Giorgia Brambilla Pisoni from the laboratory of Maurizio Molinari at the Istituto di Ricerca in Biomedicina in Bellinzona, Switzerland, presented a talk on how a membrane bound PDI-like protein preferentially intervenes during maturation of cysteine-containing membrane-associated proteins, while ignoring the same cysteine-containing ectodomains if not anchored at the ER membrane. This first example of a topology-specific redox catalyst rightfully earned Giorgia the award for the best oral presentation by a junior scientist that was sponsored by this journal, which was handed to her by Ineke Braakman at the closing session of the meeting.

Tiziana Anelli from Roberto Sitia's laboratory at the San Raffaele Scientific Institute in Milan presented a talk on the PDI family member ERp44. She summarized how ERp44 controls the intracellular localization of several important players in oxidative protein folding, such as Ero1 α [32], Peroxiredoxin 4 [33], and SUMF1 [34]. As such, ERp44 is a key regulator of the redox poise in the secretory pathway. Likewise, through interplay with



Figure 1. Award winners. Giorgia Brambilla Pisoni (left) winner of the award for the best oral presentation by a junior scientist, which was sponsored by this journal, and Masaki Okumura (right) winner of the Armenise-Harvard Foundation sponsored award for the best poster presentation receive their prizes from Ineke Braakman (photographs courtesy of Maurizio Molinari)

Ero1 α at MAMs, ERp44 contributes to the regulation of Ca²⁺ shuttling between the ER and mitochondria [35]. Tiziana furthermore addressed how ERp44 governs a pH-dependent post-ER quality control mechanism to warrant proper disulfide-linked assembly of multimeric IgM and other complex oligomeric proteins. At the lower pH of the Golgi, movements in the ERp44 tail expose the client binding site and RDEL motif, allowing retrieval of assembly intermediates via the KDEL receptor [36]. Sara Sannino from the same laboratory revealed on a poster that the ERp44 tail opening is regulated by other means than pH changes alone to ensure fidelity in the biosynthesis of proteins that travel along the secretory pathway.

ERAD

At times, even the joint efforts of ER-resident chaperones, oxidoreductases and PDI family members cannot ensure the accurate folding and assembly of certain client proteins. As a rule, these ill-fated clients are prevented from traveling any further along the secretory pathway, and, instead, are retro-translocated to the cytosol for destruction by the proteasome in a process known as ERAD. The laboratory of Kaz Nagata at the Kyoto Sangyo University in Kyoto, Japan, earlier identified a lectin EDEM1 and a PDI-like protein, ERdj5, that also is a co-chaperone of BiP. EDEM1 and ERdj5 cooperate with BiP in handing misfolded ER-clients to the retro-translocation machinery [37, 38]. Ryo Ushioda from the Nagata laboratory presented a talk on how ERdj5 contributes to the regulation of Ca²⁺ homeostasis in the ER through its redox activity. Kenji Inaba's talk highlighted how ERdj5 can adopt two very different conformations. He has previously reported a crystal structure of ERdj5 [37], but at the meeting Kenji showed a new crystal structure representing the other conformer. High speed AFM recordings of ERdj5 dynamics gave further support for ERdj5 alternating conformations. The movements that accompany the transition between the two conformations likely lie at the heart of ERdj5 function in ERAD.

The ERdj5 partner EDEM1 delivers ill-fated ER-clients to the adaptor protein SEL1L for retro-translocation [39]. The laboratory of Stefana Petrescu at the Institutul de Biochimie al Academiei Române in Bucharest, Romania, previously reported that EDEM1 facilitates degradation of misfolded tyrosinase [40]. They showed that EDEM1 has an intrinsically disordered domain (IDD) at its N-terminus that is key for the association with tryosinase and hence for subsequent delivery to SEL1L. At the meeting, Mari Chiritoiu from the Petrescu laboratory demonstrated

on a poster that binding via the IDD may be the default mechanism for EDEM1 to recognize its targets. Anett Köhler from the laboratory of Thomas Sommer at the Max Delbrück Centrum in Berlin, Germany, presented a talk on how the yeast homolog of EDEM1, Htm1p, teams up with the 'standard' PDI in yeast, Pdi1p, as there is no direct ERdj5 homolog. Once the Htm1p-Pdi1p tandem associates with ERAD target proteins, it facilitates the mannose trimming of their glycans from the Man₈GlcNAc₂ to the Man₇GlcNAc₂ form, as a key step in their subsequent delivery to the retro-translocation machinery and proteasomal clearance [41].

Retro-translocation alone is not sufficient to brand ERAD substrate proteins for proteasomal destruction. E3 ubiquitin ligases first must conjugate ubiquitin moieties to those substrates [42]. From the laboratory of John Christianson at Ludwig Cancer Research in Oxford, England, Federica Lari (in a talk) and Emma Fenech (on a poster) highlighted that there are at least 24 other E3 ubiquitin ligases in the ER membrane that may act in parallel to the archetypal ER-resident E3 ligase HRD1 [43]. Federica and Emma sketched the approaches they currently employ to map the individual contributions of this long list of ER-resident E3 ligases to ERAD and ER-homeostasis in general. Stephen High from the University of Manchester, England, presented a talk on how the quality control of hydrophobic precursor proteins may actually involve cycles of substrate ubiquitination and de-ubiquitination prior to their proteasomal destruction. Whilst the BAG6 complex helps the posttranslational delivery of tail-anchored membrane proteins to the ER, it can also promote the E3 ligase mediated ubiquitination of aberrant membrane proteins that have mislocalized to the cytosol. Surprisingly, SGTA reverses the actions of the BAG6 complex and promotes the de-ubiquitination of these same substrates [44, 45]. Hence, the fate of precursor proteins that enter this quality control cycle rests on how the tug-of-war between BAG6 and SGTA is decided, and depending on specific cellular cues the outcome may vary.

Most knowledge of ERAD centers on how ill-fated ER-clients that are either soluble or containing a bulky ectodomain are dislocated across the ER membrane to become ubiquitinated for proteasomal degradation [46]. Marius Lemberg from the Zentrum für Molekulare Biologie in Heidelberg, Germany, unveiled at the meeting in a talk a fascinating ERAD mechanism that only now is beginning to be appreciated. He showed that ER-resident intramembrane proteases, such as the rhomboid protease RHBDL4, cleave single spanning and polytopic membrane proteins and that various ERAD substrates require protease-mediated clipping within the plane of

the membrane to allow the subsequent removal of soluble cleavage fragments by ‘conventional’ ubiquitination and proteasomal destruction [47]. Nina Bergbold from his laboratory presented a poster adding further detail to this story and she highlighted that these clipping events may also contribute to abundance control of polytopic membrane proteins.

UPR

The joint efforts of the regular ER folding and ERAD machineries may become insufficient under a variety of adverse conditions, such as glucose starvation, hypoxia, expression of folding incompetent ER-clients, or the presence of ER-stress eliciting drugs [48], but also when cells commit to a professional secretory role, such as when B lymphocytes differentiate into antibody secreting plasma cells [49]. To restore ER-homeostasis cells under ER-stress conditions invoke the UPR. There are at least three UPR sensors, IRE1, PERK and ATF6, each with a stress sensing domain in the ER lumen and an effector domain in the cytosol. Once activated, the UPR sensors set the signaling cascades in motion through their cytosolic domains that aim for enhanced folding capacity of and reduced folding load in the ER [48].

The UPR sensor PERK, which was discovered in the laboratory of David Ron, now at the University of Cambridge, England, counteracts ER-stress by phosphorylation of the alpha subunit of translation initiation factor 2 (eIF2 α) [50]. As a result, global rates of protein synthesis drop, because when eIF2 is in a phosphorylated state its guanine nucleotide exchange factor, eIF2B, is inhibited. Conversely, the translation of a small subset of mRNAs with special 5' untranslated regions (5'UTR) is increased, including ATF4, which encodes a potent transcription factor that initiates a gene expression program known as the integrated stress response (ISR). Peter Walter and colleagues recently reported on an inhibitor, ISRIB, that reverses the effects of eIF2 α phosphorylation [51]. At the meeting David presented a talk in which he demonstrated that ISRIB targets an interaction between eIF2 and eIF2B in a manner that stimulates eIF2B to act as a nucleotide exchange factor of eIF2, and thereby trumps the inhibitory effect of eIF2's phosphorylated state [52].

While ISRIB pharmacologically counteracts PERK activity, negative feedback on PERK signaling in the normal physiological context is provided by the ATF4 target GADD34/PPP1R15A as it teams up with the phosphatase PP1 as a regulatory subunit [53, 54]. As

such, the PP1/GADD34 tandem dephosphorylates eIF2 α to restore protein synthesis. Ruming Chen from David Ron's laboratory presented a poster on which he showed that the PP1/GADD34 tandem needs to team up with G-actin as a third partner to dephosphorylate eIF2 α in a selective manner. Ruming's findings suggest that conditions affecting the local availability of G-actin may regulate the rate of eIF2 α dephosphorylation and thereby rates of protein synthesis [55].

Local availability through specific targeting events also plays a role in how the UPR sensor IRE1 signals in response to ER-stress. Under acute ER-stress conditions, IRE1 forms multimeric clusters in the plane of the ER membrane both in man and yeast [56, 57]. When activated IRE1 initiates an unconventional splicing event that alters the reading frame of its substrate mRNA XBP1 (in metazoans) or HAC1 (in fungi) such that it encodes a transcription factor that will drive expression of UPR target genes [48]. In yeast, Ire1 clustering is required for HAC1 mRNA to efficiently be recruited (via a targeting element in its 3' UTR) and for Ire1 to accommodate the docking of its mRNA substrate (via a positively charged region in its cytosolic linker domain). Yet, neither HAC1's targeting element nor Ire1's mRNA docking site are conserved in non-fungal species [57, 58]. Instead, XBP1 mRNA appears to be targeted to the ER membrane via a hydrophobic signal called HR2 that is encoded in the unspliced mRNA [59]. At the meeting, Tomás Aragón from the Centro de Investigación Médica Aplicada in Pamplona, Spain, revealed in a talk that HR2 translation and association of XBP1 mRNA with the ER membrane are dispensable for its splicing under acute ER-stress, suggesting that XBP1 mRNA recruitment to IRE1 clusters must then rely on other targeting mechanisms.

Tumor cells exploit the UPR as a prosurvival mechanism [60]. As hypoxia contributes to UPR activation in cancer cells, Florentina Pena from the Institutul de Biochimie al Academiei Române addressed how ER function alters upon changes in oxygen availability. At the meeting, she reported in a talk that folding and assembly of IgM are negatively affected under hypoxic conditions and that the redox state of some PDI family members is modified in a way that may impact on the UPR circuitry. Along the same lines a poster jointly presented by Raffaella Magnoni, Ana Paula Cordeiro, and Cecilie Lützen Søltoft from the Lars Ellgaard laboratory reported on the identification of a novel protein that can modify BiP such that it rewires the UPR circuitry.

From the laboratory of Eelco van Anken at the San Raffaele Scientific Institute in Milan, Milena Vitale and Laura Tadè (on posters) and Andrea Orsi (in a talk) presented how inducible expression of the secretory heavy

chain (HC) of IgM (μ_s) in absence of the light chain (LC) provides a potent UPR stimulus in HeLa cells. Early upon onset of expression, μ_s activates the UPR in a manner that recapitulates how it is activated by ER-stress eliciting drugs. Yet, prolonged μ_s expression – in contrast to these drugs – conveys no toxicity to cells, but leads to a successful re-adjustment of ER-homeostasis, which mimics chronic ER-stress in pathology. The transition from acute to chronic ER-stress responses varies dependent on the nature and levels of the proteostatic challenge and coincides for μ_s with a restoration of BiP levels being in surplus. Thus, in the acute phase μ_s seems to temporarily titrate BiP away from UPR sensors leading to their activation as has been proposed before [61, 62]. Tampering with individual UPR branches and ERAD revealed how each of these pathways contributed individually to the re-adjustment of ER-homeostasis under chronic μ_s expression driven ER-stress. Anush Bakunts from the same laboratory sketched on a poster her approaches to map μ_s driven alterations both in ER morphology by electron microscopy and in the ER proteome by mass spectrometric analysis.

Antibody folding, assembly and production

Studies on antibody folding and assembly not only serve us to better understand ER function and homeostatic control or the humoral immune response, but also to improve their production for use as therapeutics or for biotechnological purposes. Thus, antibody folding and assembly not likely will cease to inspire researchers anytime soon. IgM is the first antibody isotype that is produced during the immune response to antigens. To compensate for their low antigen affinity, they are secreted in polymeric form, namely as pentamers or hexamers. The C-terminal end of IgM, the tail piece (tp) allows unpolymerized IgM to be retained intracellularly by ERp44 [63]. At the same time, it is exploited for formation of IgM polymers in cells and sufficient for formation of C μ 4-tp polymers in vitro. Džana Pašalić from the laboratory of Johannes Buchner presented on a poster the defining elements of the tp for oligomerization. Christina Stutzer from the same laboratory presented a poster on how specific regions of λ 5 and VpreB contribute to their own dimerization to form the surrogate light chain (SLC). The SLC teams up with the HC to form the pre-B cell receptor (pre-BCR) and signaling through the pre-BCR is a key checkpoint in B cell development [64].

Yields of antibody from cultured mammalian cells have improved exponentially over the last decade thanks

to various optimizations in expression and culturing methods [65]. Fiona Chalmers from the laboratory of Neil Bulleid presented on a poster how tampering with the UPR circuitry in CHO cells offers further improvements in antibody yields. Chloe Stoye from the same laboratory showed on a poster that in CHO cells the HC of IgG folds, dimerizes and gets secreted even in the absence of the LC. This finding is surprising since from several studies it was established that the first constant (C $_H$ 1) domain of HCs, including μ_s – see above – teams up with BiP, thereby retaining the HC in the ER and thus preventing its secretion, until the LC displaces it [66]. It remains to be solved why CHO cells show exceptional leniency in LC-less IgG secretion. Specificity of antibodies toward antigens is determined by the variable domains of the HC and LC (V $_H$ and V $_L$), which explains the interest in artificial constructs consisting of these two domains alone, the so-called antibody single chain F $_V$ fragments (scF $_V$). Christine John from the Johannes Buchner laboratory presented a poster on what determines the stability of the V $_H$ /V $_L$ interface to learn how to improve on it for engineering of scF $_V$ -based antibodies.

ER-homeostasis in health & disease

B to plasma cell differentiation is but one example of how ER-homeostasis and function alter during development. To sustain skeletal growth, chondrocytes commit to producing collagen in bulk, which the ER must accommodate. Carmine Settembre from the Telethon Institute of Genetics & Medicine in Pozzuoli, Italy, presented in a talk how autophagy is crucial for maintaining ER-homeostasis in chondrocytes, and, hence, for normal bone growth [67]. In accordance, skeletal disorders may stem both from defects in collagen and from defects in lysosomal genes (which are key for autophagy [68]). At present, diseases stemming from mutations in collagen genes are considered loss-of-function disorders. In view of Carmine's findings, however, some may be caused, at least in part, also by a gain-of-function, namely that certain defects in collagen cause a burden on the ER and/or autophagic machinery that leads to homeostatic re-adjustments that turn out maladaptive.

Along these lines, a gain-of-function indeed lies at the basis of Charcot-Marie-Tooth 1B (CMT1B) neuropathy as has become evident from the work of Lawrence Wrabetz, now at the University at Buffalo, New York, and colleagues. They established that P0, an abundant peripheral myelin component produced by Schwann cells, when it lacks serine 63 (POS63del), misfolds in the

ER and elicits a maladaptive UPR [69, 70]. The laboratory of Maurizio D'Antonio continues the work on CMT1B at the San Raffaele Scientific Institute. Cristina Scapin from his laboratory presented a poster on how genetic and pharmacological inactivation of GADD34 reverses the neuropathy in the POS63del CMT1B model [71, 72]. As mentioned above, GADD34 dephosphorylates eIF2 α and thereby overrules PERK mediated translational inhibition. Cristina provided genetic evidence that the reversal of the neuropathy in mice indeed relies on the inhibition of GADD34 through that mode of action. Thus, keeping eIF2 α in a phosphorylated state is protective in CMT1B. Vera Volpi from the same laboratory presented a talk on whether tampering with ERAD might provide additional alleviation of this neuropathy.

Genetic disorders stemming from mutations in alpha 1-antitrypsin (A1AT), which is synthesized in the liver, are characterized by both loss-of-function and gain-of-function pathogenicity. Misfolding of mutant A1AT in the ER leads to diminished secretion, which may cause emphysema as A1AT serum levels drop to a level that is insufficient to protect the lungs from neutrophil elastase activity. In the liver, misfolded A1AT mutants form polymers that accumulate in inclusions and, ultimately, cause cirrhosis [73]. Riccardo Ronzoni from the laboratory of Anna Fra at the Università degli Studi di Brescia, Italy, described on a poster three rare A1AT variants and how mutation-introduced cysteines prompt the formation of multiple homotypic disulfide-linked isoforms that are retained in the ER. Riccardo further stressed that aberrant disulfide-linked complex formation may be a recurring theme in the pathogenesis of A1AT deficiencies, since also the most common A1AT mutant Z (E342K) forms covalent homodimers via C232, while the wild-type A1AT does not.

Uromodulin is a protein specifically expressed in the thick ascending limb of the nephron and various uromodulin mutations lead to tubulointerstitial kidney disease. Common to all disease-causing uromodulin mutants is that they accumulate in the ER, but how such ER retention relates to pathogenesis remains unclear [74]. Céline Schaeffer from the laboratory of Luca Rampoldi at the San Raffaele Scientific Institute presented on a poster that when a typical uromodulin mutant (C150S) is stably expressed in cells, it causes a chronic UPR without affecting cell viability. „She also identified which other stress“ pathways are invoked through transcriptional profiling of the cells. Matteo Trudu from the same laboratory highlighted in a talk that expression of mutant uromodulin in a mouse model in fact induces inflammation in the kidney. The question is whether the UPR that is caused by the ER accumulation of the mutant protein is

the instigator of the pro-inflammatory signals that lead to disease. In that light it was of interest that Kitty McCaffrey from the laboratory of Ineke Braakman presented on a poster that ER-stress can induce an IRE1-dependent type I interferon response. Indeed, links between IRE1 and immune activation are rapidly emerging [75].

Another Braakman laboratory member, Daniel Mesquita da Fonseca, presented a poster on yet another genetic disease that is associated with protein folding defects: cystic fibrosis (CF). The most common mutation that causes CF is the lack of a phenylalanine at position 508 in CFTR (Δ F508). As it is a polytopic membrane protein, CFTR biogenesis is regulated by both ER and cytosolic chaperones, including Hsp90. Folding of Δ F508 is compromised, which leads to its retention in the ER, thereby causing the pathology [76]. The use of radiolabeling and protease susceptibility assays allowed Daniel to pinpoint which precise Hsp90-assisted folding step is compromised in Δ F508. Wilson's disease (WD) shares some features with CF in that it is caused by mutations in a polytopic membrane protein that relies on both ER and cytosolic chaperones for its folding. In WD the ATP7B protein is mutated with H1069Q being the most common mutation in western populations. Like CFTR Δ F508 this ATP7B mutant misfolds and is retained in the ER [77]. Mafalda Concilli from the laboratory of Roman Polishchuk at the Telethon Institute of Genetics & Medicine in Pozzuoli presented a poster on her identification of cytosolic Hsp70 as an interactor that causes ER retention of mutant ATP7B and on how suppression of Hsp70 restores ATP7B trafficking to the trans-Golgi network to act as a copper transporter. Both CF and WD indeed are loss-of-function disorders that could be alleviated by the defective proteins reaching their proper destinations.

Loss-of-function mutations in the ER-resident selenoprotein N (SEPN1) lead to myopathies. Ester Zito from the Istituto di Ricerche Farmacologiche Mario Negri in Milano, Italy, presented in a talk how SEPN1's redox activity defends the cell from Ero1 α -generated peroxides. In particular, hyperoxidation of its luminal cysteines inactivates the SERCA2 Ca²⁺ pump. Thus, when Ero1 α activity is unchecked by SEPN1, Ca²⁺ re-uptake into the ER becomes defective, which explains the muscular dysfunction. Accordingly, in model mice lacking SEPN1, enhanced Ero1 α expression unmasks muscular dysfunction that resembles the myopathy found in patients [78]. These findings point out that inhibiting Ero1 α activity may offer a therapeutic avenue for SEPN1-related myopathies. Marianna Marino from Ester's laboratory presented a poster on how she currently explores that avenue. Stefano Bestetti from Roberto Sitia's

laboratory also addressed on a poster the importance of cellular defense against peroxides. H_2O_2 is essential for efficient cell signaling. At high (cytosolic) concentrations, however, it is extremely toxic. Stefano showed that AQP8 is the main gatekeeper of H_2O_2 transport across the plasma and ER membranes [79], but also that H_2O_2 fluxes are abrogated upon stress through modification of AQP8 by an as yet unknown mechanism.

Technological advances

At the meeting some technological advances were presented as well. For instance, Uri Weill from the laboratory of Maya Schuldiner presented on a poster the creation of a new yeast library in which all endomembrane proteins are tagged with green fluorescent protein (GFP) on their N terminus (N') thus complementing existing C' tagged libraries [80]. The new library enabled the identification of hundreds of endomembrane and secreted proteins that had never been visualized before. Moreover, the unique technology that was developed to create the library, SWAp Tag (SWAT), now enables swapping of tags in a matter of weeks with little effort. The library Uri presented may thus serve as a platform from which a wide diversity of functional libraries can be made for further systematic exploration of yeast cell biology.

Recently developed protein tags indeed may open up exciting new research strategies or applications. Alberto Danieli from the laboratory of Eelco van Anken presented on a poster how he developed an easy to use innovative approach for Correlative Light and Electron Microscopy (CLEM) making use of the HaloTag [81], which is commercialized by Promega. Alessandro Bosetti from that company presented in a talk how the NanoLuc luciferase tag can serve as a reporter for promoter analysis, tracking protein function, localization, and turnover in living cells [82]. Moreover, Alessandro sketched how a split version of this tag, referred to as NanoBiT, and a bioluminescence resonance energy transfer approach, NanoBRET, can be used to reveal real-time dynamics of protein interactions in living cells.

Considering the scope of the meeting, the audience needed little convincing that disulfide bond formation is an arduous task already under the favorable circumstances found in the ER, let alone for recombinant protein production facilities. Yet, Lloyd Ruddock from the University of Oulu, Finland, presented a talk on how his laboratory developed CyDisCo, a system, which allows efficient disulfide bond formation in the cytoplasm of *E. coli*. He reported on the successful production of over 100

disulfide bonded proteins at high yields, including IL6 and human growth hormone, but also ER folding factors and ERAD components [83-85]. The success of CyDisCo is rooted in Lloyd's profound knowledge of oxidative folding [31], while at the same time the intricacies of how CyDisCo works are beginning to reveal novel aspects of redox chemistry that facilitates oxidative folding in general.

Conclusions

Only at a stone's throw from Piazza San Marco – swarming with tourists as always – the meeting's participants found themselves oddly enough in splendid isolation on the all but deserted Certosa Island. The combination of the tranquil setting and the stunning backdrop of the enchanting old city in no small part contributed to the relaxed and collaborative atmosphere, ideally suited for the mingling of participants and the fruitful exchange of ideas until late hours.

The presentations at the meeting testified of great progress being made in the field. Live recordings of individual PDI-like proteins 'at work' engendered much enthusiasm and exemplified how the search for precise molecular mechanisms can be most rewarding from a scientific point of view. Yet, fundamental molecular insights at the same time prove to be key for how we may successfully exploit findings for clinical benefit. For instance, the delineation of the UPR circuitry to impressive detail has taken the field to a point where it starts to bring therapeutic applications within reach, as was evident from the spectacular findings on how to reverse neuropathy in a CMT1B mouse model. Likewise, the intricate mapping of intracellular trafficking pathways provided the framework for the identification of compounds that prevent otherwise deadly toxins from getting to the ER through retrograde transport. It was furthermore evident that progress in the tailoring of bacteria and mammalian cells for obtaining high quality recombinant proteins at high yields is greatly indebted to the ever maturing fundamental insights on (oxidative) protein folding mechanisms.

Along these lines, various other exciting advances that were put forward at the meeting seem to open a wealth of new perspectives. For example, particular steps in both ER folding and ERAD now appear to be specialized for subsets of clients. These findings may prove to be the stepping stone for identifying ways that allow selective disposal of harmful ER-clients in gain-of-function disorders, or instead allow selective exemption from degradation of ER-clients in loss-of-function disorders. Similarly, further mapping of pathways that control

ER-homeostasis in various cellular and developmental contexts may prove invaluable in finding methods to intervene when these pathways become maladaptive in disease. In that light it is of great interest that a variety of small-molecule modulators of ER-homeostasis are now becoming available and that their precise mode of action is being deciphered. With such tantalizing perspectives in mind, the next installment of the ER & Redox Club meeting to be organized under the auspices of Richard Zimmermann no doubt will be an equally if not more exciting event.

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References

- [1] Balch WE et al. Adapting proteostasis for disease intervention. *Science*. 2008; 319(5865):916-9. [PubMed: 18276881]
- [2] Hartl FU Bracher A Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature*. 2011; 475(7356):324-32. [PubMed: 21776078]
- [3] Worby CA Dixon JE. Unpacking the unfolded protein response. *Cell*. 2014; 158(6):1221-4. [PubMed: 25215479]
- [4] Benham AM. Protein folding and disulfide bond formation in the eukaryotic cell: meeting report based on the presentations at the European Network Meeting on Protein Folding and Disulfide Bond Formation 2009 (Elsinore, Denmark). *FEBS J*. 2009; 276(23):6905-11. [PubMed: 19860835]
- [5] Elbaz Y Schuldiner M. Staying in touch: the molecular era of organelle contact sites. *Trends Biochem Sci*. 2011; 36(11):616-23. [PubMed: 21958688]
- [6] Naón D Scorrano L. At the right distance: ER-mitochondria juxtaposition in cell life and death. *Biochim Biophys Acta*. 2014; 1843(10):2184-94. [PubMed: 24875902]
- [7] de Brito OM Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature*. 2008; 456(7222):605-10. [PubMed: 19052620]
- [8] Ast T Schuldiner M. All roads lead to Rome (but some may be harder to travel): SRP-independent translocation into the endoplasmic reticulum. *Crit Rev Biochem Mol Biol*. 2013; 48(3):273-88. [PubMed: 23530742]
- [9] Aviram N Schuldiner M. Embracing the void--how much do we really know about targeting and translocation to the endoplasmic reticulum? *Curr Opin Cell Biol*. 2014; 29(8-17. [PubMed: 24662022]
- [10] Schauble N et al. BiP-mediated closing of the Sec61 channel limits Ca²⁺ leakage from the ER. *EMBO J*. 2012; 31(15):3282-96. [PubMed: 22796945]
- [11] Geva Y Schuldiner M. The back and forth of cargo exit from the endoplasmic reticulum. *Curr Biol* 2014; 24(3):R130-6. [PubMed: 24502791]
- [12] Fossati M Colombo SF Borgese N. A positive signal prevents secretory membrane cargo from recycling between the Golgi and the ER. *EMBO J*. 2014; 33(18):2080-97. [PubMed: 25063674]
- [13] Spooner RA Lord JM. How ricin and Shiga toxin reach the cytosol of target cells: retrotranslocation from the endoplasmic reticulum. *Curr Top Microbiol Immunol*. 2012; 357(19-40. [PubMed: 21761287]
- [14] Stechmann B et al. Inhibition of retrograde transport protects mice from lethal ricin challenge. *Cell*. 2010; 141(2):231-42. [PubMed: 20403321]
- [15] Ellgaard L Helenius A. Quality control in the endoplasmic reticulum. *Nat Rev Mol Cell Biol*. 2003; 4(3):181-91. [PubMed: 12612637]
- [16] Behnke J Feige MJ Hendershot LM. BiP and its nucleotide exchange factors Grp170 and Sil1: mechanisms of action and biological functions. *J Mol Biol*. 2015; 427(7):1589-608. [PubMed: 25698114]
- [17] Otero JH et al. Dissection of structural and functional requirements that underlie the interaction of ERdj3 protein with substrates in the endoplasmic reticulum. *J Biol Chem*. 2014; 289(40):27504-12. [PubMed: 25143379]
- [18] Anelli T van Anken E. Missing links in antibody assembly control. *Int J Cell Biol*. 2013; 606703. [PubMed: 24489546]
- [19] Shimizu Y Meunier L Hendershot LM. pErp1 is significantly up-regulated during plasma cell differentiation and contributes to the oxidative folding of immunoglobulin. *Proc Natl Acad Sci U S A*. 2009; 106(40):17013-8. [PubMed: 19805157]
- [20] van Anken E et al. Efficient IgM assembly and secretion require the plasma cell induced endoplasmic reticulum protein pErp1

- Proc Natl Acad Sci U S A. 2009; 106(40):17019-24. [PubMed: 19805154]
- [21] Bulleid NJ. Disulfide bond formation in the mammalian endoplasmic reticulum. *Cold Spring Harb Perspect Biol.* 2012; 4(11):[PubMed: 23125019]
- [22] Bulleid NJ Ellgaard L. Multiple ways to make disulfides. *Trends Biochem Sci.* 2011; 36(9):485-92. [PubMed: 21778060]
- [23] Tu BP Weissman JS. Oxidative protein folding in eukaryotes: mechanisms and consequences. *J Cell Biol.* 2004; 164(3):341-6. [PubMed: 14757749]
- [24] Wang L et al. Glutathione peroxidase 7 utilizes hydrogen peroxide generated by Ero1alpha to promote oxidative protein folding. *Antioxid Redox Signal.* 2014; 20(4):545-56. [PubMed: 23919619]
- [25] Araki K Inaba K. Structure, mechanism, and evolution of Ero1 family enzymes. *Antioxid Redox Signal.* 2012; 16(8):790-9. [PubMed: 22145624]
- [26] Battle DM et al. Expression of the endoplasmic reticulum oxidoreductase Ero1alpha in gastro-intestinal cancer reveals a link between homocysteine and oxidative protein folding. *Antioxid Redox Signal.* 2013; 19(1):24-35. [PubMed: 23373818]
- [27] Benham AM et al. Ero1-PDI interactions, the response to redox flux and the implications for disulfide bond formation in the mammalian endoplasmic reticulum. *Philos Trans R Soc Lond B Biol Sci.* 2013; 368(1617):20110403. [PubMed: 23530257]
- [28] Zhang L et al. Different interaction modes for protein-disulfide isomerase (PDI) as an efficient regulator and a specific substrate of endoplasmic reticulum oxidoreductin-1alpha (Ero1alpha). *J Biol Chem.* 2014; 289(45):31188-99. [PubMed: 25258311]
- [29] Goldenberger RF Epstein CJ Anfinsen CB. Acceleration of reactivation of reduced bovine pancreatic ribonuclease by a microsomal system from rat liver. *J Biol Chem.* 1963; 238(2):628-35. [PubMed: 13948694]
- [30] Okumura M Kadokura H Inaba K. Structures and functions of protein disulfide isomerase family members involved in proteostasis in the endoplasmic reticulum. *Free Radic Biol Med.* 2015; 83: 314-22. [PubMed: 25697777]
- [31] Ellgaard L Ruddock LW. The human protein disulphide isomerase family: substrate interactions and functional properties. *EMBO Rep.* 2005; 6(1):28-32. [PubMed: 15643448]
- [32] Anelli T et al. Thiol-mediated protein retention in the endoplasmic reticulum: the role of ERp44. *EMBO J.* 2003; 22(19):5015-22. [PubMed: 14517240]
- [33] Kakinana T et al. Dynamic regulation of Ero1alpha and peroxiredoxin 4 localization in the secretory pathway. *J Biol Chem.* 2013; 288(41):29586-94. [PubMed: 23979138]
- [34] Fraldi A et al. Multistep, sequential control of the trafficking and function of the multiple sulfatase deficiency gene product, SUMF1 by PDI, ERGIC-53 and Erp44. *Hum Mol Genet.* 2008; 17(17):2610-21. [PubMed: 18508857]
- [35] Anelli T et al. Ero1alpha regulates Ca(2+) fluxes at the endoplasmic reticulum-mitochondria interface (MAM). *Antioxid Redox Signal.* 2012; 16(10):1077-87. [PubMed: 21854214]
- [36] Vavassori S et al. A pH-regulated quality control cycle for surveillance of secretory protein assembly. *Mol Cell.* 2013; 50(6):783-92. [PubMed: 23685074]
- [37] Hagiwara M et al. Structural basis of an ERAD pathway mediated by the ER-resident protein disulfide reductase ERdj5. *Mol Cell.* 2011; 41(4):432-44. [PubMed: 21329881]
- [38] Ushioda R et al. ERdj5 is required as a disulfide reductase for degradation of misfolded proteins in the ER. *Science.* 2008; 321(5888):569-72. [PubMed: 18653895]
- [39] Cormier JH et al. EDEM1 recognition and delivery of misfolded proteins to the SEL1L-containing ERAD complex. *Mol Cell.* 2009; 34(5):627-33. [PubMed: 19524542]
- [40] Marin MB et al. Tyrosinase degradation is prevented when EDEM1 lacks the intrinsically disordered region. *PloS one.* 2012; 7(8):e42998. [PubMed: 22905195]
- [41] Clerc S et al. Htm1 protein generates the N-glycan signal for glycoprotein degradation in the endoplasmic reticulum. *J Cell Biol.* 2009; 184(1):159-72. [PubMed: 19124653]
- [42] Christianson JC Ye Y. Cleaning up in the endoplasmic reticulum: ubiquitin in charge. *Nat Struct Mol Biol.* 2014; 21(4):325-35. [PubMed: 24699081]
- [43] Kikkert M et al. Human HRD1 is an E3 ubiquitin ligase involved in degradation of proteins from the endoplasmic reticulum. *J Biol Chem.* 2004; 279(5):3525-34. [PubMed: 14593114]
- [44] Wunderley L et al. SGTA regulates the cytosolic quality control of hydrophobic substrates. *J Cell Sci.* 2014; 127(21):4728-39. [PubMed: 25179605]
- [45] Leznicki P High S. SGTA antagonizes BAG6-mediated protein triage. *Proc Natl Acad Sci U S A.* 2012; 109(47):19214-9. [PubMed: 23129660]
- [46] Olzmann JA Kopito RR Christianson JC. The mammalian endoplasmic reticulum-associated degradation system. *Cold Spring Harb Perspect Biol.* 2013; 5(9):[PubMed: 23232094]
- [47] Fleig L et al. Ubiquitin-dependent intramembrane rhomboid protease promotes ERAD of membrane proteins. *Mol Cell.* 2012; 47(4):558-69. [PubMed: 22795130]
- [48] Walter P Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science.* 2011; 334(6059):1081-6. [PubMed: 22116877]
- [49] van Anken E et al. Sequential waves of functionally related proteins are expressed when B cells prepare for antibody secretion. *Immunity.* 2003; 18(2):243-53. [PubMed: 12589777]
- [50] Harding HP Zhang Y Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature.* 1999; 397(6716):271-4. [PubMed: 10400498]
- [51] Sidrauski C et al. Pharmacological brake-release of mRNA translation enhances cognitive memory. *eLife.* 2013; 2:e00498. [PubMed: 23741617]
- [52] Sekine Y et al. Stress responses. Mutations in a translation initiation factor identify the target of a memory-enhancing compound. *Science.* 2015; 348(6238):1027-30. [PubMed: 25858979]
- [53] Ma Y Hendershot LM. Delineation of a negative feedback regulatory loop that controls protein translation during endoplasmic reticulum stress. *J Biol Chem.* 2003; 278(37):34864-73. [PubMed: 12840028]
- [54] Brush MH Weiser DC Shenolikar S. Growth arrest and DNA damage-inducible protein GADD34 targets protein phosphatase 1 alpha to the endoplasmic reticulum and promotes dephosphorylation of the alpha subunit of eukaryotic translation initiation factor 2. *Mol Cell Biol.* 2003; 23(4):1292-303. [PubMed: 12556489]
- [55] Chen R et al. G-actin provides substrate-specificity to eukaryotic initiation factor 2alpha holophosphatases. *eLife.* 2015; 4:04871 [PubMed: 25774600]

- [56] Li H et al. Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. *Proc Natl Acad Sci U S A*. 2010; 107(37):16113-8. [PubMed: 20798350]
- [57] Aragón T et al. Messenger RNA targeting to endoplasmic reticulum stress signalling sites. *Nature*. 2009; 457(7230):736-40. [PubMed: 19079237]
- [58] van Anken E et al. Specificity in endoplasmic reticulum-stress signaling in yeast entails a step-wise engagement of HAC1 mRNA to clusters of the stress sensor Ire1. *eLife*. 2014; 3:e05031. [PubMed: 25549299]
- [59] Yanagitani K et al. Cotranslational targeting of XBP1 protein to the membrane promotes cytoplasmic splicing of its own mRNA. *Mol Cell*. 2009; 34(2):191-200. [PubMed: 19394296]
- [60] Lee AS Hendershot LM. ER-stress and cancer. *Cancer Biol Ther*. 2006; 5(7):721-2. [PubMed: 16880733]
- [61] Carrara M et al. Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein CH1 to initiate ER-stress signaling. *eLife*. 2015; 4:03522 [PubMed: 25692299]
- [62] Bertolotti A et al. Dynamic interaction of BiP and ER-stress transducers in the unfolded-protein response. *Nat Cell Biol*. 2000; 2(6):326-32. [PubMed: 10854322]
- [63] Anelli T et al. Sequential steps and checkpoints in the early exocytic compartment during secretory IgM biogenesis. *EMBO J*. 2007; 26(19):4177-88. [PubMed: 17805346]
- [64] Martensson IL Keenan RA Licence S. The pre-B-cell receptor. *Curr Opin Immunol*. 2007; 19(2):137-42. [PubMed: 17306522]
- [65] Feige MJ Buchner J. Principles and engineering of antibody folding and assembly. *Biochim Biophys Acta*. 2014; 1844(11):2024-31. [PubMed: 24931831]
- [66] Hendershot LM Sitia R. Immunoglobulin assembly and secretion. in: *Molecular Biology of B Cells*, Honjo T Alt FW and Neuberger MS, Eds. Elsevier Acad Press, Amsterdam. 2005; 261-73.
- [67] Shapiro IM et al. Boning up on autophagy: the role of autophagy in skeletal biology. *Autophagy*. 2014; 10(1):7-19. [PubMed: 24225636]
- [68] Settembre C et al. Signals from the lysosome: a control centre for cellular clearance and energy metabolism. *Nat Rev Mol Cell Biol*. 2013; 14(5):283-96. [PubMed: 23609508]
- [69] Pennuto M et al. Ablation of the UPR-mediator CHOP restores motor function and reduces demyelination in Charcot-Marie-Tooth 1B mice. *Neuron*. 2008; 57(3):393-405. [PubMed: 18255032]
- [70] Wrabetz L et al. Different intracellular pathomechanisms produce diverse Myelin Protein Zero neuropathies in transgenic mice. *J Neurosci*. 2006; 26(8):2358-68. [PubMed: 16495463]
- [71] D'Antonio M et al. Resetting translational homeostasis restores myelination in Charcot-Marie-Tooth disease type 1B mice. *J Exp Med*. 2013; 210(4):821-38. [PubMed: 23547100]
- [72] Das I et al. Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science*. 2015; 348(6231):239-42. [PubMed: 25859045]
- [73] Lomas DA et al. The mechanism of Z alpha 1-antitrypsin accumulation in the liver. *Nature*. 1992; 357(6379):605-7. [PubMed: 1608473]
- [74] Bernascone I et al. A transgenic mouse model for uromodulin-associated kidney diseases shows specific tubulo-interstitial damage, urinary concentrating defect and renal failure. *Hum Mol Genet*. 2010; 19(15):2998-3010. [PubMed: 20472742]
- [75] Lencer WI et al. Innate Immunity at Mucosal Surfaces: the IRE1-RIDD-RIG-I Pathway. *Trends Immunol*. 2015; [PubMed: 26093676]
- [76] Cheng SH et al. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell*. 1990; 63(4):827-34. [PubMed: 1699669]
- [77] Payne AS Kelly EJ Gitlin JD. Functional expression of the Wilson disease protein reveals mislocalization and impaired copper-dependent trafficking of the common H1069Q mutation. *Proc Natl Acad Sci U S A*. 1998; 95(18):10854-9. [PubMed: 9724794]
- [78] Marino M et al. SEPN1, an endoplasmic reticulum-localized selenoprotein linked to skeletal muscle pathology, counteracts hyperoxidation by means of redox-regulating SERCA2 pump activity. *Hum Mol Genet*. 2015; 24(7):1843-55. [PubMed: 25452428]
- [79] Bertolotti M et al. Tyrosine kinase signal modulation: a matter of H₂O₂ membrane permeability? *Antioxid Redox Signal*. 2013; 19(13):1447-51. [PubMed: 23541115]
- [80] Howson R et al. Construction, verification and experimental use of two epitope-tagged collections of budding yeast strains. *Comp Funct Genomics*. 2005; 6(1-2):2-16. [PubMed: 18629296]
- [81] Encell LP et al. Development of a dehalogenase-based protein fusion tag capable of rapid, selective and covalent attachment to customizable ligands. *Curr Chem Genomics*. 2012; 6:55-71. [PubMed: 23248739]
- [82] Hall MP et al. Engineered luciferase reporter from a deep sea shrimp utilizing a novel imidazopyrazinone substrate. *ACS Chem Biol*. 2012; 7(11):1848-57. [PubMed: 22894855]
- [83] Nguyen VD et al. Pre-expression of a sulfhydryl oxidase significantly increases the yields of eukaryotic disulfide bond containing proteins expressed in the cytoplasm of *E.coli*. *Microbial Cell Fact*. 2011; 10:1. [PubMed: 21211066]
- [84] Hatahet F Ruddock LW. Topological plasticity of enzymes involved in disulfide bond formation allows catalysis in either the periplasm or the cytoplasm. *J Mol Biol*. 2013; 425(18):3268-76. [PubMed: 23810903]
- [85] Matos CF et al. Efficient export of prefolded, disulfide-bonded recombinant proteins to the periplasm by the Tat pathway in *Escherichia coli* CyDisCo strains. *Biotech Progress*. 2014; 30(2):281-90. [PubMed: 24376243]