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MEETING REPORT

Meeting Report – proteostasis in Ericeira

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ABSTRACT

It was a sunny Ericeira, in Portugal, that received the participants of the EMBO Workshop on Proteostasis, from 17 to 21 November 2017. Most participants gave talks or presented posters concerning their most recent research results, and lively scientific discussions occurred against the backdrop of the beautiful Atlantic Ocean.

Proteostasis is the portmanteau of the words protein and homeostasis, and it refers to the biological mechanisms controlling the biogenesis, folding, trafficking and degradation of proteins in cells. An imbalance in proteostasis can lead to the accumulation of misfolded proteins or excessive protein degradation, and is associated with many human diseases. A wide variety of research approaches are used to identify the mechanisms that regulate proteostasis, typically involving different model organisms (yeast, invertebrates or mammalian systems) and different methodologies (genetics, biochemistry, biophysics, structural biology, cell biology and organismal biology). Around 140 researchers in the proteostasis field met in the Hotel Vila Galé, Ericeira, Portugal for the EMBO Workshop in Proteostasis, organized by Pedro Domingos (ITQB-NOVA, Oeiras, Portugal) and Colin Adrain (IGC, Oeiras, Portugal). In this report, we attempt to review and integrate the ideas that emerged at the workshop. Owing to space restrictions, we could not cover all talks or posters and we apologize to the colleagues whose presentations could not be discussed.

View of the meeting location. Image courtesy of Hotel Vila Galé Ericeira.

Protein biogenesis and quality control

The quality control of protein biogenesis begins when nascent polypetides emerge from the translating ribosomes. Claudio Joazeiro (Heidelberg University, Germany) covered the role of yeast and mammalian homologs of the Listerin E3 ubiquitin ligase and its cofactors in a process his group had originally discovered – the degradation of aberrant polypeptide nascent chains in stalled ribosomes (ribosome-associated quality control; RQC). He then provided further evidence strengthening the link between RQC and neurodegeneration from novel mouse models with mutations in RQC factors. Danny Nedialkova (MPI of Biochemistry, Martinsried, Germany) followed up on the theme of codon-specific ribosome pausing, which causes the aggregation of many essential proteins and impairs the ability of cells to re-establish proteostasis (Nedialkova and Leidel, 2015). Lea Sistonen (Abo Akademi University, Turku, Finland) talked about the transcriptional regulation of proteostasis mediated by heat shock factor 1 (HSF1) (Vihervaara et al., 2017) and the epigenetic regulatory mechanisms that establish the transcriptional memory of stress. Claudina Rodrigues-Pousada (ITQB-NOVA, Oeiras, Portugal) followed up on the transcriptional regulation theme. Budding yeast cells exposed to arsenic compounds adapt to this stress by stabilizing Yap8, a member of AP1 family of transcription factors. Yap8 degradation under non-stress conditions is mediated by Ubc4, Rad23 and Dsk2, whereas the ubiquitin ligase Ufd2 stabilizes Yap8 upon arsenic exposure (Ferreira et al., 2015). Eszter Zavodszky (MRC LMB, Cambridge, UK) discussed the quality control of misfolded glycosylphosphatidylinositol (GPI)-anchored proteins, whose degradation involves traversing the secretory pathway to the cell surface, accompanied by ER-resident chaperones, on their way to the lysosomes.

Protein folding in the endoplasmic reticulum and the unfolded protein response

Accumulation of misfolded proteins in the endoplasmic reticulum (ER) activates the unfolded protein response (UPR) (Walter and Ron, 2011), a network of signalling pathways that attempt to restore homeostasis to the ER. A long-standing question in the field is how the accumulation of misfolded proteins activates the UPR sensors – ATF6, PERK and IRE1 (also known as ERN1). One possibility is that misfolded peptides directly bind to the luminal domains of the UPR sensors, in particular IRE1 (Karagoz et al., 2017), to promote its dimerization and activation. An alternative possibility is that IRE1 activation is regulated by BiP recruitment to IRE1 (Bertolotti et al., 2000). Based on an *in vitro* reconstituted system, David Ron (University of Cambridge, UK) presented evidence that the ER luminal co-chaperone ERdj4 is required for the formation of a repressive complex between BiP (luminal Hsp70) and IRE1 (Amin-Wetzel et al., 2017). Presumably, activation of IRE1 occurs when the accumulation of misfolded proteins recruits BiP and promotes the disassembly of this IRE1-repressing complex.

One UPR pathway regulated by IRE1 is the XBP1 pathway, which modulates transcription of target genes during ER stress. Another UPR pathway mediated by the UPR sensor IRE1 is regulated IRE1-dependent decay (RIDD), in which mRNAs are degraded by the ribonuclease activity of IRE1 (Hollien et al., 2009;

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Hollien and Weissman, 2006). Julie Hollien (University of Utah, Salt Lake City, USA) showed that RIDD protects cells from ER stress-induced apoptosis, potentially stabilizing plasma membrane proteins when *de novo* synthesis is compromised. Bertrand Mollereau (ENS Lyon, France) demonstrated the role of RIDD pathway target fatty acid transport protein (Fatp) (Coelho et al., 2013; Dourlen et al., 2015) in lipid droplet biogenesis in *Drosophila* and mouse retinas, while Fátima Cairrão (ITQB-NOVA, Oeiras, Portugal) spoke about the post-transcriptional mechanisms that regulate *Xbp1* mRNA stability.

Another UPR sensor is PERK, which is involved in attenuation of global translation by phosphorylating the translation initiation factor eIF2α, thus causing the specific upregulation of the transcription factor ATF4. eIF2α phosphorylation and ATF4 activation also occur through the activation of other kinases, such as GCN2, upon bacterial infection. Hyung Don Ryoo (New York University, USA) showed the involvement of an additional layer of translational regulation upon ATF4 activation. Specifically, that the cap-dependent translation inhibitor 4E-BP is induced by GCN2–ATF4, which interferes with cap-dependent translation in the stressed cells. This led to the idea that mRNAs that need to be efficiently translated under conditions associated with eIF2α phosphorylation should have the means to bypass the inhibition of translation that is imposed by the GCN2–ATF4–4E-BP pathway. Indeed, they found that transcripts induced by this pathway, including those that encode anti-microbial peptides and BiP, contain IRES elements that allow them to bypass the inhibition of translation imposed by 4E-BP induction (Kang et al., 2017; Vasudevan et al., 2017).

Luigi Puglielli (University of Wisconsin, Madison, USA) described how the posttranslational Nε-lysine acetylation of ER-resident and ER cargo proteins regulates proteostasis within the secretory pathway and discussed disease phenotypes associated with defects in the acetylation machinery in the ER. Roberto Sitia (University San Raffaele, Milan, Italy) discussed the connections between proteostasis and redoxtasis, the maintenance of homeostasis of reactive oxygen species that originate from disulfide bond formation in ER cargo proteins. He described the role of ERp44, ERGIC53 and Ero1 in coupling oxidative folding, oligomerization and quality control of cargo proteins as they proceed along the early secretory compartment.

ER-associated degradation

In the ER, there is a molecular machinery that is involved in the detection of misfolded protein and their retro-translocation to the cytoplasm so that these proteins can be targeted for proteasomal degradation, a process referred to as ER-associated degradation (ERAD) (Brodsky, 2012). Proteomic and other studies have identified many proteins that form an ERAD interaction network, including the ER-resident E3 ubiquitin ligase Hrd1 (Carvalho et al., 2006; Christianson et al., 2012; Denic et al., 2006). Tom Rapoport (Harvard University, Cambridge, USA) presented *in vitro* reconstitution results showing that Hrd1 forms the long sought-after retro-translocation channel (Baldridge and Rapoport, 2016), as well as cryo-electron microscopy (EM) data indicating that the transmembrane domains of Hrd1 form a hydrophilic cone, presumably to allow for the retro-translocation of misfolded proteins. Pedro Carvalho (University of Oxford, UK) focused on novel results for a different E3 ubiquitin ligase complex – the Asi complex – that allows for the retro-translocation of misfolded proteins from the inner nuclear membrane to the nuclear matrix (Foresti et al., 2014).

Three talks, given by Liz Miller (MRC LMB, Cambridge, UK), John Christianson (University of Oxford, UK) and Matthew Shurtleff (University of California, San Francisco, USA), focused on the ER membrane complex (EMC), a multiprotein complex that had been previously identified to be associated with protein folding in the ER (Jonikas et al., 2009), and ERAD (Christianson et al., 2012). The emerging consensus is that, instead of having a direct role in ERAD, the EMC appears to be required for the biogenesis and membrane insertion of proteins with transmembrane domains with low hydrophobicity, a model recently substantiated for particular tail-anchor proteins (Guna et al., 2017).

Quality control and stress response pathways in mammalian homeostasis and disease

Organelles, including the ER, have dedicated quality control and stress response machineries. Although the biochemistry and cell biology of ERAD and/or UPR has been dissected extensively, the organismal roles of these important homeostatic pathways remain underexplored. Ling Qi (University of Michigan Medical School, Ann Arbor, USA) provided an elegant example of the physiological importance of ERAD by demonstrating its essential role in the hormonal signalling relay that maintains water homeostasis in mammals. The antidiabetic hormone arginine vasopressin (AVP), which is secreted by the hypothalamus, is a key negative regulator of water excretion by the kidneys. Pro-AVP is a thiol-rich protein prone to misfolding in the ER. When the Sel1L–Hrd1 ERAD complex is disabled by specifically knocking out Sel1L in AVP neurons in the hypothalamus, pro-AVP is retained in the ER and forms intermolecular disulfide-bonded aggregates (Shi et al., 2017). As a consequence, no AVP is secreted, and the mice develop polyuria and polydipsia, characteristics of diabetes insipidus.

In recent years, the importance of stress response pathways that ensure the robustness of the host parenchyma (i.e. non-immune compartment) in the face of infection has become apparent. This phenomenon, called disease tolerance, is distinct from the immune cell-mediated resistance mechanisms that limit the pathogen load (Soares et al., 2017). Luis Moita (IGC, Oeiras, Portugal), whose previous work showed that low doses of the anthracycline anticancer drug family conferred disease tolerance to severe sepsis (Figueiredo et al., 2013), presented unpublished data that defined the precise mechanistic basis of the salutary effects of anthracyclins. His talk also illustrated the importance of organelle-specific stress responses in mediating the hormetic effects of some commonly used clinically approved drugs. Continuing on the theme of the mechanism of action of common pro-apoptotic chemotherapeutic drugs, Seamus Martin (Trinity College, Dublin, Ireland) discussed unpublished data that revealed a surprising twist in how a group of widely used anti-mitotic chemotherapeutic agents promote inflammation through initiating ER stress via upregulation of members of the TNF ‘death receptor’ family.

Claudia Almeida (CEDOC/NOVA Medical School, Lisbon, Portugal) discussed the link between the regulators of endocytic trafficking BIN1 and CD2AP and the production of β-amyloid within the endosomes in late-onset Alzheimer’s disease (Ubelmann et al., 2017). Paola Picotti (ETH Zürich, Switzerland) described a limited proteolysis mass-spectrometry-based tool to detect and quantitatively analyse conformational changes of aggregation prone proteins, such as α-synuclein, directly in the cellular matrix and on a proteome-wide scale.

Ubiquitin and the proteasome

The proteasome is essential for degradation of ubiquitylated proteins. Hermann Steller (The Rockefeller University, New York, USA) spoke about the regulation of the 26S proteasome by tankyrase

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(TNKS)-mediated ADP-rybosylation of the PI31 protein. Genetic activation of the TNKS–PI31 pathway promotes the assembly of the 26S proteasome and prevents age-related neurodegeneration in animal models. Ugo Mayor (Ikerbasque, Bilbao, Spain) discussed proteomic methods to identify the ubiquitylated substrates of the two disease-related E3 ubiquitin ligases Parkin and UBE3A, which are involved in Parkinson's disease and Angelman syndrome, respectively. Thomas Sommer (Max Delbrück Centre, Berlin, Germany) followed up on the ubiquitylation theme by speaking about the role of the muscle-specific MuRF family of RING ubiquitin ligases, which function as adaptors in complexes containing DCAF and Cullin4.

When the ubiquitin proteasome system is overwhelmed, misfolded proteins aggregate in the vicinity of the centrosome, forming a structure named the aggresome. Suzanna Prosser (Lunenfeld-Tanenbaum Research Institute, Toronto, Canada) discussed the role of the centrosomal proteins CP110, CEP97 and CEP290 in aggresome formation.

Trafficking and protein quality control by the rhomboid superfamily

Several talks expanded our view of the functions of the enigmatic rhomboid superfamily. Rhomboids were identified as intramembrane proteases that cleave growth factors to control intercellular signalling in *Drosophila*, but over the past 5 years, rhomboid-like proteins have emerged as serving a wide variety of important catalytic and non-catalytic functions (Lemberg and Adrain, 2016). Matthew Freeman (University of Oxford, UK) reported an important physiological role for a rhomboid-like protein in the control of lipid homeostasis in mammals. Ioanna Oikonomidi (IGC, Oeiras, Portugal) focused on a novel regulator of the iRhom–ADAM17 axis, a pathway important for inflammatory and growth factor signalling (Lemberg and Adrain, 2016). Kvido Strisovsky (Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic) reported a novel physiological role for a bacterial rhomboid protease in protein quality control which impacted on ion homeostasis *in vivo*. Marius Lemberg (Heidelberg University, Germany) expanded on the role of the rhomboid protease RHBDL4 (Fleig et al., 2012), focusing on its regulation of abundance control in the ER.

Drug discovery for proteostasis-associated diseases

Protein misfolding, or a failure to clear misfolded proteins or their aggregates, lies at the heart of a range of diseases. An obvious goal in the treatment of such diseases is to enhance misfolded protein clearance, reduce translational levels to relieve the burden on chaperones, or, specifically attack the proteostatic machinery in pathogens but not their host. For loss-of-function genetic diseases, enhancing the folding of a mutant protein, or overriding quality control checkpoints that retain mutant proteins in the ER could be therapeutically beneficial. Anne Bertolotti (MRC-LMB, Cambridge, UK) focused on the challenge of specifically targeting protein phosphatase-1 (PP1), a holophosphatase with extensive cellular roles, complicating its therapeutic targeting. Fortunately, PP1 specificity is governed by a smaller number of regulatory subunits, including R15A. Anne showed that drugging R15A with small-molecule inhibitors (e.g. sephin1 or guanabenz) counteracts the proteostasis-associated phenotype in mouse models of neurological diseases (Das et al., 2015). This maintains the translation initiation factor eIF2α in a phosphorylated state, prolonging UPR-associated translational repression and reducing the burden on chaperones, thereby ameliorating the proteostasis deficit.

Larry Dick (Takeda Oncology, Cambridge, USA) described the development of inhibitors to target the proteasome in the malarial parasite *Plasmodium falciparum*. A common drug to reduce malarial parasite load is the natural product artemisinin (Tu, 2011), which kills *P. falciparum* by mechanisms that may include triggering proteotoxic stress in the parasite (Zhang et al., 2017). In turn, this imposes selective pressure on the parasite to acquire mutations to mitigate the proteotoxicity. Larry showed that inhibitors that specifically target *P. falciparum* proteasomes but spare the host proteasome may help to undermine parasite resistance to artemisinin (Dogovski et al., 2015). Jeff Brodsky (University of Pittsburgh, USA) discussed the establishment of a yeast model to characterize the biogenesis and ERAD of the K^+ channel ROMK, which is mutated in a condition called Bartter syndrome. This yeast model could be useful to identify conditions or drugs to optimize the biogenesis of ERAD-susceptible mutant proteins and to identify the machinery that degrades the mutant protein, thus providing insights for future therapies (O'Donnell et al., 2017).

Margarida Amaral (University of Lisbon, Portugal) continued on the theme of monogenetic diseases and ERAD by focusing on cystic fibrosis (CF), a debilitating condition that is caused by loss of function mutations in the Cl^- channel CFTR. As CFTR normally represses the activity of the Na^+ channel ENaC, in CF patients, the loss of CFTR also results in Na^+ hyperabsorption and airway dehydration. Margarida described cell-based functional genomics approaches that were used to identify both ENaC regulators and genes that, when downregulated, can rescue the trafficking of F508del-CFTR, the most prevalent CF-causing mutant, which is retained in the ER and prematurely degraded by ERAD. This mutant can exhibit some functional channel activity if its ER retention by the quality control machinery can be overridden, allowing it to traffic to the plasma membrane. Also focusing on CFTR, Carlos Farinha (University of Lisbon, Portugal) described how cAMP signalling stabilizes CFTR on the plasma membrane through the guanine nucleotide exchange factor EPAC1 to block CFTR endocytosis (Lobo et al., 2016).

The tumour microenvironment is often subject to a range of stresses, making it advantageous for cancers to upregulate stress pathways to mitigate stressful conditions. Xi Chen (Baylor College of Medicine, Houston, USA) discussed the requirement and mechanisms for activation of the UPR by oncogenes during tumour progression, which offers the possibility of an additional weapon to tackle aggressive cancers by drugging the UPR. Ville Paavilainen (University of Helsinki, Finland) focused on the mechanism of action of mycolactone, an immunosuppressive compound released by the human pathogen *Mycobacterium ulcerans* that impairs the Sec61-dependent protein translocation of key immunomodulatory molecules.

Modulation of protein aggregation – age and other factors

Previous work in *C. elegans* has clearly demonstrated that ageing leads to the aggregation of hundreds of proteins (David et al., 2010). By analysing the soluble and insoluble proteome, Daniel Jarosz (Stanford University, USA) has now confirmed this observation also applies to aging vertebrates by using the African turquoise killifish model system; this revealed tissue-specific changes in aggregation and proteostasis with age. Della David (DZNE, Tübingen, Germany) demonstrated that inhibition of the major protein quality control systems (namely chaperones, proteasome and autophagy) affects the aggregation of the same protein in different ways in different tissues. Taken together, this suggests that differential age-dependent aggregation in different tissues may be accounted for by not only a different repertoire of tissue-specific proteins, but also by different tissue-specific proteostasis mechanisms.

Ellen Nollen's (University of Groningen, The Netherlands) talk focused on genes that act as modifiers of aggregation, whose inactivation reduces aggregation and toxicity of polyQ (Glutamine) proteins without regulating their expression levels. Examples of such aggregation-modifying proteins include MOAG-2/LIR-3, a nuclear protein that is hijacked to the cytosol to promote aggregate formation (Sin et al., 2017), and MOAG-4/SERF2, which catalyses aggregation through direct and transient interaction with disease proteins, thus affecting the structure of the aggregate (Yoshimura et al., 2017).

One way to promote proteostasis and decrease age-related protein aggregation, both in *C. elegans* and in mammals, is to artificially and consistently activate the ER UPR. Rebecca Taylor (MRC LMB, Cambridge, UK) showed that in *C. elegans*, activation of an ER–UPR stress response pathway specifically in neurons or in the intestine of the animals not only affects longevity (Taylor and Dillin, 2013) but also reduces the toxicity associated with aggregating proteins across a variety of tissues. Interestingly, changes in lipid metabolism may underlie some of the beneficent effects of tissue-specific UPR activation. Counterintuitively, while constitutive expression of activated XBP1 promotes proteostasis in many model organisms, Claudio Hetz (University of Chile, Santiago, Chile) gave an overview over the complex involvement of the UPR in different brain diseases where, depending on the disease context and the signalling branch manipulated, distinct and even opposite effects are observed (Hetz and Saxena, 2017). Specifically, his group demonstrated that a brain-conditional knockdown of *Xbp1* can also improve pathology in murine models of age-related diseases such as amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD). He also showed that IRE1 deficiency protects against Alzheimer's disease (Duran-Aniotz et al., 2017), and discussed the development of gene therapy strategies to attenuate ER stress in disease.

Tomas Aragon (University of Navarra, Pamplona, Spain) presented results in which UPR activation predicted the death of primary neurons expressing pathologic forms of SOD1, an important factor in ALS. Xavier Le Goff (University of Rennes, France) discussed the identification of aggregation-prone mutations for the von Hippel–Lindau tumour suppression protein. Daniel Segal (Tel-Aviv University, Israel) discussed the effects of glycosylation in Tau aggregation and consequences for Alzheimer's disease-related phenotypes.

Eli Arama (Weizmann Institute, Rehovot, Israel) spoke about the destruction of paternal mitochondria after fertilization in *Drosophila*, a process that displays common features with the endocytic and autophagy pathways. Andrew Jarman (University of Edinburgh, UK) discussed the role of chaperones and other factors in the assembly of large multisubunit dynein motor complexes that are required to power beating cilia and flagella.

Crosstalk between longevity pathways and proteostasis-promoting pathways

Pathways that promote longevity are also associated with improved proteostasis. Thus, activation of longevity-promoting pathways may be beneficial for counteracting proteostasis failure. Accordingly, Sivan Henis-Korenblit (Bar-Ilan University, Ramat Gan, Israel) demonstrated that *C. elegans* mutants that are long-lived due to alterations in their reproductive system, produce an endogenous siRNA signal that enables the central proteostasis-promoting transcription factor HSF-1 to respond to proteotoxic conditions in old animals to a similar extent as in young animals. Furthermore, Thorsten Hoppe (University of Cologne, Germany) discovered that in *C. elegans*, the insulin and IGF-1 signalling pathway is actively restrained by the targeting of the insulin and IGF-1 receptor DAF-2 for endo-lysosomal degradation. Here, the insulin/IGF-1 receptor is marked for degradation by the same ubiquitin ligase that ubiquitylates misfolded proteins for their proteasomal degradation (Tawo et al., 2017). Taken together, these findings suggest that longevity-promoting pathways may be turned on by proteostasis-related stress response proteins, as an attempt to further counteract proteostasis failure.

Three poster sessions gave ample opportunity for discussion of the 64 posters presented. The FEBS Journal generously sponsored three 'best poster presentation' prizes, which were attributed to Emma Fenech (Oxford University, UK), Nivedita Natarajan (Oxford University, UK) and Yetis Gultekin (The Rockefeller University, New York, USA).

In its relaxed, friendly and collegial atmosphere, the workshop brought together experts using a wide variety of research approaches to identify the cellular and molecular mechanisms that regulate proteostasis. Novel methodologies, for example in proteomics or structural biology, raise new questions and opportunities, predicting that the pace of scientific discovery will increase in the next few years.

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Competing interests

The authors declare no competing or financial interests.

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