

# The life of proteins: the good, the mostly good and the ugly

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**The health of the proteome in the face of multiple and diverse challenges directly influences the health of the cell and the lifespan of the organism. A recent meeting held in Nara, Japan, provided an exciting platform for scientific exchange and provocative discussions on the biology of proteins and protein homeostasis across multiple scales of analysis and model systems.**

The International Conference on Protein Community brought together nearly 300 scientists in Japan to exchange ideas on how proteins in healthy humans are expressed, folded, translocated, assembled and disassembled, and on how such events can go awry, leading to a myriad of protein conformational diseases. The meeting, held in Nara, Japan, in September 2010, coincided with the 1,300<sup>th</sup> birthday of Nara, Japan's ancient capital, and provided a meditative setting for reflecting on the impact of advances in protein community research on biology and medicine. It also provided an opportunity to consider the success of the protein community program in Japan since meetings on the stress response (Kyoto, 1989) and on the life of proteins (Awaji Island, 2005). The highlights of the Nara meeting were, without question, the social periods held after long days of talks



and poster presentations. During these socials, graduate and postdoctoral students and all of the speakers sat together on tatami mats at low tables replete with refreshments and enjoyed each other's company, while participating in relaxed, spirited and open discussions.

## Nascency and nascent-chain biology

Replenishment and renewal of the 'protein community' of cellular proteins requires new proteins to be synthesized. A theme that coursed throughout multiple conference sessions was 'nascency', the period between initiation of the nascent chain and completion of its assembly into a functioning product. A nascent protein is in a particularly delicate state, prone to inappropriate interactions, misfolding and aggregation. To avoid these detrimental fates, nascent chains are guided through their maturation by molecular chaperones, modifying enzymes and targeting

and accessory factors. Considerable effort has been and continues to be devoted toward understanding the mechanistic basis of protein maturation and chaperone function.

Several new concepts and methods are bringing nascent-chain biology to the forefront of the field. Koreaki Ito (Kyoto Sangyo University, Japan), who has studied programmed ribosomal stalling by the *Escherichia coli* protein SecM, proposed that ribosomes have heterogeneous translation speeds. He presented a two-dimensional gel system to examine peptidyl-tRNA species, revealing the nonuniform nature of translation. Ito suggested that during elongation, the nascent chain is 'reviewed' both inside and outside the ribosome to adjust translation rates, by as-yet-unknown feedback mechanisms. In a global strategy to examine translation, Jonathan Weissman (University of California, San Francisco) has developed ribosome

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profiling, a deep sequencing-based method to accurately map the position of ribosomes on all translated mRNAs. Applying this method to bacterial and mammalian cells, Weissman illustrated that sites of translational pausing were readily detectable, opening the way for genome-wide analyses. By preselecting the ribosomes being analyzed, via their association with trigger factor, Weissman (in collaboration with Bernd Bukau) showed it was possible to monitor precisely when individual nascent chains engage this bacterial chaperone.

In a new example of how non-uniform translation is used by the cell, Kenji Kohno (Nara Institute of Science and Technology, Japan) showed that translational pausing may facilitate mRNA localization. A transient pause during Xbp1 translation, initiated when a short peptide segment enters the ribosomal tunnel, provides sufficient time for a preceding hydrophobic segment to 'drag' the entire ribosome-mRNA complex to the endoplasmic reticulum (ER) membrane. Xbp1 mRNA is spliced by the ER-resident protein Ire1- $\alpha$  when cells experience ER stress, showing that translational pausing influences mRNA trafficking and splicing. Analogous work from Ramanujan Hegde (US National Institutes of Health, Bethesda, Maryland) showed that transient pausing, just before termination of tail-anchored membrane proteins, may facilitate recruitment of factors for targeting. One such factor, Bat3 (also called Bag6), was suggested by Hegde to have a dual role in targeting and degradation and to function as a triage factor that facilitates degradation of improperly targeted membrane proteins. Quite satisfyingly, Weissman's ribosomal profiling method observed the pauses detected biochemically by Kohno and Hegde. Given the emerging evidence for the diverse uses and relatively widespread nature of translational pausing, it seems that nascent-chain processes are tightly regulated and choreographed.

To further illustrate the 'dance' of the nascent chain, Bernd Bukau (University of Heidelberg, Germany) described how multiple ribosome-associating maturation factors—peptide deformylase (PDF), methionine aminopeptidase (MAP) and trigger factor—share the crowded space surrounding the nascent chain. All three factors bind overlapping sites on the ribosome, but different kinetics explains their sequences of action. PDF, for example, has extremely fast on- and off-rates and does not discriminate between empty and translating ribosomes, which allows it to work first. Next, MAP acts on the deformylated nascent chains. Trigger factor, whose on-rate is very slow, binds only after ~60 residues have been synthesized. It is clear that there are tight interrelationships between trans-

lational elongation, the nascent polypeptide, the ribosome and ribosome-associating factors.

Upon release from the ribosome, nascent chains fold with the assistance of chaperones. Presentations by Masasuke Yoshida (Kyoto Sangyo University) and Hideki Taguchi (Tokyo Institute of Technology, Yokohama, Japan) addressed the function and mechanism of the GroEL chaperonin. Yoshida used various probes such as antibody accessibility and protease digestion to show that a folded substrate is not completely enclosed by GroEL but rather is transiently accessible to the environment. Most substrates spend time in a 'tethered' state that facilitates folding in the GroEL cage by avoiding nonproductive and kinetically trapped states, from which they can be released to fold rapidly or can escape and risk aggregation. Taguchi addressed the issue of defining GroEL substrates using a combination of *in vitro* and *in vivo* assays. He reinvestigated earlier work by Hartl (*Cell* 122, 209–220, 2005) and refined the classes of substrates that use the GroEL system to avoid aggregation. He suggested that GroEL may help buffer destabilizing mutations in proteins, thereby allowing their faster evolution.

Despite extensive work, little is known about how chaperones such as GroEL or GroES function to regulate the expression of nascent chains. Takashi Yura (Kyoto University, Japan) addressed chaperone-mediated feedback control in bacteria and described a new feedback-resistant mutant isolated with a transposon inserted upstream of the *ftsY* gene (encoding the bacterial signal recognition particle receptor FtsY). Yura proposed that the SRP-dependent targeting of sigma factor 32 to the bacterial inner membrane represents a newly discovered step that facilitates the inactivation and/or degradation of sigma factor 32, in order to coordinate the cellular responses to changing protein-folding states in the cytoplasm and membrane.

### Translocation across membranes

Another aspect of the protein community is the targeting of proteins from the cytosol to organelles, where they have to cross one or more membranes before reaching their final destination. Mitochondria import about 1,000 different proteins from the cytosol. Although different machineries for translocation of pre-proteins have been identified, less is known about the regulation of import. Nikolaus Pfanner (University of Freiburg, Germany) showed that the mitochondrial proteome contains numerous protein kinases and phosphatases and many more phosphorylated proteins than expected. He discussed how post-translational modification of transport components affects

the biogenesis and function of pre-protein translocases. Toshiya Endo (Nagoya University, Japan) discussed structural and dynamic aspects of protein import into mitochondria. Using an *in vivo* cross-linking approach, he showed that the cytosol receptor domain of Tom22 in the outer-membrane TOM40 complex changes its interaction with another receptor subunit, Tom20, to decode the pre-sequence targeting signal. Tom22 then changes its interaction with the inner-membrane pre-sequence receptor Tim50 to transfer the pre-sequence from Tom22 to Tim50.

Mechanisms of membrane fusion events were analyzed *in vitro* by Hisao Kondo (Kyushu University, Fukuoka, Japan) and Jodi Nunnari (University of California, Davis). Kondo used an ER reformation assay to identify new promoting and inhibitory factors for ER reassembly, and Nunnari described an *in vitro* mammalian mitochondrial fusion assay to address the significance of the two outer-membrane fusion dynamins, MFN1 and MFN2. MFN1-MFN2 *trans*-complexes showed greater efficacy in fusion as compared to either alone. Moreover, MFN2-only complexes also interacted with Bax, the proapoptotic Bcl2 protein, to regulate mitochondrial fusion, a result that provides further insight into the unique physiological roles of these mitochondrial fusion proteins. Mitochondrial fission, discussed by Katsuyoshi Mihara (Kyushu University), is associated with the recruitment of the cytoplasmic dynamin-related GTPase Drp1 to mitochondria. Much as in yeast, the mitochondrial outer-membrane protein Fis1 is thought to be a Drp1 receptor in mammals. However, Mihara showed that the mitochondrial fission and fusion protein Mff, but not Fis1, functions as an essential factor in mitochondrial recruitment of Drp1.

Although studies on membrane fusion events have revealed their importance for cellular quality control, the dynamic behavior of membranes raises questions about how the supply of membrane proteins and phospholipids is coordinated. Thomas Langer (University of Cologne, Germany) reported on a conserved protein network in the intermembrane space of yeast mitochondria that determines the phospholipid composition of mitochondrial membranes. The *i*-AAA protease Yme1, a mitochondrial quality-control enzyme, was found to regulate the steady-state levels of intrinsically unstable and lipid-specific proteins, suggesting that there is coordination between mitochondrial protein quality control and phospholipid metabolism. Masato Nakai (Osaka University, Japan) discussed the composition of the translocase of the inner membrane of the chloroplast. This large (1-MDa) complex with Tic20 as a core component was purified,

resulting in the identification of three previously uncharacterized components that are all essential for translocation.

In bacteria, protein translocation across the cytoplasmic membrane is mediated by the protein-conducting SecYEG channel (translocon) and the SecA ATPase. Because of a wealth of structural information, many of the mechanistic details of the process of protein translocation, including some that are controversial in the field, can now be tackled at the molecular level. Arnold Driessen (University of Groningen, The Netherlands) presented the results of single-molecule studies on the oligomeric state of the SecYEG complex. These SecYEG complexes were labeled with fluorescent markers, reconstituted into giant unilamellar vesicles at low protein-to-lipid ratios and subjected to fluorescence cross-correlation spectroscopy with single-molecule sensitivity. This allowed the detection of SecYEG and the determination of pre-protein dynamics in the lipid membranes as well as SecYEG complex formation. Tomoya Tsukazaki (University of Tokyo, Japan) presented the X-ray crystallographic structure of an accessory complex of the Sec translocase, the SecDF complex, thereby providing new insights about how these proteins may cooperate with the SecYEG and SecA.

How transmembrane helices are recognized for membrane insertion by the SecYEG translocon was addressed by Gunnar von Heijne (Stockholm University, Sweden), who described the use of a translation-arrest peptide as an *in vivo* sensor to map out the forces acting on a hydrophobic polypeptide segment during its passage through the ribosome-translocon complex. He observed a substantial force acting on the nascent polypeptide chain at the precise point during chain elongation when the hydrophobic segment is located within the translocon channel, a force possibly caused by the lateral partitioning of the transmembrane segment into the lipid bilayer. Masao Sakaguchi (University of Hyogo, Japan) systematically examined the effect of positive charges on polypeptide-chain movement through the translocon and showed that they arrest movement at the translocon by a mechanism involving electrostatic interaction. This translocation arrest might be the foundation for the various topogenic functions of positive charges in polypeptides.

The mechanistic details of peroxisomal protein import have remained obscure for many years, but recent developments have unmasked the major players, providing further understanding of protein import. Ralf Erdmann (Ruhr-Universität Bochum, Germany) pointed out the striking similarity

between the composition of the machinery for the import into peroxisomes and the machinery for ER-associated degradation of proteins. He discussed mechanistic aspects of the newly identified transient protein-import pore and an unusual deubiquitinating enzyme. He also proposed an export-driven import model for peroxisomal protein import, wherein the ATP- and AAA-peroxin-dependent export of the import receptor from the peroxisomal membrane to the cytosol is mechanically connected to cargo-protein import into the organelle. Yukio Fujiki (Kyushu University) addressed various aspects of peroxisome homeostasis in mammals. Through a series of biochemical experiments, he showed that matrix proteins are targeted by the cytosolic PTS-receptors, Pex5p and Pex7p, and subsequently imported via the Pex5p-docking complex comprising Pex14p and Pex13p. Pex5p then shuttles back to the cytosol with the aid of the AAA ATPase Pex1p–Pex6p complexes on Pex26p and a cytosolic factor.

#### The ER: sensing stress and folding

The ability of the ER to sense and respond to the accumulation of unfolded polypeptides represents another important facet of the protein community. Peter Walter (University of California, San Francisco) reported that short peptides bind and trigger oligomerization of the Ire1 ER-luminal domain in the unfolded protein response (UPR). He proposed that peptide ligands mimic unfolded proteins in the ER lumen, where disordered loops on the surface of misfolded proteins would similarly interact with Ire1, leading to its activation. Oligomerization of the luminal side of the ER membrane juxtaposes the Ire1 kinase and RNase domains, which, upon reaching a threshold concentration, clusters in an ordered array. Thus, the Ire1 kinase domain seems to serve as a conformational module that, akin to a rheostat, sets the sensitivity of Ire1 activation by the degree of unfolding of the protein ligands bound to the kinase ATP-binding site. This allows integration of the UPR with the metabolic state of the cell.

Like many forms of stress, persistent ER stress can lead to cell death. Feroz Papa (University of California, San Francisco) showed that Ire1- $\alpha$  controls a commitment switch into apoptosis. Under conditions of low ER stress, the kinase activity of Ire1- $\alpha$  autophosphorylates to activate XBP1 mRNA splicing and promote homeostasis. High-level activation of the Ire1- $\alpha$  kinase causes endonucleolytic decay of thousands of mRNAs encoding ER cargos, such as insulin and chaperones, and promotes apoptosis. Interestingly, the number of such ER sensors has changed over the course of

evolution from Ire1 in yeast, worm and flies to ATF6 in mammals. Kazutoshi Mori (Kyoto University) is investigating when and why this switch occurred, using the chicken DT40 cell line and the teleost fish medaka.

In the ER, oxidoreductases play major roles both for the ER-associated degradation (ERAD) of misfolded proteins and for the productive folding of newly synthesized proteins. Kaz Nagata (Kyoto Sangyo University) examined oxidoreductases by proteomic analysis and identified several that are oxidized by ERO1- $\alpha$ : ERp46, ERp57, P5 and PDI. Intriguingly, ERp72 functions as an inhibitory regulator of the ERO1- $\alpha$  oxidation system without inhibiting the oxidase activity of PDI. Kenji Inaba (Kyushu University) described the structure of human Ero1- $\alpha$  in its hyperactive and inactive forms, revealing how the enzyme generates protein disulfide bonds *de novo* in human cells and how its activity is tightly regulated to avoid hyperoxidation of the ER. Systematic biochemical studies also elucidated the mechanism by which Ero1- $\alpha$  oxidizes PDI specifically and efficiently among nearly 20 types of PDI-family member proteins. David Ron (University of Cambridge, UK) reported on a role for the ER-localized peroxiredoxin 4 (PRDX4) in disulfide bond formation. PRDX4 is redundant with ERO1, explaining the lack of phenotype in Prdx4-deficient mice. However, research on animals harboring mutations in ERO1 uncovered PRDX4's role in disulfide bond formation and reveals links between oxidative protein folding in the ER and peroxide production.

The importance of disulfide bond formation in the folding of many secretory proteins was highlighted by Hiroshi Kadokura (Nara Institute of Science and Technology), who detected oxidative folding intermediates of substrates and DsbA, a bacterial disulfide bond-forming enzyme. Analysis of these intermediates led him to propose the existence of factors that may affect the interaction between the enzyme and its substrate to regulate the formation of disulfide bonds. The role of the DnaJ-like cochaperones in ER quality control was discussed by Linda Hendershot (St. Jude Children's Research Hospital, Memphis, Tennessee), who showed how nonglycosylated ER proteins are recognized by the Hsp70 machinery. She showed that immunoglobulin heavy chains that have the potential to be secreted are associated with ERdj3. In order to be targeted for degradation, ERdj3 must be released to allow ERdj5 and ERdj4 to bind. ERdj4 is associated with proteins required for retrotranslocation to the cytosol, and its overexpression accelerates the degradation of non-glycosylated ERAD substrates.



**Stress and aging, misfolding and disease**

Aging, disease and the role of stress response pathways that upregulate chaperones have been topics of considerable interest to the protein community. Whereas robust quality control pathways and stress responses act to detect, destroy and mitigate the effects of misfolded proteins, an all-too-common outcome is their aggregation. Although almost all work to this point has focused on cell-autonomous stress responses in single eukaryotic cells, the diseases of protein misfolding occur in highly interconnected multicellular organisms.

Presentations by Rick Morimoto (Northwestern University, Evanston, Illinois) and Andrew Dillin (Salk Institute for Biological Studies, La Jolla, California) stressed the importance of complementing cell culture studies with analyses of animals. For the cytosolic heat-shock response (HSR), Morimoto showed that two thermosensory neurons in the nematode *Caenorhabditis elegans* can regulate the HSR in the worm's somatic tissues. An RNA interference screen was used to identify many of the signaling pathways involved, allowing the importance of the non-autonomous HSR to be examined. Remarkably, by permitting the autonomous HSR to operate, the worms improved their protein homeostasis and better dealt with misfolded proteins, but at the substantial cost of reduced fecundity. Also using *C. elegans*, Dillin showed that the mitochondrial unfolded protein response (mtUPR) can act non-autonomously in the cell by means of hypothesized 'mitokines'. Remarkably, induction of the mtUPR in neurons can activate it in other tissues such as the intestine, with downstream consequences for electron transport chain function and, ultimately, longevity. These studies highlighted the complexities of and differences in stress response regulation in cells that are isolated in the laboratory versus those found *in situ*.

Ulrich Hartl (Max Planck Institute of Biochemistry, Martinsried, Germany) described how appropriate chaperone levels can maintain protein homeostasis and help the cell avoid toxic aggregates. Why amyloid should be detrimental remains poorly understood, so to address this question, Hartl used synthetic  $\beta$ -sheet-forming proteins that generate artificial amyloids to identify the beta-interactome by proteomic analyses. The identification of cellular proteins that coaggregate with amyloids suggests that aggregation interferes with the basic defense mechanisms of the cell. Continuing the theme of protein aggregation, Harm Kampinga

(University of Groningen) described the non-overlapping anti-aggregation activities among the gene families encoding the large chaperones. Focusing on the diverse DNA-J family of cochaperones, Kampinga showed that DNAJB6 and DNAJB8 are the most efficient suppressors of aggregation and toxicity of polyglutamine proteins. He further showed that their activity is regulated by histone deacetylases and retained in cells with an abrogated HSR. This implies that members of the DNA-J family of chaperones could prevent disease, even in aged individuals whose HSR has declined.

The misfolding theme was addressed further by Chris Dobson (University of Cambridge), who provided an overview of misfolding and aggregation, pointing out that a protein's native state is in fact an unusual and special situation dominated by highly specific side chain interactions that force the main chain into a specific conformation. By contrast, the  $\beta$ -sheet-rich amyloid structure is a generic protein structure dominated by main chain interactions, largely irrespective of the side chains. This makes amyloid formation, associated with degenerative diseases, a possible outcome for many, if not most, cellular proteins. Furthermore, the amyloid state can be propagated by a nucleated seeding process, which Keiichi Higuchi (Shinshu University, Matsumoto, Japan) suggested could underlie transmission of certain amyloidoses among animals such as cheetahs and mice, to cause disease. This view is consistent with work by Hideki Nishitoh (University of Tokyo), who examined the toxic function of 130 different amyotrophic lateral sclerosis (ALS)-linked *SOD1* mutations. These *SOD1* mutants trigger ER stress through the interaction with Derlin-1, a component of the ERAD machinery, eventually leading to ER stress-mediated motor neuron death.

Clearance of damaged proteins is an essential component of the protein community and was represented by talks on the proteasome and on autophagy. Kunitoshi Yamanaka (Kumamoto University, Japan) described how the ubiquitin-selective AAA (ATPases associated with diverse cellular activities) chaperone p97 (also known as CDC48) plays an important role in sex determination in *C. elegans* by regulating CUL-2-mediated TRA-1A degradation by proteasomes. Keiji Tanaka (Tokyo Metropolitan Institute of Medical Science, Japan) then explained how the proteasome, which has a highly complex structure, is assembled with high precision

by multiple, dedicated chaperones. PAC1 is a chaperone that assists the initial assembly step of 20S proteasomes, the catalytic core of 26S proteasomes, and is required for embryonic development and neuronal proliferation after birth. PAC1-deficient hepatocytes were shown to contain normal levels of 26S proteasomes but deficient levels of free latent 20S proteasomes, leading to inefficient degradation of ubiquitinated proteins.

Other quality control systems, such as autophagy or organellar quality control, involve intense membrane dynamics, whose mechanisms and regulation were discussed in several talks. Yoshinori Ohsumi (Tokyo Institute of Technology, Japan) provided evidence for an Atg9-containing intermediate structure in the formation of yeast autophagosomes—the most critical event in autophagy—that results in the sequestration of damaged material. Mitophagy, an evolutionarily conserved degradation process selective for mitochondria, involves autophagy-related proteins essential for autophagosome formation. Specificity is ensured by the actions of transmembrane receptor proteins. The mitophagy-specific receptor Atg32 in budding yeast recruits Atg8 and Atg11 to the surface of mitochondria. Koji Okamoto (Osaka University) showed that peroxisome-anchored Atg32 can promote pexophagy, suggesting that this protein receptor contains modules compatible with autophagy-mediated organelle degradation. Further, Okamoto reported on the phosphorylation of Atg32, which is critical for mitophagy, and showed that phosphorylation depends on Atg1, a protein kinase required in all autophagy-related pathways.

**Prospects for the protein community**

The 2010 Protein Community Meeting was a success on multiple levels. Over half of the participants were graduate or postdoctoral students, who contributed tremendous energy and ideas to the discussions, pointing to a long and promising future for the field. As we understand more about the fundamental biology of proteins and how proteins can exist in multiple functional states that affect the health of the cell, we increasingly appreciate that protein dysregulation contributes to the complexity of human disease and may be a core problem of aging and age-associated disease. The prospects for protein community research are rich with opportunity.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.