

Damian

Individual and Collective Contributions of Chaperoning and Degradation to Protein Homeostasis in *E. coli*

Younhee Cho⁶, Xin Zhang, Kristine Faye R. Pobre, Yu Liu, David L. Powers, Jeffery W. Kelly, Lila M. Gierasch¹✉, Evan T. Powers
Cell Reports
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Summary

The folding fate of a protein in vivo is determined by the interplay between a protein's folding energy landscape and the actions of the proteostasis network, including molecular chaperones and degradation enzymes. The mechanisms of individual components of the *E. coli* proteostasis network have been studied extensively, but much less is known about how they function as a system. We used an integrated experimental and computational approach to quantitatively analyze the folding outcomes (native folding versus aggregation versus degradation) of three test proteins biosynthesized in *E. coli* under a variety of conditions. Overexpression of the entire proteostasis network benefited all three test proteins, but the effect of upregulating individual chaperones or the major degradation enzyme, Lon, varied for proteins with different biophysical properties. In sum, the impact of the *E. coli* proteostasis network is a consequence of concerted action by the Hsp70 system (DnaK/DnaJ/GrpE), the Hsp60 system (GroEL/GroES), and Lon.

Indu

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Fionn:

Nature

1) Agrochemical control of plant water use using engineered abscisic acid receptors

Sang-Youl Park, Francis C. Peterson, Assaf Mosquna, Jin Yao, Brian F. Volkman & Sean R. Cutler

Rising temperatures and lessening fresh water supplies are threatening agricultural productivity and have motivated efforts to improve plant water use and drought tolerance. During water deficit, plants produce elevated levels of abscisic acid (ABA), which improves water consumption and stress tolerance by controlling guard cell aperture and other protective responses^{1, 2}. One attractive strategy for controlling water use is to develop compounds that activate ABA receptors, but agonists approved for use have yet to be developed. In principle, an engineered ABA receptor that can be activated by an existing agrochemical could achieve this goal. Here we describe a variant of the ABA receptor PYRABACTIN RESISTANCE 1 (PYR1) that possesses nanomolar sensitivity to the agrochemical mandipropamid and demonstrate its efficacy for controlling ABA responses and drought tolerance in transgenic plants. Furthermore, crystallographic studies provide a mechanistic basis for its activity and demonstrate the relative ease with which the PYR1 ligand-binding pocket can be altered to accommodate new ligands. Thus, we have successfully repurposed an agrochemical for a new application using receptor engineering. We anticipate that this strategy will be applied to other plant receptors and represents a new avenue for crop improvement.

2) The future of the postdoc

There is a growing number of postdocs and few places in academia for them to go. But change could be on the way.

Kendall Powell

Stephanie:

Endogenous *Arabidopsis* messenger RNAs transported to distant tissues

- **Christoph J. Thieme, Monica Rojas-Triana, Ewelina Stecyk, Christian Schudoma, Wenna Zhang, Lei Yang, Miguel Miñambres, Dirk Walther, Waltraud X. Schulze, Javier Paz-Ares, Wolf-Rüdiger Scheible & Friedrich Kragler**

The concept that proteins and small RNAs can move to and function in distant body parts is well established. However, non-cell-autonomy of small RNA molecules raises the question: To what extent are protein-coding messenger RNAs (mRNAs) exchanged between tissues in plants? Here we report the comprehensive identification of 2,006 genes producing mobile RNAs in *Arabidopsis thaliana*. The analysis of variant ecotype transcripts that were present in heterografted plants allowed the identification of mRNAs moving between various organs under normal or nutrient-limiting conditions. Most of these mobile transcripts seem to follow the phloem-dependent allocation pathway transporting sugars from photosynthetic tissues to roots via the vasculature. Notably, a high number of transcripts also move in the opposite, root-to-shoot direction and are transported to specific tissues including flowers. Proteomic data on grafted plants indicate the presence of proteins from mobile RNAs, allowing the possibility that they may be translated at their destination site. The mobility of a high number of mRNAs suggests that a postulated tissue-specific gene expression profile might not be predictive for the actual plant body part in which a transcript exerts its function.

For Indu: Cutting it close: CRISPR-associated endoribonuclease structure and function

Megan L. Hochstrasser Jennifer A. Doudna  

DOI: <http://dx.doi.org/10.1016/j.tibs.2014.10.007>

For Fionn:

Heat-induced ribosome pausing triggers mRNA co-translational decay in *Arabidopsis thaliana*

1. Rémy Merret^{1,2}, Vinay K. Nagarajan^{3,†}, Marie-Christine Carpentier^{1,2,†}, Sunhee Park³, Jean-Jacques Favory^{1,2}, Julie Descombin^{1,2}, Claire Picart^{1,2}, Yee-yung Charng⁴, Pamela J. Green³, Jean-Marc Deragon^{1,2} and Cécile Bousquet-Antonelli^{1,2,*}

The reprogramming of gene expression in heat stress is a key determinant to organism survival. Gene expression is downregulated through translation initiation inhibition and release of free mRNPs that are rapidly degraded or stored. In mammals, heat also triggers 5'-ribosome pausing preferentially on transcripts coding for HSC/HSP70 chaperone targets, but the impact of such phenomenon on mRNA fate remains unknown. Here, we provide evidence that, in *Arabidopsis thaliana*, heat provokes 5'-ribosome pausing leading to the XRN4-mediated 5'-directed decay of translating mRNAs. We also show that hindering HSC/HSP70 activity at 20°C recapitulates heat effects by inducing ribosome pausing and co-translational mRNA turnover. Strikingly, co-translational decay targets encode proteins with high HSC/HSP70 binding scores and hydrophobic N-termini, two characteristics that were previously observed for transcripts most prone to pausing in animals. This work suggests for the first time that stress-induced variation of translation elongation rate is an evolutionarily conserved process leading to the polysomal degradation of thousands of 'non-aberrant' mRNAs.

Keith:

A Functional DnaK Dimer Is Essential for the Efficient Interaction with Hsp40 Heat Shock Protein

April 3, 2015 The Journal of Biological Chemistry, 290, 8849-8862.

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Highly conserved molecular chaperone Hsp70 heat shock proteins play a key role in maintaining protein homeostasis (proteostasis). DnaK, a major Hsp70 in *Escherichia coli*, has been widely used as a paradigm for studying Hsp70s. In the absence of ATP, purified DnaK forms low-ordered oligomer, whereas ATP binding shifts the equilibrium toward the monomer. Recently, we solved the crystal structure of DnaK in complex with ATP. There are two molecules of DnaK-ATP in the asymmetric unit. Interestingly, the interfaces between the two molecules of DnaK are large with good surface complementarity, suggesting functional importance of this crystallographic dimer. Biochemical analyses of DnaK protein supported the formation of dimer in solution. Furthermore, our cross-linking experiment based on the DnaK-ATP structure confirmed that DnaK forms specific dimer in an ATP-dependent manner. To understand the physiological function of the dimer, we mutated five residues on the dimer interface. Four mutations, R56A, T301A, N537A, and D540A, resulted in loss of chaperone activity and compromised the formation of dimer, indicating the functional importance of the dimer. Surprisingly, neither the intrinsic biochemical activities, the ATP-induced allosteric coupling, nor GrpE co-chaperone interaction is affected appreciably in all of the mutations except for R56A. Unexpectedly, the interaction with co-chaperone Hsp40 is significantly compromised. In summary, this study suggests that DnaK forms a transient dimer upon ATP binding, and this dimer is essential for the efficient interaction of DnaK with Hsp40.

Nathen:

Opposing effects of folding and assembly chaperones on evolvability of Rubisco

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Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the fixation of CO₂ in photosynthesis. Despite its pivotal role, Rubisco is an inefficient enzyme and thus is a key target for directed evolution. Rubisco biogenesis depends on auxiliary factors, including the GroEL/ES-type chaperonin for folding and the chaperone RbcX for assembly. Here we performed directed evolution of cyanobacterial form I Rubisco using a Rubisco-dependent *Escherichia coli* strain. Overexpression of GroEL/ES enhanced Rubisco solubility and tended to expand the range of permissible mutations. In contrast, the specific assembly chaperone RbcX had a negative effect on evolvability by preventing a subset of mutants from forming holoenzyme. Mutation F140I in the large Rubisco subunit, isolated in the absence of RbcX, increased carboxylation efficiency approximately threefold without reducing CO₂ specificity. The F140I mutant resulted in a ~55% improved photosynthesis rate in *Synechocystis* PCC6803. The requirement of specific biogenesis factors downstream of chaperonin may have retarded the natural evolution of Rubisco.

Elizabeth:

April 9, 2015

The Plant Journal Content Alert (New Articles)

Enzyme activities of Arabidopsis inositol polyphosphate kinases AtIPK2 α and AtIPK2 β are involved in pollen development, pollen tube guidance and embryogenesis

Huadong Zhan, Yujiao Zhong, Zhongnan Yang and Huijun Xia

Accepted manuscript online: 6 APR 2015 09:51PM EST | DOI: 10.1111/tpj.12846

A land plant-specific thylakoid membrane protein contributes to photosystem II maintenance in Arabidopsis thaliana

Jun Liu and Robert L. Last Accepted manuscript online: 6 APR 2015 02:36AM EST | DOI: 10.1111/tpj.12845

Regulation of the Arabidopsis CBF regulon by a complex low-temperature regulatory network (pages 193–207)

Sunchung Park, Chin-Mei Lee, Colleen J. Doherty, Sarah J. Gilmour, YongSig Kim and Michael F. Thomashow

Article first published online: 23 MAR 2015 | DOI: 10.1111/tpj.12796

Evidence is presented indicating that cold-regulation of the CBF regulon, which includes genes that impart freezing tolerance, is more complex than previously thought, involving extensive co-regulation by CBF1, CBF2, CBF3 and other ‘first-wave’ transcription factors encoded by genes induced in parallel with the CBF genes.

The NdhV subunit is required to stabilize the chloroplast NADH dehydrogenase-like complex in Arabidopsis (pages 221–231)

Xiangyuan Fan, Jiao Zhang, Wenjing Li and Lianwei Peng

Article first published online: 18 MAR 2015 | DOI: 10.1111/tpj.12807
This work identifies the chloroplast NADH dehydrogenase-like (NDH) subunit NdhV, which is located on the most fragile part of the NDH complex. NdhV interacts with and stabilizes the NDH subcomplexes A and E, especially under high-light conditions.

Ethylene mediates brassinosteroid-induced stomatal closure via G α protein-activated hydrogen peroxide and nitric oxide production in Arabidopsis (pages 280–301)

Chenyu Shi, Cheng Qi, Hongyan Ren, Aixia Huang, Shumei Hei and Xiaoping She

Article first published online: 8 APR 2015 | DOI: 10.1111/tpj.12815

The data presented herein suggest a working model for BR action in guard cell response in Arabidopsis. The findings confirm the effect of BR on stomatal movement, and elucidate the crucial role of ethylene and G proteins in the guard cell BR signalling.

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[Mitochondrial and Nuclear Accumulation of the Transcription Factor ATFS-1 Promotes OXPHOS Recovery during the UPRmt](#) *Pages 123-133* Amrita M. Nargund, Christopher J. Fiorese, Mark W. Pellegrino, Pan Deng, Cole M. Haynes