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Systemic control of protein synthesis through sequestration of translation and ribosome biogenesis factors during severe heat stress

FEBS Letters 17 October 2015

Valeria Cherkasov[a, b, 1], Tomas Grousl[a, b, 1], Patrick Theer[a], Yevhen Vainshtein[a, b], Christine Gläßer[a], Cyril Mongis[a], Günter Kramer[a, b], Georg Stoecklin[a, b], Michael Knop[a], Axel Mogk[a, b,], Bernd Bukau[a, b]

Environmental stress causes the sequestration of proteins into insoluble deposits including cytoplasmic stress granules (SGs), containing mRNA and a variety of translation factors. Here we systematically identified proteins sequestered in *Saccharomyces cerevisiae* at 46°C by a SG co-localization screen and proteomic analysis of insoluble protein fractions. We identified novel SG components including essential aminoacyl-tRNA synthetases. Moreover, we discovered nucleus-associated deposits containing ribosome biogenesis factors. Our study suggests downregulation of cytosolic protein synthesis and nuclear ribosome production at multiple levels through heat shock induced protein sequestrations.

H**sp70 and Hsp90 of *E. coli* Directly Interact for Collaboration in Protein Remodeling**

JMB, 23 October 2015

Olivier Genest[†, 1], Joel R. Hoskins[†], Andrea N. Kravats[†], Shannon M. Doyle[,], Sue Wickner

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

Hsp90 is a highly conserved molecular chaperone that remodels hundreds of client proteins, many involved in the progression of cancer and other diseases. It functions with the Hsp70 chaperone and numerous cochaperones. The bacterial Hsp90 functions with an Hsp70 chaperone, DnaK, but is independent of Hsp90 cochaperones. We explored the collaboration between *Escherichiacoli* Hsp90 and DnaK and found that the two chaperones form a complex that is stabilized by client protein binding. A J-domain protein, CbpA, facilitates assembly of the $Hsp90_{Ec}$–DnaK–client complex. We identified *E. coli* Hsp90 mutants defective in DnaK interaction *in vivo* and show that the purified mutant proteins are defective in physical and functional interaction with DnaK. Understanding how Hsp90 and Hsp70 collaborate in protein remodeling will provide the groundwork for the development of new therapeutic strategies targeting multiple chaperones and cochaperones.

Fionn

Molecular Cell

RNA Controls PolyQ Protein Phase Transitions

Huaiying Zhang1, 2, Shana Elbaum-Garfinkle2, Erin M. Langdon1, Nicole Taylor2, Patricia Occhipinti1, Andrew A. Bridges1, Clifford P. Brangwynne2, , , Amy S. Gladfelter1, ,

Summary
Compartmentalization in cells is central to the spatial and temporal control of biochemistry. In addition to membrane-bound organelles, membrane-less compartments form partitions in cells. Increasing evidence suggests that these compartments assemble through liquid-liquid phase separation. However, the spatiotemporal control of their assembly, and how they maintain distinct functional and physical identities, is poorly understood. We have previously shown an RNA-binding protein with a polyQ-expansion called Whi3 is essential for the spatial patterning of cyclin and formin transcripts in cytosol. Here, we show that specific mRNAs that are known physiological targets of Whi3 drive phase separation. mRNA can alter the viscosity of droplets, their propensity to fuse, and the exchange rates of components with bulk solution. Different mRNAs impart distinct biophysical properties of droplets, indicating mRNA can bring individuality to assemblies. Our findings suggest that mRNAs can encode not only genetic information but also the biophysical properties of phase-separated compartments.

Residue-by-Residue View of In Vitro FUS Granules that Bind the C-Terminal Domain of RNA Polymerase II

Kathleen A. Burke1, Abigail M. Janke1, Christy L. Rhine2, Nicolas L. Fawzi1, ,

Summary
Phase-separated states of proteins underlie ribonucleoprotein (RNP) granules and nuclear RNA-binding protein assemblies that may nucleate protein inclusions associated with neurodegenerative diseases. We report that the N-terminal low-complexity domain of the RNA-binding protein Fused in Sarcoma (FUS LC) is structurally disordered and forms a liquid-like phase-separated state resembling RNP granules. This state directly binds the C-terminal domain of RNA polymerase II. Phase-separated FUS lacks static structures as probed by fluorescence microscopy, indicating they are distinct from both protein inclusions and hydrogels. We use solution nuclear magnetic resonance spectroscopy to directly probe the dynamic architecture within FUS liquid phase-separated assemblies. Importantly, we find that FUS LC retains disordered secondary structure even in the liquid phase-separated state. Therefore, we propose that disordered protein granules, even those made of aggregation-prone prion-like domains, are dynamic and disordered molecular assemblies with transiently formed protein-protein contacts.

The FEBS journal

Structures of the double-ring AAA ATPase Pex1/Pex6 involved in peroxisome biogenesis Dongyan Tan1*, Neil B. Blok1,2*, Tom A. Rapoport1,2, and Thomas Walz3

Abstract
The Pex1/Pex6 complex is a member of the AAA family of ATPases that is involved in peroxisome biogenesis. Recently, cryo-electron microscopy structures have been determined of the Pex1/Pex6 complex in different nucleotide states. This Structural Snapshot describes the structural features of the complex, their implications for its function, as well as questions that still await answers.

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Formation and Maturation of Phase-Separated Liquid Droplets by RNA-Binding Proteins

Yuan Lin, David S.W. Protter, Michael K. Rosen, Roy Parker
Eukaryotic cells possess numerous dynamic membrane-less organelles, RNP granules, enriched in RNA and RNA-binding proteins containing disordered regions. We demonstrate that the disordered regions of key RNP granule components and the full-length granule protein hnRNPA1 can phase separate in vitro, producing dynamic liquid droplets. Phase separation is promoted by low salt concentrations or RNA. Over time, the dropletsmature to more stable states, as assessed by slowed fluorescence recovery after photobleaching and resistance to salt. Maturation often coincides with formation of fibrous structures. Different disordered domains can co-assemble into phase-separated droplets. These biophysical properties demonstrate a plausible mechanism by which interactions between disordered regions, coupled with RNA binding, could contribute to RNP granule assembly in vivo through promoting phase separation. Progression from dynamic liquids to stable fibers may be regulated to produce cellular structures with diverse physiochemical properties and functions. Misregulation could contribute to diseases involving aberrant RNA granules.

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October 22, 2015
Yuan Lin, David S.W. Protter, Michael K. Rosen, Roy Parker
Formation and Maturation of Phase-Separated Liquid Droplets by RNA-Binding Proteins
Molecular Cell, *Volume 60, Issue 2*, *15 October 2015*, *Pages 208-219*

Huaiying Zhang, Shana Elbaum-Garfinkle, Erin M. Langdon, Nicole Taylor, Patricia Occhipinti, Andrew A. Bridges, Clifford P. Brangwynne, Amy S. Gladfelter
RNA Controls PolyQ Protein Phase Transitions
Molecular Cell, *Volume 60, Issue 2*, *15 October 2015*, *Pages 220-230*

Kathleen A. Burke, Abigail M. Janke, Christy L. Rhine, Nicolas L. Fawzi
Residue-by-Residue View of In Vitro FUS Granules that Bind the C-Terminal Domain of RNA Polymerase II
Molecular Cell, *Volume 60, Issue 2*, *15 October 2015*, *Pages 231-241*

The Plant Journal Content Alert (New Articles)

Genome-wide analysis on *Chlamydomonas reinhardtii* reveals impact of hydrogen peroxide on protein stress responses and overlap with other stress transcriptomes

Ian K. Blaby, Crysten E. Blaby-Haas, María Esther Pérez-Pérez, Stefan Schmollinger, Sorel Fitz-Gibbon, Stéphane D. Lemaire and Sabeeha S. Merchant

Accepted manuscript online: 16 OCT 2015 10:10AM EST | DOI: 10.1111/tpj.13053

Nature Reviews Molecular Cell Biology contents November 2015 Volume 16 Number 11 pp 639-698 **Preparation of a cyanine-based fluorescent probe for highly selective detection of glutathione and its use in living cells and tissues of mice** **pp1742 - 1754**

Changes in the levels of cellular thiols (e.g., glutathione) are linked to many diseases. This protocol is for the synthesis of CPDSA, a fluorescent turn-on glutathione probe with near-infrared emission. In-cell and *in vivo* assays are also described.

Jun Yin *et al.*

Published online: 15 October 2015 | doi:10.1038/nprot.2015.109 Abstract | Full Text | PDF (2,441K)

Top

Article series: Technologies and techniques

Ribosome profiling reveals the what, when, where and how of protein synthesis

Gloria A. Brar & Jonathan S. Weissman

p651 | doi:10.1038/nrm4069

Ribosome profiling has the power to interrogate — *in vivo* and on a global scale — what is being translated, how this translation is regulated and where in the cell the translation of specific sets of proteins occurs.

Abstract | Full Text | **PDF** | Supplementary information

Chemistry & Biology: Alert 17 October-23 October

Targeting Mycobacterial Enzymes with Natural Products Review Article *Pages 1288-1300* Elwira Sieniawska

Recently discovered compounds targeting *M. tuberculosis* are soil bacteria metabolites

The ideal drug target is an enzyme with fully elucidated function and mechanism

The ideal drug target is absolutely required for the survival of the microbe

The most promising candidates for drug leads are Clp inhibitors

Cell

Wenyang Li, Mengdi Ma, Ying Feng, Hongjiang Li, Yichuan Wang, Yutong Ma, Mingzhe Li, Fengying An, Hongwei Guo

EIN2-Directed Translational Regulation of Ethylene Signaling in *Arabidopsis*

Cell, *Volume 163, Issue 3*, *22 October 2015*, *Pages 670-683*

PDF (5814 K) Supplementary content

Catharina Merchante, Javier Brumos, Jeonga Yun, Qiwen Hu, Kristina R. Spencer, Paul Enríquez, Brad M. Binder, Steffen Heber, Anna N. Stepanova, Jose M. Alonso
Gene-Specific Translation Regulation Mediated by the Hormone-Signaling Molecule EIN2
Cell, *Volume 163, Issue 3*, *22 October 2015*, *Pages 684-697*
PDF (4286 K) Supplementary content
Ethylene regulates many aspects of plant growth and development. In the presence of ethylene, the C terminus of EIN2 (EIN2C) translocates into the nucleus and activates transcription. Li et al. and Merchante et al. show that EIN2C also regulates translation through an interaction with the 3′ UTRs of transcripts.
Plant, Cell & Environment Content Alert (New Articles)
Auxin response factors John William Chandler
Accepted manuscript online: 21 OCT 2015 02:07AM EST | DOI: 10.1111/pce.12662
Lipidomic and transcriptomic analyses of ***Chlamydomonas reinhardtii*** under heat stress unveil a direct route for the conversion of membrane lipids into storage lipids
Bertrand Légeret, Miriam Schulz-Raffelt, Hoa Mai Nguyen, Pascaline Auroy, Fred Beisson, Gilles Peltier, Guillaume Blanc and Yonghua Li-Beisson
Accepted manuscript online: 19 OCT 2015 01:39AM EST | DOI: 10.1111/pce.12656
Analysis of the sodium chloride-dependent respiratory kinetics of wheat mitochondria reveals differential effects on phosphorylating and non-phosphorylating electron transport pathways
Richard P. Jacoby, M. Hafiz Che-Othman, A. Harvey Millar and Nicolas L. Taylor
Accepted manuscript online: 15 OCT 2015 04:42PM EST | DOI: 10.1111/pce.12653

Metabolism: Plugging the leak
Bryan C Dickinson Nature Chemical Biology 11, 831–832 (2015)
doi:10.1038/nchembio.1934 20 October 2015 PDF
Multiple mitochondrial components generate reactive oxygen species (ROS), but separating the consequences of each ROS-generating source from overall mitochondrial health is challenging. A new class of small-molecule inhibitors that selectively block ROS generation from one of the most active sources may provide a new approach toward achieving that goal.

FREE **Structures of the double-ring AAA ATPase Pex1/Pex6 involved in peroxisome biogenesis**
Dongyan Tan, Neil B. Blok, Tom A. Rapoport and Thomas Walz
Accepted manuscript online: 17 OCT 2015 09:43AM EST | DOI: 10.1111/febs.13569

Current Opinion in Plant Biology: Alert 12 October-18 October
Stress-induced structural changes in plant chromatin Review Article *Pages 8-16*
Aline V Probst, Ortrun Mittelsten Scheid
The Arabidopsis epitranscriptome Review Article *Pages 17-21*
Rupert G Fray, Gordon G Simpson

Transcriptome-wide measurement of plant RNA secondary structure Review Article *Pages 36-43*
Shawn W Foley, Lee E Vandivier, Pavel P Kuksa, Brian D Gregory
Unfolded protein response in plants: one master, many questions Review Article *Pages 59-66*
Cristina Ruberti, Sang-Jin Kim, Giovanni Stefano, Federica Brandizzi
Role of alternative pre-mRNA splicing in temperature signaling Review Article *Pages 97-103*
Giovanna Capovilla, Alice Pajoro, Richard GH Immink, Markus Schmid
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Elena A Vidal, José M Álvarez, Tomás C Moyano, Rodrigo A Gutiérrez
More than meets the eye: from carotenoid biosynthesis, to new insights into apocarotenoid signaling Review Article
Pages 172-179 Ryan P McQuinn, James J Giovannoni, Barry J Pogson
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Peter J Gollan, Mikko Tikkanen, Eva-Mari Aro

Nitrogen signaling and use efficiency in plants: what's new? Review Article *Pages 192-198*
Qian Liu, Xiangbin Chen, Kun Wu, Xiangdong Fu

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