Reversible, Specific, Active Aggregates of Endogenous Proteins Assemble upon Heat Stress

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Heat causes protein misfolding and aggregation and, in eukaryotic cells, triggers aggregation of proteins and RNA into stress granules. We have carried out extensive proteomic studies to quantify heat-triggered aggregation and subsequent disaggregation in budding yeast, identifying >170 endogenous proteins aggregating within minutes of heat shock in multiple subcellular compartments. We demonstrate that these aggregated proteins are not misfolded and destined for degradation. Stable-isotope labeling reveals that even severely aggregated endogenous proteins are disaggregated without degradation during recovery from shock, contrasting with the rapid degradation observed for many exogenous thermolabile proteins. Although aggregation likely inactivates many cellular proteins, in the case of a heterotrimeric aminoacyl-tRNA synthetase complex, the aggregated proteins remain active with unaltered fidelity. We propose that most heat-induced aggregation of mature proteins reflects the operation of an adaptive, autoregulatory process of functionally significant aggregate assembly and disassembly that aids cellular adaptation to thermal stress.
In this study we report the molecular genetic characterization of the Arabidopsis mitochondrial phosphopantetheinyl transferase (mtPPT), which catalyzes the phosphopantetheinylation and thus activation of mitochondrial acyl carrier protein (mtACP) of mitochondrial fatty acid synthase (mtFAS). This catalytic capability of the purified mtPPT protein (encoded by AT3G11470) was directly demonstrated in an invitro assay that phosphopantetheinylated mature Arabidopsis apo-mtACP isoforms. The mitochondrial localization of the AT3G11470-encoded proteins was validated by the ability of their N-terminal 80-residue leader sequence to guide a chimeric GFP protein to this organelle. A T-DNA-tagged null mutant mtppt-1 allele shows an embryo-lethal phenotype, illustrating a crucial role of mtPPT for embryogenesis. Arabidopsis RNAi transgenic lines with reduced mtPPT expression display typical phenotypes associated with a deficiency in the mtFAS system, namely miniaturized plant morphology, slow growth, reduced lipooylation of mitochondrial proteins, and the hyperaccumulation of photorespiratory intermediates glycine and glycolate. These morphological and metabolic alterations are reversed when these plants are grown in a non-photorespiratory condition (i.e., 1% CO2 atmosphere), demonstrating that they are a consequence of a deficiency in photorespiration due to the reduced lipooylation of the photorespiratory glycine decarboxylase.

Keith

Pathways of allosteric regulation in Hsp70 chaperones

Nat Commun. 2015 Sep 18

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Central to the protein folding activity of Hsp70 chaperones is their ability to interact with protein substrates in an ATP-controlled manner, which relies on allosteric regulation between their nucleotide-binding (NBD) and substrate-binding domains (SBD). Here we dissect this mechanism by analysing mutant variants of the Escherichia coli Hsp70 DnaK blocked at distinct steps of allosteric communication. We show that the SBD inhibits ATPase activity by interacting with the NBD through a highly conserved hydrogen bond network, and define the signal transduction pathway that allows bound substrates to trigger ATP hydrolysis. We identify variants deficient in only one direction of allosteric control and demonstrate that ATP-induced substrate release is more important for chaperone activity than substrate-stimulated ATP hydrolysis. These findings provide evidence of an unexpected dichotomic allosteric mechanism in Hsp70 chaperones and provide the basis for a comprehensive mechanical model of allosterism in Hsp70s.

Elizabeth

Sept 24, 2015
The Plant Journal Content Alert (New Articles)

A comprehensive strategy to identify long-distance mobile peptides in xylem sap
Satoru Okamoto, Takamasa Suzuki, Masayoshi Kawaguchi, Tetsuya Higashiyama and Yoshikatsu Matsubayashi
Accepted manuscript online: 2 SEP 2015 08:45PM EST | DOI: 10.1111/tpj.13015

There is a growing awareness that secreted peptides mediate organ-to-organ communication in higher plants. Xylem sap peptidomics is an effective but challenging approach to identify long-distance mobile peptides. In this study, we developed a simple, gel-free purification system that combines o-chlorophenol extraction with HPLC separation. Using this system, we successfully identified seven oligopeptides from soybean xylem sap exudate that had one or more posttranscriptional modifications: glycosylation, sulfation and/or hydroxylation. RNA sequencing and quantitative PCR analyses showed that the peptide-encoding genes are expressed in multiple tissues. We further analyzed the long-distance translocation of four of the seven peptides using gene-encoding peptides with single amino acid substitutions, and we identified these four peptides as potential root-to-shoot mobile oligopeptides. Promoter-GUS analysis showed that all four peptide-encoding genes were expressed in the inner tissues of the root endodermis. Moreover, we found that some of these peptide-encoding genes responded to biotic and/or abiotic factors. These results indicate that our purification system provides a comprehensive approach for effectively identifying endogenous small peptides and reinforce the concept that higher plants employ various peptides in root-to-shoot signaling.
Transplanting the wisdom of the mayapple

Etoposide, a topoisomerase inhibitor, is used to treat various cancers. However, etoposide isn't that easy to get. Its precursor comes from the very slow-growing mayapple plant. Lau and Sattely used bioinformatics, heterologous enzyme expression, and kinetic characterization, to work out the pathway that makes the precursor in mayapple (see the Perspective by O'Connor). They then successfully transplanted the full biosynthetic pathway into tobacco plants.

Science, this issue p. 1224; see also p. 1167

Switching off protein production

Controlling protein production is desirable, but current methods are complex, inefficient, difficult to generalize, or not quickly reversible. Chung et al. describe a small-molecule–assisted shutoff (SMASH) tag that is genetically added to a target protein and allows reversible shutoff of various proteins in multiple cell types. The tag includes a site that is cut by a protease and a degron sequence that targets the protein for rapid destruction. Active protease cuts the tag from newly synthesized protein so that it does not disrupt protein function. However, inhibiting the protease with a clinically approved drug protects the tag, and the degron sequence causes the protein to be rapidly degraded. Stopping the drug restores protein production.


Identification of novel small ncRNAs in pollen of tomato.
BMC Genomics. 2015 Sep 18;16(1):714.PMID: 26385469 [PubMed - as supplied by publisher]

Kityk R, Vogel M, Schlecht R, Bukau B, Mayer MP.
Pathways of allosteric regulation in Hsp70 chaperones.

Xu Y, Burgess P, Huang B.

Koteiche HA, Claxton DP, Mishra S, Stein RA, McDonald ET, Mchaourab HS.
Species-Specific Structural and Functional Divergence of Î±-Crystallins: Zebrafish Î±-Ba- and Rodent Î±-A<sup>ins</sup>-Crystallin Encode Activated Chaperones.
Biochemistry. 2015 Sep 17;. PMID: 26378715 [PubMed - as supplied by publisher]

Functional specialization in regulation and quality control in thermal adaptive evolution.
Genes Cells. 2015 Sep 15;. PMID: 26373241 [PubMed - as supplied by publisher]
Pest control and resistance management through release of insects carrying a male-selecting transgene


BMC Biology 2015, 13:49

Development and evaluation of new insect pest management tools is critical for overcoming over-reliance upon, and growing resistance to, synthetic, biological and plant-expressed insecticides. For transgenic crops expressing insecticidal proteins from the bacterium *Bacillus thuringiensis* (‘*Bt* crops’) emergence of resistance is slowed by maintaining a proportion of the crop as non-*Bt*
varieties, which produce pest insects unselected for resistance. While this strategy has been largely successful, multiple cases of Bt resistance have now been reported.

One new approach to pest management is the use of genetically engineered insects to suppress populations of their own species. Models suggest that released insects carrying male-selecting (MS) transgenes would be effective agents of direct, species-specific pest management by preventing survival of female progeny, and simultaneously provide an alternative insecticide resistance management strategy by introgression of susceptibility alleles into target populations. We developed a MS strain of the diamondback moth, *Plutella xylostella*, a serious global pest of crucifers. MS-strain larvae are reared as normal with dietary tetracycline, but, when reared without tetracycline or on host plants, only males will survive to adulthood. We used this strain in glasshouse-cages to study the effect of MS male *P. xylostella* releases on target pest population size and spread of Bt resistance in these populations.

**Methodology article**

Induction of rapid and selective cell necrosis in *Drosophila* using *Bacillus thuringiensis* Cry toxin and its silkworm receptor  Fumiaki Obata, Shiho Tanaka, Soshiro Kashio, Hidenobu Tsujimura, Ryoichi Sato, Masayuki Miura

*BMC Biology* 2015, 13:48

Genetic ablation of target cells is a powerful tool to study the origins and functions of cells, tissue regeneration, or pathophysiology in a human disease model *in vivo*. Several methods for selective cell ablation by inducing apoptosis have been established, using exogenous toxins or endogenous proapoptotic genes. However, their application is limited to cells with intact apoptotic machinery. We established a method for inducing rapid and selective cell necrosis by the pore-forming bacterial toxin Cry1Aa, which is specifically active in cells expressing the Cry1Aa receptor (CryR) derived from the silkworm *Bombyx mori*. We demonstrated that overexpressing CryR in *Drosophila melanogaster* tissues induced rapid cell death of CryR-expressing cells only, in the presence of Cry1Aa toxin. Cry/CryR system was effective against both proliferating cells in imaginal discs and polyploid postmitotic cells in the fat body. Live imaging analysis of cell ablation revealed swelling and subsequent osmotic lysis of CryR-positive cells after 30 min of incubation with Cry1Aa toxin. Osmotic cell lysis was still triggered when apoptosis, JNK activation, or autophagy was inhibited, suggesting that Cry1Aa-induced necrotic cell death occurred independently of these cellular signaling pathways. Injection of Cry1Aa into the body cavity resulted in specific ablation of CryR-expressing cells, indicating the usefulness of this method for *in vivo* cell ablation.

Genome Biology

Chromatin in 3D: progress and prospects for plants  Chang Liu, Detlef Weigel

*Genome Biology* 2015, 16:170 (21 August 2015)

Current Opinion in Microbiology: Alert 12 September-18 September  
How eukaryotic filamentous pathogens evade plant recognition  Review Article  *Pages 92-101*

Ely Oliveira-Garcia, Barbara Valent

Chemistry & Biology: Alert 12 September-18 September  
Previously Uncultured Marine Bacteria Linked to Novel Alkaloid Production  Original Research Article  *Pages 1270-1279*

Eun Ju Choi, Sang-Jip Nam, Lauren Paul, Deanna Beatty, Christopher A. Kauffman, Paul R. Jensen, William Fenical
The Redox Code – Review  Dean P. Jones and Helmut Sies
Antioxidants & Redox Signaling, Vol. 23, No. 9, September 2015: 734-746
Abstract | Full Text HTML | Full Text PDF (945 KB) | Full Text PDF with Links (603 KB)

Nature Protocols Contents: Volume 10 Number 10, pp 1459-1642
Whole-mount immunolocalization to study female meiosis in Arabidopsis  pp1535 - 1542
The study of meiosis in plants is considered to be gender-biased owing to the easy accessibility of male meiocytes. This protocol describes how to prepare and image female Arabidopsis meiocytes to investigate protein localization during meiosis.
Rocio Escobar-Guzman, Daniel Rodriguez-Leal, Jean-Philippe Vielle-Calzada and Arnaud Ronceret
Published online: 10 September 2015 | doi:10.1038/nprot.2015.098
Abstract | Full Text | PDF (1,034K)

Nature Reviews Molecular Cell Biology contents October 2015 Volume 16 Number 10 pp 577-638
Integrating mitochondrial translation into the cellular context
Ricarda Richter-Dennerlein, Sven Dennerlein & Peter Rehling
p586 | doi:10.1038/nrm4051
Recent findings revealed the extent to which mitochondrial translation and other cellular processes are mutually controlled. Mitochondrial translation is coordinated with the assembly of respiratory chain complexes and is positively regulated by microRNAs imported from the cytoplasm. In turn, mitochondrial translation stress activates retrograde signalling pathways that suppress cell proliferation.
Abstract | Full Text | PDF | Supplementary information

The Circadian Clock Modulates Global Daily Cycles of mRNA Ribosome Loading
Anamika Missra, Ben Ernest, Tim Lohoff, Qidong Jia, James Satterlee, Kenneth Ke, and Albrecht G. von Arnim
Plant Cell 2015 tpc.15.00546; Advance Publication September 21, 2015;
doi:10.1105/tpc.15.00546 OPEN
http://www.plantcell.org/content/early/2015/09/21/tpc.15.00546.abstract
Measurements of mRNA ribosome loading in wild-type and clock-deficient plants show that the circadian clock regulates the translation of Arabidopsis mRNAs in concert with diurnal light-dark changes.

Nature Chemical Biology Contents: October 2015, Volume 11 No 10 pp 744 - 815
N-terminal domains mediate [2Fe-2S] cluster transfer from glutaredoxin-3 to anamorsin  pp772 - 778
Lucia Banci, Simone Ciofi-Baffoni, Karolina Gajda, Riccardo Muzzioli, Riccardo Peruzzini et al.
doi:10.1038/nchembio.1892
Cooperative development of antimiobiotics: looking back to look ahead
Carl Nathan
p651 | doi:10.1038/nrmicro3523
In this Science and Society article, Carl Nathan reviews historical collaborations between industry and academic institutions that developed antimicrobials, and discusses similar strategies that have recently emerged to tackle the crisis of antimicrobial resistance.

Abstract | Full Text | PDF

The EMBO Journal Table of Contents for September 2015; Vol. 34, No. 18
Better to burn out than it is to rust: coordinating cellular redox states during aging and stress
Christopher Rongo
Published online 31.07.2015

Both the protein homeostasis (proteostasis) and the oxidation/reduction (redox) environment of the cell play critical roles in disease- and age-associated decline, yet the relationship between the two remains mysterious. In this issue of The EMBO Journal, Kirstein et al (2015) show that both the cytosol and the ER shift their redox states in response to proteotoxic stress and that stress in one compartment can alter redox state in the other. Moreover, proteotoxic stress can induce changes in redox state across tissues, suggesting that an organism-wide surveillance mechanism modulates cellular redox environment.

Proteotoxic stress and ageing triggers the loss of redox homeostasis across cellular compartments
Janine Kirstein, Daisuke Morito, Taichi Kakihana, Munechika Sugihara, Anita Minnen, Mark S Hopp, Carmen Nussbaum-Krammer, Prasad Kasturi, F Ulrich Hartl, Kazuhiro Nagata, and Richard I Morimoto
Published online 29.07.2015

Using genetically encoded sensors this study shows that the opposing redox state in ER lumen and cytosol is altered during ageing and upon disruption of proteostasis. The resulting redox imbalance can spread across tissues.

RuvbL1 and RuvbL2 enhance aggresome formation and disaggregate amyloid fibrils
Nava Zaarur, Xiaobin Xu, Patrick Lestienne, Anatoli B Meriin, Mark McComb, Catherine E Costello, Gary P Newnam, Rakhee Ganti, Nina V Romanova, Maruda Shanmugasundaram, Sara TN Silva, Tiago M Bandeiras, Pedro M Matias, Kirill S Lobachev, Igor K Lednev, Yury O Chernoff, and Michael Y Sherman
Published online 24.08.2015

http://EMBOJ.embopress.org/content/34/18/2363?etoc
The AAA+ ATPase RuvbL is required for aggresome formation and serves as a protein disaggregate that can disassemble amyloids in mammalian and yeast cells.

Kou LH, Wu HH, Liu YM, Zhang YP, Zhang JZ, Guo YP, Ma EB.
Environ Entomol. 2015 Sep 11;PMID: 26363174 [PubMed - as supplied by publisher]

Reversible, Specific, Active Aggregates of Endogenous Proteins Assemble upon Heat Stress.

Li J, Xiang CY, Yang J, Chen JP, Zhang HM.
Interaction of HSP20 with a viral RdRp changes its sub-cellular localization and distribution pattern in plants.

Conway ME, Lee C.
The redox switch that regulates molecular chaperones.

Clark JI.
Functional sequences in human alphaB crystallin.
Biochim Biophys Acta. 2015 Sep 2;.. PMID: 26341790 [PubMed - as supplied by publisher]

Marcos AT, Ramos MS, Marcos JF, Carmona L, Strauss J, Cànovas D.
Nitric oxide synthesis by nitrate reductase is regulated during development in Aspergillus.

Rossi A, Kontarakis Z, Gerri C, Nolte H, HÃ¶rner S, KrÃ¼ger M, Stainier DY.
Genetic compensation induced by deleterious mutations but not gene knockdowns.

Physiologia Plantarum
Effects of long-term individual and combined water and temperature stress on the growth of rice, wheat and maize: relationship with morphological and physiological acclimation (pages 149-165)
Juan Alejandro Perdomo, Miquel À. Conesa, Hipólito Medrano, Miquel Ribas-Carbó and Jeroni Galmés

A Mechanism for Sustained Cellulose Synthesis during Salt Stress
Anne Endler, Christopher Kesten, René Schneider, Yi Zhang, Alexander Ivakov, Anja Froehlich, Norma Funke, Staffan Persson
Abiotic stress, such as salinity, drought, and cold, causes detrimental yield losses for all major plant crop species. Understanding mechanisms that improve plants’ ability to produce biomass, which largely is constituted by the plant cell wall, is therefore of utmost importance for agricultural activities. Cellulose is a principal component of the cell wall and is synthesized by microtubule-guided cellulose synthase enzymes at the plasma membrane. Here, we identified two components of the cellulose synthase complex, which we call companion of cellulose synthase (CC) proteins. The cytoplasmic tails of these membrane proteins bind to microtubules and promote microtubule dynamics. This activity supports microtubule organization, cellulose synthase localization at the plasma membrane, and renders seedlings less sensitive to stress. Our findings offer a mechanistic model for how two molecular components, the CC proteins, sustain microtubule organization and cellulose synthase localization and thus aid plant biomass production during salt stress.