Mary

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 pro- teins and RNA into stress granules. We have carried out extensive proteomic studies to quantify heat- triggered aggregation and subsequent disaggrega- tion in budding yeast, identifying >170 endogenous proteins aggregating within minutes of heat shock in multiple subcellular compartments. We demon- strate that these aggregated proteins are not mis- folded and destined for degradation. Stable-isotope labeling reveals that even severely aggregated endogenous proteins are disaggregated without degradation during recovery from shock, contrast- ing with the rapid degradation observed for many exogenous thermolabile proteins. Although aggre- gation likely inactivates many cellular proteins, in the case of a heterotrimeric aminoacyl-tRNA syn- thetase 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 assem- bly and disassembly that aids cellular adaptation to thermal stress.

Damian

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KW - phosphopantetheinyl transferase
KW - acyl carrier protein
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KW - lipoic acid
KW - glycine decarboxylase
KW - photorespiration
KW - Arabidopsis thaliana
PY - 2015
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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 thepurified 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 tothis organelle. A T-DNA-tagged null mutant mtppt-1 allele shows an embryo-lethalphenotype, 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 lipoylation 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 aconsequence of a deficiency in photorespiration due to the reduced lipoylation of the photorespiratory glycine decarboxylase.

Keith

Pathways of allosteric regulation in Hsp70 chaperones

Nat Commun. 2015 Sep 18

Roman Kityk[1], Markus Vogel[1,w], Rainer Schlecht[1,w], Bernd Bukau[1,2] & Matthias P. Mayer[1]

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DKFZ-ZMBH-Alliance, INF282, D-69120 Heidelberg, Germany. [w] Present addresses: Bachem Holding AG, Hauptstrasse 144, 4416 Bubendorf, Switzerland (M.V.); Sanofi-Aventis Deutschland GmbH, C&BD Frankfurt Biotechnology, Industriepark Ho¨chst, Building H780, 65926 Frankfurt am Main, Germany (R.S.).

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 allostery mechanism in Hsp70 chaperones and provide the basis for a comprehensive mechanical model of allostery 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.

Science 11 September 2015; Vol. 349, No. 6253

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.

Nat. Chem. Biol 10.1038/nchembio.1869 (2015).

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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.
Nat Commun. 2015 Sep 18;6:8308.PMID: 26383706 [PubMed - as supplied by publisher]

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Functional specialization in regulation and quality control in thermal adaptive evolution.
Genes Cells. 2015 Sep 15;. PMID: 26373241 [PubMed - as supplied by publisher]

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Plant Physiol. 2015 Sep 14;. PMID: 26371235 [PubMed - as supplied by publisher]

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The network of molecular chaperones: insights in the cellular proteostasis machinery.
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Transcriptomic Analyses on the Role of Nitric Oxide in Plant Disease Resistance.
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Endothelial Nitric Oxide Synthase-Derived Nitric Oxide Prevents Dihydrofolate Reductase Degradation via Promoting S-Nitrosylation.
Arterioscler Thromb Vasc Biol. 2015 Sep 17;. PMID: 26381869 [PubMed - as supplied by publisher]

Li J, Dukowic-Schulze S, Lindquist IE, Farmer AD, Kelly B, Li T, Smith AG, Retzel EF, Mudge J, Chen C.
The plant-specific protein FEHLSTART controls male meiotic entry, initializing meiotic synchronization in Arabidopsis.
Plant J. 2015 Sep 18;. PMID: 26382719 [PubMed - as supplied by publisher]

Link H, Fuhrer T, Gerosa L, Zamboni N, Sauer U.
Real-time metabolome profiling of the metabolic switch between starvation and growth.
Nat Methods. 2015 Sep 14;.PMID: 26366986 [PubMed - as supplied by publisher]

Pest control and resistance management through release of insects carrying a male-selecting transgene

Tim Harvey-Samuel, Neil Morrison, Adam Walker, Thea Marubbi, Ju Yao, Hilda Collins, Kevin Gorman, T. Davies, Nina Alphey, Simon Warner, Anthony Shelton, Luke Alphey
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

Nature Reviews Microbiology contents October 2015 Volume 13 Number 10 pp pp 599-657

Cooperative development of antimicrobials: 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 instiutions 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

http://EMBOJ.embopress.org/content/34/18/2310?etoc

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 Hipp, Carmen Nussbaum-Krammer, Prasad Kasturi, F Ulrich Hartl, Kazuhiro Nagata, and Richard I Morimoto

Published online 29.07.2015

http://EMBOJ.embopress.org/content/34/18/2334?etoc

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 disaggregase that can disassemble amyloids in mammalian and yeast cells.

Kou LH, Wu HH, Liu YM, Zhang YP, Zhang JZ, Guo YP, Ma EB.
Molecular Characterization of Six Small Heat Shock Proteins and Their Responses Under Cadmium Stress in Oxya chinensis (Orthoptera: Acridoidea).
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Wallace EW, Kear-Scott JL, Pilipenko EV, Schwartz MH, Laskowski PR, Rojek AE, Katanski CD, Riback JA, Dion MF, Franks AM, Airoldi EM, Pan T, Budnik BA, Drummond DA.
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Cell. 2015 Sep 10;162(6):1286-98. PMID: 26359986 [PubMed - in process]

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Interaction of HSP20 with a viral RdRp changes its sub-cellular localization and distribution pattern in plants.
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Nitric oxide synthesis by nitrate reductase is regulated during development in Aspergillus.
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Genetic compensation induced by deleterious mutations but not gene knockdowns.
Nature. 2015 Aug 13;524(7564):230-3. PMID: 26168398 [PubMed - indexed for MEDLINE]

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 Cell: Alert 5 September-11 Septemberrticle first published online: 26 NOV 2014 | DOI: 10.1111/ppl.12303

A Mechanism for Sustained Cellulose Synthesis during Salt Stress Original Research Article *Pages 1353-1364*
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 upmost 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.