

Lit Lunch 3_27_15

KEITH:

Non-native, N-terminal Hsp70 Molecular Motor Recognition Elements in Transit Peptides Support Plastid Protein Translocation

March 20, 2015 The Journal of Biological Chemistry, 290, 7602-7621.

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Previously, we identified the N-terminal domain of transit peptides (TPs) as a major determinant for the translocation step in plastid protein import. Analysis of Arabidopsis TP dataset revealed that this domain has two overlapping characteristics, highly uncharged and Hsp70-interacting. To investigate these two properties, we replaced the N-terminal domains of the TP of the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase and its reverse peptide with a series of unrelated peptides whose affinities to the chloroplast stromal Hsp70 have been determined. Bioinformatic analysis indicated that eight out of nine peptides in this series are not similar to the TP N terminus. Using in vivo and in vitro protein import assays, the majority of the precursors containing Hsp70-binding elements were targeted to plastids, whereas none of the chimeric precursors lacking an N-terminal Hsp70-binding element were targeted to the plastids. Moreover, a pulse-chase assay showed that two chimeric precursors with the most uncharged peptides failed to translocate into the stroma. The ability of multiple unrelated Hsp70-binding elements to support protein import verified that the majority of TPs utilize an N-terminal Hsp70-binding domain during translocation and expand the mechanistic view of the import process. This work also indicates that synthetic biology may be utilized to create de novo TPs that exceed the targeting activity of naturally occurring sequences.

Single-molecule Analyses of the Dynamics of Heat Shock Protein 104 (Hsp104) and Protein Aggregates

March 20, 2015 The Journal of Biological Chemistry, 290, 7833-7840.

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Hsp104 solubilizes protein aggregates in cooperation with Hsp70/40. Although the framework of the disaggregase function has been elucidated, the actual process of aggregate solubilization by Hsp104-Hsp70/40 remains poorly understood. Here we developed several methods to investigate the functions of Hsp104 and Hsp70/40 from *Saccharomyces cerevisiae*, at single-molecule levels. The single-molecule methods, which provide the size distribution of the aggregates, revealed that Hsp70/40 prevented the formation of large aggregates from small aggregates and that the solubilization of the small aggregates

required both Hsp104 and Hsp70/40. We directly visualized the individual association-dissociation dynamics of Hsp104 on immobilized aggregates and found that the lifetimes of the Hsp104-aggregate complex are divided into two groups: short (~4 s) and long (~30 s). Hsp70/40 stimulated the association of Hsp104 with aggregates and increased the duration of this association. The single-molecule data provide novel insights into the functional mechanism of the Hsp104 disaggregation machine.

DAMIAN:

Effects of hyperbaric oxygen on nitric oxide generation in humans

Original Research Article *Pages 88-97*

Johan Uusijärvi, Karin Eriksson, Agneta C. Larsson, Carina Nihlén, Tomas Schiffer, Peter Lindholm, Eddie Weitzberg

Background

Hyperbaric oxygen (HBO₂) has been suggested to affect nitric oxide (NO) generation in humans. Specific NO synthases (NOSs) use L-arginine and molecular oxygen to produce NO but this signaling radical may also be formed by serial reduction of the inorganic anions nitrate and nitrite. Interestingly, commensal facultative anaerobic bacteria in the oral cavity are necessary for the first step to reduce nitrate to nitrite. The nitrate-nitrite-NO pathway is greatly potentiated by hypoxia and low pH in contrast to classical NOS-dependent NO generation.

We investigated the effects of HBO₂ on NO generation in healthy subjects including orally and nasally exhaled NO, plasma and salivary nitrate and nitrite as well as plasma cGMP and plasma citrulline/arginine ratio. In addition, we also conducted in-vitro experiments in order to investigate the effects of hyperoxia on nitrate/nitrite metabolism and NO generation by oral bacteria.

ELIZABETH:

March 27, 2015

Cell

Volume 161, Issue 1, Pages 1-176 (26 March 2015)

[Meeting the Global Food Demand of the Future by Engineering Crop Photosynthesis and Yield Potential](#)

Pages 56-66 □ Stephen P. Long, Amy Marshall-Colon, Xin-Guang Zhu [Abstract](#) [PDF \(1648 K\)](#)

Human health and well-being depend on adequate nutrition. Although the current food production is suitable for the needs of the global population, there is a significant potential for future food shortages. This Review discusses the biological limits of crop production and how engineering cellular processes such as photosynthesis can be used to improve the yield of our major crops.

[Nutrient-Sensing Mechanisms across Evolution](#) Review Article *Pages 67-83*

□ Lynne Chantranupong, Rachel L. Wolfson, David M. Sabatini [Abstract](#) [PDF \(1535 K\)](#)

All organisms sense nutrients in the environment to coordinate growth and development. Many sensing strategies, from unicellular organisms to mammals, are evolutionarily conserved.

Current Opinion in Chemical Biology: Alert 20 March-26 March

[Oxygen-evolving complex of Photosystem II: an analysis of second-shell residues and hydrogen-bonding networks](#) Review Article *Pages 152-158* Leslie Vogt, David J Vinyard, Sahr Khan, Gary W Brudvig

-An extensive and interconnected hydrogen-bonding network surrounds the OEC in PSII.

-This network stabilizes intermediates of the OEC during water oxidation.

•Three distinct channels have been identified that transport water, protons, and O₂.

-Changing second-shell residues of the OEC affects the efficiency of water oxidation.

The oxygen-evolving complex (OEC) is a Mn₄O₅Ca cluster embedded in the Photosystem II (PSII) protein complex. As the site of water oxidation, the OEC is connected to the lumen by channels that conduct water, oxygen, and/or protons during the catalytic cycle. The hydrogen-bond networks found in these channels also serve to stabilize the oxidized intermediates, known as the S states. We review recent developments in characterizing these networks via protein mutations, molecular inhibitors, and computational modeling. On the basis of these results, we highlight regions of the PSII protein in which changes have indirect effects on the S₁, S₂, and S₃ oxidation states of the OEC while still allowing photosynthetic activity.

Current Opinion in Cell Biology: Alert 19 March-25 March

ROS-dependent signal transduction [Colleen R Reczek^{1,2}](#), [Navdeep S Chandel^{1,2}](#)

Reactive oxygen species (ROS) are no longer viewed as just a toxic by-product of mitochondrial respiration, but are now appreciated for their role in regulating a myriad of cellular signaling pathways. H₂O₂, a type of ROS, is a signaling molecule that confers target specificity through thiol oxidation. Although redox-dependent signaling has been implicated in numerous cellular processes, the mechanism by which the ROS signal is transmitted to its target protein in the face of highly reactive and abundant antioxidants is not fully understood. In this review of redox-signaling biology, we discuss the possible mechanisms for H₂O₂-dependent signal transduction.

[Mitochondrial pyruvate import and its effects on homeostasis](#) Review Article *Pages 35-41*

Benoit Vanderperre, Tom Bender, Edmund RS Kunji, Jean-Claude Martinou

[Metabolic control via the mitochondrial protein import machinery](#) Review Article *Pages 42-48*

Magdalena Opalinska, Chris Meisinger

[PPARs and ERRs: molecular mediators of mitochondrial metabolism](#) Review Article *Pages 49-54*

Weiwei Fan, Ronald Evans

[Lipid-dependent regulation of the unfolded protein response](#) Review Article *Pages 67-73* Romain

Volmer, David Ron

[The mitochondrial unfolded protein response — synchronizing genomes](#) Review Article *Pages 74-81*

Virginija Jovaisaite, Johan Auwerx

[Mitochondrial division and fusion in metabolism](#) Review Article *Pages 111-118*
Madhuparna Roy, P Hemachandra Reddy, Miho Iijima, Hiromi Sesaki

Plant Cell Advance Online Publication

Cleavage of *INDOLE-3-ACETIC ACID INDUCIBLE28* mRNA by MicroRNA847 Upregulates Auxin Signaling to Modulate Cell Proliferation and Lateral Organ Growth in Arabidopsis

Jing-Jing Wang and Hui-Shan Guo

Plant Cell 2015 tpc.15.00101; First Published on March 20, 2015; doi:10.1105/tpc.15.00101

<http://www.plantcell.org/content/early/2015/03/20/tpc.15.00101.abstract>

A microRNA-auxin/indole acetic acid repressor module regulates the extent of cell competence, thereby affecting the duration of cell proliferation and the development of lateral organs.

**Nature Reviews Molecular Cell Biology contents April 2015 Volume 16 Number 4 pp 203-264
Diversity and selectivity in mRNA translation on the endoplasmic reticulum**

David W. Reid & Christopher V. Nicchitta p221 | doi:10.1038/nrm3958

Recent studies of mRNA distribution and translation show that, in addition to serving as the site of protein translocation into the endoplasmic reticulum (ER), ER-bound ribosomes translate a large fraction of mRNAs that encode cytosolic proteins. This, along with the discovery of many mechanisms for recruiting translation to the ER, suggests an expansive role for the ER in post-transcriptional gene expression.

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Stromal Expression of Heat-Shock Protein 27 Is Associated with Worse Clinical Outcome in Patients with Colorectal Cancer Lung Metastases.

PLoS One. 2015;10(3):e0120724. PMID: 25793600 [PubMed - as supplied by publisher]

BÄ¶ettinger L, Oeljeklaus S, Guiard B, Rospert S, Warscheid B, Becker T.

The mitochondrial heat shock protein 70 (Hsp70) and Hsp10 cooperate in the formation of Hsp60 complexes.

J Biol Chem. 2015 Mar 18; [Epub ahead of print] PMID: 25792736 [PubMed - as supplied by publisher]

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Fluorescence In Situ Hybridization of Small Non-Coding RNAs.

Methods Mol Biol. 2015;1296:73-83. PMID: 25791592 [PubMed - in process]

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Stress Granules Regulate Double-Stranded RNA-Dependent Protein Kinase Activation through a Complex Containing G3BP1 and Caprin1.

MBio. 2015 Mar 17;6(2). PMID: 25784705 [PubMed - in process]

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Hsf and Hsp gene families in Populus: genome-wide identification, organization and correlated expression during development and in stress responses.

BMC Genomics. 2015 Dec;16(1):1398. PMID: 25776715 [PubMed - in process]

Mao J, Chi W, Ouyang M, He B, Chen F, Zhang L.

PAB is an assembly chaperone that functions downstream of chaperonin 60 in the assembly of chloroplast ATP synthase coupling factor 1.

Proc Natl Acad Sci U S A. 2015 Mar 16; [Epub ahead of print] PMID: 25775508 [PubMed - as supplied by publisher]

Barducci A, De Los Rios P.

Non-equilibrium conformational dynamics in the function of molecular chaperones.

Curr Opin Struct Biol. 2015 Mar 12;30:161-169. PMID: 25771489 [PubMed - as supplied by publisher]

Sun H, Li J, Song W, Tao J, Huang S, Chen S, Hou M, Xu G, Zhang Y.

Nitric oxide generated by nitrate reductase increases nitrogen uptake capacity by inducing lateral root formation and inorganic nitrogen uptake under partial nitrate nutrition in rice.

J Exp Bot. 2015 Mar 17;. [Epub ahead of print] PMID: 25784715 [PubMed - as supplied by publisher]

Hancock JT, Whiteman M.

Hydrogen sulfide signaling: interactions with nitric oxide and reactive oxygen species.

Ann N Y Acad Sci. 2015 Mar 17;. [Epub ahead of print] PMID: 25782612 [PubMed - as supplied by publisher]

Zoschke R, Barkan A.

Genome-wide analysis of thylakoid-bound ribosomes in maize reveals principles of cotranslational targeting to the thylakoid membrane.

Proc Natl Acad Sci U S A. 2015 Mar 16;. PMID: 25775549 [PubMed - as supplied by publisher]

[Molecular Cell: Alert 14 March-20 March](#)

[Proteasomal Control of Cytokinin Synthesis Protects *Mycobacterium tuberculosis* against Nitric Oxide](#)

[Marie I. Samanovic¹, Shengjiang Tu², Ondřej Novák^{3,4}, Lakshminarayan M. Iyer⁵, Fiona E. McAllister⁶, L. Aravind⁵, Steven P. Gygi⁶, Stevan R. Hubbard⁷, cMiroslav Strnad^{3,4}, K. Heran Darwin¹.](#)

- Accumulation of Rv1205 sensitizes *M. tuberculosis* to nitric oxide
- Rv1205 is a homolog of LONELY GUY, which makes cytokinins
- Cytokinin breakdown products synergize with NO to kill *M. tuberculosis*

One of several roles of the *Mycobacterium tuberculosis* proteasome is to defend against host-produced nitric oxide (NO), a free radical that can damage numerous biological macromolecules. Mutations that inactivate proteasomal degradation in *Mycobacterium tuberculosis* result in bacteria that are hypersensitive to NO and attenuated for growth in vivo, but it was not known why. To elucidate the link between proteasome function, NO resistance, and pathogenesis, we screened for suppressors of NO hypersensitivity in a mycobacterial proteasome ATPase mutant and identified mutations in Rv1205. We determined that Rv1205 encodes a pupylated proteasome substrate. Rv1205 is a homolog of the plant enzyme LONELY GUY, which catalyzes the production of hormones called cytokinins. Remarkably, we report that an obligate human pathogen secretes several cytokinins. Finally, we determined that the Rv1205-dependent accumulation of cytokinin breakdown products is likely responsible for the sensitization of *Mycobacterium tuberculosis* proteasome-associated mutants to NO.

[Agrochemical control of plant water use using engineered abscisic acid receptors](#)

[Sang-Youl Park, Francis C. Peterson, Assaf Mosquana, Jin Yao, Brian F. Volkman & Sean R. Cutler](#)

Nature (2015) doi:10.1038/nature14123 [PDF](#)

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.

STEPHANIE:

Endoplasmic reticulum stress triggers ROS signalling, changes the redox state, and regulates the antioxidant defence of *Arabidopsis thaliana*

Rengin Ozgur, Ismail Turkan*, Baris Uzilday and Askim H. Sekmen Department of Biology, Faculty of Science, Ege University, Bornova, 35100, Izmir, Turkey

[J Exp Bot.](#) 2014 Mar;65(5):1377-90. doi: 10.1093/jxb/eru034. Epub 2014 Feb 20.

Endoplasmic reticulum stress triggers ROS signalling, changes the redox state, and regulates the antioxidant defence of *Arabidopsis thaliana* Rengin Ozgur, Ismail Turkan*, Baris Uzilday and Askim H. Sekmen Department of Biology, Faculty of Science, Ege University, Bornova, 35100, Izmir, Turkey * To whom correspondence should be addressed. E-mail: ismail.turkan@ege.edu.tr Received 17 September 2013; Revised 15 January 2014; Accepted 17 January 2014 Abstract Inefficient chaperone activity in endoplasmic reticulum (ER) causes accumulation of unfolded proteins and is called ER stress, which triggers the unfolded protein response. For proper oxidative protein folding, reactive oxygen species (ROS) such as H₂O₂ are produced in the ER. Although the role of ROS during abiotic stresses such as salinity is well documented, the role of ER-related ROS production and its signalling is not yet known. Moreover, how H₂O₂ production, redox regulation, and antioxidant defence are affected in salt-treated plants when ER protein-folding machinery is impaired needs to be elucidated. For this aim, changes in NADPH-oxidase-dependent ROS signalling and H₂O₂ content at sequential time intervals and after 48h of ER stress, induced by tunicamycin (Tm), salinity, and their combination were determined in *Arabidopsis thaliana*. The main root growth was inhibited by ER stress, while low levels of Tm caused an increase in lateral root density. Salt stress and Tm induced the expression of ER-stress-related genes (bZIP17, bZIP28, bZIP60, TIN1, BiP1, BiP3) and ERO1. Tm induced expression of RBOHD and RBOHF, which led to an early increase in H₂O₂ and triggered ROS signalling. This study is the first report that ER stress induces the antioxidant system and the Asada–Halliwell pathway of *A. thaliana* in a similar way to salinity. ER stress caused oxidative damage, as evident by increased H₂O₂ accumulation, lipid peroxidation, and protein oxidation. As a result, this study shows that ER stress triggers ROS signalling, changes the redox state, and regulates the antioxidant defence of *A. thaliana*.

NATHEN:

Novel Allosteric Mechanism on Protein–DNA Interactions underlying the Phosphorylation-Dependent Regulation of Ets1 Target Gene Expressions Original Research Article

Pages 1655-1669

Masaaki Shiina, Keisuke Hamada, Taiko Inoue-Bungo, Mariko Shimamura, Akiko Uchiyama, Shiho Baba, Ko Sato, Masaki Yamamoto, Kazuhiro Ogata

Cooperative assemblies of transcription factors (TFs) on target gene enhancers coordinate cell proliferation, fate specification, and differentiation through precise and complicated transcriptional mechanisms. Chemical modifications, such as phosphorylation, of TFs induced by cell signaling further modulate the dynamic cooperativity of TFs. In this study, we found that various Ets1-containing TF–DNA complexes respond differently to calcium-induced phosphorylation of Ets1, which is known to inhibit Ets1–DNA binding. Crystallographic analysis of a complex comprising Ets1, Runx1, and CBF β at the TCR α enhancer revealed that Ets1 acquires robust binding stability in the Runx1 and DNA-complexed state, via allosteric mechanisms. This allows phosphorylated Ets1 to be retained at the TCR α enhancer with Runx1, in contrast to other Ets1 target gene enhancers including mb-1 andstromelysin-1. This study provides a structure-based model for cell-signaling-dependent regulation of target genes, mediated via chemical modification of TFs.

MINSOO:

1. Plant Cell Physiol (2015) 56 (3): 389-400.

Yuriko Osakabe and Keishi Osakabe

Genome Editing with Engineered Nucleases in Plants

Review article

2. Plant Cell Physiol (2015) 56 (3): 481-496.

Physiological and Transcriptional Responses to High Temperature in *Arthrospira (Spirulina) platensis* C1

Jaruta Panyakampol¹, Supapon Cheevadhanarak^{1,2,3}, Sawanee Sutheeworapong², Jeerayut Chaijaruwanich⁴, Jittisak Senachak³, Wipawan Siangdung², Wattana Jeamton², Morakot Tanticharoen⁵ and Kalyanee Paithoonrangsid^{3,*}

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Abstract

Arthrospira (Spirulina) platensis is a well-known commercial cyanobacterium that is used as a food and in feed supplements. In this study, we examined the physiological changes and whole-genome expression in *A. platensis* C1 exposed to high temperature. We found that **photosynthetic activity was significantly decreased after the temperature was shifted from 35°C to 42°C for 2 h.** A reduction in biomass production and protein content, concomitant with the accumulation of carbohydrate content, was observed after prolonged exposure to high temperatures for 24 h. Moreover, the results of the expression profiling in response to high temperature at the designated time points (8 h) revealed two distinct phases of the responses. The first was **the immediate response phase, in which the transcript levels of genes involved in different mechanisms, including genes for heat shock proteins;** genes involved in signal transduction and carbon and nitrogen metabolism; and genes encoding inorganic ion transporters for magnesium, nitrite and nitrate, were either transiently induced or repressed by the high temperature. In the second phase, **the long-term response phase, both the induction and repression of the expression of genes with important roles in translation and photosynthesis were observed.** Taken together, the results of our physiological and transcriptional studies suggest that dynamic changes in the transcriptional profiles of these thermal-responsive genes might play a role in maintaining cell homeostasis under high temperatures, as reflected in the growth and biochemical composition, particularly the protein and carbohydrate content, of *A. platensis* C1.

Genome of *A. platensis* C1 was sequenced (Cheevadhanarak et al. 2012)

INDU:

1: Zhang J, Khan SA, Hasse C, Ruf S, Heckel DG, Bock R. Pest control. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. *Science*. 2015 Feb 27;347(6225):991-4. doi: 10.1126/science.1261680. PubMed PMID: 25722411.

2: Jovanovic M, Rooney MS, Mertins P, Przybylski D, Chevrier N, Satija R, Rodriguez EH, Fields AP, Schwartz S, Raychowdhury R, Mumbach MR, Eisenhaure T, Rabani M, Gennert D, Lu D, Delorey T, Weissman JS, Carr SA, Hacohen N, Regev A. Immunogenetics. Dynamic profiling of the protein life cycle in response to pathogens. *Science*. 2015 Mar 6;347(6226):1259038. doi: 10.1126/science.1259038. Epub 2015 Feb 12. PubMed PMID: 25745177.

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FIONN:

Trends in plant science

Autophagy: a multifaceted intracellular system for bulk and selective recycling.
Li F1, Vierstra RD.

Abstract

Plants have evolved sophisticated mechanisms to recycle intracellular constituents. One gaining in appreciation is autophagy, which involves specialized vesicles engulfing and delivering unwanted cytoplasmic material to the vacuole for breakdown. Central to this process is the ubiquitin-fold protein autophagy (ATG)-8, which becomes tethered to the developing autophagic membranes by lipidation. Here, we review data showing that the ATG8 moiety provides a docking site not only for proteins that help shape the enclosing vesicles and promote their fusion with the tonoplast, but also for a host of receptors that recruit appropriate autophagic cargo. The identity of these receptors has dramatically altered the view of autophagy as being a relatively nonspecific mechanism to one that may selectively sequester aggregated proteins, protein complexes, organelles, and even invading pathogens.