

Lit Lunch 8/1/14

Elizabeth:

The Plant Journal Content Alert: 79, 3 (August 2014)

HSP90s are required for NLR immune receptor accumulation in Arabidopsis (pages 427–439)

Shuai Huang, Jacqueline Monaghan, Xionghui Zhong, Ling Lin, Tongjun Sun, Oliver Xiaoou Dong and Xin Li

Article first published online: 30 JUN 2014 | DOI: 10.1111/tpj.12573

[Large-scale gene expression profiling data for the model moss *Physcomitrella patens* aid understanding of developmental progression, culture and stress conditions \(pages 530–539\)](#)

Manuel Hiss, Oliver Laule, Rasa M. Meskauskiene, Muhammad A. Arif, Eva L. Decker, Anika Erxleben, Wolfgang Frank, Sebastian T. Hanke, Daniel Lang, Anja Martin, Christina Neu, Ralf Reski, Sandra Richardt, Mareike Schallenberg-Rüdinger, Peter Szövényi, Theodor Tiko, Gertrud Wiedemann, Luise Wolf, Philip Zimmermann and Stefan A. Rensing

Article first published online: 9 JUL 2014 | DOI: 10.1111/tpj.12572

Nature Protocols Contents: Volume 9 Number 8, pp 1771-2004

Flux profiling of photosynthetic carbon metabolism in intact plants pp1803 – 1824

Full characterization of the metabolic state of a biological system involves quantifying the flux of the biochemical reactions. In this protocol, *Arabidopsis* rosettes are treated with $^{13}\text{CO}_2$, and ^{13}C -labeled metabolites are analyzed by GC- and LC-MS. Robert Heise *et al.*

Published online: 03 July 2014 | doi:10.1038/nprot.2014.115 [Abstract](#) | [Full Text](#) | [PDF \(1,313K\)](#)

Nature Biotechnology |

Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew Nature Biotechnology (2014)

doi:10.1038/nbt.2969

The complete works of Charles Darwin Online:

http://darwin-online.org.uk/BeagleLibrary/Beagle_Library_Introduction.htm

Nature contents: 24 July 2014

Structure of an Rrp6–RNA exosome complex bound to poly(A) RNA

The exosome complex contains two catalytic subunits which degrade RNA in either a distributive (Rrp6) or a processive (Rrp44) manner—previous structures indicated how RNA could be directed to Rrp44, but the path taken to Rrp6 was unclear; here the location of the Rrp6 catalytic domain and the RNA 3' end are determined and it is found that the RNA lies in an opposite orientation from that of the Rrp44-containing exosome structure, suggesting that the fate of an RNA may be influenced by the manner in which cofactors present it.

Nature Reviews Molecular Cell Biology contents August 2014 Volume 15 Number 8 pp 497-558

Small RNAs break out: the molecular cell biology of mobile small RNAs

Peter Sarkies & Eric A. Miska p525 | doi:10.1038/nrm3840

Mobile RNAs function in antiviral defence, cell signalling and gene expression regulation, and might also mediate transgenerational epigenetic inheritance. Genetic and molecular studies in plants and nematodes have begun to provide insights into the mechanisms underlying RNA movement, its functions and the nature of mobile RNA molecules.

Nature (2014) doi:10.1038/nature13453

Serial time-resolved crystallography of photosystem II using a femtosecond X-ray laser

Photosynthesis, a process catalysed by plants, algae and cyanobacteria converts sunlight to energy thus sustaining all higher life on Earth. Two large membrane protein complexes, photosystem I and II (PSI and PSII), act in series to catalyse the light-driven reactions in photosynthesis. PSII catalyses the light-driven water splitting process, which maintains the Earth's oxygenic atmosphere¹. In this process, the oxygen-evolving complex (OEC) of PSII cycles through five states, S_0 to S_4 , in which four electrons are sequentially extracted from the OEC in four light-driven charge-separation events. Here we describe time resolved experiments on PSII nano/microcrystals from *Thermosynechococcus elongatus* performed with the recently developed² technique of serial femtosecond crystallography. Structures have been determined from PSII in the dark S_1 state and after double laser excitation (putative S_3 state) at 5 and 5.5 Å resolution, respectively. The results provide evidence that PSII undergoes significant conformational changes at the electron acceptor side and at the Mn_4CaO_5 core of the OEC. These include an elongation of the metal cluster, accompanied by changes in the protein environment, which could allow for binding of the second substrate water molecule between the more distant protruding Mn (referred to as the 'dangler' Mn) and the Mn_3CaO_x cubane in the S_2 to S_3 transition, as predicted by spectroscopic and computational studies^{3,4}. This work shows the great potential for time-resolved serial femtosecond crystallography for investigation of catalytic processes in biomolecules.

Insights into Secondary Metabolism from a Global Analysis of Prokaryotic Biosynthetic Gene Clusters

Pages 412-421

Peter Cimermancic, Marnix H. Medema, Jan Claesen, Kenji Kurita, Laura C. Wieland Brown, Konstantinos Mavrommatis, Amrita Pati, Paul A. Godfrey, Michael Koehrsen, Jon Clardy, Bruce W. Birren, Eriko Takano, Andrej Sali, Roger G. Linington, Michael A. Fischbach

Although biosynthetic gene clusters (BGCs) have been discovered for hundreds of bacterial metabolites, our knowledge of their diversity remains limited. Here, we used a novel algorithm to systematically identify BGCs in the extensive extant microbial sequencing data. Network analysis of the predicted BGCs revealed large gene cluster families, the vast majority uncharacterized. We experimentally characterized the most prominent family, consisting of two subfamilies of hundreds of BGCs distributed throughout the Proteobacteria; their products are aryl polyenes, lipids with an aryl head group conjugated to a polyene tail. We identified a distant relationship to a third subfamily of aryl polyene BGCs, and together the three subfamilies represent the largest known family of biosynthetic gene clusters, with more than 1,000 members. Although these clusters are widely divergent in sequence, their small molecule products are remarkably conserved, indicating for the first time the important roles these compounds play in Gram-negative cell biology.

Jackrel ME, Shorter J.

Potentiated Hsp104 variants suppress toxicity of diverse neurodegenerative disease-linked proteins. *Dis Model Mech.* 2014 Jul 25; PMID: 25062688

Stein KC, True HL.
Structural variants of yeast prions show conformer-specific requirements for chaperone activity. *Mol Microbiol.* 2014 Jul 24; PMID 25060529

Gerashchenko MV, Gladyshev VN.
Translation inhibitors cause abnormalities in ribosome profiling experiments.
Nucleic Acids Res. 2014 Jul 23;. [Epub ahead of print]
PMID: 25056308 [PubMed - as supplied by publisher]

Wang J, Pareja KA, Kaiser CA, Sevier CS.
Redox signaling via the molecular chaperone BiP protects cells against endoplasmic reticulum-derived oxidative stress.
Elife (Cambridge). 2014 Jul 22;;e03496. [Epub ahead of print]
PMID: 25053742 [PubMed - as supplied by publisher]

Zhang X, Liu Y, Genereux JC, Nolan C, Singh M, Kelly JW.
The Heat-Shock Response Transcriptional Program Enables High-Yield and High-Quality Recombinant Protein Production in *Escherichia coli*.
ACS Chem Biol. 2014 Jul 22; PMID: 25051296

Lee KW, Choi GJ, Kim KY, Ji HJ, Park HS, Kim YG, Lee BH, Lee SH.
Transgenic Expression of MsHsp23 Confers Enhanced Tolerance to Abiotic Stresses in Tall Fescue.
Asian-Australas J Anim Sci. 2012 Jun;25(6):818-23. PMID: 25049632 [PubMed]

Ramundo S, Rochaix JD.
Loss of chloroplast ClpP elicits an autophagy-like response in *Chlamydomonas*.
Autophagy. 2014 Jul 17;10(9). PMID: 25046108 [PubMed - as supplied by publisher]

Hanssum A, Zhong Z, Rousseau A, Krzyzosiak A, Sigurdardottir A, Bertolotti A.
An Inducible Chaperone Adapts Proteasome Assembly to Stress.
Mol Cell. 2014 Jul 16;. [Epub ahead of print]
PMID: 25042801 [PubMed - as supplied by publisher]

Zimmer-Prados LM, Moreira AS, Magalhaes JR, Fran sa MG.
Nitric oxide increases tolerance responses to moderate water deficit in leaves of *Phaseolus vulgaris* and *Vigna unguiculata* bean species.
Physiol Mol Biol Plants. 2014 Jul;20(3):295-301.
PMID: 25049456 [PubMed]

Journal of Plant Physiology: Alert 21 July-27 July
[Nitric-oxide inhibits nyctinastic closure through cGMP in *Albizia lophantha* leaflets](#) *Pages 1299-1305* Carmen Bergareche, Luisa Moysset, Alcira Paola Angelo, Samira Chellik, Esther Sim n

Plant, Cell & Environment Content Alert: 37, 9 (September 2014)

Histone chaperone ASF1 is involved in gene transcription activation in response to heat stress in *Arabidopsis thaliana* (pages 2128–2138)

MINJIE WENG, YUE YANG, HAIYANG FENG, ZONGDE PAN, WEN-HUI SHEN, YAN ZHU and AIWU DONG

Article first published online: 19 MAR 2014 | DOI: 10.1111/pce.12299

This work reports on a previously unknown new role of the histone chaperone ASF1 in plant thermotolerance. The *Arabidopsis A tasf1ab* double mutant exhibits both basal and acquired thermotolerance defects, linked with impaired induction of some heat response genes. Heat induced gene expression is accompanied by AtASF1A/B protein enrichment, histone H3 eviction, RNA polymerase II accumulation, and H3K56 acetylation in chromatin regions of the target genes (e.g. *H sfA2* and *H sa32*). The data suggest that ASF1 may be involved in nucleosome dissociation regulation in plant response to environmental heat stress.

Tracing Compartmentalized NADPH Metabolism in the Cytosol and Mitochondria of Mammalian Cells *Molecular Cell, Volume 55, Issue 2, 17 July 2014, Pages 253-263*

Eukaryotic cells compartmentalize biochemical processes in different organelles, often relying on metabolic cycles to shuttle reducing equivalents across intracellular membranes. NADPH serves as the electron carrier for the maintenance of redox homeostasis and reductive biosynthesis, with separate cytosolic and mitochondrial pools providing reducing power in each respective location. This cellular organization is critical for numerous functions but complicates analysis of metabolic pathways using available methods. Here we develop an approach to resolve NADP(H)-dependent pathways present within both the cytosol and the mitochondria. By tracing hydrogen in compartmentalized reactions that use NADPH as a cofactor, including the production of 2-hydroxyglutarate by mutant isocitrate dehydrogenase enzymes, we can observe metabolic pathway activity in these distinct cellular compartments. Using this system we determine the direction of serine/glycine interconversion within the mitochondria and cytosol, highlighting the ability of this approach to resolve compartmentalized reactions in intact cells.

Molecular Cell: Alert 20 July-26 July

Visualization of Transient Protein-Protein Interactions that Promote or Inhibit Amyloid Assembly *Pages 214-226*

Theodoros K. Karamanos, Arnout P. Kalverda, Gary S. Thompson, Sheena E. Radford
Cytosolic Quality Control of Mislocalized Proteins Requires RNF126 Recruitment to Bag6 *Pages 227-237*

Monica C. Rodrigo-Brenni, Erik Gutierrez, Ramanujan S. Hegde

Approximately 30% of eukaryotic proteins contain hydrophobic signals for localization to the secretory pathway. These proteins can be mislocalized in the cytosol due to mutations in their targeting signals, certain stresses, or intrinsic inefficiencies in their translocation. Mislocalized proteins (MLPs) are protected from aggregation by the Bag6 complex and degraded by a poorly characterized proteasome-dependent pathway. Here, we identify the ubiquitin ligase RNF126 as a key component of the MLP degradation pathway. In vitro reconstitution and fractionation studies reveal that RNF126 is the primary Bag6-dependent ligase. RNF126 is recruited to the N-terminal Ubl domain of Bag6 and preferentially ubiquitinates juxtahydrophobic lysine residues on Bag6-associated clients. Interfering with RNF126 recruitment in vitro prevents ubiquitination, and RNF126 depletion in cells partially stabilizes a Bag6 client. Bag6-dependent

ubiquitination can be recapitulated with purified components, paving the way for mechanistic analyses of downstream steps in this cytosolic quality control pathway.

Functional Role of Tia1/Pub1 and Sup35 Prion Domains: Directing Protein Synthesis Machinery to the Tubulin Cytoskeleton *Pages 305-318*

Xiang Li, Joseph B. Rayman, Eric R. Kandel, Irina L. Derkatch

Tia1/Pub1 is a stress granule component carrying a Q/N-rich prion domain. We provide direct evidence that Tia1 forms a prion in yeast. Moreover, Tia1/Pub1 acts cooperatively with release factor Sup35/eRF3 to establish a two-protein self-propagating state. This two-protein prion driven by the Q/N-rich prion domains of Sup35 and Tia1/Pub1 can be visualized as distinctive line structures along tubulin cytoskeleton. Furthermore, we find that tubulin-associated complex containing Pub1 and Sup35 oligomers normally exists in yeast, and its assembly depends on prion domains of Pub1 and Sup35. This Sup35/Pub1 complex, which also contains *TUB1* mRNA and components of translation machinery, is important for the integrity of the tubulin cytoskeleton: *PUB1* disruption and Sup35 depletion from the complex lead to cytoskeletal defects. We propose that the complex is implicated in protein synthesis at the site of microtubule assembly. Thus our study identifies the role for prion domains in the assembly of multiprotein complexes.

20S proteasome activity is modified via S-glutathionylation based on intracellular redox status of the yeast *Saccharomyces cerevisiae*: Implications for the degradation of oxidized proteins *Pages 65-71*

Marilene Demasi, Adrian Hand, Erina Ohara, Cristiano L.P. Oliveira, Renata N. Bicev, Clelia A. Bertocini, Luis E.S. Netto

Protein S-glutathionylation is a post-translational modification that controls many cellular pathways. Recently, we demonstrated that the $\alpha 5$ -subunit of the 20S proteasome is S-glutathionylated in yeast cells grown to the stationary phase in rich medium containing glucose, stimulating 20S core gate opening and increasing the degradation of oxidized proteins. In the present study, we evaluated the correlation between proteasomal S-glutathionylation and the intracellular redox status. The redox status was controlled by growing yeast cells in distinct carbon sources which induced respiratory (glycerol/ethanol) or fermentative (glucose) metabolism. Cells grown under glycerol/ethanol displayed higher reductive power when compared to cells grown under glucose. When purified from cells grown in glucose, 20S proteasome $\alpha 5$ -subunit exhibited an intense anti-glutathione labeling. A higher frequency of the open catalytic chamber gate was observed in the S-glutathionylated preparations as demonstrated by transmission electron microscopy. Therefore, cells that had been grown in glucose displayed an increased ability to degrade oxidized proteins. The results of the present study suggest that 20S proteasomal S-glutathionylation is a relevant adaptive response to oxidative stress that is capable to sense the intracellular redox environment, leading to the removal of oxidized proteins via a process that is not dependent upon ubiquitylation and ATP consumption.

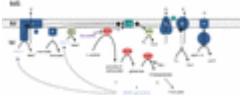
June 19

Biochemical characterization of proline dehydrogenase in *Arabidopsis* mitochondria (pages 2794–2804)

Peter Schertl, Cécile Cabassa, Kaouthar Saadallah, Marianne Bordenave, Arnould

Savouré and Hans-Peter Braun

Article first published online: 14 MAY 2014 | DOI: 10.1111/febs.12821



Proline protects plant cells under different stress conditions. The first enzyme of proline catabolism is proline dehydrogenase (ProDH). Here, we investigate effects of ProDH induction on mitochondrial metabolism. Besides enzymes of the proline catabolic pathway, D-lactate dehydrogenase is induced. Lactate was identified as a competitive inhibitor of ProDH which might regulate ProDH during the plant stress response.

Roy U, Woods AG, Sokolowska I, Darie CC.

Utility of Computational Structural Biology in Mass Spectrometry.

Adv Exp Med Biol. 2014;806:107-128.

PMID: 24952181 [PubMed - as supplied by publisher]

Zuo RJ, Zhao YC, Lei W, Wang TS, Wang BC, Yang ZM.

Crystallin β acts as a molecular guard in mouse decidualization: Regulation and function during early pregnancy.

FEBS Lett. 2014 Jun 18;. [Epub ahead of print]

PMID: 24951838 [PubMed - as supplied by publisher]

Sachelaru I, Petriman NA, Kudva R, Koch HG.

Dynamic Interaction of the Sec Translocon with the Chaperone PpiD.

J Biol Chem. 2014 Jun 20;. [Epub ahead of print]

PMID: 24951590 [PubMed - as supplied by publisher]

Leach MD, Cowen LE.

Membrane fluidity and temperature sensing are coupled via circuitry comprised of Ole1, Rsp5, and Hsf1 in *Candida albicans*.

Eukaryot Cell. 2014 Jun 20;. [Epub ahead of print]

PMID: 24951438 [PubMed - as supplied by publisher]

Kirschke E, Goswami D, Southworth D, Griffin PR, Agard DA.

Glucocorticoid receptor function regulated by coordinated action of the hsp90 and hsp70 chaperone cycles.

Cell. 2014 Jun 19;157(7):1685-97.

PMID: 24949977 [PubMed - in process]

Adjibade P, Mazroui R.

Control of mRNA turnover: Implication of cytoplasmic RNA granules.

Semin Cell Dev Biol. 2014 Jun 16;. [Epub ahead of print]

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Hipp MS, Park SH, Hartl FU.

Proteostasis impairment in protein-misfolding and -aggregation diseases.

Trends Cell Biol. 2014 Jun 16;. [Epub ahead of print]

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Zblewska K, Krajewska J, Zolkiewski M, KÄ™dziarska-Mieszkowska S.
Role of the disaggregase ClpB in processing of proteins aggregated as inclusion bodies.
Arch Biochem Biophys. 2014 Jun 2;555-556C:23-27. [Epub ahead of print]
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Replica exchange molecular dynamics simulations provide insight into substrate recognition by small heat shock proteins.
Biophys J. 2014 Jun 17;106(12):2644-55.
PMID: 24940782 [PubMed - in process]

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Interplay between trigger factor and other protein biogenesis factors on the ribosome.
Nat Commun. 2014 Jun 18;5:4180.
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Fusion of protein aggregates facilitates asymmetric damage segregation.
PLoS Biol. 2014 Jun;12(6):e1001886.
PMID: 24936793 [PubMed - in process]

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OP10 Proteomic analysis of endogenous S-sulfhydration in Arabidopsis thaliana.
Nitric Oxide. 2013 Sep 1;31 Suppl 2:S23.
PMID: 24948248 [PubMed - in process]

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PMID: 24948247 [PubMed - in process]

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Free radical-mediated systemic immunity in plants.
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PMID: 24929297 [PubMed - as supplied by publisher]
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TOPLESS mediates brassinosteroid-induced transcriptional repression through interaction with BZR1.
Nat Commun. 2014 Jun 18;5:4140.
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Plant Cell. 2014 Jun 17;. [Epub ahead of print]
PMID: 24938288 [PubMed - as supplied by publisher]

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Translation Elongation Factor 1A Mutants With Altered Actin Bundling Activity Show Reduced Aminoacyl-tRNA Binding and Alter Initiation Via eIF2 $\hat{\pm}$ Phosphorylation.
J Biol Chem. 2014 Jun 16;. [Epub ahead of print]
PMID: 24936063 [PubMed - as supplied by publisher]

[Redox and nitric oxide homeostasis are affected in tomato \(*Solanum lycopersicum*\) roots under salinity-induced oxidative stress](#) Original Research Article *Pages 1028-1035* Jamel Manai, Houda Gouia, Francisco J. Corpas

Cox D, Carver JA, Ecroyd H.

Preventing $\hat{\pm}$ -synuclein aggregation: The role of the small heat-shock molecular chaperone proteins.

Biochim Biophys Acta. 2014 Jun 25;. [Epub ahead of print]

PMID: 24973551 [PubMed - as supplied by publisher]

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The C-terminal Tails of the Bacterial Chaperonin GroEL Stimulate Protein Folding by Directly Altering the Conformation of a Substrate Protein.

J Biol Chem. 2014 Jun 25;. [Epub ahead of print]

PMID: 24970895 [PubMed - as supplied by publisher]

Nagasawa T, Matsushima-Nishiwaki R, Toyoda H, Matsuura J, Kumada T, Kozawa O. Heat shock protein 20 (HSPB6) regulates apoptosis in human hepatocellular carcinoma cells: Direct association with Bax.

Oncol Rep. 2014 Jun 23;. [Epub ahead of print]

PMID: 24969689 [PubMed - as supplied by publisher]

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Interplay between Chaperones and Protein Disorder Promotes the Evolution of Protein Networks.

PLoS Comput Biol. 2014 Jun;10(6):e1003674.

PMID: 24968255 [PubMed - in process]

Ben-Lulu S, Ziv T, Admon A, Weisman-Shomer P, Benhar M.

A substrate trapping approach identifies proteins regulated by reversible S-nitrosylation.

Mol Cell Proteomics. 2014 Jun 27;. [Epub ahead of print]

PMID: 24973421 [PubMed - as supplied by publisher]

Zhang J, Zhao X, Sun P, Ma Z.

PSNO: Predicting Cysteine S-Nitrosylation Sites by Incorporating Various Sequence-Derived Features into the General Form of Chou's PseAAC.

Int J Mol Sci. 2014 Jun 25;15(7):11204-19.

PMID: 24968264 [PubMed - in process]

Archives of Biochemistry and Biophysics: Alert 24 June-30 June

[Role of the disaggregase ClpB in processing of proteins aggregated as inclusion bodies](#) Original Research Article

Pages 23-27 Kamila Zblewska, Joanna Krajewska, Michal Zolkiewski, Sabina Kędzierska-Mieszkowska

Wheat cultivars selected for high F_v/F_m under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter

Dew Kumari Sharma, Sven Bode Andersen, Carl-Otto Ottosen and Eva Rosenqvist
Accepted manuscript online: 24 JUN 2014 05:21AM EST | DOI: 10.1111/pp1.12245

Shiber A, Ravid T.

Chaperoning Proteins for Destruction: Diverse Roles of Hsp70 Chaperones and their Co-Chaperones in Targeting Misfolded Proteins to the Proteasome.
Biomolecules. 2014 Jul 17;4(3):704-24.
PMID: 25036888 [PubMed]

Taipale M, Tucker G, Peng J, Krykbaeva I, Lin ZY, Larsen B, Choi H, Berger B, Gingras AC, Lindquist S.

A quantitative chaperone interaction network reveals the architecture of cellular protein homeostasis pathways.
Cell. 2014 Jul 17;158(2):434-48.
PMID: 25036637 [PubMed - in process]

Marcion G, Seigneuric R, Chavanne E, Artur Y, Briand L, Hadi T, Gobbo J, Garrido C, Neiers F.

C-terminal amino acids are essential for human heat shock protein 70 dimerization.
Cell Stress Chaperones. 2014 Jul 17;. [Epub ahead of print]
PMID: 25030382 [PubMed - as supplied by publisher]

Holmes WM, Mannakee BK, Gutenkunst RN, Serio TR.

Loss of amino-terminal acetylation suppresses a prion phenotype by modulating global protein folding.
Nat Commun. 2014 Jul 15;5:4383.
PMID: 25023910 [PubMed - in process]

Clark GB, Morgan RO, Fernandez MP, Salmi ML, Roux SJ.

Breakthroughs spotlighting roles for extracellular nucleotides and apyrases in stress responses and growth and development.
Plant Sci. 2014 Aug;225C:107-116. Review.
PMID: 25017166 [PubMed - as supplied by publisher]

Cheng S, Shi T, Wang XL, Liang J, Wu H, Xie L, Li Y, Zhao YL.

Features of S-nitrosylation based on statistical analysis and molecular dynamics simulation: cysteine acidity, surrounding basicity, steric hindrance and local flexibility.
Mol Biosyst. 2014 Jul 17;. [Epub ahead of print]
PMID: 25030274 [PubMed - as supplied by publisher]

Dever TE, Gutierrez E, Shin BS.
The hypusine-containing translation factor eIF5A.
Crit Rev Biochem Mol Biol. 2014 Jul 17;:1-13. [Epub ahead of print]
PMID: 25029904 [PubMed - as supplied by publisher]

PabÃ³n-Mora N, Wong GK, Ambrose BA.
Evolution of fruit development genes in flowering plants.
Front Plant Sci. 2014;5:300. PMID: 25018763 [PubMed]

[J. Biol. Chem. doi:10.1074/jbc.M114.578625](https://doi.org/10.1074/jbc.M114.578625)

The inefficient enzyme RuBisCO fixes CO₂, enabling life, but improvements of its catalytic activity would facilitate applications in carbon capture and metabolic engineering. Structures are available for the open (apo) and closed (ligand-bound) states of two of the three known forms (I and III), but only an open structure exists for form II of the homolog from *Rhodospirillum rubrum*. Satagopan *et al.* now report the closed structure of a form II RuBisCO from *Rhodopseudomonas palustris* with a transition state analog. This RuBisCO, which forms a hexamer, unlike the dimeric *R. rubrum* homolog, cants at a different angle compared to the form I and III oligomers, creating a distinct subunit interface. Mutations to nonconserved amino acids neighboring the active site such as I165A were generally deleterious, indicating roles in enzyme function even if not directly in catalysis. The C terminus, representing a second point of divergence between the three forms, could not be exchanged between forms, though adding a C-terminal extension derived from the *R. rubrum* enzyme enhanced the activity of the *R. palustris* enzyme substantially. Finally, the slow reactivation of the I165A mutant after inhibition suggested that accessory proteins needed for regulation of the eukaryotic and cyanobacterial enzymes may not be required for *R. palustris*. These results highlight similarities and differences in enzyme structure and function that may lead to new mechanistic understanding of this important family of enzymes.

DrugTargetSeqR: a genomics- and CRISPR-Cas9–based method to analyze drug targets pp626 - 628

Corynn Kasap, Olivier Elemento and Tarun M Kapoor
Finding the biological targets of small molecules remains an important challenge in chemical biology and drug discovery. A method involving high-throughput sequencing, mutational analysis and clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 genome editing identifies the targets and potential modes of compound resistance for two anticancer agents.

Chen YC, Umanah GKE, Dephore N, Andrabi SA, Gygi SP, Dawson TM, Dawson VL, Rutter J (2014) *Msp1/ATAD1 maintains mitochondrial function by facilitating the degradation of mislocalized tail-anchored proteins. EMBO J 33: 1548–1564* [Abstract](#)
[Full Article \(HTML\)](#)

Okreglak V, Walter P (2014) *The conserved AAA-ATPase Msp1 confers organelle specificity to tail-anchored proteins. Proc Natl Acad Sci USA 111: 8019–8024*
Science 18 July 2014

[Greening the food pyramid](#) Scientists say dietary guidelines should recommend environmentally sustainable choices

the International Wheat Genome Sequencing Consortium (IWGSC) developed a strategy of physical mapping and sequencing the individual chromosomes and chromosome arms of the bread wheat genome. In this special issue of *Science*, four Research Articles are presented in full online (www.sciencemag.org/extra/wheatgenome), with abstracts in print on p. 286 and a News story on p. 251. These papers present major advances toward obtaining a reference sequence and enhancing our understanding of the bread wheat genome.

The IWGSC produced a survey of the gene content and composition of all 21 chromosomes and identified 124,201 gene loci, with more than 75,000 positioned along the chromosomes. Comparing the bread wheat gene sequences with gene repertoires from its closest extant relatives (representing the species that donated the A, B, and D progenitor genomes) showed limited gene loss during the evolution of the hexaploid wheat genome but frequent gene duplications after these genomes came together. Gene expression patterns revealed that none of the subgenomes dominated gene expression.

Choulet *et al.* describe the sequencing, assembly, annotation, and analysis of the reference sequence of the largest wheat chromosome, 3B, which at nearly 1 gigabase is more than seven times larger than the entire sequence of the model plant *Arabidopsis thaliana*. Relying on a physical map derived from the chromosome 3B-specific bacterial artificial chromosome (BAC) library (*1*), more than 8000 BAC clones were sequenced and assembled into a pseudomolecule—a nearly complete representation of the entire chromosome.

Science, this issue p. [325](#)

Food Security - **How to optimize global food production** [Andrew M. Sugden](#)

Keeping societies stable and managing Earth's resources sustainably depend on doing a good, steady job producing and distributing food. West *et al.* asked what combinations of crops and regions offer the best chance of progress. Their analysis focused on reducing greenhouse gas emissions, nutrient pollution, water use, and food waste. They identify regions that are likely to yield the best balance between applying fertilizer to increase crop yields versus the resulting environmental impact.

Science, this issue p. [328](#) **Alternative Splicing- Evolving from an enzyme and into a regulator** [Valda Vinson](#)

Proteins, the work-horses of the cell, are made on a messenger RNA (mRNA) template. An enzyme called aminoacyl tRNA synthetases (AARSs) attaches the correct amino acid to a transfer RNA so that mRNA is accurately translated. Over evolution, additional sequences have been added to AARSs. Lo *et al.* found a large number of AARS variants in which the domain responsible for enzyme function was deleted. Ninety-four such variants had diverse signaling activities. Thus, AARSs are used both as enzymes and alternately as regulators of signaling pathways.

Plant Journal

Analysis of an Arabidopsis Heat-sensitive Mutant Reveals that Chlorophyll Synthase is Involved in Reutilization of Chlorophyllide during Chlorophyll Turnover

Yao-Pin Lin, Tsung-yuan Lee, Ayumi Tanaka and Yee-yung Charng

Accepted manuscript online: 8 JUL 2014 11:16AM EST | DOI: 10.1111/tpj.12611

Plant, Cell & Environment Content Alert (New Articles)

[In comparison with nitrate nutrition, ammonium nutrition increases growth of the](#)

[frostbite1 Arabidopsis mutant](#)

ANNA PODGÓRSKA, MONIKA OSTASZEWSKA, PER GARDESTRÖM, ALLAN G. RASMUSSEN and BOŽENA SZAL

Accepted manuscript online: 8 JUL 2014 03:57AM EST | DOI: 10.1111/pce.12404

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Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in *Arabidopsis thaliana* (pages 348–359)

Friedrich Fauser, Simon Schiml and Holger Puchta
Article first published online: 17 JUN 2014 | DOI: 10.1111/tpj.12554

S-Nitrosoglutathione Reductase Deficiency-Induced S-Nitrosylation Results in Neuromuscular Dysfunction

Costanza Montagna, Giuseppina Di Giacomo, Salvatore Rizza, Simone Cardaci, Elisabetta Ferraro, Paolo Grumati, Daniela De Zio, Emiliano Maiani, Carolina Muscoli, Filomena Lauro, Sara Ilari, Sergio Bernardini, Stefano Cannata, Cesare Gargioli, Maria R. Ciriolo, Francesco Cecconi, Paolo Bonaldo, and Giuseppe Filomeni

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Correction: In Vivo Substrates of the Lens Molecular Chaperones α -Crystallin and β -Crystallin.
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Indu:

1. *Science.* 2014 Jun 6;344(6188):1160-4. doi: 10.1126/science.1250778.

A mutually assured destruction mechanism attenuates light signaling in *Arabidopsis*.

Ni W(1), Xu SL(2), Tepperman JM(1), Stanley DJ(3), Maltby DA(3), Gross JD(3), Burlingame AL(3), Wang ZY(4), Quail PH(5).

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After light-induced nuclear translocation, phytochrome photoreceptors interact with and induce rapid phosphorylation and degradation of basic helix-loop-helix transcription factors, such as PHYTOCHROME-INTERACTING FACTOR 3 (PIF3), to regulate gene expression. Concomitantly, this interaction triggers feedback reduction of phytochrome B (phyB) levels. Light-induced phosphorylation of PIF3 is necessary for the degradation of both proteins. We report that this PIF3 phosphorylation induces, and is necessary for, recruitment of LRB [Light-Response

Bric-a-Brack/Tramtrack/Broad (BTB)] E3 ubiquitin ligases to the PIF3-phyB complex. The recruited LRBs promote concurrent polyubiquitination and degradation of both PIF3 and phyB in vivo. These data reveal a linked signal-transmission and attenuation mechanism involving mutually assured destruction of the receptor and its immediate signaling partner.

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2. Science. 2014 Jun 20;344(6190):1389-92. doi: 10.1126/science.1252634. Epub 2014 May 22.

Life-span extension by a metacaspase in the yeast *Saccharomyces cerevisiae*.

Hill SM(1), Hao X(1), Liu B(2), Nyström T(2).

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Comment in

Science. 2014 Jun 20;344(6190):1341-2.

Single-cell species harbor ancestral structural homologs of caspase proteases, although the evolutionary benefit of such apoptosis-related proteins in unicellular organisms is unclear. Here, we found that the yeast metacaspase Mca1 is recruited to the insoluble protein deposit (IPOD) and juxtannuclear quality-control compartment (JUNQ) during aging and proteostatic stress. Elevating MCA1 expression counteracted accumulation of unfolded proteins and aggregates and extended life span in a heat shock protein Hsp104 disaggregase- and proteasome-dependent manner. Consistent with a role in protein quality control, genetic interaction analysis revealed that MCA1 buffers against deficiencies in the Hsp40 chaperone YDJ1 in a caspase cysteine-dependent manner. Life-span extension and aggregate management by Mca1 was only partly dependent on its conserved catalytic cysteine, which suggests that Mca1 harbors both caspase-dependent and independent functions related to life-span control.

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PMID: 24855027 [PubMed - indexed for MEDLINE]

3. Science. 2014 Jul 4;345(6192):98-101. doi: 10.1126/science.1254312.

Cell death. Opposing unfolded-protein-response signals converge on death receptor 5 to control apoptosis.

Lu M(1), Lawrence DA(1), Marsters S(1), Acosta-Alvear D(2), Kimmig P(2), Mendez AS(2), Paton AW(3), Paton JC(3), Walter P(4), Ashkenazi A(5).

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Protein folding by the endoplasmic reticulum (ER) is physiologically critical; its disruption causes ER stress and augments disease. ER stress activates the unfolded protein response (UPR) to restore homeostasis. If stress persists, the UPR induces apoptotic cell death, but the mechanisms remain elusive. Here, we report that unmitigated ER stress promoted apoptosis through cell-autonomous, UPR-controlled activation of death receptor 5 (DR5). ER stressors induced DR5 transcription via the UPR mediator CHOP; however, the UPR sensor IRE1 α transiently catalyzed DR5 mRNA decay, which allowed time for adaptation. Persistent ER stress built up intracellular DR5 protein, driving ligand-independent DR5 activation and apoptosis engagement via caspase-8. Thus, DR5 integrates opposing UPR signals to couple ER stress and apoptotic cell fate.

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PMID: 24994655 [PubMed - indexed for MEDLINE]

Dan:

Heat shock factor HSF2a involved in gametophyte development of *Arabidopsis thaliana* and its expression is controlled by a heat-inducible long non-coding antisense RNA

Heat stress transcription factors (HSFs) are central regulators of the heat stress response. Plant HSFs of subgroup B lack a conserved sequence motif present in the transcriptional activation domain of class A-HSFs. *Arabidopsis* members were found to be involved in non-heat shock functions. In the present analysis we investigated the expression, regulation and function of *HSF2a*. *HSF2a* expression was counteracted by a natural long non-coding antisense RNA, *asHSF2a*. In leaves, the antisense RNA gene is only expressed after heat stress and dependent on the activity of HSF1a/HSF1b. *HSF2a* and *asHSF2a* RNAs were also present in the absence of heat stress in the female gametophyte. Transgenic overexpression of *HSF2a* resulted in a complete knock down of the *asHSF2a* expression. Conversely, *asHSF2a* overexpression leads to the absence of *HSF2a* RNA. The knockdown of *HSF2a* by *asHSF2a* correlated with an improved, knockdown of *asHSF2a* by *HSF2a* overexpression with an impaired biomass production early in vegetative development. In both cases the development of female gametophytes was impaired. A T-DNA knock-out line did not segregate homozygous mutant plants, only heterozygotes *hsf2a-tt1/+* were viable. Approximately 50 % of the female gametophytes were arrested in early development, before mitosis 3, resulting in 45 % of sterile ovules. Our analysis indicates that the “Yin–Yang” regulation of gene expression at the *HSF2a* locus influences vegetative and gametophytic development in *Arabidopsis*.

Nathen:

Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in *Arabidopsis thaliana*

Engineered nucleases can be used to induce site-specific double-strand breaks (DSBs) in plant genomes. Thus, homologous recombination (HR) can be enhanced and targeted mutagenesis can be achieved by error-prone non-homologous end-joining (NHEJ). Recently, the bacterial CRISPR/Cas9 system was used for DSB induction in plants to promote HR and NHEJ. Cas9 can also be engineered to work as a nickase inducing single-strand breaks (SSBs). Here we show that only the nuclease but not the nickase is an efficient tool for NHEJ-mediated mutagenesis in plants. We demonstrate the stable inheritance of nuclease-induced targeted mutagenesis events in the ADH1 and TT4 genes of *Arabidopsis thaliana* at frequencies from 2.5 up to 70.0%. Deep sequencing analysis revealed NHEJ-mediated DSB repair in about a third of all reads in T1 plants. In contrast, applying the nickase resulted in the reduction of mutation frequency by at least 740-fold. Nevertheless, the nickase is able to induce HR at similar efficiencies as the nuclease or the homing endonuclease I-SceI. Two different types of somatic HR mechanisms, recombination between tandemly arranged direct repeats as well as gene conversion using the information on an inverted repeat could be enhanced by the nickase to a similar extent as by DSB-inducing enzymes. Thus, the Cas9 nickase has the potential to become an important tool for genome engineering in plants. It should not only be applicable for HR-mediated gene targeting systems but also by the combined action of two nickases as DSB-inducing agents excluding off-target effects in homologous genomic regions.

Friedrich Fauser Simon Schiml† and Holger Puchta*

Proteome-wide remodeling of protein location and function by stress

KiYoung Leea,b,1,2, Min-Kyung Sungc,1, Jihyun Kima,b, Kyung Kima,b, Junghyun Byuna,b, Hyojung Paika, Bongkeun Kimc Won-Ki Huhc,2, and Trey Ikerde, e,2

Protein location and function can change dynamically depending on many factors, including environmental stress, disease state, age, developmental stage, and cell type. Here, we describe an integrative computational framework, called the conditional function predictor (CoFP; <http://nbm.ajou.ac.kr/cofp/>), for predicting changes in subcellular location and function on a proteome-wide scale. The essence of the CoFP approach is to cross-reference general knowledge about a protein and its known network of physical interactions, which typically pool measurements from diverse environments, against gene expression profiles that have been measured under specific conditions of interest. Using CoFP, we predict condition-specific subcellular locations, biological processes, and molecular functions of the yeast proteome under 18 specified conditions. In addition to highly accurate retrieval of previously known gold standard protein locations and functions, CoFP predicts previously unidentified condition-dependent locations and functions for nearly all yeast proteins. Many of these predictions can be confirmed using high-resolution cellular imaging. We show that, under DNA-damaging conditions, Tsr1, Caf120, Dip5, Skg6, Lte1, and Nnf2 change subcellular location and RNA polymerase I subunit A43, Ino2, and Ids2 show changes in DNA binding. Beyond specific predictions, this work reveals a global landscape of changing protein location and function, highlighting a surprising number of proteins that translocate from the mitochondria to the nucleus or from endoplasmic reticulum to Golgi apparatus under stress

Stephanie:

Inbreeding interferes with the heat-shock response

Kristin Franke¹ and Klaus Fischer¹

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Abstract

Inbreeding is typically detrimental to individual fitness, with negative effects being often exaggerated in stressful environments. However, the causal mechanisms underlying inbreeding depression in general and the often increased susceptibility to stress in particular are not well understood. We here test whether inbreeding interferes with the heat-shock response, comprising an important component of the stress response which may therefore underscore sensitivity to stress. To this end we subjected the tropical butterfly *Bicyclus anynana* to a full-factorial design with three temperatures and three levels of inbreeding, and measured the expression of heat-shock protein (HSP) 70 via qPCR. HSP70 expression increased after exposure to heat as compared with cold or control conditions. Most strikingly, inbreeding strongly interfered with the heat-shock response, with inbred individuals showing a very weak upregulation of HSP70 only. Our results thus indicate that, in our study organism, interference with the heat-shock response may be one mechanism underlying reduced fitness of inbred individuals, especially when exposed to stressful conditions. However, these indications need to be corroborated using a broader range of different temperatures, genes and taxa.

2) eEF1By is a positive regulator of NF- κ B signaling pathway – Journal: Biochemical and Biophysical Research Communications

Damian:

Corpas, Francisco J

Peroxisomal plant nitric oxide synthase protein is imported by a peroxisomal targeting signal Type 2 (PTS2) in a process that depends of calmodulin

How is NO made? Light-mediated? pH mediated? Or L-Arginine/other polyamine? Mammalian NOS uses 5 electrons with NADPH and O₂ as cofactors; calcium, calmodulin, FAD, FMN, and biopterin are also involved. Plant peroxisomes are sites of concentrated oxidative metabolism (beta oxidation, photorespiration, IAA/JA synthesis, GSH/ASC cycle enzymes).

Pea plant peroxisome NOS activity depends on Arginine, NADPH, CaM, tetrahydrobiopterin. Using EPR to detect the NO signal, it is found that peroxisome signal traces are similar to commercial NOS. An antibody against iNOS colocalizes with catalase. An NO fluorescent probe colocalizes with the peroxisomal PTS1. It is also known in animals that iNOS in mammals localizes to peroxisomes (2002 paper). PTS1 and 2 are at the C- and N-terminus and are targeting peptides for getting proteins to peroxisomes. PEX5 facilitates import of PTS1-containing proteins, while PEX5 and PEX7 are needed for PTS2 signals. The pex12 and pex13 mutants make significantly less NO than wild-type (according to fluorescent NO signal). Knockdown of pex5 or pex7

causes complete and partial disruption of GFP-PTS1 signal. However, both lines exhibit aberrant NO signal, suggesting the NOS depends on a PTS2 signal. It appears that there is less NO when plants are treated with calcium. Why might that be? Calcium channel blocking causes all NO signal to disappear.

If the plant NOS is peroxisomally-targeted, does that mean that it is the exclusive NOS, the primary NOS, or just one NOS? Probably the primary one, perhaps maybe the only one, at least according to Francisco Corpas.

Mata-Pérez, Capilla

Detection and characterization of endogenous nitrated fatty acids in olive oil. Potential role as cellular mediators.

Nitro-fatty acids facilitate anti-inflammatory response by inhibiting COX acting on arachidonic acid. This investigation mostly just showed that nitrated fatty acids are present in plants, more highly enriched in certain organs (peel and mesocarp), and suggested that olive oil may be beneficial to human health since it contains nitrated fatty acids. Nitro-fatty acids are more stable as NO/NO₂ donors than are other nitroso compounds, according to the speaker. Nitration is favored under hypoxic conditions, while peroxidation is favored by high oxygen concentrations. Membranes could serve as an NO reservoir since the internal part of membranes is hydrophobic and free of nucleophilic groups like cysteines. What role might they play in plants? Incubation of roots with nitro-fatty acids certainly increase the NO fluorescent probe signal.

Nitroalkylation of cysteine residues is known to occur in animals and probably occurs in plants. Incubation of linoleic acid from olive extracts with acidic conditions and nitrates led to the formation of nitrated fatty acids as detected by mass spec.

It is still not clear, in this talk or in anything I have read yet, how NO₂ can come off a fatty acid and promote trans-nitrosylation of cysteine residues. It is an attractive hypothesis that nitrated fatty acids in a membrane could serve as a reservoir of NO.

Gupta, Kapuganti J.

The requirement of nitric oxide for oxygen and reactive oxygen species homeostasis in plants under aerobic conditions

Nitric oxide can be made from NR-derived NO₂ in mitochondria (Gupta et al, 2011), as well as hydroxylamine via oxidative pathways. Hypoxia leads to higher citrate levels and alternative oxidase activation via NO. However, NO is also made under normoxia. Over-expression of non-symbiotic hemoglobins leads to lower NO levels. Hb-OE have higher respiration rates, presumably because less NO is around to inhibit respiration. It follows that O₂ levels are also lower in Hb OE roots. Carbohydrate uptake, as measured by labeled CO₂ released, is more efficient in the Hb OE line. Ironically, ROS levels are actually *higher* in Hb OE lines. Hb OE plants have shorter roots as well. Maybe not so ironic though, since higher respiration rates would suggest higher concentrations of ROS. Roots are studied since they are non-photosynthetic organs and therefore do not produce a whole lot of oxygen on their own.

Gross, Felicitas

Nitric oxide production in *Arabidopsis thaliana*

All measurements were made in roots. This study aimed to identify putative NOSs in plants. A knockout screen was conducted, both for known reductive and known oxidative pathway enzymes that may be important for NO production. Roots were stained with DAF-FM DA; NO production was stimulated with 2,6-dichloro-isonicotinic acid (INA). Relative fluorescence intensity was then measured, with Col-0 set as the standard. Knock-out of copper amine oxidase 8 led to significant reduction in fluorescence. CAO8 preferentially oxidizes putrescine, perhaps releasing NO in the process. Basal NO production is not affected in this mutant, but induced NO production is affected. H₂O₂ synthesis is also impaired in this mutant. Polyamine content is not different between the mutant and Col-0, however.

Cochrane, Devin

Contribution of terminal mitochondrial oxidases to nitric oxide turnover

Nitrate can be used as an electron acceptor during hypoxia, leading to the production of NO. This allows ATP production and redox balance (NADH/NAD) to proceed semi-normally. Hemoglobins re-oxidize NO back to NO₃⁻ to allow the pathway to continue cyclically. Alternative oxidase (AOX) acts as an alternative to Complex III. Does AOX participate in the NO/Hb cycle? Or is this the work of Complex IV? Study conducted in tobacco. Leaf NO emission was measured. Also, known targets of NO were studied (such as aconitase). NO emission was lower in AOX knockout. However, cyanide completely inhibited NO emission, suggesting C IV makes NO, but not AOX. The lower NO levels in the *aox* mutant therefore may be attributable to heightened Hb expression and/or a breakdown in ROS/RNS balance, but this is the subject of further investigation.

Ling, Tengfang

Host S-nitrosylation inhibits a bacterial type III effector during hypersensitive response

Animals are known to decrease bacterial and viral virulence through S-nitrosylation of the infectious agent's proteins. This investigation wanted to address if a similar mechanism exists in plants. Indeed, the *P. syringae* virulence protein HopAII is inhibited by S-nitrosylation in vitro. Mutation of a conserved cysteine to serine diminishes this inhibition, and infection happens much more vigorously. Endogenously, the HopAII protein is S-nitrosylated during hypersensitive response, but the C-S mutant is not. MAPK pathways that transduce pathogen attack signal to promote a defense response are also not activated by the C-S mutant.

Meilhoc, Eliane

Sinorhizobium meliloti proteins protect the plant glutamine synthetase against inactivation by nitric oxide in

Medicago truncatula symbiotic nodules

Rhizobia infect root hairs and then create a thread that penetrates into the inner root cortex, eventually forming root nodules. Senescence of nodules (turn from pink to green) involves death of both plant and bacterial cells. NO can be detected at the very early, middle, and mature phases of symbiosis, across the "infection thread" of bacteria. Not

only this, but NO is necessary for good nodulation and proper nodule senescence. Many bacteria can oxidize NO via flavohemoglobins or nitric oxide reductases. Iron sulfur cluster repair proteins fix the proteins oxidized by NO. Other bacteria go dormant to resist NO (such as *M. tuberculosis*). A screen (transcriptomic microarray) was conducted to identify NO-upregulated genes in *S. melliloti* after it was subjected to treatment with the NO donor spermine NONOate (SpNN). Nodule NO levels were significantly higher when the upregulated genes HMP and NorB, or NnrS (NO repair and avoidance proteins) were knocked out. Senescence also happened much earlier in these mutants. Fluorescent probes specific for living and dead bacteria indicated that the number of dead bacteria within nodules was much higher in the hmp mutant at 3 and 5 weeks post infection. Plants infected with these mutant proteins accumulate NO-mediated tyrosine-nitrated glutamine synthetase (GS). GS levels are higher in nodules because assimilated nitrogen needs to be incorporated into the amino acid pool. GS is significantly more nitrated in the nnrS mutant, and is also much less active. This suggests the bacterial proteins protect the plant protein. How this relates to overall nodule health, however, is not yet known.

Romero-Puertas, Maria C.

Unravelling the function of nitric oxide in the host recognition of endosymbiotic and pathogenic fungi in tomato plants

Arbuscular mycorrhiza (AM) infect ~80% of plant roots and allow for better conductance of water and nutrients, while the fungi get the fixed carbon of photosynthesis for their part. AM infection is similar to nodulation in terms of how the fungal hyphae penetrate into the inner root cortex. Shortly after first contact between roots and hyphae, there is a strong NO burst. The question is, does the NO burst happen for only the endosymbiotic fungi, or for pathogenic fungi also? *Fusarium oxysporum* is a pathogenic species. The same NO burst happens, but it is sustained rather than tapering off. In tomato, Hb1 is upregulated during AM infection. NO donors such as GSNO were also found to upregulate Hb1 expression. Hb2 and truncated Hb were not as responsive. Hb expression plummets during an *F. oxysporum* infection, while it rises during AM infection.

Dariusz, Abramowski

Synthesis and functioning of nitric oxide in resistance of potato leaves to *Phytophthora infestans*

H₂O₂, GSNOR activity, and peroxynitrite levels all increased during infection. Flavanoid, GSH, and ascorbate levels all increased during infection.

Vandelle, Elodie

Study of cGMP function in NO-mediated hypersensitive disease resistance response in *Arabidopsis*

Thaliana

Basal and hypersensitive responses are general and specific reactions to pathogens, respectively. HR-induced cell death entails H₂O₂ and NO bursts. Systemic acquired

resistance occurs through SA, which is activated by NO. What role is played by cGMP? To answer this, an ELISA-like cGMP detection method was envisaged. CGMP levels increase, decrease, and then increase again after infection (biphasic), but this only occurs in the presence of NO, as cPTIO and constitutive expression of a bacterial NO catabolic enzyme both erased the trend. Guanlylate cyclases that are NO dependent? Not quite worked out yet in plants. There are candidates, but their NO-dependent GC activity is significantly lower than that of the mammalian homologs. Higher cGMP levels (brought about through constitutive expression of a GC) does not seem to improve resistance to pathogens. Instead, SA-dependent responses were suppressed, while JA-dependent responses were favored. Perhaps cGMP is part of the “switch” mechanism from SA to JA responses?

Lamotte, Olivier

NO signaling in cryptogein-induced immune responses in tobacco

Cryptogein is a microbe-associated molecular pattern (MAMP). Treatment with Cryptogein leads to NO burst. The proteins calmodulin and CDC48 are S-nitrosylated upon cryptogein treatment. CDC48 is an AAA ATPase that unfolds ubiquitinated proteins in preparation for their degradation. GSNO and cryptogein both bring about an increase in CDC48. CDC48 activity decreases upon NO treatment. Calmodulin is non-enzymatic, but it binds calcium, which alters its tertiary structure to modulate its interaction with other proteins. CaM contains an evolutionarily-conserved cysteine. PyMol indicates this cysteine's sidechain is solvent-exposed. GSNO treatment of CaM caused no structural change apart from what GSH could do. Calcium-binding isn't affected by GSNO either. On the other hand, it does alter the structure of CaM if CaM is already bound to Calcium before the GSNO is added.

Loake, Gary

Interplay between S-nitrosylation and SUMOylation during plant immune function

S-nitrosylation can turn on G-proteins in humans, while denitrosylation activates caspase 3. And RBOH NADPH oxidase is nitrosylated at a cysteine near the FAD cofactor, rendering the enzyme inactive. Knockout of SUMO E1 or E2 is embryo-lethal, while SIZ1 (SUMO E3) knock-out enhances pathogen resistance, but makes for a dwarf phenotype. Another E3 (HPY2) knock-out leads to meristematic irregularities. Incubation of plants with GSNO led to the disappearance of SUMO conjugates via western blot. In vitro, SCE1 (the E2) can be S-nitrosylated. Mutation of the catalytic cysteine to serine had no effect, though. Mass spec determined that cysteine 139 (which is not catalytic) is the one that gets modified. Indeed, the C139S mutant could no longer be modified. SCE1 activity was, indeed, reduced by nitrosylation. In vitro and in vivo, the C139S mutant can still form a SUMO-thioester. Conversely, S-nitrosylation of C139 did not inhibit SUMO E2 activity. What does it do, then? Apparently, it inhibits SUMO E3 activity (using PCNA as the model substrate). Pathogen insult (Pst and Pst avrB) cause S-nitrosylation of SCE1. Interestingly, plants expressing the C139S mutant are substantially more

pathogen-susceptible, and PR1 expression is a lot lower during pathogen attack. Thus, on the whole, SUMOylation is a negative regulator of disease resistance.

Alche, Juan de Dios

Protein S-nitrosylation in olive pollen during in vitro germination.

There is a ROS burst in pollen development just at the point of dehiscence, but not much before or after. On the other hand, there is sustained ROS in ovules (as assessed by a number of fluorescent reporters).

Begara-Morales, Juan Carlos

Regulation of ascorbate-glutathione cycle by nitric oxide-related post-translational modifications

Tyrosine nitration of Ascorbate Peroxidase (catabolizes H₂O₂ at the expense of ascorbate) from pea via the donor SIN-1 caused it to lose activity. In contrast, its activity went up when incubated with GSNO. MDAR (Monodehydroascorbate reductase) followed a similar trend, albeit this time, GSNO also caused it to lose activity, as did H₂O₂. Cytosolic and chloroplastic glutathione reductase were not particularly affected by SIN-1, although western blots indicated they were nitrated. Similar effects observed with GSNO (and the proteins were S-nitrosylated). It was confirmed that APX is also S-nitrosylated in vivo under salinity stress.

Chaki, Mounira

Computer-based identification of candidate proteins for S-nitrosylation: a tool which can replace the biotin switch assay?

Biotin switch (BST) assays have a lot of technical hurdles, and are prone to false positives. We would benefit from having an algorithm for the identification of S-nitrosated cysteines in silico. Among existing programs, some more sensitive than others, more error prone than others, etc. GPS-SNO was used to predict S-nitrosylation within the entire predicted Arabidopsis proteome. Only the top 10% of entries (in terms of confidence) were carefully scrutinized (representing 5-17% of the predicted proteome, depending on the subcellular compartment). GSP-SNO successfully predicted 61% of the S-nitrosylated sites identified experimentally with the BST.

Krasuska, Urszula

Nitro-oxidative stress as a mode of action of non-protein amino acids during inhibition of root growth of tomato seedlings

Many non-proteogenic amino acids are common in plants because they are the byproducts of phytotoxic secondary metabolism—meta-tyrosine and canavanine, to name just two. “Allelopathic molecules” are those that are released by one plant and inhibit the

survival and thriving of other plants, usually through uptake by the roots of the affected organism. In this investigation, tomato seedlings were treated with non-proteogenic amino acids. Canavanine and m-Tyr both inhibited root elongation. Root cell viability also decreased, and H₂O₂ increased. NO emission was greatly enhanced by 5-MT (5-methyl-tryptophan), but was reduced somewhat at high concentrations of m-Tyr. At lower m-Tyr concentrations, there is a transient NO burst peaking at 24 hrs post-treatment. As might be guessed, catalase and peroxidase activities are higher upon m-Tyr treatment.

Gaupels, Frank

Differential sensitivity of Arabidopsis natural accessions to nitrogen dioxide NO₂ (nitronium) is produced by peroxidases and hemoglobins; growth-promoting at very low, but growth-inhibiting at ppm or higher concentrations. Different Arabidopsis accessions vary from strongly inhibited to nearly insensitive to NO₂ (Col-0 is about in the middle). 212 natural accessions were fumigated with 10 or 20ppm NO₂ for 90 minutes (NO and O₃ gas mixed at the proper concentrations to afford NO₂). There is a strong correlation between stomatal conductance after fumigation and subsequent survival. Stronger stomatal conductance, less survival. Also, NO₂ conversion to NO₂- (nitrite) and S-nitrosothiols follows the same trend. Breaks from the trend (e.g., accession Shakhara, strong stomatal conductance, low nitrite levels) might refer to high nitrite reductase levels. Accessions that were NO₂-sensitive, tolerant, or similar to Col-0? They did not respond the same way to O₃ alone—O₃ sensitivity, tolerance, or similarity to Col-0 was not a predictor of how the accessions would respond to NO₂.

Raghavendra, Agepati

Nitric oxide is an important secondary messenger during stomatal closure by microbial and chemical elicitors and may form a part of plant immunity response against pathogens

Blume, Yaroslav

Tubulin tyrosine nitration regulates microtubule organization in plant cells Tyrosination of alpha tubulin dramatically alters the quaternary structure of microtubules, particularly during catastrophe. Nitrotyrosination also has an effect. Anti-nitrotyrosine antibodies successfully immunoprecipitated nitrotyrosinated alpha-tubulin. 3-nitrotyrosine affected root hair morphology. Looking more closely, it's clear that NO donors affect the shape of MT arrays. SNP and SNAP-treated roots have tangled MT arrays, while cPTIO treatment caused depolymerization of arrays. SNP also caused the number of preprophase bands, mitotic spindles, and pragnoplasts per root section to decrease, suggesting that NO affects rates of cell division. Tyrosination and nitrotyrosination affect the manner in which MTs are bound by kinesins. It is not known if tyrosine nitration on MTs occurs alongside, or as an alternative of, other RNS modifications.

Ciacka, Katarzyna

Polyamine metabolism during NO-induced dormancy removal and germination of apple embryos

Polyamine treatment of apple seed (putrescine and spermidine) enhance germination rates, along with NO. NO release was detected after polyamine treatment. This was accompanied by H₂O₂ release. This makes sense, since polyamine oxidase (PAO) catabolizes polyamines and releases H₂O₂ in the process, and PAO was transcriptionally upregulated. Upon germination, free polyamine content decreases.

Feigl, Gábor

Zinc induced a nitro-oxidative stress in roots of brassica species

Brassica juncea and Brassica napus were compared in this study for sensitivity to zinc stress (zinc sulfate, 250-300 µM). B. napus transports more zinc into the shoots vs. the roots than does B. juncea. Lateral root number increased substantially for B. juncea with more zinc, but not so in B. napus. Lignin amounts did not differ, but callose deposition was higher with more zinc, with the strongest response in B. napus. B. napus roots were less viable at high zinc, while B. juncea was not noticeably affected. H₂O₂ levels were similar across the board unless at the highest concentration, but then, only in B. napus was it significantly higher. NO accumulated to higher levels in B. juncea as zinc increased. Protein tyrosine nitration (as assessed by anti-TYR-NO₂ antibodies) appeared to increase upon zinc stress in both plant species. It is argued that zinc tolerance is determined more by oxidative stress than by nitrosative stress.