Lit Lunch 10/18/13

#### INDU

1. Science. 2013 Sep 20;341(6152):1384-7. doi: 10.1126/science.1242736. Epub 2013 Sep 5.

# Nonlegumes respond to rhizobial Nod factors by suppressing the innate immune response.

Liang Y, Cao Y, Tanaka K, Thibivilliers S, Wan J, Choi J, Kang Ch, Qiu J, Stacey G.

Division of Plant Science, National Center for Soybean Biotechnology, Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA.

Virtually since the discovery of nitrogen-fixing Rhizobium-legume symbioses, researchers have dreamed of transferring this capability into nonlegume crop species (for example, corn). In general, nonlegumes were assumed to lack the ability to respond to the rhizobial lipo-chitin Nod factors, which are the essential signal molecules that trigger legume nodulation. However, our data indicate that Arabidopsis thaliana plants, as well as other nonlegumes, recognize the rhizobial Nod factor via a mechanism that results in strong suppression of microbe-associated molecular pattern (MAMP)-triggered immunity. The mechanism of action leads to reduced levels of pattern-recognition receptors on the plasma membrane involved in MAMP recognition.

2. Science. 2013 Sep 20;341(6152):1235005. doi: 10.1126/science.1235005. Epub 2013 Aug 15.

#### DAMIAN

1) Plant hemoglobins can be maintained in functional form by reduced flavins in the nuclei and confer differential tolerance to nitro-oxidative stress

Martha Sainz1, Carmen Pérez-Rontomé1, Javier Ramos1, Jose Miguel Mulet2, Euan K. James3, Ujjal Bhattacharjee4, Jacob W. Petrich4 and Manuel Becana1,\*

2) Long-term ammonium nutrition of *Arabidopsis* increases the extrachloroplastic NAD(P)H/NAD(P)+ ratio and mitochondrial reactive oxygen species level in leaves but does not impair photosynthetic capacity

#### ANNA PODGÓRSKA1, KATARZYNA GIECZEWSKA1, KATARZYNA ŁUKAWSKA-KUZ'MA1, ALLAN G. RASMUSSON2, PER GARDESTRÖM3 & BOZ<sup>-</sup> ENA SZAL

#### FIONN

#### 1) Arabidopsis J-Protein J20 Delivers the First Enzyme of the Plastidial Isoprenoid Pathway to Protein Quality Control

Pablo Pulidoa, Gabriela Toledo-Ortiza,1, Michael A. Phillipsa, Louwrance P. Wrightb and Manuel Rodríguez-Concepcióna,2

Abstract:

Plastids provide plants with metabolic pathways that are unique among eukaryotes, including the methylerythritol 4-phosphate pathway for the production of isoprenoids essential for photosynthesis and plant growth. Here, we show that the first enzyme of the pathway, deoxyxylulose 5-phosphate synthase (DXS), interacts with the J-protein J20 in Arabidopsis thaliana. J-proteins typically act as adaptors that provide substrate specificity to heat shock protein 70 (Hsp70), a molecular chaperone. Immunoprecipitation experiments showed that J20 and DXS are found together in vivo and confirmed the presence of Hsp70 chaperones in DXS complexes. Mutants defective in J20 activity accumulated significantly increased levels of DXS protein (but no transcripts) and displayed reduced levels of DXS enzyme activity, indicating that loss of J20 function causes posttranscriptional accumulation of DXS in an inactive form. Furthermore, J20 promotes degradation of DXS following a heat shock. Together, our data indicate that J20 might identify unfolded or misfolded (damaged) forms of DXS and target them to the Hsp70 system for proper folding under normal conditions or degradation upon stress.

#### KEITH

#### 1) Quantification of Interaction Strengths between Chaperones and Tetratricopeptide Repeat Domain-containing Membrane Proteins

The Journal of Biological Chemistry, 288, 30614-30625

Regina Schweiger, Jürgen Soll, Kirsten Jung, Ralf Heermann and Serena Schwenkert

Biology I, Botany Biology I, Microbiology, Munich Center for Integrated Protein Science, Ludwig-Maximilians-Universität München, Grosshaderner Strasse 2–4, D-82152 Planegg-Martinsried, Germany

The three tetratricopeptide repeat domain-containing docking proteins Toc64, OM64, and AtTPR7 reside in the chloroplast, mitochondrion, and endoplasmic reticulum of

Arabidopsis thaliana, respectively. They are suggested to act during post-translational protein import by association with chaperone-bound preprotein complexes. Here, we performed a detailed biochemical, biophysical, and computational analysis of the interaction between Toc64, OM64, and AtTPR7 and the five cytosolic chaperones HSP70.1, HSP90.1, HSP90.2, HSP90.3, and HSP90.4. We used surface plasmon resonance spectroscopy in combination with Interaction Map® analysis to distinguish between chaperone oligomerization and docking protein-chaperone interactions and to calculate binding affinities for all tested interactions. Complementary to this, we applied pulldown assays as well as microscale thermophoresis as surface immobilization independent techniques. The data revealed that OM64 prefers HSP70 over HSP90, whereas Toc64 binds all chaperones with comparable affinities. We could further show that AtTPR7 is able to bind HSP90 in addition to HSP70. Moreover, differences between the HSP90 isoforms were detected and revealed a weaker binding for HSP90.1 to AtTPR7 and OM64, showing that slight differences in the amino acid composition or structure of the chaperones influence binding to the tetratricopeptide repeat domain. The combinatory approach of several methods provided a powerful toolkit to determine binding affinities of similar interaction partners in a highly quantitative manner.

#### 2) Identification of Amino Acids Conferring Chain Length Substrate Specificities on Fatty Alcohol-forming Reductases FAR5 and FAR8 from *Arabidopsis thaliana*

The Journal of Biological Chemistry, 288, 30345-30355.

Micaëla G. Chacón, Ashley E. Fournier<sup>‡</sup>, Frances Tran<sup>‡</sup>, Franziska Dittrich-Domergue, Ian P. Pulsifer, Frédéric Domergue and Owen Rowland

Institute of Biochemistry, Department of Biology, Carleton University, Ottawa, Ontario K1S 5B6, Canada and the Laboratoire de Biogenèse Membranaire, Université Bordeaux Ségalen, CNRS-UMR 5200, Bâtiment A3-INRA Bordeaux Aquitaine BP81, 71 Avenue Edouard Bourlaux, 33883 Villenave D'Ornon Cedex, France

Fatty alcohols play a variety of biological roles in all kingdoms of life. Fatty acyl reductase (FAR) enzymes catalyze the reduction of fatty acyl-coenzyme A (CoA) or fatty acyl-acyl carrier protein substrates to primary fatty alcohols. FAR enzymes have distinct substrate specificities with regard to chain length and degree of saturation. FAR5 (At3g44550) and FAR8 (At3g44560) from Arabidopsis thaliana are 85% identical at the amino acid level and are of equal length, but they possess distinct specificities for 18:0 or 16:0 acyl chain length, respectively. We used Saccharomyces cerevisiae as a heterologous expression system to assess FAR substrate specificity determinants. We identified individual amino acids that affect protein levels or 16:0-CoA versus 18:0-CoA

specificity by expressing in yeast FAR5 and FAR8 domain-swap chimeras and sitespecific mutants. We found that a threonine at position 347 and a serine at position 363 were important for high FAR5 and FAR8 protein accumulation in yeast and thus are likely important for protein folding and stability. Amino acids at positions 355 and 377 were important for dictating 16:0-CoA versus 18:0-CoA chain length specificity. Simultaneously converting alanine 355 and valine 377 of FAR5 to the corresponding FAR8 residues, leucine and methionine, respectively, almost fully converted FAR5 specificity from 18:0-CoA to 16:0-CoA. The reciprocal amino acid conversions, L355A and M377V, made in the active FAR8-S363P mutant background converted its specificity from 16:0-CoA to 18:0-CoA. This study is an important advancement in the engineering of highly active FAR proteins with desired specificities for the production of fatty alcohols with industrial value.

#### STEPHANIE

#### 1) Identification and functional characterization of silicon transporters in soybean using comparative genomics of major intrinsic proteins in Arabidopsis and rice

Rupesh K. Deshmukh<sup>1</sup>, Julien Vivancos<sup>1</sup>, Valérie Guérin<sup>1</sup>, Humira Sonah<sup>1</sup>, Caroline Labbé<sup>1</sup>, François Belzile<sup>1</sup> and Richard R. Bélanger<sup>1</sup>-(1) Département de Phytologie, Faculté des Sciences de l'Agriculture et de l'Alime

Département de Phytologie, Faculté des Sciences de l'Agriculture et de l'Alimentation, Centre de Recherche en Horticulture, Université Laval, Quebec, Canada

Silicon (Si) confers several benefits to many plant species when absorbed as silicic acid through nodulin 26-like intrinsic proteins (NIPs). The NIPs belong to major intrinsic protein (MIP) family, members of which form channels with high selectivity to control transport of water and different solutes. Here, comparative genomic analysis of the MIPs was performed to investigate the presence of Si transporter MIPs in soybean. Thorough analysis of phylogeny, gene organization, transcriptome profiling and protein modeling was performed to characterize MIPs in rice, Arabidopsis and soybean. Based on several attributes, two putative Si transporter genes, GmNIP2-1 and GmNIP2-2, were identified, characterized and cloned from soybean. Expression of both genes was detected in shoot and root tissues, and decreased as Si increased. The protein encoded by GmNIP2-2 showed functionality for Si transport when expressed in Xenopus oocytes, thus confirming the genetic capability of soybean to absorb the element. Comparative analysis of MIPs in plants provides opportunities to decipher gene evolution, functionality and selectivity of nutrient uptake mechanisms. Exploitation of this strategy has helped to uncover unique features of MIPs in soybean. The identification and functional characterization of Si transporters can be exploited to optimize the benefits that plants can derive from Si absorption.

### 2) Useless Peer Review?

A study shows that the methods by which scientists evaluate each other's work are errorprone and poor at measuring merit.

#### ELIZABETH

#### Physiologia Plantarum Content Alert (New Articles)

An assessment of the biotechnological use of hemoglobin modulation in cereals Kim H. Hebelstrup, Jay K. Shah, Catherine Simpson, Jan K. Schjoerring, Julien Mandon, Simona M. Cristescu, Frans J. M. Harren, Michael W. Christiansen, Luis A. J. Mur and Abir U. Igamberdiev Accepted manuscript online: 4 OCT 2013 10:59AM EST | DOI: 10.1111/ppl.12115

Non-symbiotic hemoglobin (nsHb) genes are ubiquitous in plants, but their biological functions have mostly been studied in model plant species rather than in crops. nsHb influences cell signaling and metabolism by modulating the levels of nitric oxide (NO). Class 1 nsHb is upregulated under hypoxia and is involved in various biotic and abiotic stress responses. Ectopic over-expression of nsHb in Arabidopsis thaliana accelerates development, whilst targeted over-expression in seeds can increase seed yield. Such observations suggest that manipulating nsHb could be a valid biotechnological target. We studied the effects of overexpression of class 1 nsHb in the monocotyledonous crop plant barley (Hordeum vulgare cv. Golden Promise). nsHb was shown to be involved in NO metabolism in barley, as ectopic overexpression reduced the amount of NO released during hypoxia. Further, as in Arabidopsis, nsHb overexpression compromised basal resistance towards pathogens in barley. However, unlike Arabidopsis, nsHb ectopic overexpression delayed growth and development in barley, and specific over-expression reduced seed yield. Thus, nsHb overexpression in barley does not seem to be an efficient strategy for increasing yield in cereal crops. These findings highlight the necessity for using actual crop plants rather than laboratory model plants when assessing the effects of biotechnological approaches to crop improvement.

Current Opinion in Plant Biology: Alert 7 October-13 October

**ROS signaling loops — production, perception, regulation** Review Article

Pages 575-582 Michael Wrzaczek, Mikael Brosché, Jaakko Kangasjärvi

Ambient temperature signalling in plants Review Article

Pages 661-666 Philip A Wigge Satomura A, Katsuyama Y, Miura N, Kuroda K, Tomio A, Bamba T, Fukusaki E, Ueda M. Acquisition of thermotolerant yeast Saccharomyces cerevisiae by breeding via stepwise adaptation. Biotechnol Prog. 2013 Sep 20;. [Epub ahead of print] PMID: 24115578 [PubMed - as supplied by publisher]

Matassa DS, Amoroso MR, Agliarulo I, Maddalena F, Sisinni L, Paladino S, Romano S, Romano MF, Sagar V, Loreni F, Landriscina M, Esposito F. Translational control in the stress adaptive response of cancer cells: a novel role for the heat shock protein TRAP1. Cell Death Dis. 2013 Oct 10;4:e851. PMID: 24113185 [PubMed - as supplied by publisher]

Wolfe KJ, Ren HY, Trepte P, Cyr DM. The Hsp70/90 cochaperone, Stil, suppresses proteotoxicity by regulating spatial quality control of amyloid-like proteins. Mol Biol Cell. 2013 Oct 9;. [Epub ahead of print] PMID: 24109600 [PubMed - as supplied by publisher] Zhu L, Cai Z, Zhang Y, Li Y. Engineering stress tolerance of Escherichia coli by stress-inducedmutagenesis (SIM) based adaptive evolution. Biotechnol J. 2013 Sep 19;. [Epub ahead of print] PMID: 24106039 [PubMed - as supplied by publisher] Pulido P, Toledo-Ortiz G, Phillips MA, Wright LP, RodrÃguez-ConcepciÃ3n Μ. Arabidopsis J-Protein J20 Delivers the First Enzyme of the Plastidial Isoprenoid Pathway to Protein Quality Control. Plant Cell. 2013 Oct 8;. [Epub ahead of print] PMID: 24104567 [PubMed - as supplied by publisher] Liu ZB, Wang JM, Yang FX, Yang L, Yue YF, Xiang JB, Gao M, Xiong FJ, Lv D, Wu XJ, Liu N, Zhang X, Li XF, Yang Y. A novel membrane-bound E3 ubiquitin ligase enhances the thermal resistance in plants. Plant Biotechnol J. 2013 Sep 16;. [Epub ahead of print] PMID: 24102799 [PubMed - as supplied by publisher] Paul S, Mahanta S. Association of heat-shock proteins in various neurodegenerative disorders: is it a master key to open the therapeutic door? Mol Cell Biochem. 2013 Oct 5;. [Epub ahead of print] PMID: 24096700 [PubMed - as supplied by publisher] Li YF, Wang Y, Tang Y, Kakani VG, Mahalingam R. Transcriptome analysis of heat stress response in switchgrass (Panicum virgatum L.). BMC Plant Biol. 2013 Oct 6;13(1):153. [Epub ahead of print] PMID: 24093800 [PubMed - as supplied by publisher] Verdier J, Lalanne D, Pelletier S, Torres-Jerez I, Righetti K, Bandyopadhyay K, Leprince O, Chatelain E, Vu BL, Gouzy J, Gamas P, Udvardi MK, Buitink J. A Regulatory Network-Based Approach Dissects Late Maturation Processes Related to the Acquisition of Desiccation Tolerance and Longevity of Medicago truncatula Seeds. Plant Physiol. 2013 Oct;163(2):757-774. PMID: 23929721 [PubMed - as supplied by publisher] Chmielowska-BÄ...k J, Deckert J. Nitric oxide mediates Cd-dependent induction of signaling- associated genes. Plant Signal Behav. 2013 Oct 8;8(12). [Epub ahead of print] PMID: 24105301 [PubMed - as supplied by publisher] KubienovÃ; L, TichÃ; T, JahnovÃ; J, LuhovÃ; L, MieslerovÃ; B, Petřivalský M.

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Reisz JA, Bechtold E, King SB, Poole LB, Furdui CM. Thiol-Blocking Electrophiles Interfere with Labeling and Detection of Protein Sulfenic Acids. FEBS J. 2013 Sep 18;. [Epub ahead of print] PMID: 24103186 [PubMed - as supplied by publisher]

#### Molecular Cell: Alert 7 October-13 October

<u>The Translational Landscape of the Mammalian Cell Cycle</u> *Available online 10 October 2013* Craig R. Stumpf, Melissa V. Moreno, Adam B. Olshen, Barry S. Taylor, Davide Ruggero

Global Analysis of Eukaryotic mRNA Degradation Reveals Xrn1-DependentBuffering of Transcript LevelsOriginal Research ArticlePages 52-62Mai Sun, Björn Schwalb, Nicole Pirkl, Kerstin C. Maier, Arne Schenk, HenrikFailmezger, Achim Tresch, Patrick Cramer

Physiologia Plantarum Content Alert: 149, 3 (November 2013) Nitric oxide mediates cold- and dehydration-induced expression of a novel *MfHyPRP* that confers tolerance to abiotic stress (pages 310–320)

Jiali Tan, Chunliu Zhuo and Zhenfei Guo

Article first published online: 15 MAR 2013 | DOI: 10.1111/ppl.12032

Current Biology: Alert 5 October-11 October

Cytokinin Induces Cell Division in the Quiescent Center of the Arabidopsis Root

Apical Meristem Original Research Article

Available online 10 October 2013

Wenjing Zhang, Ranjan Swarup, Malcolm Bennett, G. Eric Schaller, Joseph J. Kieber

#### Fission Yeast Does Not Age under Favorable Conditions, but Does So after

Stress Original Research

Pages 1844-1852

Miguel Coelho, Aygül Dereli, Anett Haese, Sebastian Kühn, Liliana Malinovska, Morgan E. DeSantis, James Shorter, Simon Alberti, Thilo Gross, Iva M. Tolić-Nørrelykke

#### **Defining the Site of Light Perception and Initiation of Phototropism in** <u>Arabidopsis</u>

Pages 1934-1938

Tobias Preuten, Tim Hohm, Sven Bergmann, Christian Fankhauser The Plant Journal Content Alert (New Articles)

Plant hemoglobins can be maintained in functional form by reduced flavins in the nuclei and confer differential tolerance to nitro-oxidative stress

Martha Sainz, Carmen Pérez-Rontomé, Javier Ramos, Jose Miguel Mulet, Euan K. James, Ujjal Bhattacharjee, Jacob W. Petrich and Manuel Becana Accepted manuscript online: 9 OCT 2013 08:22AM EST | DOI: 10.1111/tpj.12340

The Plant Journal Content Alert: 76, 3 (November 2013)

# An integrative model of the control of ovule primordia formation (pages 446–455)

Francesca Galbiati, Dola Sinha Roy, Sara Simonini, Mara Cucinotta, Luca Ceccato, Candela Cuesta, Maria Simaskova, Eva Benkova, Yuri Kamiuchi, Mitsuhiro Aida, Dolf Weijers, Rüdiger Simon, Simona Masiero and Lucia Colombo

Article first published online: 19 SEP 2013 | DOI: 10.1111/tpj.12309

Upon hormonal signaling, ovules develop as lateral organs from the placenta. Ovule numbers ultimately determine the number of seeds that develop, and thereby contribute to the final seed yield in crop plants. We demonstrate here that *CUP-SHAPED* 

*COTYLEDON 1* (*CUC1*), *CUC2* and *AINTEGUMENTA* (*ANT*) have additive effects on ovule primordia formation. We show that expression of the *CUC1* and *CUC2* genes is required to redundantly regulate expression of *PINFORMED1* (*PIN1*), which in turn is required for ovule primordia formation. Furthermore, our results suggest that the auxin response factor *MONOPTEROS* (*MP/ARF5*) may directly bind *ANT*, *CUC1* and *CUC2* and promote their transcription. Based on our findings, we propose an integrative model to describe the molecular mechanisms of the early stages of ovule development.

FEBS Journal Content Alert (New Articles)

## In Vivo Characterisation of the Role of Tissue-specific Translation Elongation Factor eEF1A2 in Protein Synthesis Reveals Insights into Muscle Atrophy

Jennifer Doig, Lowri A. Griffiths, David Peberdy, Permphan Dharmasaroja, Maria Vera, Faith J.C. Davies, Helen J. Newbery, David Brownstein and Catherine M. Abbott

Accepted manuscript online: 2 OCT 2013 10:28PM EST | DOI: 10.1111/febs.12554

Plant, Cell & Environment Content Alert: 36, 11 (November 2013)

### Meristem temperature substantially deviates from air temperature even in moderate environments: is the magnitude of this deviation speciesspecific? (pages 1950–1960)

Meristem temperature ( $T_{\text{meristem}}$ ) drives plant development but is hardly ever quantified. Instead, air temperature  $(T_{air})$  is usually used as its approximation. Meristems are enclosed within apical buds. Bud structure and function may differ across species. Therefore,  $T_{\text{meristem}}$  may deviate from  $T_{\text{air}}$  in a species-specific way. Environmental variables (air temperature, vapour pressure deficit, radiation, and wind speed) were systematically varied to quantify the response of  $T_{\text{meristem}}$ . This response was related to observations of bud structure and transpiration. Tomato and cucumber plants were used as model plants as they are morphologically distinct and usually growing in similar environments.  $T_{\text{meristem}}$  substantially deviated from  $T_{\text{air}}$  in a species-specific manner under moderate environments. This deviation ranged between -2.6 and 3.8 °C in tomato and between -4.1 and 3.0 °C in cucumber. The lower  $T_{\text{meristem}}$  observed in cucumber was linked with the higher transpiration of the bud foliage sheltering the meristem when compared with tomato plants. We here indicate that for properly linking growth and development of plants to temperature in future applications, for instance in climate change scenarios studies,  $T_{\text{meristem}}$  should be used instead of  $T_{\text{air}}$ , as a species-specific trait highly reliant on various environmental factors.

#### Long-term ammonium nutrition of *Arabidopsis* increases the extrachloroplastic NAD(P)H/NAD(P)<sup>+</sup> ratio and mitochondrial reactive oxygen species level in leaves but does not impair photosynthetic capacity (pages 2034–2045)

ANNA PODGÓRSKA, KATARZYNA GIECZEWSKA, KATARZYNA ŁUKAWSKA-KUŹMA, ALLAN G. RASMUSSON, PER GARDESTRÖM and BOŻENA SZAL Article first published online: 6 MAY 2013 | DOI: 10.1111/pce.12113

Ammonium nutrition has been suggested to be associated with alterations in the oxidation-reduction state of leaf cells. Herein, we show that ammonium nutrition in *Arabidopsis thaliana* increases leaf NAD(P)H/NAD(P)<sup>+</sup> ratio, reactive oxygen species content, and accumulation of biomolecules oxidized by free radicals. We also showed that ammonium nutrition changes mitochondrial electron transport chain activity, increasing mitochondrial reactive oxygen species production. Our results indicate that the functional impairment associated with ammonium nutrition is mainly associated with redox reactions outside the chloroplast.

#### Journal of Plant Physiology: Alert 30 September-6 October

**Light intensity-dependent retrograde signalling in higher plants** Original Research Article

Pages 1501-1516 Magdalena Szechyńska-Hebda, Stanisław Karpiński

Kim DH, Xu ZY, Hwang I. AtHSP17.8 overexpression in transgenic lettuce gives rise to dehydration and salt stress resistance phenotypes through modulation of ABA-mediated signaling. Plant Cell Rep. 2013 Oct 1;. [Epub ahead of print] PMID: 24081610 [PubMed - as supplied by publisher]

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Jonathan RL, Cynthia MC, Annamalai P, Guillén G, Olivares JE, Stefano G, LledÃas F, Ramos F, Ghabrial SA, Brandizzi F, Rocha-Sosa M, DÃaz C, Sanchez F. Nodulin 22, a Novel Small Heat Shock Protein of the Endoplasmic Reticulum, is linked to the Unfolded Protein Response in Common Bean. Mol Plant Microbe Interact. 2013 Sep 27;. [Epub ahead of print] PMID: 24073881 [PubMed - as supplied by publisher]

Matsubayashi M, Teramoto-Kimata I, Uni S, Lillehoj HS, Matsuda H, Furuya M, Tani H, Sasai K. Elongation factor-1î± is a novel protein associated with host cell invasion and a potential protective antigen of Cryptosporidium parvum. J Biol Chem. 2013 Sep 30;. [Epub ahead of print] PMID: 24085304 [PubMed - as supplied by publisher]

## Mitochondrial Respiratory Supercomplex Association Limits Production of Reactive Oxygen Species from Complex I

Antioxidants & Redox Signaling, Vol. 19, No. 13, November 2013: 1469-1480.

*Aims:* The mitochondrial respiratory chain is recognized today to be arranged in supramolecular assemblies (supercomplexes). Besides conferring a kinetic advantage (substrate channeling) and being required for the assembly and stability of Complex I, indirect considerations support the view that supercomplexes may also prevent excessive formation of reactive oxygen species (ROS) from the respiratory chain. In the present study, we have directly addressed this issue by testing the ROS generation by Complex I in two experimental systems in which the supramolecular organization of the respiratory assemblies is impaired by: (i) treatment either of bovine heart mitochondria or liposome-reconstituted supercomplex I-III with dodecyl maltoside; (ii) reconstitution of Complexes I and III at high phospholipids to protein ratio. *Results:* The results of our investigation provide experimental evidence that the production of ROS is strongly increased in either model, supporting the view that disruption or prevention of the association between Complex I and Complex III by different means enhances the generation of superoxide from Complex I. *Innovation:* Dissociation of supercomplexes may link oxidative stress

and energy failure in a vicious circle. *Conclusion:* Our findings support a central role of mitochondrial supramolecular structure in the development of the aging process and in the etiology and pathogenesis of most major chronic diseases. *Antioxid. Redox Signal.* 19, 1469–1480.

#### In Science Oct 18

#### **Changing the Code**

Easily and efficiently expanding the genetic code could provide tools to genome engineers with broad applications in medicine, energy, agriculture, and environmental safety. **Lajoie** *et al.* (p. 357) replaced all known UAG stop codons with synonymous UAA stop codons in *Escherichia coli* MG1655, as well as release factor 1 (RF1; terminates translation at UAG), thereby eliminating natural UAG translation function without impairing fitness. This made it possible to reassign UAG as a dedicated codon to genetically encode nonstandard amino acids while avoiding deleterious incorporation at native UAG positions. The engineered *E. coli* incorporated nonstandard amino acids into its proteins and showed enhanced resistance to bacteriophage T7. In a second paper, **Lajoie** *et al.* (p. 361) demonstrated the recoding of 13 codons in 42 highly expressed essential genes in *E. coli.* Codon usage was malleable, but synonymous codons occasionally were nonequivalent in unpredictable ways.

#### Seeing the Trees in the Forest

Despite botanical exploration over two centuries, knowledge of the species composition and quantitative distribution of the trees of the Amazonian forest has remained decidedly patchy. **Ter Steege** *et al.* (p. <u>1243092</u>) report the results from a network of 1170 tree plots arrayed across the Amazon Basin and Guiana Shield, in which the species of all trees with stem diameter >10 centimeters were identified. The tree flora comprised a total of about 16,000 species. However, just 227 very common Amazonian species accounted for half of the trees in the Amazon—the world's most diverse forest.

#### **Capturing Binding Location and Speed**

Transcription factor–binding sites in chromatin can be mapped by the chromatin immunoprecipitation (ChIP) assay, which analyzes formaldehyde-fixed chromatin fragments obtained from cells. However, the standard ChIP assay does not provide information about how stable the inter-actions are. Other approaches, including live-cell imaging, can reveal aspects of the dynamic behavior of transcription factors but are limited either in location precision or time resolution. **Poorey** *et al.* (p. <u>369</u>, published online 3 October) developed a model to explain how the ChIP signal relates to formaldehyde cross-linking time, and they developed a method to measure chromatin site–specific binding dynamics with high temporal resolution.

#### Random Sample

CreatureCast, a series of video shorts based at Brown University that often features littleknown invertebrates, hits the big time with a partnership with *The New York Times*, which may feature a video each week on its online science page. http://creaturecast.org/

#### < Science Express Index

Science DOI: 10.1126/science.1241934 Causes and Effects of N-Terminal Codon Bias in Bacterial Genes

### Daniel B. Goodman<sup>1,<sup>2</sup>,<sup>3</sup></sup>, George M. Church<sup>1,<sup>2</sup></sup>, Sriram Kosuri<sup>1</sup>,\*

Most amino acids are encoded by multiple codons, and codon choice has strong effects on protein expression. Rare codons are enriched at the N terminus of genes in most organisms, although the causes and effects of this bias are unclear. Here, we measure expression from >14,000 synthetic reporters in *Escherichia coli* and show that using N-terminal rare codons instead of common ones increases expression by ~14-fold (median 4-fold). We quantify how individual N-terminal codons affect expression and show that these effects shape the sequence of natural genes. Finally, we demonstrate that reduced RNA structure and not codon rarity itself is responsible for expression increases. Our observations resolve controversies over the roles of N-terminal codon bias and suggest a straightforward method for optimizing heterologous gene expression in bacteria.