Lit Lunch 6 -5 - 2013

Gene Expression Is Circular: Factors for mRNA Degradation Also Foster mRNA Synthesis

Gal Haimovich, Daniel A. Medina, Sebastien Z. Causse, Manuel Garber, Gonzalo Millán-Zambrano, Oren Barkai, Sebastián Chávez, José E. Pérez-Ortín, Xavier Darzacq, Mordechai Choder

Cell - 23 May 2013 (Vol. 153, Issue 5, pp. 1000-1011)

Maintaining proper mRNA levels is a key aspect in the regulation of gene expression. The balance between mRNA synthesis and decay determines these levels. We demonstrate that most yeast mRNAs are degraded by the cytoplasmic 5′-to-3′ pathway (the "decaysome"), as proposed previously. Unexpectedly, the level of these mRNAs is highly robust to perturbations in this major pathway because defects in various decaysome components lead to transcription downregulation. Moreover, these components shuttle between the cytoplasm and the nucleus, in a manner dependent on proper mRNA degradation. In the nucleus, they associate with chromatin—preferentially ~30 bp upstream of transcription start-sites—and directly stimulate transcription initiation and elongation. The nuclear role of the decaysome in transcription is linked to its cytoplasmic role in mRNA decay; linkage, in turn, seems to depend on proper shuttling of its components. The gene expression process is therefore circular, whereby the hitherto first and last stages are interconnected.

The metabolic flux phenotype of heterotrophic Arabidopsis cells reveals a complex response to changes in nitrogen supply.

Masakapalli SK, Kruger NJ, Ratcliffe RG.

Plant J. 2013 May;74(4):569-82. doi: 10.1111/tpj.12142. Epub 2013 Feb 28.

The extent to which individual plants utilise nitrate and ammonium, the two principal nitrogen sources in the rhizosphere, is variable and many species require a balance between the two forms for optimal growth. The effects of nitrate and ammonium on gene expression, enzyme activity and metabolite composition have been documented extensively with the aim of understanding the way in which plant cells respond to the different forms of nitrogen, but ultimately the impact of these changes on the organisation and operation of the central metabolic network can only be addressed by analysing the fluxes supported by the network. Accordingly steady-state metabolic flux analysis was used to define the metabolic phenotype of a heterotrophic Arabidopsis thaliana cell culture grown in Murashige and Skoog and ammonium-free media, treatments that

influenced growth and biomass composition. Fluxes through the central metabolic network were deduced from the redistribution of label into metabolic intermediates and end products observed when cells were labelled with [1-(13) C]-, [2-(13) C]- or [(13) C6]glucose, in tandem with (14) C-measurements of the net accumulation of biomass. Analysis of the flux maps showed that: (i) flux through the oxidative pentose phosphate pathway varied independently of the reductant demand for biosynthesis, (ii) non-plastidic processes made a significant and variable contribution to the provision of reducing power for the plastid, and (iii) the inclusion of ammonium in the growth medium increased cell maintenance costs, in agreement with the futile cycling model of ammonium toxicity. These conclusions highlight the complexity of the metabolic response to a change in nitrogen nutrition.

Transitive RNA silencing signals induce cytosine methylation of a transgenic but not an endogenous target.

Vermeersch L, De Winne N, Nolf J, Bleys A, Kovařík A, Depicker A.

Plant J. 2013 Jun;74(5):867-79. doi: 10.1111/tpj.12172. Epub 2013 May 6.

Post-transcriptional gene silencing of a primary target gene in plants can coincide with the production of secondary small interfering RNAs (siRNAs) of coding sequences adjacent to the target region and with de novo RNA-directed DNA methylation (RdDM) thereof. Here, we analyzed the susceptibility of transgenic and endogenous targets to RdDM induced by primary and secondary silencing signals. In three different configurations, primary silencing signals were able to direct in trans methylation of chimeric transgenes and the CATALASE2 (CAT2) endogene; however, extensive spreading of methylation occurred only in the transgene, resulting in the methylation of the flanking CAT2 sequence, whereas methylation of the CAT2 endogene was restricted to the target region and the enclosed introns. The secondary silencing signals arising from this transgenic primary target simultaneously silenced a secondary transgene target and the CAT2 endogene, but were only capable of directing RdDM to the transgene. Our data indicate that RdDM is correlated with the in situ generation of secondary siRNAs, occurring in P35S-driven transgenes but not in most endogenes. We conclude that although both endogenes and transgenes are equally sensitive to transitive silencing, differences exist in their susceptibility to undergo secondary RdDM.

1)
Journal: **Molecular Cell**

Title: eIF5A Promotes Translation of Polyproline Motifs

Abstract: Translation factor eIF5A, containing the unique amino acid hypusine, was originally shown to stimulate Met-puromycin synthesis, a model assay for peptide bond formation. More recently, eIF5A was

shown to promote translation elongation; however, its precise requirement in protein synthesis remains elusive. We use in vivo assays in yeast and in vitro reconstituted translation assays to reveal a specific requirement for eIF5A to promote peptide bond formation between consecutive Pro residues. Addition of eIF5A relieves ribosomal stalling during translation of three consecutive Pro residues in vitro, and loss of eIF5A function impairs translation of polyproline-containing proteins in vivo. Hydroxyl radical probing experiments localized eIF5A near the E site of the ribosome with its hypusine residue adjacent to the acceptor stem of the P site tRNA. Thus, eIF5A, like its bacterial ortholog EFP, is proposed to stimulate the peptidyl transferase activity of the ribosome and facilitate the reactivity of poor substrates like Pro.

2)
Journal: **Evolution**

Title: RECURRENT AND RECENT SELECTIVE SWEEPS IN THE piRNA PATHWAY

Abstract: Uncontrolled transposable element (TE) insertions and excisions can cause chromosome breaks and mutations with dramatic deleterious effects. The PIWI interacting RNA (piRNA) pathway functions as an adaptive TE silencing system during germline development. Several essential piRNA pathway proteins appear to be rapidly evolving, suggesting that TEs and the silencing machinery may be engaged in a classical “evolutionary arms race.” Using a variety of molecular evolutionary and population genetic approaches, we find that the piRNA pathway genes rhino, krimper, and aubergine show patterns suggestive of extensive recurrent positive selection across Drosophila species.We speculate that selection on these proteins reflects crucial roles in silencing unfamiliar elements during vertical and horizontal transmission of TEs into na¨ıve populations and species, respectively.

1. Science. 2013 Apr 26;340(6131):491-5. doi: 10.1126/science.1234273. Epub 2013 Feb 14.

Simultaneous femtosecond X-ray spectroscopy and diffraction of photosystem II at room temperature.

Kern J, Alonso-Mori R, Tran R, Hattne J, Gildea RJ, Echols N, Glöckner C,

Hellmich J, Laksmono H, Sierra RG, Lassalle-Kaiser B, Koroidov S, Lampe A, Han G, Gul S, Difiore D, Milathianaki D, Fry AR, Miahnahri A, Schafer DW, Messerschmidt M, Seibert MM, Koglin JE, Sokaras D, Weng TC, Sellberg J, Latimer MJ, Grosse-Kunstleve RW, Zwart PH, White WE, Glatzel P, Adams PD, Bogan MJ, Williams GJ, Boutet S, Messinger J, Zouni A, Sauter NK, Yachandra VK, Bergmann U, Yano J.

Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.

Comment in
Nat Methods. 2013 Apr;10(4):287.

Intense femtosecond x-ray pulses produced at the Linac Coherent Light Source (LCLS) were used for simultaneous x-ray diffraction (XRD) and x-ray emission spectroscopy (XES) of microcrystals of photosystem II (PS II) at room temperature. This method probes the overall protein structure and the electronic structure of the Mn4CaO5 cluster in the oxygen-evolving complex of PS II. XRD data are presented from both the dark state (S1) and the first illuminated state (S2) of PS II. Our simultaneous XRD-XES study shows that the PS II crystals are intact during our measurements at the LCLS, not only with respect to the structure of PS II, but also with regard to the electronic structure of the highly radiation-sensitive Mn4CaO5 cluster, opening new directions for future dynamics studies.

PMID: 23413188 [PubMed - indexed for MEDLINE]

2. Science. 2013 Apr 26;340(6131):475-8. doi: 10.1126/science.1232578.

Direct proteomic quantification of the secretome of activated immune cells.

Meissner F, Scheltema RA, Mollenkopf HJ, Mann M.

Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried, Germany.

Protein secretion allows communication of distant cells in an organism and controls a broad range of physiological functions. We describe a quantitative, high-resolution mass spectrometric workflow to detect and quantify proteins that are released from immune cells upon receptor ligation. We quantified the time-resolved release of 775 proteins, including 52 annotated cytokines from only 150,000 primary Toll-like receptor 4-activated macrophages per condition.

Achieving low picogram sensitivity, we detected secreted proteins whose abundance increased by a factor of more than 10,000 upon stimulation. Secretome to transcriptome comparisons revealed the transcriptionally decoupled release of lysosomal proteins. From genetic models, we defined secretory profiles that depended on distinct intracellular signaling adaptors and showed that secretion of many proinflammatory proteins is safeguarded by redundant mechanisms, whereas signaling adaptor synergy promoted the release of anti-inflammatory proteins.

PMID: 23620052 [PubMed - indexed for MEDLINE]

Plant physiology

ROOT ULTRAVIOLET B-SENSITIVE1/WEAK AUXIN RESPONSE3 Is Essential for Polar Auxin Transport in Arabidopsis

Hong Yu, Michael Karampelias, Stephanie Robert, Wendy Ann Peer, Ranjan Swarup, Songqing Ye,Lei Ge, Jerry Cohen, Angus Murphy, Jirí Friml, and Mark Estelle

The phytohormone auxin regulates virtually every aspect of plant development. To identify new genes involved in auxin activity, a genetic screen was performed for Arabidopsis (Arabidopsis thaliana) mutants with altered expression of the auxinresponsive reporter DR5rev:GFP. One of the mutants recovered in the screen, designated as weak auxin response3 (wxr3), exhibits much lower DR5rev:GFP expression when treated with the synthetic auxin 2,4-dichlorophenoxyacetic acid and displays severe defects in root development. The wxr3 mutant decreases polar auxin transport and results in a disruption of the asymmetric auxin distribution. The levels of the auxin transporters AUXIN1 and PIN-FORMED are dramatically reduced in the wxr3 root tip. Molecular analyses demonstrate that WXR3 is ROOT ULTRAVIOLET B-SENSITIVE1 (RUS1), a member of the conserved Domain of Unknown Function647 protein family found in diverse eukaryotic organisms. Our data suggest that RUS1/WXR3 plays an essential role in the regulation of polar auxin transport by maintaining the proper level of auxin transporters on the plasma membrane.

RNA-Seq of Arabidopsis Pollen Uncovers Novel Transcription and Alternative Splicing

Ann E. Loraine*, Sheila McCormick, April Estrada, Ketan Patel , and Peng Qin

Pollen grains of Arabidopsis (Arabidopsis thaliana) contain two haploid sperm cells enclosed in a haploid vegetative cell. Upon germination, the vegetative cell extrudes a pollen tube that carries the sperm to an ovule for fertilization. Knowing the identity, relative abundance, and splicing patterns of pollen transcripts will improve our understanding of pollen and allow investigation of tissue-specific splicing in plants. Most Arabidopsis pollen transcriptome studies have used the ATH1 microarray, which does not

assay splice variants and lacks specific probe sets for many genes. To investigate the pollen transcriptome, we performed high-throughput sequencing (RNA-Seq) of Arabidopsis pollen and seedlings for comparison. Gene expression was more diverse in seedling, and genes involved in cell wall biogenesis were highly expressed in pollen. RNA-Seq detected at least 4,172 proteincoding genes expressed in pollen, including 289 assayed only by nonspecific probe sets. Additional exons and previously unannotated 59 and 39 untranslated regions for pollen-expressed genes were revealed. We detected regions in the genome
not previously annotated as expressed; 14 were tested and 12 were confirmed by polymerase chain reaction. Gapped read alignments revealed 1,908 high-confidence new splicing events supported by 10 or more spliced read alignments. Alternative splicing patterns in pollen and seedling were highly correlated. For most alternatively spliced genes, the ratio of variants in pollen and seedling was similar, except for some encoding proteins involved in RNA splicing. This study highlights the robustness of splicing patterns in plants and the importance of ongoing annotation and visualization of RNA-Seq data using interactive tools such as Integrated Genome Browser.

Assembly, analysis and architecture of atypical ubiquitin chains

Nature Structural & Molecular Biology **20**, 555–565 (2013)

Manuela K Hospenthal, Stefan M V Freund & David Komander

Medical Research Council Laboratory of Molecular Biology, Cambridge, UK

Ubiquitin (Ub) chains regulate many cellular processes, but several chain types including Lys6 linkages have remained unstudied. Here we analyze the bacterial effector E3 ligase NleL (non-Lee–encoded effector ligase) from enterohemorrhagic *Escherichia coli* (EHEC) O157:H7, which assembles Lys6- and Lys48-linked Ub polymers. Using linkage-specific human deubiquitinases (DUBs) we show that NleL generates heterotypic Ub chains, and branched chains are efficiently hydrolyzed by DUBs. USP family DUBs cleave Lys6-linked polymers exclusively from the distal end, whereas a DUB with preference for Lys6 can cleave Lys6-linked polymers at any position in the chain. We used NleL to generate large quantities of Lys6-linked polyUb. Crystallographic and NMR spectroscopy analyses revealed that an asymmetric interface between Ile44 and Ile36 hydrophobic patches of neighboring Ub moieties is propagated in longer Lys6-linked Ub chains. Interactions via the Ile36 patch can displace Leu8 from the Ile44 patch, leading to marked structural perturbations of Ub.

Dengue likes it hot

Nature Structural & Molecular Biology **20**, 546 (2013)

(Proc. Natl. Acad. Sci. USA http://dx.doi.org/10.1073/pnas.1304300110, published online 8 April 2013

Inês Chen

Dengue fever is a mosquito-borne viral infection that affects over 300 million people each year. The dengue viral particle undergoes conformational changes during its maturation process, going from a 'spiky' immature form to a 'smooth' mature virion, whose cryo-EM structure is available; crystal structures of the envelope glycoprotein E have also been solved. One puzzling observation was that several epitopes recognized by neutralizing monoclonal antibodies are not exposed in the smooth, mature viral particle at room temperature (20 °C). Now work from Rossmann and colleagues may help solve this mystery. The authors incubated mature dengue virus particles at 37 °C, the temperature inside the human host, and then examined them by using cryo-EM. In contrast to the smooth particles seen at room temperature, the viral particles had a 'bumpy' appearance at 37 °C. These were different from the spiky immature form, which was the same at 20 or 37 °C. The transition from smooth to bumpy mature particles occurs between 31 and 35 °C, and this conformational change is irreversible. The authors determined the three-dimensional structure of the bumpy particle and fitted in the crystal structure of the glycoprotein-E dimer, which revealed a different arrangement of the dimers as compared to the smooth form. Notably, the bumpy structure is similar to a fusogenic intermediate form of dengue virus that was proposed over a decade ago. This indicates that the bumpy mature dengue virus is the predominant form in the human body and the one that actually infects human cells. Supporting this notion, the bumpy particles showed higher infectivity than did the smooth particles in a cell culture assay. This hypothesis needs to be further investigated, but a change to a 'ready-to-infect' form of dengue virus, triggered by the temperature shift upon entry into the human host, would constitute a remarkable example of host adaptation and have clear implications for vaccine development.

Positive Feedback Regulation of Human Inducible Nitric-oxide Synthase Expression by Ras Protein *S*-Nitrosylation[*]

May 31, 2013 *The Journal of Biological Chemistry*, 288, 15677-15686.

Martin Lee[1] and Jonathan C. Choy

Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

The production of nitric oxide (NO) by inducible NO synthase (iNOS) regulates many aspects of physiology and pathology. The expression of iNOS needs to be tightly regulated to balance the broad

ranging properties of NO. We have investigated the feedback regulation of cytokine-induced iNOS expression by NO in human cells. The pharmacological inhibition of iNOS activity reduced iNOS protein levels in response to cytokine stimulation in a human epithelial cell line (A549 cells) as well as in primary human astrocytes and bronchial epithelial cells. The addition of exogenous NO using a NO donor prevented the reduction in iNOS levels caused by blockade of iNOS activity. Examination of signaling pathways affected by iNOS indicated that NO S-nitrosylated Ras. Transfection of cells with a S-nitrosylation-resistant Ras mutant reduced iNOS protein levels, indicating a role for this Ras modification in the amplification of iNOS levels. Further, the induction of iNOS protein levels correlated with the late activation of the phosphatidylinositol 3-kinase/Akt and mammalian target of rapamycin (mTOR) pathways, and inhibition of these signaling molecules reduced iNOS levels. Altogether, our findings reveal a previously unknown regulatory pathway that amplifies iNOS expression in human cells.

Nature biotech

When bad science makes good headlines: *Bt* maize and regulatory bans

Nature

Reconfiguration of the proteasome during chaperone-mediated assembly

Soyeon Park, Xueming Li, Ho Min Kim, Chingakham Ranjit Singh, Geng Tian, Martin A. Hoyt, Scott Lovell, Kevin P. Battaile, Michal Zolkiewski, Philip Coffino, Jeroen Roelofs, Yifan Cheng & Daniel Finley

The proteasomal ATPase ring, comprising Rpt1–Rpt6, associates with the heptameric α-ring of the proteasome core particle (CP) in the mature proteasome, with the Rpt carboxy-terminal tails inserting into pockets of the α-ring1, 2, 3, 4. Rpt ring assembly is mediated by four chaperones, each binding a distinct Rpt subunit5, 6, 7, 8, 9, 10. Here we report that the base subassembly of the *Saccharomyces cerevisiae* proteasome, which includes the Rpt ring, forms a high-affinity complex with the CP. This complex is subject to active dissociation by the chaperones Hsm3, Nas6 and Rpn14. Chaperone-mediated dissociation was abrogated by a non-hydrolysable ATP analogue, indicating that chaperone action is coupled to nucleotide hydrolysis by the Rpt ring. Unexpectedly, synthetic Rpt tail peptides bound α-pockets with poor specificity, except for Rpt6, which uniquely bound the α2/α3-pocket. Although the Rpt6 tail is not visualized within an α-pocket in mature proteasomes2, 3, 4, it inserts into the α2/α3-pocket in the base–CP complex and is important for complex formation. Thus, the Rpt–CP interface is reconfigured when the lid complex joins the nascent proteasome to form the mature holoenzyme.

Crystal structure of the integral membrane diacylglycerol kinase

Dianfan Li, Joseph A. Lyons, Valerie E. Pye, Lutz Vogeley, David Aragão, Colin P. Kenyon, Syed T. A. Shah, Christine Doherty, Margaret Aherne & Martin Caffrey

Diacylglycerol kinase catalyses the ATP-dependent phosphorylation of diacylglycerol to phosphatidic acid for use in shuttling water-soluble components to membrane-derived oligosaccharide and lipopolysaccharide in the cell envelope of Gram-negative bacteria1. For half a century, this 121-residue kinase has served as a model for investigating membrane protein enzymology1, 2, 3, 4, 5, 6, folding7, 8, assembly9, 10, 11, 12 and stability1, 13. Here we present crystal structures for three functional forms of this unique and paradigmatic kinase, one of which is wild type. These reveal a homo-trimeric enzyme with three transmembrane helices and an amino-terminal amphiphilic helix per monomer. Bound lipid substrate and docked ATP identify the putative active site that is of the composite, shared site type. The crystal structures rationalize extensive biochemical and biophysical data on the enzyme. They are, however, at variance with a published solution NMR model14 in that domain swapping, a key feature of the solution form, is not observed in the crystal structures.

J Mol Biol. 2013 May 27;425(10):1683-96. doi: 10.1016/j.jmb.2013.02.011. Epub 2013 Feb 14.

An unusual dimeric small heat shock protein provides insight into the mechanism of this class of chaperones.

Basha E, Jones C, Blackwell AE, Cheng G, Waters ER, Samsel KA, Siddique M, Pett V, Wysocki V, Vierling E.

Source

Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ 85721, USA; Department of Botany, Tanta University, Tanta 31527, Egypt.

Abstract

Small heat shock proteins (sHSPs) are virtually ubiquitous stress proteins that are also found in many normal tissues and accumulate in diseases of protein folding. They generally act as ATP-independent chaperones to bind and stabilize denaturing proteins that can be later reactivated by ATP-dependent Hsp70/DnaK, but the mechanism of substrate capture by sHSPs remains poorly understood. A majority of sHSPs form large oligomers, a property that has been linked to their effective chaperone action. We describe AtHsp18.5 from Arabidopsis thaliana, demonstrating that it is dimeric and exhibits robust chaperone activity, which adds support to the model that suboligomeric sHSP forms are a substrate binding species. Notably, like oligomeric sHSPs, when bound to substrate, AtHsp18.5 assembles into large complexes, indicating that

reformation of sHSP oligomeric contacts is not required for assembly of sHSP-substrate complexes. Monomers of AtHsp18.5 freely exchange between dimers but fail to coassemble in vitro with dodecameric plant cytosolic sHSPs, suggesting that AtHsp18.5 does not interact by coassembly with these other sHSPs in vivo. Data from controlled proteolysis and hydrogen-deuterium exchange coupled with mass spectrometry show that the N- and C-termini of AtHsp18.5 are highly accessible and lack stable secondary structure, most likely a requirement for substrate interaction. Chaperone activity of a series of AtHsp18.5 truncation mutants confirms that the N-terminal arm is required for substrate protection and that different substrates interact differently with the N-terminal arm. In total, these data imply that the core α-crystallin domain of the sHSPs is a platform for flexible arms that capture substrates to maintain their solubility.

Proc Natl Acad Sci U S A. 2013 May 21;110(21):8513-8. doi: 10.1073/pnas.1217988110. Epub 2013 May 6.

Heat shock protein (Hsp) 70 is an activator of the Hsp104 motor.

Lee J, Kim JH, Biter AB, Sielaff B, Lee S, Tsai FT.

Source

Verna and Marrs McLean Department of Biochemistry and Molecular Biology and Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030.

Abstract

Heat shock protein (Hsp) 104 is a ring-forming, protein-remodeling machine that harnesses the energy of ATP binding and hydrolysis to drive protein disaggregation. Although Hsp104 is an active ATPase, the recovery of functional protein requires the species-specific cooperation of the Hsp70 system. However, like Hsp104, Hsp70 is an active ATPase, which recognizes aggregated and aggregation-prone proteins, making it difficult to differentiate the mechanistic roles of Hsp104 and Hsp70 during protein disaggregation. Mapping the Hsp70-binding sites in yeast Hsp104 using peptide array technology and photo-cross-linking revealed a striking conservation of the primary Hsp70-binding motifs on the Hsp104 middle-domain across species, despite lack of sequence identity. Remarkably, inserting a Strep-Tactin binding motif at the spatially conserved

Hsp70-binding site elicits the Hsp104 protein disaggregating activity that now depends on Strep-Tactin but no longer requires Hsp70/40. Consistent with a Strep-Tactin-dependent activation step, we found that full-length Hsp70 on its own could activate the Hsp104 hexamer by promoting intersubunit coordination, suggesting that Hsp70 is an activator of the Hsp104 motor.

June 5, 2013

Plant, Cell & Environment Content Alert: 36, 7 (July 2013)

Mapping quantitative trait loci for freezing tolerance in a recombinant inbred line population of *Arabidopsis thaliana* accessions Tenela and C24 reveals REVEILLE1 as negative regulator of cold acclimation (pages 1256–1267)

MEIKE MEISSNER, ELENA ORSINI, MORITZ RUSCHHAUPT, ALBRECHT E. MELCHINGER, DIRK K. HINCHA and ARND G. HEYER

Article first published online: 17 JAN 2013 | DOI: 10.1111/pce.12054

A recombinant inbred line population for the accessions C24 and Tenela (Te), showing large variation in freezing tolerance, was established to study genetic determinants of cold acclimation capacity in *Arabidopsis thaliana*. Mapping of quantitative trait loci (QTL) revealed three QTL regions on chromosomes 2, 4 and 5. With the aid of gene expression data, the Myb family transcription factor *REVEILLE1* (At5g17300) on chromosome 5 was identified as a novel negative regulator of freezing tolerance in Arabidopsis.

High-resolution temperature responses of leaf respiration in snow gum (*Eucalyptus pauciflora*) reveal high-temperature limits to respiratory function (pages 1268–1284)

ODHRAN S. O'SULLIVAN, K. W. LASANTHA K. WEERASINGHE, JOHN R. EVANS, JOHN J. G. EGERTON, MARK G. TJOELKER and OWEN K. ATKIN

Article first published online: 24 JAN 2013 | DOI: 10.1111/pce.12057

The response of leaf energy metabolism to temperature is central to predicting the impact of environmental gradients and future climate regimes on carbon exchange in forests. Here, we tested whether *Eucalyptus pauciflora* (an evergreen, broadleaved tree) growing in thermally contrasting environments (including winter-acclimated trees encased in ice at high altitudes) exhibit generalizable temperature response functions of leaf respiration and fluorescence. We found that leaf energy metabolism was surprisingly heat tolerant (with maximal rates of respiration occurring at 51–57°C), with temperature responses varying seasonally. Collectively, our results: (1) highlight high-temperature limits of energy metabolism in *E. pauciflora*; and, (2) provide a framework for improving representation of *T*-responses of leaf respiration in predictive models.

The nitrate transporter NRT2.1 functions in the ethylene response to nitrate deficiency in Arabidopsis (pages 1328–1337)

DONGCHAO ZHENG, XIAO HAN, YI AN, HONGWEI GUO, XINLI XIA and WEILUN YIN

Article first published online: 4 FEB 2013 | DOI: 10.1111/pce.12062

Molecular interaction between *NRT2.1* transcript levels and the ethylene signaling pathway under nitrate deficiency is still elusive. Here, we report a low nitrate (LN) treatment-induced rapid burst of ethylene production and regulated expression of ethylene signaling components *CTR1*, *EIN3* and *EIL1* in wild-type *Arabidopsis thaliana* (Col-0) seedlings, and enhanced ethylene response reporter *EBS:GUS* activity in both Col-0 and the ethylene mutants *ein3-1eil1-1* and *ctr1-1*. Comparison of ethylene production and *EBS:GUS* activity between *nrt1.1*, *nrt2.1* mutants and Col-0 indicated that this up-regulation of *NRT2.1* expression caused a positive effect on ethylene biosynthesis/signaling under LN treatment, and ethylene down-regulated *NRT2.1* expression and reduced the high-affinity nitrate uptake. Together, these findings uncover a negative feedback loop between *NRT2.1* expression and ethylene biosynthesis/signaling under nitrate deficiency, which may contribute to finely tuning of plant nitrate acquisition during

exploring dynamic soil conditions.

DÃaz-ChÃ¡vez J, Fonseca-SÃ¡nchez MA, Arechaga-Ocampo E, Flores-PÃ©rez A, Palacios-RodrÃguez Y, DomÃnguez-GÃ³mez G, Marchat LA, Fuentes-Mera L, Mendoza-HernÃ¡ndez G, Gariglio P, LÃ³pez-Camarillo C.
Proteomic Profiling Reveals That Resveratrol Inhibits HSP27 Expression and Sensitizes Breast Cancer Cells to Doxorubicin Therapy.
PLoS One. 2013;8(5):e64378.
PMID: 23724044 [PubMed - in process]

Corsepius NC, Lorimer GH.
Measuring how much work the chaperone GroEL can do.
Proc Natl Acad Sci U S A. 2013 May 30;. [Epub ahead of print]
PMID: 23723348 [PubMed - as supplied by publisher]

Keown JR, Griffin MD, Mertens HD, Pearce FG.
Small Oligomers of Ribulose-bisphosphate carboxylase/oxygenase (Rubisco) Activase are required for Biological Activity.
J Biol Chem. 2013 May 29;. [Epub ahead of print]
PMID: 23720775 [PubMed - as supplied by publisher]

Ng AH, Fang NN, Comyn SA, Gsponer J, Mayor T.
System-Wide Analysis Reveals Intrinsically Disordered Proteins are Prone to Ubiquitylation after Misfolding Stress.
Mol Cell Proteomics. 2013 May 28;. [Epub ahead of print]
PMID: 23716602 [PubMed - as supplied by publisher]

Schmollinger S, Schulz-Raffelt M, Strenkert D, Veyel D, Vallon O, Schroda M.
Dissecting the heat stress response in Chlamydomonas by pharmaceutical and RNAi approaches reveals conserved and novel aspects.
Mol Plant. 2013 May 27;. [Epub ahead of print]
PMID: 23713078 [PubMed - as supplied by publisher]

Socha RD, Tokuriki N.
Q1Modulating Protein Stability: Directed Evolution Strategies for Improved Protein Function.
FEBS J. 2013 May 26;. [Epub ahead of print]
PMID: 23711026 [PubMed - as supplied by publisher]

Piri N, Kwong JM, Caprioli J.
Crystallins in Retinal Ganglion Cell Survival and Regeneration.
Mol Neurobiol. 2013 May 25;. [Epub ahead of print]
PMID: 23709342 [PubMed - as supplied by publisher]

Qi R, Sarbeng EB, Liu Q, Le KQ, Xu X, Xu H, Yang J, Wong JL, Vorvis C, Hendrickson WA, Zhou L, Liu Q.
Allosteric opening of the polypeptide-binding site when an Hsp70 binds ATP.
Nat Struct Mol Biol. 2013 May 26;. [Epub ahead of print]
PMID: 23708608 [PubMed - as supplied by publisher]

Kovacs I, Lindermayr C.
Nitric oxide-based protein modification: formation and site-specificity of protein S-nitrosylation.
Front Plant Sci. 2013;4:137.
PMID: 23717319 [PubMed]

Vaishnav A, Jain S, Kasotia A, Kumari S, Gaur RK, Choudhary DK.
Effect of nitric oxide signaling in bacterial-treated soybean plant under salt stress.
Arch Microbiol. 2013 May 25;. [Epub ahead of print]
PMID: 23708508 [PubMed - as supplied by publisher]

Nakamura T, Tu S, Akhtar MW, Sunico CR, Okamoto S, Lipton SA.
Aberrant protein s-nitrosylation in neurodegenerative diseases.
Neuron. 2013 May 22;78(4):596-614.
PMID: 23719160 [PubMed - in process]

Rodnina MV.
The ribosome as a versatile catalyst: reactions at the peptidyl transferase center.
Curr Opin Struct Biol. 2013 May 24;. [Epub ahead of print]
PMID: 23711800 [PubMed - as supplied by publisher]

Leprivier G, Remke M, Rotblat B, Dubuc A, Mateo AR, Kool M, Agnihotri S, El-Naggar A, Yu B, Prakash Somasekharan S, Faubert B, Bridon G, Tognon CE, Mathers J, Thomas R, Li A, Barokas A, Kwok B, Bowden M, Smith S, Wu X, Korshunov A, Hielscher T, Northcott PA, Galpin JD, Ahern CA, Wang Y, McCabe MG, Collins VP, Jones RG, Pollak M, Delattre O, Gleave ME, Jan E, Pfister SM, Proud CG, Derry WB, Taylor MD, Sorensen PH.
The eEF2 Kinase Confers Resistance to Nutrient Deprivation by Blocking Translation Elongation.
Cell. 2013 May 23;153(5):1064-79.
PMID: 23706743 [PubMed - in process]

Nano-suiting up for SEM

A polymer 'nano-suit' allows living organisms to survive the harsh conditions of scanning electron microscopy.

Scanning electron microscopy (SEM) produces finely detailed images of microscopic structures. This technique is performed under high vacuum to prevent air molecules from scattering the electron beam. The electron beam itself also causes damage to the sample. Such extreme conditions mean that biological specimens must be chemically preserved before they can be

imaged, and such preservation can introduce image artifacts and precludes the observation of living organisms.

Recent evidence, however, has suggested that some uniquely hardy organisms can endure SEM conditions. Takahiko Hariyama of the Hamamatsu University School of Medicine and his colleagues investigated this further, placing a series of living organisms into an SEM to see how long they would survive. Most organisms were dehydrated under high-vacuum conditions and collapsed in a dramatic fashion. Surprisingly, however, they found that *Drosophila* larvae tolerated SEM conditions, living for up to 60 minutes while maintaining their morphology and, after the analysis, continuing to develop normally.

What is so special about *Drosophila* larvae? This immature form of the fruit fly has a soft cuticle that is covered by a coat of extracellular substances (ECSs), made up of amphiphilic molecules. Hariyama's team discovered that when the larvae were subjected to SEM electron bombardment, their ECS coat was cross-linked to form a durable polymer 'nano-suit'. This nano-suit, which could also be generated by plasma irradiation, provided protection from the SEM conditions much as a space suit protects an astronaut from the extreme environment of space.

Hariyama's team found that other organisms with ECS coats, including the fly maggot and the Japanese honey bee, also formed this nano-suit upon experiencing electron bombardment. Species without an ECS coat, however, were not protected. The researchers thus devised a solution mimicking the ECS composition, containing a nontoxic, amphiphilic detergent called Tween 20. They dunked various species (including a flatworm, an ant, an amphipod and two species of mosquito larvae) in the detergent solution and found that all then survived SEM high vacuum and could be imaged while actively moving for up to 60 minutes, all the while retaining their morphology. The approach may thus broaden the applicability of SEM to image living creatures.

References

1. Takaku, Y. *et al*. A thin polymer membrane, nano-suit, enhancing survival across the continuum between air and high vacuum. Proc. Natl. Acad. Sci. USA 110, 7631–7635 (2013).

Nature Methods

Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data

Chen-Shan Chin, et al

Nature Methods 10,563–569(2013)

We present a hierarchical genome-assembly process (HGAP) for high-quality *de novo* microbial genome assemblies using only a single, long-insert shotgun DNA library in conjunction with Single Molecule, Real-Time (SMRT) DNA sequencing. Our method uses the longest reads as seeds to recruit all other reads for construction of highly accurate preassembled reads through a

directed acyclic graph–based consensus procedure, which we follow with assembly using off-the-shelf long-read assemblers. In contrast to hybrid approaches, HGAP does not require highly accurate raw reads for error correction. We demonstrate efficient genome assembly for several microorganisms using as few as three SMRT Cell zero-mode waveguide arrays of sequencing and for BACs using just one SMRT Cell. Long repeat regions can be successfully resolved with this workflow. We also describe a consensus algorithm that incorporates SMRT sequencing primary quality values to produce *de novo* genome sequence exceeding 99.999% accuracy.

Electron counting and beam-induced motion correction enable near-atomic-resolution single-particle cryo-EM

Xueming Li, Paul Mooney, Shawn Zheng, Christopher R Booth, Michael B Braunfeld, Sander Gubbens, David A Agard & Yifan Cheng

Nature Methods 10,584–590(2013) doi:10.1038/nmeth.2472

In recent work with large high-symmetry viruses, single-particle electron cryomicroscopy (cryo-EM) has achieved the determination of near-atomic-resolution structures by allowing direct fitting of atomic models into experimental density maps. However, achieving this goal with smaller particles of lower symmetry remains challenging. Using a newly developed single electron–counting detector, we confirmed that electron beam–induced motion substantially degrades resolution, and we showed that the combination of rapid readout and nearly noiseless electron counting allow image blurring to be corrected to subpixel accuracy, restoring intrinsic image information to high resolution (Thon rings visible to ~3 Å). Using this approach, we determined a 3.3-Å-resolution structure of an ~700-kDa protein with D7 symmetry, the *Thermoplasma acidophilum* 20S proteasome, showing clear side-chain density. Our method greatly enhances image quality and data acquisition efficiency—key bottlenecks in applying near-atomic-resolution cryo-EM to a broad range of protein samples.

Measuring mRNA copy number in individual *Escherichia coli* cells using single-molecule fluorescent *in situ* hybridization

Nature Protocols 8,1100–1113(2013)doi:10.1038/nprot.2013.066

We present a protocol for measuring the absolute number of mRNA molecules from a gene of interest in individual, chemically fixed *Escherichia coli* cells. A set of fluorescently labeled oligonucleotide probes is hybridized to the target mRNA, such that each mRNA molecule is decorated by a known number of fluorescent dyes. Cells are then imaged using fluorescence microscopy. The copy number of the target mRNA is estimated from the total intensity of fluorescent foci in the cell, rather than from counting discrete 'spots' as in other currently available protocols. Image analysis is performed using an automated algorithm. The measured mRNA copy number distribution obtained from many individual cells can be used to extract the parameters of stochastic gene activity, namely the frequency and size of transcription bursts from

the gene of interest. The experimental procedure takes 2 d, with another 2–3 d typically required for image and data analysis.

The EMBO Journal - Table of Contents alert Volume 32 Issue 11

Real-time observation of the conformational dynamics of mitochondrial Hsp70 by spFRET

Time-resolved single molecule data of nucleotide and substrate binding of the Hsp70 chaperone Ssc1 reveals unique nucleotide-binding domain/substrate binding domain interactions and domain dynamics.

Martin Sikor, Koyeli Mapa, Lena Voith von Voithenberg, Dejana Mokranjac and Don C Lamb

The EMBO Journal (2013), **32**, 1639 - 1649; 10.1038/emboj.2013.89

Current Biology: Alert 11 May-17 May

Phyllotaxis and Rhizotaxis in *Arabidopsis* Are Modified by Three PLETHORA Transcription Factors Original Research Article

Available online 16 May 2013

Hugo Hofhuis, Marta Laskowski, Yujuan Du, Kalika Prasad, Stephen Grigg, Violaine Pinon, Ben Scheres

Journal of Plant Physiology: Alert 10 May-16 May

Rice chloroplast-localized heat shock protein 70, OsHsp70CP1, is essential for chloroplast development under high-temperature conditions Original Research Article

Pages 854-863

Sung-Ryul Kim, Gynheung An

May 23 2013 Journal articles - Elizabeth

Futile Protein Folding Cycles in the ER Are Terminated by the Unfolded Protein O-Mannosylation Pathway

Chengchao Xu, Songyu Wang, Guillaume Thibault, and Davis T. W. Ng

Science 24 May 2013: 978-981.

Proteins that fail to fold up properly after many tries are tagged with mannose and voted off the island. [Also see Perspective by Kleizen and Braakman]

Current Opinion in Structural Biology: Alert 14 May-20 May

Prediction of contacts from correlated sequence substitutions Review Article

Available online 14 May 2013

William R Taylor, Russell S Hamilton, Michael I Sadowski

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 May 16;. [Epub ahead of print]
PMID: 23681509 [PubMed - as supplied by publisher]

Pallas V, GÃ³mez G.
Phloem RNA-binding proteins as potential components of the long-distance RNA transport system.
Front Plant Sci. 2013;4:130.
PMID: 23675378 [PubMed]

Zhang T, Kedzierska-Mieszkowska S, Liu H, Cheng C, Ganta RR, Zolkiewski M.
Aggregate-Reactivation Activity of the Molecular Chaperone ClpB from Ehrlichia chaffeensis.
PLoS One. 2013;8(5):e62454.
PMID: 23667479 [PubMed - in process]

Xu Y, Liu Y, Lee JG, Ye Y.
A ubiquitin-like domain recruits an oligomeric chaperone to a retrotranslocation complex in endoplasmic reticulum-associated degradation.
J Biol Chem. 2013 May 12;. [Epub ahead of print]
PMID: 23665563 [PubMed - as supplied by publisher]

Powers ET, Balch WE.
Diversity in the origins of proteostasis networks--a driver for protein function in evolution.
Nat Rev Mol Cell Biol. 2013 Apr;14(4):237-48.
PMID: 23463216 [PubMed - indexed for MEDLINE]

Ludidi N.
Measurement of nitric oxide in plant tissue using difluorofluorescein and oxyhemoglobin.
Methods Mol Biol. 2013;1016:253-9.
PMID: 23681585 [PubMed - in process]

Vieux EF, Wohlever ML, Chen JZ, Sauer RT, Baker TA.
Distinct quaternary structures of the AAA+ Lon protease control substrate degradation.
Proc Natl Acad Sci U S A. 2013 May 14;. [Epub ahead of print]
PMID: 23674680 [PubMed - as supplied by publisher]

Sauer M, Robert S, Kleine-Vehn J.
Auxin: simply complicated.
J Exp Bot. 2013 May 13;. [Epub ahead of print]
PMID: 23669571 [PubMed - as supplied by publisher]

Kuersten S, Radek A, Vogel C, Penalva LO.
Translation regulation gets its 'omics' moment.
Wiley Interdiscip Rev RNA. 2013 May 15;. [Epub ahead of print]
PMID: 23677826 [PubMed - as supplied by publisher]

Nature contents: 23 May 2013

Mitonuclear protein imbalance as a conserved longevity mechanism ►

Riekelt H. Houtkooper, Laurent Mouchiroud, Dongryeol Ryu *et al.*

Mitochondrial ribosomal proteins have been identified as longevity regulators in *C. elegans* and mammalian systems, their role in longevity is linked to mitonuclear protein imbalance and the mitochondrial unfolded protein response.

The Norway spruce genome sequence and conifer genome evolution OPEN ►

Björn Nystedt, Nathaniel R. Street, Anna Wetterbom *et al.*

The draft genome of the Norway spruce (*P. abies*) is presented; this is the first gymnosperm genome to be sequenced and reveals a large genome size (20 Gb) resulting from the accumulation of transposable elements, and comparative sequencing of five other gymnosperm genomes provides insights into conifer genome evolution.

Reconfiguration of the proteasome during chaperone-mediated assembly ►

Soyeon Park, Xueming Li, Ho Min Kim *et al.*

The proteasome degrades ubiquitin-conjugated substrates; here, structural and functional insights from studies in yeast reveal that it is reconfigured during chaperone-mediated assembly.

Lu MX, Hua J, Cui YD, Du YZ.
Five small heat shock protein genes from Chilo suppressalis: characteristics of gene, genomic organization, structural analysis, and transcription profiles.
Cell Stress Chaperones. 2013 May 25;. [Epub ahead of print]
PMID: 23702967 [PubMed - as supplied by publisher]

Schlehe JS, Journel MS, Taylor KP, Amodeo KD, Lavoie MJ.
The mitochondrial disease associated protein Ndufaf2 is dispensable for Complex-1 assembly but critical for the regulation of oxidative stress.
Neurobiol Dis. 2013 May 20;. [Epub ahead of print]
PMID: 23702311 [PubMed - as supplied by publisher]

Li D, Wei T, Abbott CM, Harrich D.
The unexpected roles of eukaryotic translation elongation factors in RNA virus replication and pathogenesis.
Microbiol Mol Biol Rev. 2013 Jun;77(2):253-66.
PMID: 23699257 [PubMed - in process]

Lim SD, Cho HY, Park YC, Ham DJ, Lee JK, Jang CS.
The rice RING finger E3 ligase, OsHCI1, drives nuclear export of multiple substrate proteins and its heterogeneous overexpression enhances acquired thermotolerance.
J Exp Bot. 2013 May 22;. [Epub ahead of print]
PMID: 23698632 [PubMed - as supplied by publisher]

Arrigo AP, Gibert B.
Protein interactomes of three stress inducible small heat shock proteins: HspB1, HspB5 and HspB8.

Int J Hyperthermia. 2013 May 22;. [Epub ahead of print]
PMID: 23697380 [PubMed - as supplied by publisher]

Datu AK, Bag J.
Enhanced Translation of mRNAs Encoding Proteins Involved in mRNA Translation during Recovery from Heat Shock.
PLoS One. 2013;8(5):e64171.
PMID: 23696868 [PubMed - in process]

Athanasiou D, AguilÃ M, Bevilacqua D, Novoselov SS, Parfitt DA, Cheetham ME.
The cell stress machinery and retinal degeneration.
FEBS Lett. 2013 May 15;. [Epub ahead of print]
PMID: 23684651 [PubMed - as supplied by publisher]

Priya S, Sharma SK, Goloubinoff P.
Molecular chaperones as enzymes that catalytically unfold misfolded polypeptides.
FEBS Lett. 2013 May 16;. [Epub ahead of print]
PMID: 23684649 [PubMed - as supplied by publisher]

Arrigo AP.
Human small heat shock proteins: Protein interactomes of homo- and hetero-oligomeric complexes: An update.
FEBS Lett. 2013 May 15;. [Epub ahead of print]
PMID: 23684648 [PubMed - as supplied by publisher]

Lima S, Guo MS, Chaba R, Gross CA, Sauer RT.
Dual molecular signals mediate the bacterial response to outer-membrane stress.
Science. 2013 May 17;340(6134):837-41.
PMID: 23687042 [PubMed - in process]

Michel AM, Baranov PV.
Ribosome profiling: a Hi-Def monitor for protein synthesis at the genome-wide scale.
Wiley Interdiscip Rev RNA. 2013 May 20;. [Epub ahead of print]
PMID: 23696005 [PubMed - as supplied by publisher]