Lit Lunch 2_27_15

Damian:

Leghemoglobin is nitrated in functional legume nodules in a tyrosine residue within the heme cavity by a nitrite/peroxide-dependent mechanism

1. Martha Sainz[1], Laura Calvo-Begueria[1],Carmen Pérez-Rontomé[1], Stefanie Wienkoop[2], Joaquín Abián[3], Christiana Staudinger[3], Silvina Bartesaghi[4,5], Rafael Radi[4] and Manuel Becana[1,*]

Article first published online: 24 FEB 2015 DOI: 10.1111/tpj.12762

Summary

Protein tyrosine (Tyr) nitration is a post-translational modification yielding 3-nitrotyrosine (NO_2–Tyr). Formation of NO_2–Tyr is generally considered as a marker of nitro-oxidative stress and is involved in some human pathophysiological disorders, but has been poorly studied in plants. Leghemoglobin (Lb) is an abundant hemeprotein of legume nodules that plays an essential role as an O_2 transporter. Liquid chromatography coupled to tandem mass spectrometry was used for a targeted search and quantification of NO_2–Tyr in Lb. For all Lbs examined, Tyr30, located in the distal heme pocket, is the major target of nitration. Lower amounts were found for NO_2–Tyr25 and NO_2–Tyr133. Nitrated Lb and other as yet unidentified nitrated proteins were also detected in nodules of plants not receiving NO_3^- and were found to decrease during senescence. This demonstrates formation of nitric oxide (˙NO) and NO_2^- by alternative means to nitrate reductase, probably via a ˙NO synthase-like enzyme, and strongly suggests that nitrated proteins perform biological functions and are not merely metabolic byproducts. *In vitro* assays with purified Lb revealed that Tyr nitration requires NO_2^- + H_2O_2 and that peroxynitrite is not an efficient inducer of nitration, probably because Lb isomerizes it to NO_3^-. Nitrated Lb is formed via oxoferryl Lb, which generates nitrogen dioxide and tyrosyl radicals. This mechanism is distinctly different from that involved in heme nitration. Formation of NO_2–Tyr in Lb is a consequence of active metabolism in functional nodules, where Lb may act as a sink of toxic peroxynitrite and may play a protective role in the symbiosis.

Nathen:

Dynamics of gene circuits shapes evolvability

Alba Jiméneza , Andreea Munteanua , and James Sharpea,b,1 a European Molecular Biology Laboratory-CRG Systems Biology Program, Centre for Genomic Regulation (CRG), and Universitat Pompeu Fabra, 08003 Barcelona, Spain; and b Institucio Catalana de Recerca i Estudis Avancats, 08010 Barcelona, Spain

To what extent does the dynamical mechanism producing a specific biological phenotype bias the ability to evolve into novel phenotypes? We use the interpretation of a morphogen gradient into a single stripe of gene expression as a model phenotype. Although there are thousands of three-gene circuit topologies that can robustly develop a stripe of gene expression, the vast majority of these circuits use one of just six fundamentally different dynamical mechanisms. Here we explore the potential for gene circuits that use each of these six mechanisms to evolve novel phenotypes such as multiple stripes, inverted stripes, and gradients of

gene expression. Through a comprehensive and systematic analysis, we find that circuits that use alternative mechanisms differ in the likelihood of reaching novel phenotypes through mutation. We characterize the phenotypic transitions and identify key ingredients of the evolutionary potential, such as sensitive interactions and phenotypic hubs. Finally, we provide an intuitive understanding on how the modular design of a particular mechanism favors the access to novel phenotypes. Our work illustrates how the dynamical mechanism by which an organism develops constrains how it can evolve. It is striking that these dynamical mechanisms and their impact on evolvability can be observed even for such an apparently simple patterning task, performed by just three node circuits.

Stephanie:

NIK1-mediated translation suppression functions as a plant antiviral immunity mechanism

Zorzatto, C., Machado, J. P. B., Lopes, K. V. G., Nascimento, K. J. T., Pereira, W. A., Brustolini, O. J. B., Reis, P. A. B., Calil, I. P., Deguchi, M., Sachetto-Martins, G. et al. (2015). NIK1-mediated translation suppression functions as a plant antiviral immunity mechanism. *Nature* advance online publication.

Plants and plant pathogens are subject to continuous co-evolutionary pressure for dominance, and the outcomes of these interactions can substantially impact agriculture and food security[1, 2, 3]. In virus–plant interactions, one of the major mechanisms for plant antiviral immunity relies on RNA silencing, which is often suppressed by co-evolving virus suppressors, thus enhancing viral pathogenicity in susceptible hosts[1]. In addition, plants use the nucleotide-binding and leucine-rich repeat (NB-LRR) domain-containing resistance proteins, which recognize viral effectors to activate effector-triggered immunity in a defence mechanism similar to that employed in non-viral infections[2, 3]. Unlike most eukaryotic organisms, plants are not known to activate mechanisms of host global translation suppression to fight viruses[1, 2]. Here we demonstrate in *Arabidopsis* that the constitutive activation of NIK1, a leucine-rich repeat receptor-like kinase (LRR-RLK) identified as a virulence target of the begomovirus nuclear shuttle protein (NSP)[4, 5, 6], leads to global translation suppression and translocation of the downstream component RPL10 to the nucleus, where it interacts with a newly identified MYB-like protein, L10-INTERACTING MYB DOMAIN-CONTAINING PROTEIN (LIMYB), to downregulate translational machinery genes fully. LIMYB overexpression represses ribosomal protein genes at the transcriptional level, resulting in protein synthesis inhibition, decreased viral messenger RNA association with polysome fractions and enhanced tolerance to begomovirus. By contrast, the loss of *LIMYB* function releases the repression of translation-related genes and increases susceptibility to virus infection. Therefore, LIMYB links immune receptor LRR-RLK activation to global translation suppression as an antiviral immunity strategy in plants.

Fionn:

Plant journal

A Chaperone Function of NO CATALASE ACTIVITY1 Is Required to Maintain Catalase Activity and for Multiple Stress Responses in Arabidopsis

Jing Lia,1, Juntao Liua,1, Guoqiang Wanga, Joon-Yung Chab, Guannan Lia, She Chenc, Zhen Lia, Jinghua Guod, Caiguo Zhanga, Yongqing Yanga, Woe-Yeon Kimb, Dae-Jin Yunb, Karen S. Schumakere, Zhongzhou Chena and Yan Guoa,f,2

Abstract

Catalases are key regulators of reactive oxygen species homeostasis in plant cells. However, the regulation of catalase activity is not well understood. In this study, we isolated an Arabidopsis thaliana mutant, no catalase activity1-3 (nca1-3) that is hypersensitive to many abiotic stress treatments. The mutated gene was identified by map-based cloning as NCA1, which encodes a protein containing an N-terminal RING-finger domain and a C-terminal tetratricopeptide repeat-like helical domain. NCA1 interacts with and increases catalase activity maximally in a 240-kD complex in planta. In vitro, NCA1 interacts with CATALASE2 (CAT2) in a 1:1 molar ratio, and the NCA1 C terminus is essential for this interaction. CAT2 activity increased 10-fold in the presence of NCA1, and zinc ion binding of the NCA1 N terminus is required for this increase. NCA1 has chaperone protein activity that may maintain the folding of catalase in a functional state. NCA1 is a cytosol-located protein. Expression of NCA1 in the mitochondrion of the nca1-3 mutant does not rescue the abiotic stress phenotypes of the mutant, while expression in the cytosol or peroxisome does. Our results suggest that NCA1 is essential for catalase activity.

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Keith:

K63 polyubiquitination is a new modulator of the oxidative stress response

Nature Structural & Molecular Biology **22**, 116–123 (2015)

Gustavo M Silva, Daniel Finley & Christine Vogel

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Daniel Finley

Ubiquitination is a post-translational modification that signals multiple processes, including protein degradation, trafficking and DNA repair. Polyubiquitin accumulates globally during the oxidative stress response, and this has been mainly attributed to increased ubiquitin conjugation and perturbations in protein degradation. Here we show that the unconventional Lys63 (K63)-linked polyubiquitin accumulates in the yeast *Saccharomyces cerevisiae* in a highly sensitive and regulated manner as a result of exposure to peroxides. We demonstrate that hydrogen peroxide inhibits the deubiquitinating enzyme Ubp2, leading to accumulation of K63 conjugates assembled by the Rad6 ubiquitin conjugase and the Bre1 ubiquitin ligase. Using linkage-specific isolation methods and stable isotope labeling by amino acids in cell culture (SILAC)-based quantitative proteomics, we identified >100 new K63-polyubiquitinated targets, which were substantially enriched in ribosomal proteins. Finally, we demonstrate that impairment of K63 ubiquitination during oxidative stress affects polysome stability and protein expression, rendering cells more sensitive to stress, and thereby reveal a new redox-regulatory role for this modification.

February 26, 2015

Chemistry & Biology: Alert 14 February-20 February
Phosphomimics Destabilize Hsp27 Oligomeric Assemblies and Enhance Chaperone Activity
Pages 186-195 Blagojce Jovcevski, Megan A. Kelly, Anthea P. Rote, Tracey Berg, Heidi Y. Gastall, Justin L.P. Benesch, J. Andrew Aquilina, Heath Ecroyd

Cellular Signalling: Alert 14 February-20 February
Ratio of phosphorylated HSP27 to nonphosphorylated HSP27 biphasically acts as a determinant of cellular fate in gemcitabine-resistant pancreatic cancer cells *Pages 807-817*
Dongxu Kang, Hye Jin Choi, Sujin Kang, So Young Kim, Yong-sic Hwang, Suyeon Je, Zhezhu Han, Joo-Hang Kim, Jae J. Song

Science 27 February 2015: Vol. 347 no. 6225 pp. 950-951 DOI: 10.1126/science.aaa7722
Insecticidal RNA, the long and short of it
Steve Whyard
Insects cost the agricultural sector billions of dollars every year in lost crop yields and insecticide expenditures. The continued use of chemical insecticides has inadvertently selected for more resistant pest strains, prompting higher doses and more frequent applications to control them. The advent of transgenic plants, such as those expressing insecticidal *Bacillus thuringiensis* (Bt) toxins, reduces the use of

chemicals while offering protection to some crops (*1*), but not all insects are affected by Bt toxins, and continued use of Bt technologies will eventually see the rise of Bt-resistant insects. To stay ahead of the pests will require additional technologies. On page 991 of this issue, Zhang *et al.* (*2*) describe a clever modification to an existing transgenic plant technology that produces insecticidal RNAs. The trick is to express lethal RNA in the plant's photosynthetic organelles, the chloroplasts.

Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids
Jiang Zhang, Sher Afzal Khan, Claudia Hasse, Stephanie Ruf, David G. Heckel, and Ralph Bock
Science 27 February 2015: 991-994. [DOI:10.1126/science.1261680]

Journal of Evolutionary Biology Content Alert (New Articles)
Experimental evolution in fluctuating environments: Tolerance measurements at constant temperatures incorrectly predict the ability to tolerate fluctuating temperatures
Tarmo Ketola and Kati Saarinen
Accepted manuscript online: 22 FEB 2015 10:51PM EST | DOI: 10.1111/jeb.12606

Zalatan, J.G. *et al*. Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds. Cell 160, 339–350 (2015).
Eukaryotic cells execute complex transcriptional programs in which specific loci throughout the genome are regulated in distinct ways by targeted regulatory assemblies. We have applied this principle to generate synthetic CRISPR-based transcriptional programs in yeast and human cells. By extending guide RNAs to include effector protein recruitment sites, we construct modular scaffold RNAs that encode both target locus and regulatory action. Sets of scaffold RNAs can be used to generate synthetic multigene transcriptional programs in which some genes are activated and others are repressed. We apply this approach to flexibly redirect flux through a complex branched metabolic pathway in yeast. Moreover, these programs can be executed by inducing expression of the dCas9 protein, which acts as a single master regulatory control point. CRISPR-associated RNA scaffolds provide a powerful way to construct synthetic gene expression programs for a wide range of applications, including rewiring cell fates or engineering metabolic pathways.

A Chaperone Function of NO CATALASE ACTIVITY1 Is Required to Maintain Catalase Activity and for Multiple Stress Responses in Arabidopsis
Jing Li, Juntao Liu, Guoqiang Wang, Joon-Yung Cha, Guannan Li, She Chen, Zhen Li, Jinghua Guo, Caiguo Zhang, Yongqing Yang, Woe-Yeon Kim, Dae-Jin Yun, Karen S. Schumaker, Zhongzhou Chen, and Yan Guo
Plant Cell 2015 tpc.114.135095; First Published on February 19, 2015; doi:10.1105/tpc.114.135095
http://www.plantcell.org/content/early/2015/02/19/tpc.114.135095.abstract
Arabidopsis protein NCA1 interacts with catalases in the cytosol and increases catalase activity through maintaining catalase folding state, which is required for stress responses.

Leghemoglobin is nitrated in functional legume nodules in a tyrosine residue within the heme cavity by a nitrite/peroxide-dependent mechanism (pages 723–735)
Martha Sainz, Laura Calvo-Begueria, Carmen Pérez-Rontomé, Stefanie Wienkoop, Joaquín Abián, Christiana Staudinger, Silvina Bartesaghi, Rafael Radi and Manuel Becana
Article first published online: 24 FEB 2015 | DOI: 10.1111/tpj.12762

Significance Statement
We found that in functional legume nodules grown with or without nitrate, leghemoglobin, a crucial protein in symbiotic nitrogen fixation, is preferentially nitrated in a tyrosine within the heme pocket by a

mechanism involving oxoferryl heme and nitrogen dioxide rather than peroxynitrite. Therefore, a low concentration of nitrated leghemoglobin is compatible with nodule activity, and we propose that leghemoglobins may act as a sink of toxic peroxynitrite and have a protective role in the symbiosis.

C. Riedel, R. Gabizon, C.A.M. Wilson, K. Hamadani, K. Tsekouras, S. Marqusee, S. Pressé, C. Bustamante Nature, 517 (2015), pp. 227–230
New findings by Riedel et al. (2015) suggest that the heat released by catalysis, such as the highly exothermic breakdown of hydrogen peroxide to water and oxygen by the enzyme catalase, has a direct effect on enzyme diffusion. By examining single-molecule behavior of catalase and other enzymes, they show that the heat generated by catalysis increases enzyme diffusion by producing an asymmetric pressure wave that applies a force that is centered away from the protein's center of mass. To imagine a single catalytic event, the force of a recoiling gun might be one analogy, and though an enzyme's cumulative motion would still be Brownian. The authors suggest that the heat-driven pressure waves may lead to a partial unfolding of part of an enzyme that could temporarily halt its activity, or intriguingly, this force might affect the processivity of molecular complexes such as RNA or DNA polymerase.

Quantitative evolutionary dynamics using high-resolution lineage tracking

Sasha F. Levy, Jamie R. Blundell, Sandeep Venkataram, Dmitri A. Petrov, Daniel S. Fisher & Gavin Sherlock
Nature (2015) doi:10.1038/nature14279 Received 11 September 2014

Evolution of large asexual cell populations underlies ~30% of deaths worldwide, including those caused by bacteria, fungi, parasites, and cancer. However, the dynamics underlying these evolutionary processes remain poorly understood because they involve many competing beneficial lineages, most of which never rise above extremely low frequencies in the population. To observe these normally hidden evolutionary dynamics, we constructed a sequencing-based ultra high-resolution lineage tracking system in *Saccharomyces cerevisiae* that allowed us to monitor the relative frequencies of ~500,000 lineages simultaneously. In contrast to some expectations, we found that the spectrum of fitness effects of beneficial mutations is neither exponential nor monotonic. Early adaptation is a predictable consequence of this spectrum and is strikingly reproducible, but the initial small-effect mutations are soon outcompeted by rarer large-effect mutations that result in variability between replicates. These results suggest that early evolutionary dynamics may be deterministic for a period of time before stochastic effects become important.

Nature Volume 518 Number 7540 pp456-568

N^6-methyladenosine-dependent RNA structural switches regulate RNA–protein interactions

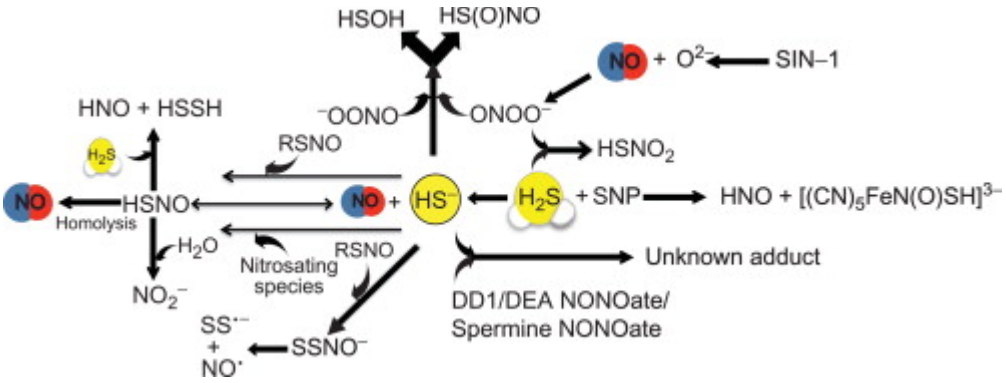
Nian Liu, Qing Dai, Guanqun Zheng, Chuan He, Marc Parisien + et al.
The binding motifs for many RNA-binding proteins are normally buried within structured regions; now, the N^6-methyladenosine modification is shown to act as a switch to remodel these regions, expose the motif, and thereby facilitate binding of RNA-binding proteins.

News & Views by Theler & Allain

Methods in Enzymology Volume 554, 2015, Pages 271–297 Hydrogen Sulfide in Redox Biology, Part A

Chapter Fifteen – H_2S Regulation of Nitric Oxide Metabolism

Gopi K. Kolluru , Shuai Yuan, Xinggui Shen, Christopher G. Kevil



Novel adducts from NO–H_2S interactions: Sulfide radical HS^- can react with NO to form thionitrous acid (HSNO). Reaction of HS^- either with a thiol (RSNO) or with nitrosating species can also from HSNO. Alternatively, HS^- can react with RSNO to form nitrosopersulfide ($SSNO^-$), which further disintegrates into NO and disulfide radical ($SS^{\bullet-}$). HSNO can undergo hemolysis to form NO or react with H_2S to form a disulfide (HSSH) and HNO. It can also form nitrite upon hydrolysis (H_2O). NO donors can directly or indirectly involve to form new adducts. SIN-1 can generate both NO and O_2^-O2− that leads to formation of peroxynitrite ($ONOO^-$). Peroxynitrite can react with either H_2S or its radical HS^- to form sulfinyl nitrite ($HSNO_2$ or HS(O)NO). HS^- can also form HSOH upon reacting with $ONOO^-$. Other NO donors such as DD1, DEA NONOate, or Spermine NONOate are known to react with HS^- to form an unknown adduct. Sodium nitroprusside (SNP) ($Na_2[Fe(CN)_5\ NO]$) reacts with H_2S to form nitroxyl (HNO) and an intermediate $[(CN)_5FeN(O)SH]^{3-}$.

Molecular Strategies for Targeting Antioxidants to Mitochondria: Therapeutic Implications

Nadezda Apostolova and Victor M. Victor

Antioxidants & Redox Signaling, Vol. 22, No. 8, March 2015: 686-729.
Abstract | Full Text HTML | Full Text PDF (1794 KB) | Full Text PDF with Links (1290 KB)

Nature Volume 518 Number 7539 pp274-450

Evolution of Darwin's finches and their beaks revealed by genome sequencing

Sangeet Lamichhaney, Jonas Berglund, Markus Sällman Almén, Khurram Maqbool, Manfred Grabherr
Comprehensive genome sequencing of 120 individuals representing all of the Darwin's finch species and two close relatives reveals important discrepancies with morphology-based taxonomy, widespread hybridization, and a gene, *ALX1*, underlying variation in beak shape.

Archives of Biochemistry and Biophysics: Alert 17 February-23 February
Enhancing the heat stability and kinetic parameters of the maize endosperm ADP-glucose pyrophosphorylase using iterative saturation mutagenesis Original Research Article
Pages 28-37
Susan K. Boehlein, Janine R. Shaw, Jon D. Stewart, Bradford Sullivan, L. Curtis Hannah

Journal of Plant Physiology: Alert 16 February-22 February
Modulation of alternative oxidase to enhance tolerance against cold stress of chickpea by chemical treatments Original Research Article *Pages 95-101*
Serkan Erdal, Mucip Genisel, Hulya Turk, Rahmi Dumlupinar, Yavuz Demir

Fermentation and alternative oxidase contribute to the action of amino acid biosynthesis-inhibiting herbicides Original Research Article *Pages 102-112*
Amaia Zulet, Miriam Gil-Monreal, Ana Zabalza, Joost T. van Dongen, Mercedes Royuela

Parameters of photosynthetic energy partitioning Review Article
Pages 131-147 Dušan Lazár

Gersch M, Famulla K, Dahmen M, GÃ¶bl C, Malik I, Richter K, Korotkov VS, Sass P, RÃ¼bsamen-Schaeff H, Madl T, BrÃ¶tz-Oesterhelt H, Sieber SA.
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