Patrick

1. Role of sulfiredoxin as a peroxiredoxin-2 denitrosylase in human iPSC-derived dopaminergic neurons

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Abstract

Nitric oxide (NO) regulates plant growth and development as well as responses to stress that enhanced its endogenous production. Arabidopsis plants exposed to a pulse of exogenous NO gas were used for untargeted global metabolomic analyses thus allowing the identification of metabolic processes affected by NO. At early time points after treatment, NO scavenged superoxide anion and induced the nitration and the S-nitrosylation of proteins. These events preceded an extensive though transient metabolic reprogramming at 6 h after NO treatment, which included enhanced levels of polyamines, lipid catabolism and accumulation of phospholipids, chlorophyll breakdown, protein and nucleic acid turnover and increased content of sugars. Accordingly, lipid-related structures such as root cell membranes and leaf cuticle altered their permeability upon NO treatment. Besides, NO-treated plants displayed degradation of starch granules, which is consistent with the increased sugar content observed in the metabolomic survey. The metabolic profile was restored to baseline levels at 24 h post-treatment, thus pointing up the plasticity of plant metabolism in response to nitroxidative stress conditions.

2. Nitric oxide triggers a transient metabolic reprogramming in Arabidopsis

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Nitric oxide (NO) regulates plant growth and development as well as responses to stress that enhanced its endogenous production. Arabidopsis plants exposed to a pulse of exogenous NO gas were used for untargeted global metabolomic analyses thus allowing the identification of metabolic processes affected by NO. At early time points after treatment, NO scavenged superoxide anion and induced the nitration and the S-nitrosylation of proteins. These events preceded an extensive though transient metabolic reprogramming at 6 h after NO treatment, which included enhanced levels of polyamines, lipid catabolism and accumulation of phospholipids, chlorophyll breakdown, protein and nucleic acid turnover and increased content of sugars. Accordingly, lipid-related structures such as root cell membranes and leaf cuticle altered their permeability upon NO treatment. Besides, NO-treated plants displayed degradation of starch granules, which is consistent with the increased sugar content observed in the metabolomic survey. The metabolic profile was restored to baseline levels at 24 h post-treatment, thus pointing up the plasticity of plant metabolism in response to nitroxidative stress conditions.

Minsoo

1. Plant Cell. 2016 Oct 19. pii: tpc.00398.2016. [Epub ahead of print]

The reverse-transcriptase/RNA-maturase protein MatR is required for the splicing of various group II introns in Brassicaceae mitochondria.

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Group II introns are large catalytic RNAs that are ancestrally related to nuclear spliceosomal introns. Sequences corresponding to group II RNAs are found in many prokaryotes and are particularly prevalent within plants organellar genomes. Proteins encoded within the introns themselves (maturases) facilitate the splicing of their own host pre-RNAs. Mitochondrial introns in plants have diverged considerably in sequence and have lost their maturases. In angiosperms, only a single maturase has been retained in the mitochondrial DNA: the matR gene found within NADH dehydrogenase 1 (nad1) intron 4. Its conservation across land plants and RNA editing events, which restore conserved amino acids, indicates that matR encodes a functional protein. However, the biological role of MatR remains unclear. Here, we performed an in vivo investigation of the roles of MatR in Brassicaceae. Directed-knockdown of matR expression via synthetically designed ribozymes altered the processing of various introns, including nad1 i4. Pull-down experiments further indicated that MatR is associated with nad1 i4 and several other intron-containing pre-mRNAs. MatR may thus represent an intermediate link in the gradual evolutionary transition from the intron-specific maturases in bacteria into their versatile spliceosomal descendants in the nucleus. The similarity between maturases and the core spliceosomal Prp8 protein further support this intriguing theory.

2. Plant Physiol. 2016 Oct 27. pii: pp.01519.2016. [Epub ahead of print]

Plant specific Preprotein and Amino Acid Transporter proteins are required for tRNA import into mitochondria.

Murcha M(1), Kubiszewski-Jakubiak S(2), Teixeira PF(3), Gügel IL(4), Kmiec B(3),

Narsai R(5), Ivanova A(6), Megel C(7), Schock A(4), Kraus S(4), Berkowitz O(8), Glaser E(9), Philippar K(10), Drouard L(11), Soll J(12), Whelan J(13).

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A variety of eukaryotes, in particular plants, do not contain the required number of tRNA to support the translation of mitochondria-encoded genes and thus need to import tRNA from the cytosol. This study identified two Arabidopsis thaliana proteins, Tric1 and Tric2, which on simultaneous inactivation by T-DNA insertion lines displayed a severely delayed and chlorotic growth phenotype, and significantly reduced tRNA import capacity into isolated mitochondria. The predicted tRNA binding domain of Tric1 and Tric2, a sterile-α-motif at the C-terminal end of the protein was required to restore tRNA uptake ability in mitochondria of complemented plants. The purified predicted tRNA binding domain binds the T-arm of the tRNA for alanine, with conserved lysine residues required for binding. T-DNA inactivation of both Tric proteins further resulted in an increase in the in vitro rate of in organello protein synthesis, which was mediated by a re-organisation of the nuclear transcriptome, in particular of genes encoding a variety of proteins required for mitochondrial gene expression at both the transcriptional and translational levels. The characterization of Tric1/2 provides mechanistic insight into the process of tRNA import into mitochondria and supports the theory that the tRNA import pathway has resulted from the repurposing of a pre-existing protein import apparatus.

3. J Exp Bot. 2016 Nov;67(21):6061-6075. Epub 2016 Oct 6.

Characterization of a novel β-barrel protein (AtOM47) from the mitochondrial outer membrane of Arabidopsis thaliana.

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In plant cells, mitochondria are major providers of energy and building blocks for growth and development as well as abiotic and biotic stress responses. They are encircled by two lipid membranes containing proteins that control mitochondrial function through the import of macromolecules and metabolites. Characterization of a novel β-barrel protein, OUTER MEMBRANE PROTEIN 47 (OM47), unique to the green lineage and related to the voltage-dependent anion channel (VDAC) protein family, showed that OM47 can complement a VDAC mutant in yeast. Mutation of OM47 in Arabidopsis thaliana by T-DNA insertion had no effect on the import of proteins, such as the β-barrel proteins translocase of the outer membrane 40 (TOM40) or sorting and assembly machinery 50 (SAM50), into mitochondria. Molecular and physiological analyses revealed a delay in chlorophyll breakdown, higher levels of starch, and a delay in the induction of senescence marker genes in the mutant lines. While there was a reduction of >90% in OM47 protein in mitochondria isolated from 3-week-old om47 mutants, in mitochondria isolated from 8-week-old plants OM47 levels were similar to that of the wild type. This recovery was achieved by an up-regulation of OM47 transcript abundance in the mutants. Combined, these results highlight a role in leaf senescence for this plant-specific β-barrel protein, probably mediating the recovery and recycling of chloroplast breakdown products by transporting metabolic intermediates into and out of mitochondria.

Ian

Regulation of Anticancer Styrylpyrone Biosynthesis in the Medicinal Mushroom *Inonotus obliquus* Requires Thioredoxin Mediated Transnitrosylation of S-nitrosoglutathione Reductase

Yanxia Zhao, Meihong He, Jianing Ding, Qi Xi, Gary J. Loake & Weifa Zheng

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Mary

Reconstitution of a *Mycobacterium tuberculosis* proteostasis network highlights essential cofactor interactions with chaperone DnaK

PNAS, October 25, 2016

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During host infection, *Mycobacterium tuberculosis* (Mtb) encounters several types of stress that impair protein integrity, including reactive oxygen and nitrogen species and chemotherapy. The resulting protein aggregates can be resolved or degraded by molecular machinery conserved from bacteria to eukaryotes. Eukaryotic Hsp104/Hsp70 and their bacterial homologs ClpB/DnaK are ATP-powered chaperones that restore toxic protein aggregates to a native folded state. DnaK is essential in *Mycobacterium smegmatis*, and ClpB is involved in asymmetrically distributing damaged proteins during cell division as a mechanism of survival in Mtb, commending both proteins as potential drug targets. However, their molecular partners in protein reactivation have not been characterized in mycobacteria. Here, we reconstituted the activities of the Mtb ClpB/DnaK bichaperone system with the cofactors DnaJ1, DnaJ2, and GrpE and the small heat shock protein Hsp20. We found that DnaJ1 and DnaJ2 activate the ATPase activity of DnaK differently. A point mutation in the highly conserved HPD motif of the DnaJ proteins abrogates their ability to activate DnaK, although the DnaJ2 mutant still binds to DnaK. The purified Mtb ClpB/DnaK system reactivated a heat-denatured model substrate, but the DnaJ HPD mutants inhibited the reaction. Finally, either DnaJ1 or DnaJ2 is required for mycobacterial viability, as is the DnaK-activating activity of a DnaJ protein. These studies lay the groundwork for strategies to target essential chaperone–protein interactions in Mtb, the leading cause of death from a bacterial infection.

Keith

The C-terminal Extension of *Mycobacterium tuberculosis* Hsp16.3 Regulates its Oligomerization, Subunit Exchange Dynamics and Chaperone Function

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Mycobacterium tuberculosis is a human pathogen that secretes a major immunodominant antigen namely Hsp16.3 throughout the course of infection. It belongs to small heat shock protein family and exhibits molecular chaperone function which is important for the growth and survival of *M. tuberculosis* in host cell macrophages. The importance of the N-terminal region on the structure and chaperone function of Hsp16.3

is well understood. However, the effect of the C-terminal region on these properties is far from clear. Therefore, we cloned, over-expressed and purified wild-type and seven C-terminal truncated mutant proteins of Hsp16.3. Mutants with deletions of 1 and 2 C-terminal extension (CTE) residues have a structure and chaperone function similar to wild-type protein. Intriguingly, deletion of 3 residues from CTE triggers perturbation of the tertiary structure, dissociation of oligomeric assembly (dodecamer to octamer/dimer), enhancement in subunit exchange dynamics as well as improvement in the chaperone function of Hsp16.3. Interestingly, these structural modulations (except oligomeric dissociation) as well as chaperoning strength reaches its apex upon truncation of the entire CTE (**1**41RSTN144). Further deletions from C-terminal region beyond the CTE increases only the degree of oligomeric dissociation and the complete removal of this region make the protein into dimer. Overall, our study suggests a “new structural element” in the C-terminal region i.e. C-terminal extension which plays an important role in the oligomerization, subunit exchange dynamics and chaperone

BAG3 is a modular, scaffolding protein that physically links heat shock protein 70 (Hsp70) to the small heat shock proteins

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Small heat shock proteins (sHsps) are a family of ATP-independent molecular chaperones that are important for binding and stabilizing unfolded proteins. In this task, the sHsps have been proposed to coordinate with ATP-dependent chaperones, including heat shock protein 70 (Hsp70). However, it isn't yet clear how these two important components of the chaperone network are linked. We report that the Hsp70 co-chaperone, BAG3, is a modular, scaffolding factor to bring together sHsps and Hsp70s. Using domain deletions and point mutations, we confirmed that BAG3 uses both of its IPV motifs to interact with sHsps, including Hsp27 (HspB1), αB-crystallin (HspB5), Hsp22 (HspB8) and Hsp20 (HspB6). BAG3 does not appear to be a passive scaffolding factor; rather, its binding promoted de-oligomerization of Hsp27, likely by competing for the self-interactions that normally stabilize large oligomers. BAG3 bound to Hsp70 at the same time as either Hsp22, Hsp27 or αB-crystallin, suggesting that it might physically bring the chaperone families together into a complex. Indeed, addition of BAG3 coordinated the ability of Hsp22 and Hsp70 to refold denatured luciferase *in vitro*. Together, these results suggest that BAG3 physically and functionally links Hsp70 and sHsps.

Elizabeth

Nov 29

Plant Journal

IRE1, a component of the unfolded protein response signaling pathway, protects pollen development in Arabidopsis from heat stress (pages 193–204)
Yan Deng, Renu Srivastava, Teagen D. Quilichini, Haili Dong, Yan Bao, Harry T. Horner and Stephen H. Howell
Version of Record online: 26 AUG 2016 | DOI: 10.1111/tpj.13239
Significance Statement
The Unfolded Protein Response (UPR) in plants is activated by various environmental stresses during vegetative development, but is constitutively active in flowers. Here we show that a major component of the UPR signaling pathway, IRE1, a dual protein kinase/ribonuclease, protects plant reproduction, the most vulnerable stage in a plant's life cycle and that the ribonuclease function of IRE1 was critical for this protection. The *ire1* mutant is male sterile at elevated temperatures because the tapetum in its anthers fails to properly deposit the pollen coat.

The Vigna unguiculata Gene Expression Atlas (VuGEA) from de novo assembly and quantification of RNA-seq data provides insights into seed maturation mechanisms (pages 318–327)
Shaolun Yao, Chuan Jiang, Ziyue Huang, Ivone Torres-Jerez, Junil Chang, Heng Zhang, Michael Udvardi, Renyi Liu and Jerome Verdier
Version of Record online: 14 NOV 2016 | DOI: 10.1111/tpj.13279
Significance Statement
Black-eyed peas are widely grown in semi-arid regions and have nutritious seeds, but there are limited genomics resources for this crop. To facilitate analyses, we generated a gene expression atlas, with a specific focus on pod and seed development.

Rapid identification of lettuce seed germination mutants by bulked segregant analysis and whole genome sequencing (pages 345–360)
Heqiang Huo, Isabelle M. Henry, Eric R. Coppoolse, Miriam Verhoef-Post, Johan W. Schut, Han de Rooij, Aat Vogelaar, Ronny V.L. Joosen, Leo Woudenberg, Luca Comai and Kent J. Bradford
Version of Record online: 15 SEP 2016 | DOI: 10.1111/tpj.13267
Significance Statement
Bulked segregant analysis and whole genome sequencing are more efficient than conventional mapping strategies, which require large populations. To demonstrate the advantages of this approach, we used two independent allelic mutants to identify the causal mutations conferring thermotolerant seed germination in lettuce

New BAR tools for mining expression data and exploring *Cis*-elements in *Arabidopsis thaliana* (pages 490–504)
Ryan S. Austin, Shu Hiu, Jamie Waese, Matthew Ierullo, Asher Pasha, Ting Ting Wang, Jim Fan, Curtis Foong, Robert Breit, Darrell Desveaux, Alan Moses and Nicholas J. Provart
Version of Record online: 5 OCT 2016 | DOI: 10.1111/tpj.13261

Significance Statement
Identifying gene sets that are specifically expressed in certain tissues or in response to an environmental stimulus is useful for designing reporter constructs, for generating gene expression markers, and for understanding gene regulatory networks. Here we describe two tools for easily identifying such gene sets and demonstrate the usefulness of these tools using genotoxic stress and pathogen response as test cases.

Plant Cell
The Plastoglobule-Localized Metallopeptidase PGM48 is a Positive Regulator of Senescence in Arabidopsis thaliana
Nazmul H. Bhuiyan, Giulia Friso, Elden Rowland, Kristina Majsec, and Klaas Jan van Wijk
Plant Cell 2016 tpc.16.00745; Advance Publication November 28, 2016;
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