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Generation of nitric oxide by olive (Olea europaea L.) pollen during in vitro germination and assessment of the S-nitroso- and nitro-proteomes by computational predictive methods

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Abstract

Nitric oxide is recognized as a signaling molecule involved in a broad range of physiological processes in plants including sexual reproduction. NO has been detected in the pollen grain at high levels and regulates pollen tube growth. Previous studies demonstrated that NO as well as ROS are produced in the olive reproductive tissues in a stage- and tissue-specific manner. The aim of this study was to assess the production of NO throughout the germination of olive (Olea europaea L.) pollen in vitro. The NO fluorescent probe DAF-2DA was used to image NO production in situ, which was correlated to pollen viability. Moreover, by means of a fluorimetric assay we showed that growing pollen tubes release NO. GSNO -a mobile reservoir of NO, formed by the Snitrosylation of NO with reduced glutathione (GSH) - was for the first time detected and quantified at different stages of pollen tube growth using a LC-ES/MS analysis. Exogenous NO donors inhibited both pollen germination and pollen tube growth and these effects were partially reverted by the specific NO-scavenger c-PTIO. However, little is known about how NO affects the germination process. With the aim of elucidating the putative relevance of protein S-nitrosylation and Tyr-nitration as important post-translational modifications in the development and physiology of the olive pollen, a de novo assembled and annotated reproductive transcriptome from olive was challenged in silico for the putative capability of transcripts to become potentially modified by S-nitrosylation/Tyr-nitration according to well-established criteria. Numerous gene products with these characteristics were identified, and a broad discussion as regards to their potential role in plant reproduction was built after their functional classification. Moreover, the importance of both Snitrosylation/Tyr-nitrations was experimentally assessed and validated by using Western blotting, immunoprecipitation and proteomic approaches.

Corey

Abstract

Thiol-dependent redox regulation of enzyme activity plays a central role in the rapid acclimation of chloroplast metabolism to ever fluctuating light availability. This regulatory mechanism relies on ferredoxin (Fdx) reduced by the photosynthetic electron transport chain, which fuels reducing power to thioredoxins (Trxs) via a Fdx-dependent Trx reductase (FTR). In addition, chloroplasts harbour an NADPH-dependent Trx reductase, which has a joint Trx domain at the C-terminus, termed NTRC. Thus, a relevant issue in chloroplast function is to establish the relationship between these two redox systems and its impact on plant development. To address this issue we generated Arabidopsis thaliana mutants combining the deficiency of NTRC with those of Trxs f, which participate in metabolic redox regulation, and Trx x, which has antioxidant function. The ntrc-trxf1f2 and, to a lower extent, the ntrc-trxx mutants showed severe growth retarded phenotypes, decreased photosynthesis performance and almost abolished light-dependent reduction of fructose-1,6-bisphosphatase. Moreover, the combined deficiency of both redox systems provokes aberrant chloroplast ultrastructure. Remarkably, both the ntrc-trxf1f2 and the ntrc-trxx mutants showed high mortality at the seedling stage, which was overcome by addition of an exogenous carbon source. Based on these results, we propose that NTRC plays a pivotal role in chloroplast redox regulation, being necessary for the activity of diverse Trxs with unrelated functions. The interaction between the two thiol-redox systems is indispensable to sustain photosynthesis performed by cotyledons chloroplasts, which is essential for early plant development.

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Eukaryotic translation initiation factor 2B-beta(eIF2Bβ), a new class of plant virus resistance gene

Summary

Recessive resistances to plant viruses in the *Potyvirus* genus have been found to be based on mutations in the plant eukaryotic translation initiation factors, *eIF4E* and *eIF4G* or their isoforms. Here we report that natural, monogenic recessive resistance to the *Potyvirus* Turnip mosaic virus (TuMV) has been found in a number of mustard (*Brassica juncea*) accessions. Bulked segregant analysis and sequencing of resistant and susceptible plant lines indicated the resistance is controlled by a single recessive

gene, recessive TuMV resistance 03 (retr03), an allele of the eukaryotic translation initiation factor 2B-beta (eIF2B β). Silencing of eIF2B β in a TuMV-susceptible mustard plant line and expression of eIF2B β from a TuMV-susceptible line in a TuMV-resistant mustard plant line confirmed the new resistance mechanism. A functional copy of a specific allele of eIF2B β is required for efficient TuMV infection. eIF2B β represents a new class of virus resistance gene conferring resistance to any pathogen. eIF2B acts as a guanine nucleotide exchange factor (GEF) for its GTP-binding protein partner eIF2 via interaction with eIF2·GTP at an early step in translation initiation. Further genotyping indicated that a single non-synonymous substitution (A120G) in the N-terminal region of eIF2B β was responsible for the TuMV resistance. A reproducible marker has been developed, facilitating marker-assisted selection for TuMV resistance in B. juncea. Our findings provide a new target for seeking natural resistance to potyviruses and new opportunities for the control of potyviruses using genome editing techniques targeted on $eIF2B\beta$.

1. Cell. 2017 Jun 15;169(7):1249-1262.e13. doi: 10.1016/j.cell.2017.05.036.

Microbial Genetic Composition Tunes Host Longevity.

Han B(1), Sivaramakrishnan P(2), Lin CJ(1), Neve IAA(1), He J(3), Tay LWR(3), Sowa JN(1), Sizovs A(4), Du G(3), Wang J(4), Herman C(5), Wang MC(6).

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Homeostasis of the gut microbiota critically influences host health and aging. Developing genetically engineered probiotics holds great promise as a new therapeutic paradigm to promote healthy aging. Here, through screening 3,983 Escherichia coli mutants, we discovered that 29 bacterial genes, when deleted, increase longevity in the host Caenorhabditis elegans. A dozen of these bacterial mutants also protect the host from age-related progression of tumor growth and amyloid-beta accumulation. Mechanistically, we discovered that five bacterial mutants promote longevity through increased secretion of the polysaccharide colanic acid (CA), which regulates mitochondrial dynamics and unfolded protein response (UPR(mt)) in the host. Purified CA polymers are sufficient to promote longevity via ATFS-1, the host UPR(mt)-responsive transcription factor.
Furthermore, the mitochondrial changes and longevity effects induced by CA are conserved across different species. Together, our results identified molecular targets for developing pro-longevity microbes and a bacterial metabolite acting on host mitochondria to promote longevity.

2. Science. 2017 Jun 15. pii: eaan1052. doi: 10.1126/science.aan1052. [Epub ahead of print]

Ratchet-like polypeptide translocation mechanism of the AAA+ disaggregase Hsp104.

Gates SN(1)(2), Yokom AL(1)(2), Lin J(3), Jackrel ME(3), Rizo AN(2), Kendsersky NM(3)(4), Buell CE(3), Sweeny EA(3), Mack KL(3)(5), Chuang E(3)(4), Torrente MP(3)(6), Su M(1), Shorter J(3)(4)(5), Southworth DR(7)(2).

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Hsp100 polypeptide translocases are conserved AAA+ machines that maintain proteostasis by unfolding aberrant and toxic proteins for refolding or proteolytic degradation. The Hsp104 disaggregase from S. cerevisiae solubilizes stress-induced amorphous aggregates and amyloid. The structural basis for substrate recognition and translocation is unknown. Using a model substrate (casein), we report cryo-EM structures at near-atomic resolution of Hsp104 in different translocation states. Substrate interactions are mediated by conserved, pore-loop tyrosines that contact an 80 Å-long unfolded polypeptide along the axial channel. Two protomers undergo a ratchet-like conformational change that advances pore-loop-substrate interactions by two-amino acids. These changes are coupled to activation of specific ATPase sites and, when transmitted around the hexamer, reveal a processive rotary translocation mechanism and a remarkable flexibility in Hsp104-catalyzed disaggregation.

Proline isomerization in the C-terminal region of HSP27

Cell Stress Chaperones. 2017 Jul;22(4):639-651

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In mammals, small heat-shock proteins (sHSPs) typically assemble into interconverting, polydisperse oligomers. The dynamic exchange of sHSP oligomers is regulated, at least in part, by molecular interactions between the α -crystallin domain and the C-terminal region(CTR). Here we report solution-state nuclear magnetic resonance (NMR) spectroscopy investigations of the conformation and dynamics of the disordered and flexible CTR of human HSP27, a systemically expressed sHSP. We observed multiple NMR signals for residues in the vicinity of proline 194, and we determined that, while all observed forms are highly disordered, the extra resonances arise from cis-trans peptidyl-prolyl isomerization about the G193-P194 peptide bond. The cis-P194 state is populated to near 15% at physiological temperatures, and, although both cis- and trans-P194 forms of the CTR are flexible and dynamic, both states show a residual but differing tendency to adopt β -strand conformations. In NMR spectra of an isolated CTR peptide, we observed similar evidence for isomerization involving proline 182, found within the IPI/V motif. Collectively, these data indicate a potential role for cis-trans prolineisomerization in regulating the oligomerization of sHSPs.

Dynamical Structures of Hsp70 and Hsp70-Hsp40 Complexes.

Alderson TR¹, Kim JH², Markley JL².

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2 National Magnetic Resonance Facility at Madison, Biochemistry Department, University of Wisconsin-Madison, Madison, WI 53706, USA.

Protein misfolding and aggregation are pathological events that place a significant amount of stress on the maintenance of protein homeostasis (proteostasis). For prevention and repair of protein misfolding and aggregation, cells are equipped with robust mechanisms that mainly rely on molecular chaperones. Two classes of molecular chaperones, heat shock protein 70 kDa (Hsp70) and Hsp40, recognize and bind to misfolded proteins, preventing their toxic biomolecular aggregation and enabling refolding or targeted degradation. Here, we review the current state of structural biology of Hsp70 and Hsp40-Hsp70 complexes and examine the link between their structures, dynamics, and functions. We highlight the power of nuclear magnetic resonance spectroscopy to untangle complex relationships behind molecular chaperones and their mechanism(s) of action.

Elizabeth

June 16

Science 15 June 2017; Vol. 356, No. 6343

Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi

Yina Jiang , Wanxiao Wang, Qiujin Xie, Na Liu, Lixia Liu, Dapeng Wang, Xiaowei Zhang, Chen Yang, Xiaoya Chen, Dingzhong Tang, Ertao Wang

Arbuscular mycorrhizal (AM) fungi facilitate plant uptake of mineral nutrients and draw organic nutrients from the plant. Organic nutrients are thought to be supplied primarily in the form of sugars. Here we show that the AM fungus *Rhizophagus irregularis* is a fatty acid auxotroph and that fatty acids synthesized in the host plants are transferred to the fungus to sustain mycorrhizal colonization. The transfer is dependent on RAM2 (REQUIRED FOR ARBUSCULAR MYCORRHIZATION 2) and the ATP binding cassette transporter—mediated plant lipid export pathway. We further show that plant fatty acids can be transferred to the pathogenic fungus *Golovinomyces cichoracerum* and are required for colonization by pathogens. We suggest that the mutualistic mycorrhizal and pathogenic fungi similarly recruit the fatty acid biosynthesis program to facilitate host invasion.

Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant

Leonie H. Luginbuehl, Guillaume N. Menard, Smita Kurup, Harrie Van Erp, Guru V. Radhakrishnan, Andrew Breakspear, Giles E. D. Oldroyd, Peter J. Eastmond

Plants form beneficial associations with arbuscular mycorrhizal fungi, which facilitate nutrient acquisition from the soil. In return, the fungi receive organic carbon from the plants. The transcription factor RAM1 (REQUIRED FOR ARBUSCULAR MYCORRHIZATION 1) is crucial for this symbiosis, and we demonstrate that it is required and sufficient for the induction of a lipid biosynthetic pathway that is expressed in plant cells accommodating fungal arbuscules. Lipids are transferred from the plant to mycorrhizal fungi, which are fatty acid auxotrophs, and this lipid export requires the glycerol-3-phosphate acyltransferase RAM2, a direct target of RAM1. Our work shows that in addition

to sugars, lipids are a major source of organic carbon delivered to the fungus, and this is necessary for the production of fungal lipids.

Physiologia Plantarum Content Alert (New Articles)

Minireviews

Non-housekeeping, non-essential GroEL (chaperonin) has acquired novel structure and function beneficial under stress in cyanobacteria

Hitoshi Nakamoto and Kouji Kojima

Accepted manuscript online: 9 JUN 2017 04:20AM EST | DOI: 10.1111/ppl.12595

Plant Breeding Content Alert: 136, 3 (June 2017)

The use of genetic, manual and chemical methods to control pollination in vegetable hybrid seed production: a review (pages 287–299)

Noemí Colombo and Claudio Rómulo Galmarini

Version of Record online: 28 MAR 2017 | DOI: 10.1111/pbr.12473

Breeding for improved drought tolerance in Chickpea (Cicer arietinm L.) (pages 300–318)

FREE Muhammad Amir Maqbool, Muhammad Aslam and Hina Ali Version of Record online: 10 APR 2017 | DOI: 10.1111/pbr.12477

The EMBO Journal Table of Contents for 14 June 2017; Vol. 36, No. 12

Getting stress out of stressed-out stress granules

Pratibha Siwach and Daniel Kaganovich Published online 23.05.2017 Accumulation of misfolded proteins, including ALS disease-associated variants, in cytoplasmic stress granules induces their conversion from a functional, dynamic state towards solid, pathological aggregates targeted by autophagy.

http://EMBOJ.embopress.org/content/36/12/1647?etoc

An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function

Daniel Mateju, Titus M Franzmann, Avinash Patel, Andrii Kopach, Edgar E

Boczek, Shovamayee Maharana, Hyun O Lee, Serena Carra, Anthony A Hyman,

and Simon Alberti Published online 04.04.2017

The presence of misfolded protein, such as ALS-linked variants of SOD1, in stress granules (SGs) leads to a gradual loss of dynamics, suggesting that SG loss-of-function could be a factor in aggregation-linked disease.

http://EMBOJ.embopress.org/content/36/12/1669?etoc

Nature Reviews Microbiology contents July 2017 Volume 15 Number 7 pp 381-447 Oxidative stress, protein damage and repair in bacteria

Benjamin Ezraty, Alexandra Gennaris, Frédéric Barras & Jean-François Collet p385 | doi:10.1038/nrmicro.2017.26

Oxidative damage can have a devastating effect on the structure and activity of proteins, leading to cell death. This Review discusses how bacteria repair oxidized proteins and highlights the importance of these repair systems in physiology and virulence.

Abstract | Full Text | PDF

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Non-housekeeping, non-essential GroEL (chaperonin) has acquired novel structure and function beneficial under stress in cyanobacteria.

Physiol Plant. 2017 Jun 9; [Epub ahead of print] PMID: 28597961 [PubMed - as supplied by publisher]

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Plant Cell Advance Publication

Technology Turbocharges Functional Genomics

C. Robin Buell and Daniel F. Voytas

Plant Cell 2017 tpc.17.00443; Advance Publication June 5, 2017; doi:10.1105/tpc.17.00443 OPEN

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Cellulose synthesis and cell expansion are regulated by different mechanisms in growing Arabidopsis Alexander A. Ivakov, Anna Flis, Federico Apelt, Maximilian Fuenfgeld, Ulrike Scherer, Mark Stitt, Friedrich Kragler, Kris

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The UPR^{ER}: Sensor and Coordinator of Organismal Homeostasis Review Article

Pages 761-771

Ashley E. Frakes, Andrew Dillin

AMPK: Mechanisms of Cellular Energy Sensing and Restoration of Metabolic Balance Review Article

Pages 789-800

Daniel Garcia, Reuben J. Shaw

Nature Structural & Molecular Biology Contents: 2017 Volume #24 pp 491 - 553

RNA base-pairing drives phase transitions p502

Anke Sparmann

doi:10.1038/nsmb.3425

Human CTP synthase filament structure reveals the active enzyme conformation pp507 - 514

Eric M Lynch, Derrick R Hicks, Matthew Shepherd, James A Endrizzi, Allison Maker et al.

doi:10.1038/nsmb.3407

The human enzyme CTP synthase forms polymeric filaments with increased catalytic activity, in contrast to the inactive filaments formed by bacterial CTP synthase. Cryo-EM and crystallographic analyses explain the structural bases for those different behaviors.

Current Opinion in Genetics & Development: Alert 19 May-26 May

Designer protein disaggregases to counter neurodegenerative disease Review Article

Pages 1-8 James Shorter

Ribosome profiling and dynamic regulation of translation in mammals Review Article

Pages 120-127

Cédric Gobet, Felix Naef

The Plant Journal Content Alert: 90, 5 (June 2017)

Translating knowledge about abiotic stress tolerance to breeding programmes (pages 898–917)

Matthew Gilliham, Jason A. Able and Stuart J. Roy

Version of Record online: 8 FEB 2017 | DOI: 10.1111/tpj.13456

Significance statement

Abiotic stress routinely reduces the yield of all our major crops. However, most crop-breeding programs do not specifically target improved abiotic stress tolerance. In this review we highlight how breeding for abiotic stress tolerance can improve crop productivity. We discuss barriers to research translation and propose that funding structures that serve both fundamental research and breeding are needed to meet food security goals in the face of a changing climate.

Improving phosphorus use efficiency: a complex trait with emerging opportunities (pages 868–885)

Sigrid Heuer, Roberto Gaxiola, Rhiannon Schilling, Luis Herrera-Estrella, Damar López-Arredondo, Matthias Wissuwa, Emmanuel Delhaize and Hatem Rouached

Version of Record online: 3 FEB 2017 | DOI: 10.1111/tpj.13423

Significance Statement

Phosphorus is an essential plant nutrient and its deficiency severely limits agricultural yields. Phosphorus fertilizers are increasingly required to obtain high yields and prevent soil degradation. Here we review our current understanding of plant responses to phosphorus deficiency, emphasizing molecular and technological approaches that are being used to develop crops with enhanced phosphorus use efficiency

Reactive oxygen species, abiotic stress and stress combination (pages 856–867)

Feroza K. Choudhury, Rosa M. Rivero, Eduardo Blumwald and Ron Mittler

Version of Record online: 1 NOV 2016 | DOI: 10.1111/tpj.13299

Significance Statement

Reactive oxygen species (ROS) primarily function as signal transduction molecules that regulate different pathways during acclimation to stress, but are also toxic byproducts of stress metabolism. Different subcellular compartments contain different sets of ROS-producing and ROS-scavenging pathways, and thus ROS levels can have different signatures in different cellular compartments. Here we review recent work on ROS and abiotic stress, and propose that different stresses, such as drought, heat, salinity and high light, result in different ROS signatures that determine the specificity of the acclimation response to a particular stress.

Differences and commonalities of plant responses to single and combined stresses (pages 839–855)

Haina Zhang and Uwe Sonnewald

Version of Record online: 2 MAY 2017 | DOI: 10.1111/tpi.13557

Significance Statement

In natural or agricultural environments, plants are constantly exposed to a wide range of biotic and abiotic stress conditions. In this review, we will summarize current knowledge on plant responses to different individual and combined stress conditions and try to find a common thread potentially underlying these responses

Eukaryotic translation initiation factor 2B-beta (eIF2Bβ), a new class of plant virus resistance gene (pages 929–940)

Jannat Shopan, Haipeng Mou, Lili Zhang, Changtong Zhang, Weiwei Ma, John A. Walsh, Zhongyuan Hu, Jinghua Yang and Mingfang Zhang

Version of Record online: 4 APR 2017 | DOI: 10.1111/tpj.13519

Significance Statement

We identified for the first time the $eIF2B\beta$ gene conferring resistance to Turnip mosaic virus in *B. juncea*, representing a new class of plant virus resistance gene. Our findings provide a new target for seeking natural resistance to potyviruses and new opportunities for the control of potyviruses using genome editing techniques targeted on $eIF2B\beta$.

Effects of eIFiso4G1 mutation on seed oil biosynthesis (pages 966–978)

Qiang Li, Wenyun Shen, Qian Zheng, Yifang Tan, Jie Gao, Jinxiong Shen, Yangdou Wei, Ljerka Kunst and Jitao Zou Version of Record online: 29 APR 2017 | DOI: 10.1111/tpj.13522

Significance Statement

Seed oil biosynthesis requires coordination of multiple lipid pathways at both the transcriptional and metabolic levels, occurring in both the plastids and the cytosol. Here, we characterized an Arabidopsis mutant with a novel seed fatty acid phenotype. We show that this mutation, in a gene encoding a translation initiation factor, perturbs plastid transcription and leads to readjusted cytosolic and plastidic metabolic coordination in storage lipid biosynthesis, thereby impacting seed oil composition.

Nature Microbiology - Table of Contents alert, Volume 2, June 2017

Widespread distribution of encapsulin nanocompartments reveals functional diversity

Tobias W. Giessen & Pamela A. Silver

Nature Microbiology 2, Article number: 17029 | doi:10.1038/nmicrobiol.2017.29

Journal of Agronomy and Crop Sci... Content Alert (New Articles)

<u>Foliage-applied sodium nitroprusside and hydrogen peroxide improves resistance against terminal drought in</u> bread wheat

M. Farooq, A. Nawaz, M. A. M. Chaudhary and A. Rehman

Version of Record online: 24 MAY 2017 | DOI: 10.1111/jac.12215

Escandón M, Valledor L, Pascual J, Pinto G, Cañal MJ, Meijón M.

System-wide analysis of short-term response to high temperature in Pinus radiata.

J Exp Bot. 2017 Jun 22; PMID: 28645179 [PubMed - as supplied by publisher]

Bersweiler A, D'Autréaux B, Mazon H, Kriznik A, Belli G, Delaunay-Moisan A, Toledano MB, Rahuel-Clermont S. A scaffold protein that chaperones a cysteine-sulfenic acid in H<sub>2</sub>0<sub>2</sub> signaling.

Nat Chem Biol. 2017 Jun 19;. PMID: 28628095 [PubMed - as supplied by publisher]

Jimenez-Quesada MJ, Carmona R, Lima-Cabello E, Traverso JÃ

□, Castro

Generation of nitric oxide by olive (Olea europaea L.) pollen during in vitro germination and assessment of the S-nitroso- and nitro-pollen proteomes by computational predictive methods.

Nitric Oxide. 2017 Jun 20;. PMID: 28645873 [PubMed - as supplied by publisher]

Singh PK, Indoliya Y, Chauhan AS, Singh SP, Singh AP, Dwivedi S, Tripathi RD, Chakrabarty D. Nitric oxide mediated transcriptional modulation enhances plant adaptive responses to arsenic stress.

Sci Rep. 2017 Jun 15;7(1):3592.PMID: 28620222 [PubMed - in process]

Current Opinion in Plant Biology: Alert 14 June-21 June

Alternative electron transport pathways in photosynthesis: a confluence of regulation Review Article

Pages 78-86

Jean Alric, Xenie Johnson

The HSP90 chaperone machinery

Florian H. Schopf, Maximilian M. Biebl & Johannes Buchner

p345 | doi:10.1038/nrm.2017.20

The heat shock protein 90 (HSP90) chaperone machinery is a key regulator of proteostasis. Recent progress has shed light on the interactions of HSP90 with its clients and co-chaperones, and on their functional implications. This opens up new avenues for the development of drugs that target HSP90, which could be valuable for the treatment of cancers and protein-misfolding diseases.

Simsek, D. *et al*. The mammalian ribo-interactome reveals ribosome functional diversity and heterogeneity. Cell 169, 1051–1065 (2017)

Liu, Z. *et al.* Plasma membrane CRPK1-mediated phosphorylation of 14-3-3 proteins induces their nuclear import to fine-tune CBF signaling during cold response. Mol. Cell http://dx.doi.org/10.1016/j.molcel.2017.02.016 (2017)

Current Opinion in Cell Biology: Alert 13 June-20 June

An emerging role for the ribosome as a nexus for post-translational modifications Review Article

Pages 92-101

Deniz Simsek, Maria Barna

<u>Translational control and the cancer cell response to stress</u> Review Article

Pages 102-109

Nathaniel Robichaud, Nahum Sonenberg

The Plant Journal Content Alert: 91, 1 (July 2017)

<u>Emp10</u> encodes a mitochondrial PPR protein that affects the *CiS*-splicing of *nad2* intron 1 and seed development in maize (pages 132–144)

Manjun Cai, Shuzhen Li, Feng Sun, Qin Sun, Hailiang Zhao, Xuemei Ren, Yanxin Zhao, Bao-Cai Tan, Zuxin Zhang and Fazhan Qiu Version of Record online: 4 MAY 2017 | DOI: 10.1111/tpj.13551