Indu

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Minsoo

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Mitochondrial pleomorphy in plant cells is driven by contiguous ER dynamics.

Jaipargas EA(1), Barton KA(1), Mathur N(1), Mathur J(1).

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Mitochondria are pleomorphic, double membrane-bound organelles involved in cellular energetics in all eukaryotes. Mitochondria in animal and yeast cells are typically tubular-reticulate structures and several micro-meters long but in green plants they are predominantly observed as 0.2-1.5 μm punctae. While fission and fusion, through the coordinated activity of several conserved proteins, shapes mitochondria, the endoplasmic reticulum (ER) has recently been identified as an additional player in this process in yeast and mammalian cells. The mitochondria-ER relationship in plant cells remains largely uncharacterized. Here, through live-imaging of the entire range of mitochondria pleomorphy we uncover the underlying basis for the predominantly punctate mitochondrial form in plants. We demonstrate that mitochondrial morphology changes in response to light and cytosolic sugar levels in an ER mediated manner. Whereas, large ER polygons and low dynamics under dark conditions favor mitochondrial fusion and elongation, small ER polygons result in increased fission and predominantly small

mitochondria. Hypoxia also reduces ER dynamics and increases mitochondrial fusion
to produce giant mitochondria. By observing elongated mitochondria in normal plants and fission-impaired Arabidopsis nmt1-2 and drp3a mutants we also establish that thin extensions called matrixules and a beads-on-a-string mitochondrial phenotype are direct consequences of mitochondria-ER interactions.

2. Front Plant Sci. 2015 Oct 28;6:922.

Impacts of high ATP supply from chloroplasts and mitochondria on the leaf metabolism of Arabidopsis thaliana.

Liang C(1), Zhang Y(2), Cheng S(1), Osorio S(3), Sun Y(1), Fernie AR(2), Cheung CY(4), Lim BL(5).

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(1)School of Biological Sciences, The University of Hong Kong Pokfulam, Hong Kong. (2)Max Planck Institute of Molecular Plant Physiology Potsdam-Golm, Germany. (3)Departamento de Biología Molecular y Bioquímica, Instituto de Hortofruticultura Subtropical y Mediterranea, Universidad de Málaga-Consejo Superior de Investigaciones Científicas Málaga, Spain. (4)Department of Chemical and Biomolecular Engineering, National University of Singapore Singapore, Singapore. (5)School of Biological Sciences, The University of Hong Kong Pokfulam, Hong Kong ; State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong Shatin, Hong Kong.

Chloroplasts and mitochondria are the major ATP producing organelles in plant leaves. Arabidopsis thaliana **purple acid phosphatase 2 (AtPAP2) is a phosphatase
dually targeted to the outer membranes of both organelles and it plays a role in
the import of selected nuclear-encoded proteins into these two organelles.** Overexpression (OE) of AtPAP2 in A. thaliana accelerates plant growth and promotes flowering, seed yield, and biomass at maturity. Measurement of ADP/ATP/NADP(+)/NADPH contents in the leaves of 20-day-old OE and wild-type (WT)
lines at the end of night and at 1 and 8 h following illumination in a 16/8 h photoperiod revealed that **the ATP levels and ATP/NADPH ratios were significantly
increased in the OE line at all three time points.** The AtPAP2 OE line is therefore a good model to investigate the impact of high energy on the global molecular status of Arabidopsis. In this study, transcriptome, proteome, and metabolome profiles of the high ATP transgenic line were examined and compared with those of WT plants. A comparison of OE and WT at the end of the night provide valuable information on the impact of higher ATP output from mitochondria

on plant physiology, as mitochondrial respiration is the major source of ATP in the dark in leaves. Similarly, comparison of OE and WT following illumination will provide information on the impact of higher energy output from chloroplasts on plant physiology. OE of AtPAP2 was found to significantly affect the transcript and protein abundances of genes encoded by the two organellar genomes. For example, **the protein abundances of many ribosomal proteins encoded by the**
chloroplast genome were higher in the AtPAP2 OE line under both light and dark
conditions, while the protein abundances of multiple components of the photosynthetic complexes were lower. RNA-seq data also showed that the transcription of the mitochondrial genome is greatly affected by the availability of energy. These data reflect that the transcription and translation of organellar genomes are tightly coupled with the energy status. This study thus provides comprehensive information on the impact of high ATP level on plant physiology, from organellar biology to primary and secondary metabolism.

3. Proc Natl Acad Sci U S A. 2015 Nov 17. pii: 201511748. [Epub ahead of print]

Pentatricopeptide-repeat family protein RF6 functions with hexokinase 6 to rescue
rice cytoplasmic male sterility.

Huang W(1), Yu C(1), Hu J(1), Wang L(1), Dan Z(1), Zhou W(1), He C(1), Zeng Y(1), Yao G(1), Qi J(1), Zhang Z(1), Zhu R(1), Chen X(2), Zhu Y(3).

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Cytoplasmic male sterility (CMS) has been extensively used for hybrid seed production in many major crops. Honglian CMS (HL-CMS) is one of the three major types of CMS in rice and has contributed greatly to food security worldwide. The **HL-CMS trait is associated with an aberrant chimeric mitochondrial transcript, atp6-orfH79**, which causes pollen sterility and can be rescued by two nonallelic restorer-of-fertility (Rf) genes, Rf5 or Rf6. Here, **we report the identification**

of Rf6, which encodes a novel pentatricopeptide repeat (PPR) family protein with
a characteristic duplication of PPR motifs 3-5. RF6 is targeted to mitochondria, where it physically associates with hexokinase 6 (OsHXK6) and promotes the processing of the aberrant CMS-associated transcript atp6-orfH79 at nucleotide 1238, which ensures normal pollen development and restores fertility. The duplicated motif 3 of RF6 is essential for RF6-OsHXK6 interactions, processing of the aberrant transcript, and restoration of fertility. Furthermore, reductions in the level of OsHXK6 result in atp6-orfH79 transcript accumulation and male sterility. Together these results reveal a novel mechanism for CMS restoration by which RF6 functions with OsHXK6 to restore HL-CMS fertility. The present study also provides insight into the function of hexokinase 6 in regulating mitochondrial RNA metabolism and may facilitate further exploitation of heterosis in rice.

Keith

Suppression of HSP27 increases the anti‐tumor effects of quercetin in human leukemia U937 cells

MOLECULAR MEDICINE REPORTS 13: 689-696, 2016

XI CHEN, XIU-SHUAI DONG, HAI-YAN GAO, YONG-FANG JIANG, YING-LAN JIN, YU-YING CHANG, LI-YAN CHEN and JING-HUA WANG

Department of Hematology, The Second Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang 150086, P.R. China

Quercetin, a natural flavonoid, inhibits the growth of leukemia cells and induces apoptosis. Heat shock protein 27 (HSP27) has been reported to promote the development of leukemia by protecting tumor cells from apoptosis through various mechanisms. The present study investigated the effects of small hairpin (sh)RNA-mediated HSP27 knockdown on the anti‐cancer effects of quercetin in U937 human leukemia cells. Cells were transfected with recombinant lentiviral vector pCMV‐G‐NR‐U6‐shHSP27 (shHSP27), which expressed shRNA specifically targeting the HSP27 gene, alone or in combination with quercetin. The results showed that shHSP27 and quercetin synergistically inhibited U937 cell proliferation and induced apoptosis by decreasing the Bcl2-to-Bax ratio. Furthermore, this combined treatment significantly suppressed the infiltration of tumor cells and the expression of angiogenesis‐associated proteins HIF1α and VEGF. Compared with shHSP27 or quercetin alone, shHSP27 plus quercetin markedly decreased the protein expression of cyclinD1 and thus blocked the cell cycle at G1 phase. The Notch/AKT/mTOR signaling pathway is important in tumor aggressiveness; quercetin plus shHSP27 significantly decreased Notch 1 expression and the phosphorylation levels of the downstream signaling proteins AKT and mTOR. The

inhibitory effects of quercetin plus shHSP27 on this pathway may thus have been responsible for the cell cycle arrest, inhibition of proliferations and infiltration as well as enhancement of apoptosis. Therefore, these findings collectively suggested that suppression of HSP27 expression amplified the anti‐cancer effects of quercetin in U937 human leukemia cells, and that quercetin in combination with shHSP27 represents a promising therapeutic strategy for human leukemia

AsHSP17, a creeping bentgrass small heat shock protein modulates plant photosynthesis and ABA-dependent and independent signaling to attenuate plant response to abiotic stress

Xinbo Sun 1,3,4, Chunyu Sun 2,3, Zhigang Li 3, Qian Hu 3, Liebao Han 1*, Hong Luo 3

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Heat shock proteins (HSPs) are molecular chaperones that accumulate in response to heat and other abiotic stressors. Small heat shock proteins (sHSPs) belong to the most ubiquitous HSP subgroup with molecular weights ranging from 12 to 42 kDa. We have cloned a new sHSP gene, *AsHSP17* from creeping bentgrass (*Agrostis stolonifera*) and studied its role in plant response to environmental stress. *AsHSP17* encodes a protein of 17 kDa. Its expression was strongly induced by heat in both leaf and root tissues, and by salt and abscisic acid (ABA) in roots. Transgenic *Arabidopsis* plants constitutively expressing *AsHSP17* exhibited enhanced sensitivity to heat and salt stress accompanied by reduced leaf chlorophyll content and decreased photosynthesis under both normal and stressed conditions compared to wild type. Overexpression of *AsHSP17* also led to hypersensitivity to exogenous ABA and salinity during germination and post-germinative growth. Gene expression analysis indicated that AsHSP17 modulates expression of photosynthesis-related genes and regulates ABA biosynthesis, metabolism and ABA signaling as well as ABA-independent stress signaling. Our results suggest that AsHSP17 may function as a protein chaperone to negatively regulate plant responses to adverse environmental stresses through modulating photosynthesis and ABA-dependent and independent signaling pathways.

The effect of electrical stimulation on *post mortem* myofibrillar protein degradation and small heat shock protein kinetics in bull beef

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Department Agri-industry, Food and Nutrition, “Luiz de Queiroz” College of

Agriculture, — University of São Paulo, P.O. Box 9, Padua Dias 11, Piracicaba, SP, Brazil. Plant and Food Research, Food Industry Science Centre, Private Bag 11600, Palmerston North, New Zealand. Food Assurance and Meat Quality, AgResearch Limited, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand.

This study aimed to determine the effect of electrical stimulation and ultimate pH (pH_u) on shear force, myofibrillar protein degradation and small heat shock protein (sHSP) concentrations in *M. longissimus lumborum* (LL). The LL from both sides of carcasses (n = 15) was excised with low voltage electrical stimulation (ES) applied to an LL muscle from one side, while the opposing LL muscle was not stimulated (NS). Muscles were categorised into low ($pH_u < 5.8$), intermediate ($5.8 \leq pH_u < 6.2$) and high pH_u ($pH_u \geq 6.2$) and aged for up to 28 days *post mortem* at − 1.5 °C. High pH_u meat tenderised faster which corresponded with the faster degradation of titin and desmin in this group compared with low and intermediate pH_u meat. Electrical stimulation significantly affected the variable levels of αβ-crystallin and HSP20 with higher concentrations of these sHSP in ES muscles at later ageing timepoints compared with NS muscles.

Damian

Meyer, Kate D., Patil, Deepak P., Zhou, J., Zinoviev, A., Skabkin, Maxim A., Elemento, O., . . . Jaffrey, Samie R. 5′ UTR m6A Promotes Cap-Independent Translation. Cell. **163**(4): 999-1010.

Protein translation typically begins with the recruitment of the 43S ribosomal complex to the 5? cap of mRNAs by a cap-binding complex. However, some transcripts are translated in a cap-independent manner through poorly understood mechanisms. Here, we show that mRNAs containing N6-methyladenosine (m6A) in their 5? UTR can be translated in a cap-independent manner. A single 5? UTR m6A directly binds eukaryotic initiation factor 3 (eIF3), which is sufficient to recruit the 43S complex to initiate translation in the absence of the cap-binding factor eIF4E. Inhibition of adenosine methylation selectively reduces translation of mRNAs containing 5?UTR m6A. Additionally, increased m6A levels in the Hsp70 mRNA regulate its cap-independent translation following heat shock. Notably, we find that diverse cellular stresses induce a transcriptome-wide redistribution of m6A, resulting in increased numbers of mRNAs with 5? UTR m6A. These data show that 5? UTR m6A bypasses 5? cap-binding proteins to promote translation under stresses.

Hsc70-4 Deforms Membranes to Promote Synaptic Protein Turnover by Endosomal Microautophagy

Cited in Scopus: 0

Valerie Uytterhoeven, Elsa Lauwers, Ine Maes, Katarzyna Miskiewicz, Manuel N. Melo, Jef Swerts, Sabine Kuenen, Rafaël Wittocx, and others

Neuron, Vol. 88, Issue 4, p735–748
Published in issue: November 18, 2015

Pausing on Polyribosomes: Make Way for Elongation in Translational Control
Cited in Scopus: 0

Joel D. Richter, Jeff Coller
Cell, Vol. 163, Issue 2, p292–300
Published in issue: October 08, 2015

Histone acetyltransferase GCN5 is essential for heat stress-responsive gene activation and thermotolerance in Arabidopsis
Zhaorong Hu, Na Song, Mei Zheng, Xinye Liu, Zhenshan Liu, Jiewen Xing, Junhua Ma, Weiwei Guo, Yingyin Yao, Huiru Peng, Mingming Xin, Dao-Xiu Zhou, Zhongfu Ni and Qixin Sun
Accepted manuscript online: 18 NOV 2015 12:35AM EST | DOI: 10.1111/tpj.13076

Cooperative Protein Folding by Two Protein Thiol Disulfide Oxidoreductases and Ero1 in Soybean
Motonori Matsusaki, Aya Okuda, Taro Masuda, Katsunori Koishihara, Ryuta Mita, Kensuke Iwasaki, Kumiko Hara, Yurika Naruo, Akiho Hirose, Yuichiro Tsuchi, and Reiko Urade
Plant Physiol. pp.15.01781; First Published on December 8, 2015;
doi:10.1104/pp.15.01781 **OPEN**
http://www.plantphysiol.org/content/early/2015/12/08/pp.15.01781.abstract

Nitro-fatty acids in plant signaling: nitro-linolenic acid induces the molecular chaperone network in Arabidopsis
Capilla Mata-Pérez, Beatriz Sánchez-Calvo, Maria de las Nieves Padilla-Serrano, Juan C Begara-Morales, Francisco Luque, Manuel Melguizo, Jaime Jiménez-Ruiz, Jesús Fierro-Risco, Antonio Peñas-Sanjuán, Raquel Valderrama, Francisco Javier Corpas, and Juan B Barroso
Plant Physiol. pp.15.01671; First Published on December 1, 2015;
doi:10.1104/pp.15.01671 **OPEN**
http://www.plantphysiol.org/content/early/2015/12/01/pp.15.01671.abstract

Mary

ClpB N-terminal domain plays a regulatory role in protein disaggregation.
Rosenzweig R1, Farber P2, Velyvis A3, Rennella E3, Latham MP4, Kay LE5.

Abstract
ClpB/Hsp100 is an ATP-dependent disaggregase that solubilizes and reactivates protein aggregates in cooperation with the DnaK/Hsp70 chaperone system. The ClpB-substrate interaction is mediated by conserved tyrosine residues located in flexible loops in nucleotide-binding domain-1 that extend into the ClpB central pore. In addition to the tyrosines, the ClpB N-terminal domain (NTD) was suggested

to provide a second substrate-binding site; however, the manner in which the NTD recognizes and binds substrate proteins has remained elusive. Herein, we present an NMR spectroscopy study to structurally characterize the NTD-substrate interaction. We show that the NTD includes a substrate-binding groove that specifically recognizes exposed hydrophobic stretches in unfolded or aggregated client proteins. Using an optimized segmental labeling technique in combination with methyl-transverse relaxation optimized spectroscopy (TROSY) NMR, the interaction of client proteins with both the NTD and the pore-loop tyrosines in the 580-kDa ClpB hexamer has been characterized. Unlike contacts with the tyrosines, the NTD-substrate interaction is independent of the ClpB nucleotide state and protein conformational changes that result from ATP hydrolysis. The NTD interaction destabilizes client proteins, priming them for subsequent unfolding and translocation. Mutations in the NTD substrate-binding groove are shown to have a dramatic effect on protein translocation through the ClpB central pore, suggesting that, before their interaction with substrates, the NTDs block the translocation channel. Together, our findings provide both a detailed characterization of the NTD-substrate complex and insight into the functional regulatory role of the ClpB NTD in protein disaggregation.

Elizabeth

Current Opinion in Structural Biology: Alert 3 December-9 December
Protein stability: computation, sequence statistics, and new experimental methods Review Article
Pages 161-168 Thomas J Magliery
Mitochondrial machineries for insertion of membrane proteins Review Article
Pages 92-102 Maria Bohnert, Nikolaus Pfanner, Martin van der Laan
Gaining mass: the structure of respiratory complex I — from bacterial towards mitochondrial versions Review Article *Pages 135-145* James A Letts, Leonid A Sazanov
Nature Biotechnology Contents: Volume 33 pp 1213 - 1300
20 years of bio-lox **p1213** doi:10.1038/nbt.3438
Here's hoping that a proposed shakeup of US regulations will mean that new biotech products avoid AquaAdvantage salmon's two-decade upstream struggle to regulatory approval.
A face-lift for biotech rules begins **pp1221 - 1222** Emily Waltz
doi:10.1038/nbt1215-1221
Cas9 gets a classmate **pp1240 - 1241**
Erik J Sontheimer and Scot A Wolfe doi:10.1038/nbt.3426
Cpf1 is one of a growing number of class II CRISPR-Cas effectors that expand both our understanding of bacterial immunity and our genome-editing toolset.
Despite the enormous interest in CRISPR-Cas (clustered, regularly interspaced, short palindromic repeats–CRISPR-associated protein) systems, much remains to be discovered about the evolutionary origins and functional diversity of the RNA-guided DNA endonucleases involved. Comprehensive bioinformatic ana-lyses of bacterial and archaeal genomes to identify divergent CRISPR-Cas systems are

shedding light on the origins of CRISPR and revealing new variants with beneficial properties for applications in biomedicine and agriculture. In a recent issue of *Cell*, Zetsche and colleagues[1] functionally characterize one newly discovered CRISPR system, the Cpf1 family of candidate interference effectors, and identify it as a new group of RNA-guided DNA endonuclease complexes. Their analyses provide a small taste of the incredible diversity of CRISPR-based defense systems that exist in nature and that can potentially be repurposed into new genome-engineering and gene-regulation tools.

Plant Cell Table of Contents for November 2015; Vol. 27, No. 11

The EF-Hand Ca^{2+} Binding Protein MICU Choreographs Mitochondrial Ca^{2+} Dynamics in Arabidopsis

Stephan Wagner, Smrutisanjita Behera, Sara De Bortoli, David C. Logan, Philippe Fuchs, Luca Carraretto, Enrico Teardo, Laura Cendron, Thomas Nietzel, Magdalena Füßl, Fabrizio G. Doccula, Lorella Navazio, Mark D. Fricker, Olivier Van Aken, Iris Finkemeier, Andreas J. Meyer, Ildikò Szabò, Alex Costa, and Markus Schwarzländer

Plant Cell 2015 27: 3190-3212. First Published on November 3, 2015; doi:10.1105/tpc.15.00509

http://www.plantcell.org/content/27/11/3190.abstract

The mitochondrial Ca2+ uptake protein At-MICU shapes mitochondrial Ca2+ dynamics, providing molecular in vivo evidence for the existence and function of a mitochondrial uniporter complex in plants. MInsoo covered this previously

Genome Biol. **16**, 258 (2015)

CRISPR–Cas9 allows researchers to easily engineer mutations in genomes and has been tested in some crops, including rice and wheat. Cristobal Uauy and Wendy Harwood at the John Innes Centre in Colney, UK, used the system in barley and the brassica species to knock out the function of genes encoding certain plant hormones that are involved in growth and seed development — both important agronomic traits.

The team generated heritable mutations and the modified plants contained no foreign genes. However, the editing system occasionally introduced unwanted, off-target genetic changes.

Shiota, T. *et al*. Science 349, 1544–1548 (2015).

Molecular architecture of the active mitochondrial protein gate

Mitochondria fulfill central functions in cellular energetics, metabolism, and signaling. The outer membrane translocator complex (the TOM complex) imports most mitochondrial proteins, but its architecture is unknown. Using a cross-linking approach, we mapped the active translocator down to single amino acid residues, revealing different transport paths for preproteins through the Tom40 channel. An N-terminal segment of Tom40 passes from the cytosol through the channel to recruit chaperones from the intermembrane space that guide the transfer of hydrophobic preproteins. The translocator contains three Tom40 β-barrel channels sandwiched between a central α-helical Tom22 receptor cluster and external

regulatory Tom proteins. The preprotein-translocating trimeric complex exchanges with a dimeric isoform to assemble new TOM complexes. Dynamic coupling of α-helical receptors, β-barrel channels, and chaperones generates a versatile machinery that transports about 1000 different proteins.

Plant Journal

Chloroplastic thioredoxin *m* functions as a major regulator of Calvin cycle enzymes during photosynthesis *in vivo* (pages 900–913) Yuki Okegawa and Ken Motohashi online: 7 DEC 2015 | DOI: 10.1111/tpj.13049

Thioredoxins regulate the activity of chloroplast enzymes by reducing disulfide bonds in a light-dependent manner. Previous *in vitro* studies indicated that *f*-type thioredoxins are the most efficient redox regulators; however, *f*-type thioredoxin mutants did not show any obvious phenotypes. Here, we used *in vivo* studies to show that the more abundant *m*-type thioredoxins are more important regulators of Calvin Cycle enzymes. These results highlight the need for *in vivo* studies.

The RNA helicase, eIF4A-1, is required for ovule development and cell size homeostasis in Arabidopsis (pages 989–1004) Maxwell S. Bush, Natalie Crowe, Tao Zheng and John H. Doonan online: 7 DEC 2015 | DOI: 10.1111/tpj.13062

While transcriptional control mechanisms in cell cycle progression and development have been extensively dissected, less is known about the role of translation. Here we show that an *Arabidopsis* TDNA mutant with reduced levels of the translation initiation factor eIF4A exhibits several growth defects suggesting that eIF4A-1 is required for normal cell cycle progression and coordination of cell division with cell growth.

Current Opinion in Plant Biology: Alert 28 November-4 December

Endocytic and autophagic pathways crosstalk in plants Review Article *Pages 39-47*
Xiaohong Zhuang, Yong Cui, Caiji Gao, Liwen Jiang

Novel links in the plant TOR kinase signaling network Review Article *Pages 83-91*
Yan Xiong, Jen Sheen•

- TOR integrates nutrient and energy signaling to promote cell division and growth.
- Powerful chemical tools are developed for probing plant TOR functions.
- Both conserved and unique TOR effectors are identified in the plant system.

Nutrient and energy sensing and signaling mechanisms constitute the most ancient and fundamental regulatory networks to control growth and development in all life forms. The target of rapamycin (TOR) protein kinase is modulated by diverse nutrient, energy, hormone and stress inputs and plays a central role in regulating cell proliferation, growth, metabolism and stress responses from yeasts to plants and animals. Recent chemical, genetic, genomic and metabolomic analyses have enabled significant progress toward molecular understanding of the TOR signaling network in multicellular plants. This review discusses the applications of new chemical tools to probe plant TOR functions and highlights recent findings and predictions on TOR-mediate biological processes. Special focus is placed on novel and evolutionarily conserved TOR kinase effectors as positive and negative signaling regulators that control transcription, translation and metabolism to support cell

proliferation, growth and maintenance from embryogenesis to senescence in the plant system.

The role of auxin signaling in early embryo pattern formation Review Article *Pages 99-105* Margot E Smit, Dolf Weijers
Peptide signaling in pollen tube guidance Review Article *Pages 127-136* Masahiro M Kanaoka, Tetsuya Higashiyama

The Plant Journal Content Alert (New Articles)

The PPR-SMR protein PPR53 enhances the stability and translation of specific chloroplast RNAs in maize

Reimo Zoschke, Kenneth P. Watkins, Rafael G. Miranda and Alice Barkan
Accepted manuscript online: 8 DEC 2015 07:29AM EST | DOI: 10.1111/tpj.13093

Yagi, Yusuke; Nakamura, Takahiro; Small, Ian
The potential for manipulating RNA with pentatricopeptide repeat proteins
The Plant Journal **2014**, vol. 78, p. 772
The pentatricopeptide repeat (PPR) protein family, which is particularly prevalent in plants, includes many sequence-specific RNA-binding proteins involved in all aspects of organelle RNA metabolism, including RNA stability, processing, editing and translation. PPR proteins consist of a tandem array of 2-30 PPR motifs, each of which aligns to one nucleotide in the RNA target. The amino acid side chains at two or three specific positions in each motif confer nucleotide specificity in a predictable and programmable manner. Thus, PPR proteins appear to provide an extremely promising opportunity to create custom RNA-binding proteins with tailored specificity. We summarize recent progress in understanding RNA recognition by PPR proteins, with a particular focus on potential applications of PPR-based tools for manipulating RNA, and on the challenges that remain to be overcome before these tools may be routinely used by the scientific community.

Cellular Signalling: Alert 4 December-10 December
Differential submitochondrial localization of PINK1 as a molecular switch for mediating distinct mitochondrial signaling pathways Original Research Article *Pages 2543-2554*
Dana Fallaize, Lih-Shen Chin, Lian Li
Mutations in mitochondrial kinase PINK1 cause Parkinson disease (PD), but the submitochondrial site(s) of PINK1 action remains unclear. Here, we report that three-dimensional structured illumination microscopy (3D-SIM) enables super-resolution imaging of protein submitochondrial localization. Dual-color 3D-SIM imaging analysis revealed that PINK1 resides in the cristae membrane and intracristae space but not on the outer mitochondrial membrane (OMM) of healthy mitochondria. Under normal physiological conditions, PINK1 colocalizes with its substrate TRAP1 in the cristae membrane and intracristae space. In response to mitochondrial depolarization, PINK1, but not TRAP1, translocates to the OMM. The PINK1 translocation to the OMM of depolarized mitochondria is independent of new protein synthesis and requires combined action of PINK1 transmembrane domain and C-terminal region. We found that mitochondrial depolarization-induced PINK1 OMM translocation is required for recruitment of parkin to the OMM of damaged

mitochondria. Our findings suggest that differential submitochondrial localization of PINK1 serves as a molecular switch for mediating two distinct mitochondrial signaling pathways in maintenance of mitochondrial homeostasis. Furthermore, our study provides evidence for the involvement of deregulated PINK1 submitochondrial localization in PD pathogenesis.

Conserved mRNA-binding proteomes in eukaryotic organisms **pp1027 - 1033**
Ana M Matiá-Gonzalez, Emma E Laing and André P Gerber doi:10.1038/nsmb.3128
Comprehensive identification of mRNA-binding proteins in *S. cerevisiae* and *C. elegans* reveals their evolutionary conservation; strikingly, most components of the glycolytic pathway and proteasome are detected, thus possibly indicating an ancient mechanism for metabolic control.

RNA-binding proteins (RBPs) are essential for post-transcriptional regulation of gene expression. Recent high-throughput screens have dramatically increased the number of experimentally identified RBPs; however, comprehensive identification of RBPs within living organisms is elusive. Here we describe the repertoire of 765 and 594 proteins that reproducibly interact with polyadenylated mRNAs in *Saccharomyces cerevisiae* and *Caenorhabditis elegans*, respectively. Furthermore, we report the differential association of mRNA-binding proteins (mRPBs) upon induction of apoptosis in *C. elegans* L4-stage larvae. Strikingly, most proteins composing mRBPomes, including components of early metabolic pathways and the proteasome, are evolutionarily conserved between yeast and *C. elegans*. We speculate, on the basis of our evidence that glycolytic enzymes bind distinct glycolytic mRNAs, that enzyme-mRNA interactions relate to an ancient mechanism for post-transcriptional coordination of metabolic pathways that perhaps was established during the transition from the early 'RNA world' to the 'protein world'.
Functional Dynamics within the Human Ribosome Regulate the Rate of Active Protein Synthesis Original Research Article *Pages 475-486*
Angelica Ferguson, Leyi Wang, Roger B. Altman, Daniel S. Terry, Manuel F. Juette, Benjamin J. Burnett, Jose L. Alejo, Randall A. Dass, Matthew M. Parks, C. Theresa Vincent, Scott C. Blanchard
A Regression-Based Analysis of Ribosome-Profiling Data Reveals a Conserved Complexity to Mammalian Translation Original Research Article *Pages 816-827*
Alexander P. Fields, Edwin H. Rodriguez, Marko Jovanovic, Noam Stern-Ginossar, Brian J. Haas, Philipp Mertins, Raktima Raychowdhury, Nir Hacohen, Steven A. Carr, Nicholas T. Ingolia, Aviv Regev, Jonathan S. Weissman
•ORF-RATER robustly identifies and quantifies translation from ribosome profiling data
•ORF-RATER reveals thousands of novel micropeptides and variants of mammalian proteins
•Hundreds of novel CDSs show evidence of protein-coding conservation among mammals
•Many ORFs are translated in both mice and humans but lack protein-coding conservation

A fundamental goal of genomics is to identify the complete set of expressed proteins. Automated annotation strategies rely on assumptions about protein-coding sequences (CDSs), e.g., they are conserved, do not overlap, and exceed a minimum length. However, an increasing number of newly discovered proteins violate these rules. Here we present an experimental and analytical framework, based on ribosome profiling and linear regression, for systematic identification and quantification of translation. Application of this approach to lipopolysaccharide-stimulated mouse dendritic cells and HCMV-infected human fibroblasts identifies thousands of novel CDSs, including micropeptides and variants of known proteins, that bear the hallmarks of canonical translation and exhibit translation levels and dynamics comparable to that of annotated CDSs. Remarkably, many translation events are identified in both mouse and human cells even when the peptide sequence is not conserved. Our work thus reveals an unexpected complexity to mammalian translation suited to provide both conserved regulatory or protein-based functions.

Defining Hsp70 Subnetworks in Dengue Virus Replication Reveals Key Vulnerability in Flavivirus Infection Original Research Article *Pages 1108-1123*
Shuhei Taguwa, Kevin Maringer, Xiaokai Li, Dabeiba Bernal-Rubio, Jennifer N. Rauch, Jason E. Gestwicki, Raul Andino, Ana Fernandez-Sesma, Judith Frydman
•The Hsp70 chaperone network mediates distinct steps of the dengue virus life cycle
•DENV cycle requires Hsp70 for viral entry, RNA replication, and virion production
•Hsp70 function at each step of DENV cycle is specified by different DNAJ proteins
•Drug inhibitor of Hsp70 potently blocks DENV infection in human and mosquito cells

Nature Plants

Article | 09 November 2015

Impact of the plastidial stringent response in plant growth and stress responses
The ppGpp-dependent control of cell activities, namely the stringent response, has been elusive in eukaryotes. *Arabidopsis* mutant analysis now shows that plant stringent response controls organelle function and contributes to systematic growth

The Plant Journal Content Alert (New Articles)

Activation of Autophagy by Unfolded Proteins during Endoplasmic Reticulum Stress
Xiaochen Yang, Renu Srivastava, Stephen H. Howell and Diane C. Bassham
Accepted manuscript online: 29 NOV 2015 11:11PM EST | DOI: 10.1111/tpj.13091

FEBS Journal Content Alert (New Articles)
State-of-the-Art Reviews

Mitochondria and the hallmarks of cancer
Evangelos Giampazolias and Stephen W.G. Tait Accepted manuscript online: 25 NOV 2015 09:12PM EST | DOI:

10.1111/febs.13603

PLOS Genetics Volume 11(11) November 2015

Insect Resistance to *Bacillus thuringiensis* Toxin Cry2Ab Is Conferred by Mutations in an ABC Transporter Subfamily A Protein

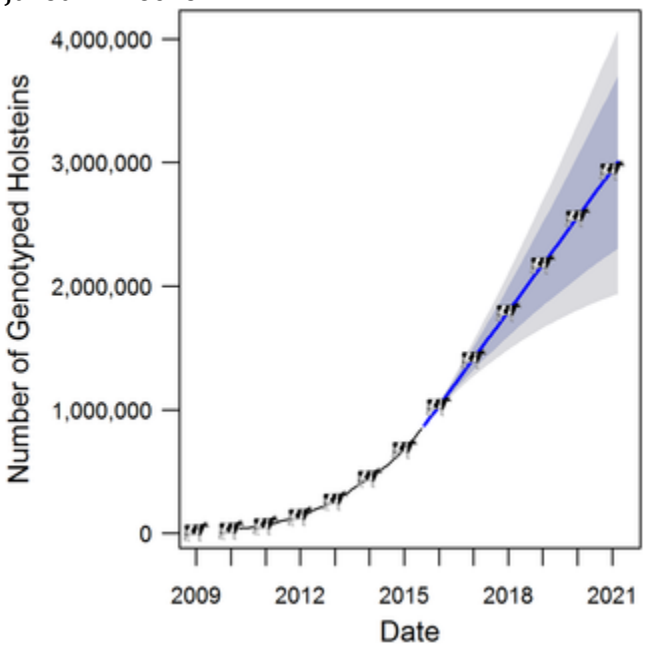
Wee Tek Tay, Rod J. Mahon, David G. Heckel, Thomas K. Walsh, Sharon Downes, William J. James, Sui-Fai Lee, Annette Reineke, Adam K. Williams, Karl H. J. Gordon

Tabashnik BE (2015) ABCs of Insect Resistance to Bt. PLoS Genet 11(11): e1005646. doi:10.1371/journal.pgen.1005646

Scientists have reported one or more field populations with >50% resistant individuals and reduced efficacy of the Bt crop that has practical consequences for pest control. Six of these eight cases entail insects resistant to Cry1 toxins: four against Bt corn (*Spodoptera frugiperda* resistance to Cry1F in Puerto Rico, Brazil, and the continental United States and *Busseola fusca* resistance to Cry1Ab in South Africa) and two against Bt cotton (resistance to Cry1Ac of *Helicoverpa zea* in the US and *Pectinophora gossypiella* in India). The other two cases of practical resistance are *Diabrotica virgifera virgifera* resistance to Cry3Bb and mCry3A in the midwestern US. The orange circle indicates >50% resistant individuals with reduced efficacy expected (but not reported) for *H. zea* resistance to Cry2Ab in multi-toxin Bt cotton in the US. The number of cases with >50% resistant individuals (red or orange) increased from one in 2005 to nine in 2013—the most recent year for which monitoring data are generally available.

Agricultural Genomics: Commercial Applications Bring Increased Basic Research Power

Jared E. Decker



Adaptation to High Ethanol Reveals Complex Evolutionary Pathways

Karin Voordeckers, Jacek Kominek, Anupam Das, Adriana Espinosa-Cantú, Dries De Maeyer, Ahmed Arslan, Michiel Van Pee, Elisa van der Zande, Wim Meert, Yudi Yang,

Bo Zhu, Kathleen Marchal, Alexander DeLuna, Vera Van Noort, Rob Jelier, Kevin J. Verstrepen

Tolerance to high levels of ethanol is an ecologically and industrially relevant phenotype of microbes, but the molecular mechanisms underlying this complex trait remain largely unknown. Here, we use long-term experimental evolution of isogenic yeast populations of different initial ploidy to study adaptation to increasing levels of ethanol. Whole-genome sequencing of more than 30 evolved populations and over 100 adapted clones isolated throughout this two-year evolution experiment revealed how a complex interplay of *de novo* single nucleotide mutations, copy number variation, ploidy changes, mutator phenotypes, and clonal interference led to a significant increase in ethanol tolerance. Although the specific mutations differ between different evolved lineages, application of a novel computational pipeline, PheNetic, revealed that many mutations target functional modules involved in stress response, cell cycle regulation, DNA repair and respiration. Measuring the fitness effects of selected mutations introduced in non-evolved ethanol-sensitive cells revealed several adaptive mutations that had previously not been implicated in ethanol tolerance, including mutations in *PRT1*, *VPS7*0 and *MEX67*. Interestingly, variation in *VPS70* was recently identified as a QTL for ethanol tolerance in an industrial bio-ethanol strain. Taken together, our results show how, in contrast to adaptation to some other stresses, adaptation to a continuous complex and severe stress involves interplay of different evolutionary mechanisms. In addition, our study reveals functional modules involved in ethanol resistance and identifies several mutations that could help to improve the ethanol tolerance of industrial yeasts.

Evolution of Robustness to Protein Mistranslation by Accelerated Protein Turnover

Dorottya Kalapis, Ana R. Bezerra, Zoltán Farkas, Peter Horvath, Zoltán Bódi, Andreea Daraba, Béla Szamecz, Ivo Gut, Mónica Bayes, Manuel A. S. Santos, Csaba Pál

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Translational errors occur at high rates, and they influence organism viability and the onset of genetic diseases. To investigate how organisms mitigate the deleterious effects of protein synthesis errors during evolution, a mutant yeast strain was engineered to translate a codon ambiguously (mistranslation). It thereby overloads the protein quality-control pathways and disrupts cellular protein homeostasis. This strain was used to study the capacity of the yeast genome to compensate the deleterious effects of protein mistranslation. Laboratory evolutionary experiments revealed that fitness loss due to mistranslation can rapidly be mitigated. Genomic analysis demonstrated that adaptation was primarily mediated by large-scale chromosomal duplication and deletion events, suggesting that errors during protein synthesis promote the evolution of genome architecture. By altering the dosages of numerous, functionally related proteins simultaneously, these genetic changes introduced large phenotypic leaps that enabled rapid adaptation to mistranslation. Evolution increased the level of tolerance to mistranslation through acceleration of ubiquitin-proteasome–mediated protein degradation and protein synthesis. As a consequence of rapid elimination of erroneous protein products, evolution reduced

the extent of toxic protein aggregation in mistranslating cells. However, there was a strong evolutionary trade-off between adaptation to mistranslation and survival upon starvation: the evolved lines showed fitness defects and impaired capacity to degrade mature ribosomes upon nutrient limitation. Moreover, as a response to an enhanced energy demand of accelerated protein turnover, the evolved lines exhibited increased glucose uptake by selective duplication of hexose transporter genes. We conclude that adjustment of proteome homeostasis to mistranslation evolves rapidly, but this adaptation has several side effects on cellular physiology. Our work also indicates that translational fidelity and the ubiquitin-proteasome system are functionally linked to each other and may, therefore, co-evolve in nature.

[Evolutionary Conservation and Diversification of Puf RNA Binding Proteins and Their mRNA Targets]

Gregory J. Hogan, Patrick O. Brown, Daniel Herschlag

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Reprogramming of a gene's expression pattern by acquisition and loss of sequences recognized by specific regulatory RNA binding proteins may be a major mechanism in the evolution of biological regulatory programs. We identified that RNA targets of Puf3 orthologs have been conserved over 100–500 million years of evolution in five eukaryotic lineages. Focusing on Puf proteins and their targets across 80 fungi, we constructed a parsimonious model for their evolutionary history. This model entails extensive and coordinated changes in the Puf targets as well as changes in the number of Puf genes and alterations of RNA binding specificity including that: 1) Binding of Puf3 to more than 200 RNAs whose protein products are predominantly involved in the production and organization of mitochondrial complexes predates the origin of budding yeasts and filamentous fungi and was maintained for 500 million years, throughout the evolution of budding yeast. 2) In filamentous fungi, remarkably, more than 150 of the ancestral Puf3 targets were gained by Puf4, with one lineage maintaining both Puf3 and Puf4 as regulators and a sister lineage losing Puf3 as a regulator of these RNAs. The decrease in gene expression of these mRNAs upon deletion of Puf4 in filamentous fungi (*N*. *crassa*) in contrast to the increase upon Puf3 deletion in budding yeast (*S*. *cerevisiae*) suggests that the output of the RNA regulatory network is different with Puf4 in filamentous fungi than with Puf3 in budding yeast. 3) The coregulated Puf4 target set in filamentous fungi expanded to include mitochondrial genes involved in the tricarboxylic acid (TCA) cycle and other nuclear-encoded RNAs with mitochondrial function not bound by Puf3 in budding yeast, observations that provide additional evidence for substantial rewiring of post-transcriptional regulation. 4) Puf3 also expanded and diversified its targets in filamentous fungi, gaining interactions with the mRNAs encoding the mitochondrial electron transport chain (ETC) complex I as well as hundreds of other mRNAs with nonmitochondrial functions. The many concerted and conserved changes in the RNA targets of Puf proteins strongly support an extensive role of RNA binding proteins in coordinating gene expression, as originally proposed by Keene. Rewiring of Puf-coordinated mRNA targets and transcriptional control of the same genes occurred at different points in evolution, suggesting that there have been distinct adaptations via RNA binding proteins and transcription factors. The changes

in Puf targets and in the Puf proteins indicate an integral involvement of RNA binding proteins and their RNA targets in the adaptation, reprogramming, and function of gene expression.

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