

Literature Lunch 7-3-13

Liyuan:

Dissecting the heat stress response in *Chlamydomonas* by pharmaceutical and RNAi approaches reveals conserved and novel aspects

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Abstract

To study how conserved fundamental concepts of the heat stress response (HSR) are in photosynthetic eukaryotes, we applied pharmaceutical and antisense/amiRNA approaches to the unicellular green alga *Chlamydomonas reinhardtii*. The *Chlamydomonas* HSR appears to be triggered by the accumulation of unfolded proteins, as it was induced at ambient temperatures by feeding cells with the arginine analog canavanine. The protein kinase inhibitor staurosporine strongly retarded the HSR, demonstrating the importance of phosphorylation during activation of the HSR also in *Chlamydomonas*. While the removal of extracellular calcium by the application of EGTA and BAPTA inhibited the HSR in moss and higher plants, only the addition of BAPTA, but not of EGTA retarded the HSR and impaired thermotolerance in *Chlamydomonas*. The addition of cycloheximide, an inhibitor of cytosolic protein synthesis, abolished the attenuation of the HSR, indicating that protein synthesis is necessary to restore proteostasis. HSP90 inhibitors induced a stress response when added at ambient conditions and retarded attenuation of the HSR at elevated temperatures. In addition, we detected a direct physical interaction between cytosolic HSP90A/HSP70A and heat shock factor 1, but surprisingly this interaction persisted after the onset of stress. Finally, the expression of antisense constructs targeting chloroplast HSP70B resulted in a delay of the cell's entire HSR, thus suggesting the existence of a retrograde stress signaling cascade that is desensitized in HSP70B-antisense strains.

Emerging concept for the role of photorespiration as an important part of abiotic stress response

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ABSTRACT

When plants are exposed to stress, generation of reactive oxygen species (ROS) is often one of the first responses. In order to survive, cells attempt to down-regulate the production of ROS, while at the same time scavenging ROS. Photorespiration is now appreciated as an important part of stress responses in green tissues for preventing ROS accumulation. Photorespiratory reactions can dissipate excess reducing equivalents and energy either directly (using ATP, NAD(P)H and reduced ferredoxin) or indirectly (e.g., via alternative oxidase (AOX) and providing an internal CO₂ pool).

Photorespiration, however, is also a source of H₂O₂ that is possibly involved in signal transduction, resulting in modulation of gene expression. We propose that photorespiration can assume a major role in the readjustment of redox homeostasis. Protection of photosynthesis from photoinhibition through photorespiration is well known. Photorespiration can mitigate oxidative stress under conditions of drought/water stress, salinity, low CO₂ and chilling. Adjustments to even mild disturbances in redox status, caused by a deficiency in ascorbate, AOX or chloroplastic NADP-malate dehydrogenase, comprise increases in photorespiratory components such as catalase, P-protein of glycine decarboxylase complex (GDC) and glycine content. The accumulation of excess reducing equivalents or ROS in plant cells also affects mitochondria. Therefore, a strong interaction between the chloroplast redox status and photorespiration is not surprising, but highlights interesting properties evident in plant cells. We draw attention to the fact that a complex network of multiple and dynamic systems, including photorespiration, prevents oxidative damage while optimising photosynthesis. Further experiments are necessary to identify and validate the direct targets of redox signals among photorespiratory components.

Keith:

The Antiprion Compound 6-Aminophenanthridine Inhibits the Protein Folding Activity of the Ribosome by Direct Competition

June 28, 2013 The Journal of Biological Chemistry, 288, 19081-19089.

Yanhong Pang, Sriram Kurella, Cécile Voisset, Dibyendu Samanta, Debapriya Banerjee, Ariane Schabe, Chanchal Das Gupta, Hervé Galons, Marc Blondel, Suparna Sanjal

Domain V of the 23S/25S/28S rRNA of the large ribosomal subunit constitutes the active center for the protein folding activity of the ribosome (PFAR). Using in vitro transcribed domain V rRNAs from *Escherichia coli* and *Saccharomyces cerevisiae* as the folding modulators and human carbonic anhydrase as a model protein, we demonstrate that PFAR is conserved from prokaryotes to eukaryotes. It was shown previously that 6-aminophenanthridine (6AP), an antiprion compound, inhibits PFAR. Here, using UV cross-linking followed by primer extension, we show that the protein substrates and 6AP interact with a common set of nucleotides on domain V of 23S rRNA. Mutations at the interaction sites decreased PFAR and resulted in loss or change of the binding pattern for both the protein substrates and 6AP. Moreover, kinetic analysis of human carbonic anhydrase refolding showed that 6AP decreased the yield of the refolded protein but did not affect the rate of refolding. Thus, we conclude that 6AP competitively occludes the protein substrates from binding to rRNA and thereby inhibits PFAR. Finally, we propose a scheme clarifying the mechanism by which 6AP inhibits PFAR.

Allosteric opening of the polypeptide-binding site when an Hsp70 binds ATP

Nature Structural & Molecular Biology **20**, 900–907 (2013)

Ruifeng Qi, Evans Boateng Sarbeng, Qun Liu, Katherine Quynh Le, Xinping Xu, Hongya Xu, Jiao Yang, Jennifer Li Wong, Christina Vorvis, Wayne A Hendrickson, Lei Zhou & Qinglian Liu

Virginia Commonwealth University, VA. Brookhaven National Laboratory, NY. Columbia University, NY.

The 70-kilodalton (kDa) heat-shock proteins (Hsp70s) are ubiquitous molecular chaperones essential for cellular protein folding and proteostasis. Each Hsp70 has two functional domains: a nucleotide-binding domain (NBD), which binds and hydrolyzes **ATP**, and a substrate-binding domain (SBD), which binds extended polypeptides. NBD and SBD interact little when in the presence of **ADP**; however, **ATP** binding allosterically couples the polypeptide- and ATP-binding sites. **ATP** binding promotes polypeptide release; polypeptide rebinding stimulates **ATP** hydrolysis. This allosteric coupling is poorly understood. Here we present the crystal structure of an intact **ATP**-bound **Hsp70** from *Escherichia coli* at 1.96-Å resolution. The **ATP**-bound NBD adopts a unique conformation, forming extensive interfaces with an SBD that has changed radically, having its α -helical lid displaced and the polypeptide-binding channel of its β -subdomain restructured. These conformational changes, together with our biochemical assays, provide a structural explanation for allosteric coupling in **Hsp70** activity.

Dynamic enzyme docking to the ribosome coordinates N-terminal processing with polypeptide folding

Nature Structural & Molecular Biology **20**, 843–850 (2013)

Arzu Sandikci, Felix Gloge, Michael Martinez, Matthias P Mayer, Rebecca Wade, Bernd Bukau & Günter Kramer

Newly synthesized polypeptides undergo various cotranslational maturation steps, including N-terminal enzymatic processing, chaperone-assisted folding and membrane targeting, but the spatial and temporal

coordination of these steps is unclear. We show that *Escherichia coli* methionine aminopeptidase (MAP) associates with ribosomes through a charged loop that is crucial for nascent-chain processing and cell viability. MAP competes with peptide deformylase (PDF), the first enzyme to act on nascent chains, for binding sites at the ribosomal tunnel exit. PDF has extremely fast association and dissociation kinetics, which allows it to frequently sample ribosomes and ensure the processing of nascent chains after their emergence. Premature recruitment of the chaperone trigger factor, or polypeptide folding, negatively affect processing efficiency. Thus, the fast ribosome association kinetics of PDF and MAP are crucial for the temporal separation of nascent-chain processing from later maturation events, including chaperone recruitment and folding.

Yichen:

J Mol Biol. 2013 Jul 24;425(14):2463-79. doi: 10.1016/j.jmb.2013.03.041. Epub 2013 Apr 2.

Structural Studies on the Forward and Reverse Binding Modes of Peptides to the Chaperone DnaK.

Zahn M, Berthold N, Kieslich B, Knappe D, Hoffmann R, Sträter N.

Source

Institute of Bioanalytical Chemistry, Center for Biotechnology and Biomedicine, University of Leipzig, 04103 Leipzig, Germany.

Abstract

Hsp70 chaperones have been implicated in assisting protein folding of newly synthesized polypeptide chains, refolding of misfolded proteins, and protein trafficking. For these functions, the chaperones need to exhibit a significant promiscuity in binding to different sequences of hydrophobic peptide stretches. To characterize the structural basis of sequence specificity and flexibility of the *Escherichia coli* Hsp70 chaperone DnaK, we have analyzed crystal structures of the substrate binding domain of the protein in complex with artificially designed peptides as well as small proline-rich antimicrobial peptides. The latter peptides from mammals and insects were identified to target DnaK after cell penetration. Interestingly, the complex crystal structures reveal two different peptide binding modes. The peptides can bind either in a forward or in a reverse direction to the conventional substrate binding cleft of DnaK in an extended conformation. Superposition of the two binding modes shows a remarkable similarity in the side chain orientations and hydrogen bonding pattern despite the reversed peptide orientation. The DnaK chaperone has evolved to bind peptides in both orientations in the substrate binding cleft with comparable energy without rearrangements of the protein. Optimal hydrophobic interactions with binding pockets -2 to 0 appear to be the main determinant for the orientation and sequence position of peptide binding.

Indu:

Articles from Science:

1. Science. 2013 May 31;340(6136):1113-7. doi: 10.1126/science.1234914. Epub 2013 Apr 25.

Structure of RSV fusion glycoprotein trimer bound to a prefusion-specific neutralizing antibody.

McLellan JS, Chen M, Leung S, Graepel KW, Du X, Yang Y, Zhou T, Baxa U, Yasuda E, Beaumont T, Kumar A, Modjarrad K, Zheng Z, Zhao M, Xia N, Kwong PD, Graham BS.

Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA. mclellanja@niaid.nih.gov

The prefusion state of respiratory syncytial virus (RSV) fusion (F) glycoprotein is the target of most RSV-neutralizing activity in human sera, but its metastability has hindered characterization. To overcome this obstacle, we identified prefusion-specific antibodies that were substantially more potent than the prophylactic antibody palivizumab. The cocrystal structure for one of these antibodies, D25, in complex with the F glycoprotein revealed D25 to lock F in its prefusion state by binding to a quaternary epitope at the trimer apex. Electron microscopy showed that two other antibodies, AM22 and 5C4, also bound to the newly identified site of vulnerability, which we named antigenic site \emptyset . These studies should enable design of improved vaccine antigens and define new targets for passive prevention of RSV-induced disease.

PMID: 23618766 [PubMed - indexed for MEDLINE]

2. Science. 2013 May 31;340(6136):1094-7. doi: 10.1126/science.1234116.

Mechanisms of age-dependent response to winter temperature in perennial flowering of *Arabis alpina*.

Bergonzi S, Albani MC, Ver Loren van Themaat E, Nordström KJ, Wang R, Schneeberger K, Moerland PD, Coupland G.

Max Planck Institute for Plant Breeding Research, Cologne, Germany.

Perennial plants live for more than 1 year and flower only after an extended vegetative phase. We used *Arabis alpina*, a perennial relative of annual *Arabidopsis thaliana*, to study how increasing age and exposure to winter cold (vernalization) coordinate to establish competence to flower. We show that the

APETALA2 transcription factor, a target of microRNA miR172, prevents flowering before vernalization. Additionally, miR156 levels decline as *A. alpina* ages, causing increased production of SPL (SQUAMOSA PROMOTER BINDING PROTEIN LIKE) transcription factors and ensuring that flowering occurs in response to cold. The age at which plants respond to vernalization can be altered by manipulating miR156 levels. Although miR156 and miR172 levels are uncoupled in *A. alpina*, miR156 abundance represents the timer controlling age-dependent flowering responses to cold.

PMID: 23723236 [PubMed - indexed for MEDLINE]

3. Science. 2013 May 31;340(6136):1097-100. doi: 10.1126/science.1234340.

Molecular basis of age-dependent vernalization in *Cardamine flexuosa*.

Zhou CM, Zhang TQ, Wang X, Yu S, Lian H, Tang H, Feng ZY, Zozomova-Lihová J, Wang JW.

National Key Laboratory of Plant Molecular Genetics (NKLPMG), Institute of Plant Physiology and Ecology (SIPPE), Shanghai Institutes for Biological Sciences (SIBS), Shanghai, P R China.

Plants flower in response to many varied cues, such as temperature, photoperiod, and age. The floral transition of *Cardamine flexuosa*, a herbaceous biennial-to-perennial plant, requires exposure to cold temperature, a treatment known as vernalization. *C. flexuosa* younger than 5 weeks old are not fully responsive to cold treatment. We demonstrate that the levels of two age-regulated microRNAs, miR156 and miR172, regulate the timing of sensitivity in response to vernalization. Age and vernalization pathways coordinately regulate flowering through modulating the expression of *CfSOC1*, a flower-promoting MADS-box gene. The related annual *Arabidopsis thaliana*, which has both vernalization and age pathways, does not possess an age-dependent vernalization response. Thus, the recruitment of age cue in response to environmental signals contributes to the evolution of life cycle in plants.

PMID: 23723237 [PubMed - indexed for MEDLINE]

Stephanie:

Plant Molecular Biology

1) Alternative splicing is required for RCT1-mediated disease resistance in *Medicago truncatula*.

Abstract:

RCT1 is a TIR-NBS-LRR-type resistance (R) gene in *Medicago truncatula* that confers resistance to multiple races of *Colletotrichum trifolii*, a hemi-biotrophic fungal pathogen that causes anthracnose disease in *Medicago* and other closely related legumes. RCT1 undergoes

alternative splicing at both coding and 3'-untranslated regions, thereby producing multiple transcript variants in its expression profile. Alternative splicing of RCT1 in the coding region results from the retention of intron 4. Because intron 4 lies downstream of the LRR-encoding exons and contains an in-frame stop codon, the alternative transcript is predicted to encode a truncated protein consisting of the entire portion of the TIR, NBS, and LRR domains but lacks the C-terminal domain of the full-length RCT1 protein encoded by the regular transcript. Here we provide evidence that the RCT1-mediated disease resistance requires the combined presence of the regular and alternative transcripts. Neither the regular nor the alternative RCT1 transcript alone is sufficient to confer resistance against the pathogen. This study, in addition to the reports on the tobacco N and Arabidopsis RPS4 genes, adds another significant example showing the involvement of alternative splicing in R gene-mediated plant immunity.

Plant Molecular Biology

2) Overexpression of *AtWRKY30* enhances abiotic stress tolerance during early growth stages in *A. thaliana*

Abstract: AtWRKY30 belongs to a higher plant transcription factor superfamily, which responds to pathogen attack. In previous studies, the AtWRKY30 gene was found to be highly and rapidly induced in Arabidopsis thaliana leaves after oxidative stress treatment. In this study, electrophoretic mobility shift assays showed that AtWRKY30 binds with high specificity and affinity to the WRKY consensus sequence (W-box), and also to its own promoter. Analysis of the AtWRKY30 expression pattern by qPCR and using transgenic Arabidopsis lines carrying AtWRKY30 promoter- β -glucuronidase fusions showed transcriptional activity in leaves subjected to biotic or abiotic stress. Transgenic Arabidopsis plants constitutively overexpressing AtWRKY30 (35S::W30 lines) were more tolerant than wild-type plants to oxidative and salinity stresses during seed germination. The results presented here show that AtWRKY30 is responsive to several stress conditions either from abiotic or biotic origin, suggesting that AtWRKY30 could have a role in the activation of defence responses at early stages of Arabidopsis growth by binding to W-boxes found in promoters of many stress/developmentally regulated genes.

Ariel:

1.

FEBS Lett. 2013 Apr 17;587(8):1073-80. doi: 10.1016/j.febslet.2013.01.021. Epub 2013 Jan 20.

One size does not fit all: the oligomeric states of β B crystallin.

Delbecq SP, Klevit RE.

Source Department of Biochemistry, Box 357350, University of Washington, Seattle, WA 98195-7350, USA.

Abstract

Small Heat Shock Proteins (sHSPs) are a diverse family of molecular chaperones that delay protein aggregation through interactions with non-native and aggregate-prone protein states. This function has been shown to be important to cellular viability and sHSP function/dysfunction is implicated in many diseases, including Alzheimer's and Alexander disease. Though their gene products are small, many sHSPs assemble into a distribution of large oligomeric states that undergo dynamic subunit exchange. These inherent properties present significant experimental challenges for characterizing sHSP oligomers. Of the human sHSPs, β B crystallin is a paradigm example of sHSP oligomeric properties. Advances in our understanding of sHSP structure, oligomeric distribution, and dynamics have prompted the proposal of several models for the oligomeric states of β B. The aim of this review is to highlight characteristics of β B

crystallin (?B) that are key to understanding its structure and function. The current state of knowledge, existing models, and outstanding questions that remain to be addressed are presented.

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Publication Types, MeSH Terms, Substances, Grant Support

2. FEBS Lett. 2013 Jun 27;587(13):1959-69. doi: 10.1016/j.febslet.2013.05.011. Epub 2013 May 15.

Human small heat shock proteins: Protein interactomes of homo- and hetero-oligomeric complexes: An update.

Arrigo AP. Source Apoptosis, Cancer and Development Laboratory, Lyon Cancer Research Center, INSERM U1052-CNRS UMR5286, Claude Bernard University Lyon 1, Lyon, France. Electronic address: parrigo@me.com.

Abstract

Small heat shock proteins (sHsps) regulate a large number of fundamental cellular processes and are involved in many pathological diseases. They share complex oligomerization and phosphorylation properties allowing them to interact and modulate the activity of many client proteins. Here, the up-to-date protein interactome of the ten human sHsps is presented as an illustration of their multiple cellular functions. In addition of forming homo-oligomers, some of these proteins interact with each other and form hetero-oligomeric complexes that could bear new protein targets recognition abilities. Here, novel informations are presented on how the formation of HspB1/HspB5 complex can stimulate the activity of the oxidoresistance promoting enzyme glucose 6-phosphate dehydrogenase through its interaction with newly formed highly phosphorylated HspB1 homo-oligomers.

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Nathen:

Hsp90 Regulates Nongenetic Variation in Response to Environmental Stress

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*

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<http://dx.doi.org/10.1016/j.molcel.2013.01.026>

SUMMARY

Nongenetic cell-to-cell variability often plays an important role for the survival of a clonal population in the face of fluctuating environments. However, the underlying mechanisms

regulating such non-genetic heterogeneity remain elusive in most organisms. We report here that a clonal yeast population exhibits morphological heterogeneity when the level of Hsp90, a molecular chaperone, is reduced. The morphological heterogeneity is driven by the dosage of Cdc28 and Cla4, a key regulator of septin formation. Low Hsp90 levels reduce Cla4 protein stability and cause a subpopulation of cells to switch to a filamentous form that has been previously suggested to be beneficial under certain hostile environments. Moreover, Hsp90-dependent morphological heterogeneity can be induced by environmental stress and is conserved across diverse yeast species. Our results suggest that Hsp90 provides an evolutionarily conserved mechanism that links environmental stress to the induction of morphological diversity.

A Cotranslational Ubiquitination Pathway for Quality Control of Misfolded Proteins

Feng Wang¹, Larissa A. Durfee¹, Jon M. Huibregtse¹, , ¹ Institute for Cellular and Molecular Biology, Section of Molecular Genetics and Molecular Biology, University of Texas at Austin, Austin, TX 78712, USA <http://dx.doi.org/10.1016/j.molcel.2013.03.009>

Summary

Previous studies have indicated that 6%–30% of newly synthesized proteins are rapidly degraded by the ubiquitin-proteasome system; however, the relationship of ubiquitination to translation for these proteins has been unclear. We report that cotranslational ubiquitination (CTU) is a robust process, with 12%–15% of nascent polypeptides being ubiquitinated in human cells. CTU products contained primarily K48-linked polyubiquitin chains, consistent with a proteasomal targeting function. While nascent chains have been shown previously to be ubiquitinated within stalled complexes (CTU^s), the majority of nascent chain ubiquitination occurred within active translation complexes (CTU^a). CTU^a was increased in response to agents that induce protein misfolding, while CTU^s was increased in response to agents that lead to translational errors or stalling. These results indicate that ubiquitination of nascent polypeptides occurs in two contexts and define CTU^a as a component of a quality control system that marks proteins for destruction while they are being synthesized.

Journal: Molecular Cell

Fionn:

Plant cell

Advanced Proteomic Analyses Yield a Deep Catalog of Ubiquitylation Targets in *Arabidopsis*^[W]

Do-Young Kim^a, Mark Scalf^b, Lloyd M. Smith^b and Richard D. Vierstra^{a,1}

Abstract

The posttranslational addition of ubiquitin (Ub) profoundly controls the half-life, interactions, and/or trafficking of numerous intracellular proteins. Using stringent two-step affinity methods to

purify Ub-protein conjugates followed by high-sensitivity mass spectrometry, we identified almost 950 ubiquitylation substrates in whole *Arabidopsis thaliana* seedlings. The list includes key factors regulating a wide range of biological processes, including metabolism, cellular transport, signal transduction, transcription, RNA biology, translation, and proteolysis. The ubiquitylation state of more than half of the targets increased after treating seedlings with the proteasome inhibitor MG132 (carbobenzoxy-Leu-Leu-Leu-al), strongly suggesting that Ub addition commits many to degradation by the 26S proteasome. Ub-attachment sites were resolved for a number of targets, including six of the seven Lys residues on Ub itself with a Lys-48>Lys-63>Lys-11>>>Lys-33/Lys-29/Lys-6 preference. However, little sequence consensus was detected among conjugation sites, indicating that the local environment has little influence on global ubiquitylation. Intriguingly, the level of Lys-11-linked Ub polymers increased substantially upon MG132 treatment, revealing that they might be important signals for proteasomal breakdown. Taken together, this proteomic analysis illustrates the breadth of plant processes affected by ubiquitylation and provides a deep data set of individual targets from which to explore the roles of Ub in various physiological and developmental pathways.

Nature biotech US Congress moves to 'protect' GM crop plantings Laura DeFrancesco

Damian:

PolyQ Proteins Interfere with Nuclear Degradation of Cytosolic Proteins by Sequestering the Sis1p Chaperone

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<http://dx.doi.org/10.1016/j.cell.2013.06.003>

Cell 154, 1–12, July 3, 2013 ©2013 Elsevier Inc.

SUMMARY

Dysfunction of protein quality control contributes to the cellular pathology of polyglutamine (polyQ) expansion diseases and other neurodegenerative disorders associated with aggregate deposition. Here we analyzed how polyQ aggregation interferes with the clearance of misfolded proteins by the ubiquitin-proteasome system (UPS). We show in a yeast model that polyQ-expanded proteins inhibit the UPS-mediated degradation of misfolded cytosolic carboxypeptidase Y* fused to green fluorescent protein (GFP) (CG*) without blocking ubiquitylation or proteasome function. Quantitative proteomic analysis reveals that the polyQ aggregates sequester the low-abundant and essential Hsp40 chaperone Sis1p. Overexpression of Sis1p restores CG* degradation. Surprisingly, we find that Sis1p, and its homolog DnaJB1 in mammalian cells, mediates the delivery of misfolded proteins into the nucleus for proteasomal degradation. Sis1p shuttles between cytosol and nucleus, and its cellular level limits the capacity

of this quality control pathway. Upon depletion of Sis1p by polyQ aggregation, misfolded proteins are barred from entering the nucleus and form cytoplasmic inclusions.

MIDGET connects COP1-dependent development with endoreduplication in *Arabidopsis thaliana*

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Received 18 December 2012; revised 2 April 2013; accepted 7 April 2013; published online 10 April 2013.

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The Plant Journal (2013) 75, 67–79 doi: 10.1111/tpj.12199

SUMMARY

In *Arabidopsis thaliana*, loss of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) function leads to constitutive photomorphogenesis in the dark associated with inhibition of endoreduplication in the hypocotyl, and a post-germination growth arrest. MIDGET (MID), a component of the TOPOISOMERASE VI (TOPOVI) complex, is essential for endoreduplication and genome integrity in *A. thaliana*. Here we show that MID and COP1 interact *in vitro* and *in vivo* through the amino terminus of COP1. We further demonstrate that MID supports sub-nuclear accumulation of COP1. The MID protein is not degraded in a COP1-dependent fashion in darkness, and the phenotypes of single and double mutants prove that MID is not a target of COP1 but rather a necessary factor for proper COP1 activity with respect to both, control of COP1-dependent morphogenesis and regulation of endoreduplication. Our data provide evidence for a functional connection between COP1 and the TOPOVI in plants linking COP1-dependent development with the regulation of endoreduplication.

Function of type–2 *Arabidopsis* hemoglobin in the auxin-mediated formation of embryogenic cells during morphogenesis

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²Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 1391 Sandford St., London, ON, N5V 4T3, Canada,

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⁴Department of Molecular Biology and Genetics, University of Aarhus, Forsøgsvej 1, 4200, Slagelse, Denmark

The Plant Journal (2013) 74, 946–958 doi: 10.1111/tpj.12181

SUMMARY

Suppression of *Arabidopsis* GLB2, a type–2 nonsymbiotic hemoglobin, enhances somatic embryogenesis by increasing auxin production. In the *glb2* knock-out line (*GLB2*_{–/–}), polarization of PIN1 proteins and auxin maxima occurred at the base of the cotyledons of the zygotic explants, which are the sites of embryogenic tissue formation. These changes were also accompanied by a transcriptional upregulation of WUSCHEL (*WUS*) and SOMATIC EMBRYOGENESIS RECEPTOR KINASE (*SERK1*), which are markers of embryogenic

competence. The increased auxin levels in the GLB2_{-/-} line were ascribed to the induction of several key enzymes of the tryptophan and IAA biosynthetic pathways, including ANTHRANILATE SYNTHASE (a subunit; ASA1), CYTOCHROME P79B2 (CYP79B2) and AMIDASE1 (AMI1). The effects of GLB2 suppression on somatic embryogenesis and IAA synthesis are mediated by increasing levels of nitric oxide (NO) within the embryogenic cells, which repress the expression of the transcription factor MYC2, a well-characterized repressor of the auxin biosynthetic pathway. A model is proposed in which the suppression of GLB2 reduces the degree of NO scavenging by oxyhemoglobin, thereby increasing the cellular NO concentration. The increased levels of NO repress the expression of MYC2, relieving the inhibition of IAA synthesis and increasing cellular IAA, which is the inductive signal promoting embryogenic competence. Besides providing a model for the induction phase of embryogenesis in vitro, these studies propose previously undescribed functions for plant hemoglobins

Elizabeth:

June 24

Teramoto S, Shimura H, Tanaka R, Shimada Y, Miyamoto N, Arai H, Urabe T, Hattori N.

Human-derived physiological heat shock protein 27 complex protects brain after focal cerebral ischemia in mice.

PLoS One. 2013;8(6):e66001.

PMID: 23785464 [PubMed - in process]

Wu TM, Lin WR, Kao YT, Hsu YT, Yeh CH, Hong CY, Kao CH.

Identification and characterization of a novel chloroplast/mitochondria co-localized glutathione reductase 3 involved in salt stress response in rice. Plant Mol Biol. 2013 Jun 20;. [Epub ahead of print]

PMID: 23783412 [PubMed - as supplied by publisher]

Sandikci A, Gloge F, Martinez M, Mayer MP, Wade R, Bukau B, Kramer G.

Dynamic enzyme docking to the ribosome coordinates N-terminal processing with polypeptide folding.

Nat Struct Mol Biol. 2013 Jun 16;. [Epub ahead of print]

PMID: 23770820 [PubMed - as supplied by publisher]

López-Sánchez LM, López-Pedrerera C, Rodríguez-Ariza A.

PROTEOMIC APPROACHES TO EVALUATE PROTEIN S-NITROSYLATION IN DISEASE.

Mass Spectrom Rev. 2013 Jun 15;:0. [Epub ahead of print]

PMID: 23775552 [PubMed - as supplied by publisher]

Jiang WB, Huang HY, Hu YW, Zhu SW, Wang ZY, Lin WH.

Brassinosteroid Regulates Seed Size and Shape in Arabidopsis.

Plant Physiol. 2013 Jun 14;. [Epub ahead of print]

PMID: 23771896 [PubMed - as supplied by publisher]

Cell: Alert 17 June-23 June

[Eukaryotic Stress Granules Are Cleared by Autophagy and Cdc48/VCP Function](#) Original Research

Pages 1461-1474

J. Ross Buchan, Regina-Maria Kolaitis, J. Paul Taylor, Roy Parker

Summary

Stress granules and P bodies are conserved cytoplasmic aggregates of nontranslating messenger ribonucleoprotein complexes (mRNPs) implicated in the regulation of mRNA translation and

decay and are related to RNP granules in embryos, neurons, and pathological inclusions in some degenerative diseases. Using baker's yeast, 125 genes were identified in a genetic screen that affected the dynamics of P bodies and/or stress granules. Analyses of such mutants, including *CDC48* alleles, provide evidence that stress granules can be targeted to the vacuole by autophagy, in a process termed granulophagy. Moreover, stress granule clearance in mammalian cells is reduced by inhibition of autophagy or by depletion or pathogenic mutations in valosin-containing protein (*VCP*), the human ortholog of *CDC48*. Because mutations in *VCP* predispose humans to amyotrophic lateral sclerosis, frontotemporal lobar degeneration, inclusion body myopathy, and multisystem proteinopathy, this work suggests that autophagic clearance of stress granule related and pathogenic RNP granules that arise in degenerative diseases may be important in reducing their pathology.

[Rate-Limiting Steps in Yeast Protein Translation](#) Original Research **Pages 1589-1601**

Premal Shah, Yang Ding, Malwina Niemczyk, Grzegorz Kudla, Joshua B. Plotkin

Summary

Deep sequencing now provides detailed snapshots of ribosome occupancy on mRNAs. We leverage these data to parameterize a computational model of translation, keeping track of every ribosome, tRNA, and mRNA molecule in a yeast cell. We determine the parameter regimes in which fast initiation or high codon bias in a transgene increases protein yield and infer the initiation rates of endogenous *Saccharomyces cerevisiae* genes, which vary by several orders of magnitude and correlate with 5' mRNA folding energies. Our model recapitulates the previously reported 5'-to-3' ramp of decreasing ribosome densities, although our analysis shows that this ramp is caused by rapid initiation of short genes rather than slow codons at the start of transcripts. We conclude that protein production in healthy yeast cells is typically limited by the availability of free ribosomes, whereas protein production under periods of stress can sometimes be rescued by reducing initiation or elongation rates.

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Single and Interactive Effects of Temperature and Light Quality on Four Canola Cultivars (pages 286–298)

K. L. I. Slauenwhite and M. M. Qaderi

Article first published online: 4 DEC 2012 | DOI: 10.1111/jac.12014

Agronomic Optimum Seeding Rate for Irrigated Maize in Texas is Concomitant to Growing Season Mean Daily Minimum Temperature (pages 299–307)

W. H. Thompson, D. Pietsch, J. M. Blumenthal, A. M. H. Ibrahim and D. D. Baltensperger

Article first published online: 27 DEC 2012 | DOI: 10.1111/jac.12015

Extensive transcriptional heterogeneity revealed by isoform profiling

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Nature 497, 127–131 (02 May 2013)

doi:10.1038/nature12121

Transcript function is determined by sequence elements arranged on an individual RNA molecule. Variation in transcripts can affect messenger RNA stability, localization and translation¹, or produce truncated proteins that differ in localization² or function³. Given the existence of overlapping, variable transcript isoforms, determining the functional impact of the transcriptome requires identification of full-length transcripts, rather than just the genomic regions that are transcribed^{4,5}. Here, by jointly determining both transcript ends for millions of RNA molecules, we reveal an extensive layer of isoform diversity previously hidden among overlapping RNA molecules. Variation in transcript boundaries seems to be the rule rather than the exception, even within a single population of yeast cells. Over 26 major transcript isoforms per protein-coding gene were expressed in yeast. Hundreds of short coding RNAs and truncated versions of proteins are concomitantly encoded by alternative transcript isoforms, increasing protein diversity. In addition, approximately 70% of genes express alternative isoforms that vary in post-transcriptional regulatory elements, and tandem genes frequently produce overlapping or even bicistronic transcripts. This extensive transcript diversity is generated by a relatively simple eukaryotic genome with limited splicing, and within a genetically homogeneous population of cells. Our findings have implications for genome compaction, evolution and phenotypic diversity between single cells. These data also indicate that isoform diversity as well as RNA abundance should be considered when assessing the functional repertoire of genomes.

The *Capsella rubella* genome and the genomic consequences of rapid mating system evolution **OPEN** pp831 - 835

Tanja Slotte, Khaled M Hazzouri, J Arvid Ågren, Daniel Koenig, Florian Maumus, Ya-Long Guo, Kim Steige, Adrian E Platts, Juan S Escobar, L Killian Newman, Wei Wang, Terezie Mandáková, Emilio Vello, Lisa M Smith, Stefan R Henz, Joshua Steffen, Shohei Takuno, Yaniv Brandvain, Graham Coop, Peter Andolfatto, Tina T Hu, Mathieu Blanchette, Richard M Clark, Hadi Quesneville, Magnus Nordborg, Brandon S Gaut, Martin A Lysak, Jerry Jenkins, Jane Grimwood, Jarrod Chapman, Simon Prochnik, Shengqiang Shu, Daniel Rokhsar, Jeremy Schmutz, Detlef Weigel & Stephen I Wright

doi: 10.1038/ng.2669

Stephen Wright, Detlef Weigel and colleagues report the whole-genome sequence of *Capsella rubella*, a highly selfing crucifer found throughout much of southern and western Europe. They compare mixed-stage flower bud transcriptomes from *C. rubella* and *C. grandiflora*, finding a shift in expression of genes associated with flowering phenotypes and providing insights into the transition to selfing.

Chemistry & Biology

Volume 20, Issue 6, Pages 743-856 (20 June 2013)

[Visualization of Redox-Controlled Protein Fold in Living Cells](#)

Pages 747-752

Lucia Banci, Letizia Barbieri, Enrico Luchinat, Erica Secci