

Ian

[Free Radic Biol Med.](#) 2016 Sep 27. pii: S0891-5849(16)30429-4. doi: 10.1016/j.freeradbiomed.2016.09.015. [Epub ahead of print]

## **Ferriheme catalyzes nitric oxide reaction with glutathione to form S-nitrosogluthathione: A novel mechanism for formation of S-nitrosothiols.**

[Nagababu E](#)<sup>1</sup>.

[PLoS Genet.](#) 2016 Sep 29;12(9):e1006255. doi: 10.1371/journal.pgen.1006255. eCollection 2016.

## **Arabidopsis CaM1 and CaM4 Promote Nitric Oxide Production and Salt Resistance by Inhibiting S-Nitrosogluthathione Reductase via Direct Binding.**

[Zhou S](#)<sup>1</sup>, [Jia L](#)<sup>1</sup>, [Chu H](#)<sup>1</sup>, [Wu D](#)<sup>1</sup>, [Peng X](#)<sup>1</sup>, [Liu X](#)<sup>1</sup>, [Zhang J](#)<sup>1</sup>, [Zhao J](#)<sup>1</sup>, [Chen K](#)<sup>2</sup>, [Zhao L](#)<sup>1</sup>.

Alyssa

## **CRISPR-Barcoding for Intratumor Genetic Heterogeneity Modeling and Functional Analysis of Oncogenic Driver Mutations**

Intratumor genetic heterogeneity underlies the ability of tumors to evolve and adapt to different environmental conditions. Using CRISPR/Cas9 technology and specific DNA barcodes, we devised a strategy to recapitulate and trace the emergence of subpopulations of cancer cells containing a mutation of interest. We used this approach to model different mechanisms of lung cancer cell resistance to EGFR inhibitors and to assess effects of combined drug therapies. By overcoming intrinsic limitations of current approaches, CRISPR-barcoding also enables investigation of most types of genetic modifications, including repair of oncogenic driver mutations. Finally, we used highly complex barcodes inserted at a specific genome location as a means of simultaneously tracing the fates of many thousands of genetically labeled cancer cells. CRISPR-barcoding is a straightforward and highly flexible method that should greatly facilitate the functional investigation of specific mutations, in a context that closely mimics the complexity of cancer.

Patrick

### **1. Cytokinin Response Factor 6 Represses Cytokinin-Associated Genes during Oxidative Stress**

Paul J. Zwack, Inge De Clercq, Timothy C. Hooton, H. Tucker Hallmark, Andrej Hurny,

Erika A. Keshishian, Alyssa M. Parish, Eva Benkova, M. Shahid Mukhtar, Frank Van Breusegem, and Aaron M. Rashotte\*  
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Department of Biology, University of Alabama, Birmingham, AL 35294 (T.C.H., M.S.M.); and Institute of Science and Technology Austria (IST Austria), 3400 Klosterneuburg, Austria (A.H., E.B.)

Cytokinin is a phytohormone that is well known for its roles in numerous plant growth and developmental processes, yet it has also been linked to abiotic stress response in a less defined manner. *Arabidopsis* (*Arabidopsis thaliana*) Cytokinin Response Factor 6 (CRF6) is a cytokinin-responsive AP2/ERF-family transcription factor that, through the cytokinin signaling pathway, plays a key role in the inhibition of dark-induced senescence. CRF6 expression is also induced by oxidative stress, and here we show a novel function for CRF6 in relation to oxidative stress and identify downstream transcriptional targets of CRF6 that are repressed in response to oxidative stress. Analysis of transcriptomic changes in wild-type and *crf6* mutant plants treated with H<sub>2</sub>O<sub>2</sub> identified CRF6-dependent differentially expressed transcripts, many of which were repressed rather than induced. Moreover, many repressed genes also show decreased expression in 35S:CRF6 overexpressing plants. Together, these findings suggest that CRF6 functions largely as a transcriptional repressor. Interestingly, among the H<sub>2</sub>O<sub>2</sub> repressed CRF6-dependent transcripts was a set of five genes associated with cytokinin processes: (signaling) ARR6, ARR9, ARR11, (biosynthesis) LOG7, and (transport) ABCG14. We have examined mutants of these cytokinin-associated target genes to reveal novel connections to oxidative stress. Further examination of CRF6-DNA interactions indicated that CRF6 may regulate its targets both directly and indirectly. Together, this shows that CRF6 functions during oxidative stress as a negative regulator to control this cytokinin-associated module of CRF6-dependent genes and establishes a novel connection between cytokinin and oxidative stress response.

## 2. Reactive Oxygen Species Tune Root Tropic Responses

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ORCID IDs: 0000-0003-3498-4362 (D.S.); 0000-0002-7196-6529 (G.M.); 0000-0003-1977-1211 (H.F.).

The default growth pattern of primary roots of land plants is directed by gravity. However, roots possess the ability to sense and respond directionally to other chemical and physical stimuli, separately and in combination. Therefore, these root tropic responses must be antagonistic to gravitropism. The role of reactive oxygen species (ROS) in gravitropism of maize and *Arabidopsis* (*Arabidopsis thaliana*) roots has been previously described. However, which cellular signals underlie the integration of the different environmental stimuli, which lead to an appropriate root tropic response, is currently unknown.

In gravity-responding roots, we observed, by applying the ROS-sensitive fluorescent dye dihydrorhodamine-123 and confocal microscopy, a transient asymmetric ROS distribution, higher at the concave side of the root. The asymmetry, detected at the distal elongation zone, was built in the first 2 h of the gravitropic response and dissipated after another 2 h. In contrast, hydrotropically responding roots show no transient asymmetric distribution of ROS. Decreasing ROS levels by applying the antioxidant ascorbate, or the ROS-generation inhibitor diphenylene iodonium attenuated gravitropism while enhancing hydrotropism. *Arabidopsis* mutants deficient in Ascorbate Peroxidase 1 showed attenuated hydrotropic root bending. Mutants of the root-expressed NADPH oxidase *RBOH C*, but not *rbohD*, showed enhanced hydrotropism and less ROS in their roots apices (tested in tissue extracts with Amplex Red). Finally, hydrostimulation prior to gravistimulation attenuated the gravistimulated asymmetric ROS and auxin signals that are required for gravity-directed curvature. We suggest that ROS, presumably H<sub>2</sub>O<sub>2</sub>, function in tuning root tropic responses by promoting gravitropism and negatively regulating hydrotropism.

**Keith**

## **Expression of Cataract-linked $\gamma$ -crystallin Variants in Zebrafish Reveals a Proteostasis Network that Senses Protein Stability.**

J Biol Chem. 2016 Oct 21.

Wu SY1, Zou P1, Fuller AW1, Mishra S1, Wang Z1, Schey KL1, Mchaourab HS2.

1,2 Vanderbilt University, United States.

The refractivity and transparency of the ocular lens is dependent on the stability and solubility of the crystallins in the fiber cells. A number of mutations of lens crystallins have been associated with dominant cataracts in humans and mice. Of particular interest were  $\gamma$ B- and  $\gamma$ D-crystallin mutants linked to dominant cataracts in mouse models. While thermodynamically destabilized and aggregation-prone, these mutants

were found to have weak affinity to the resident chaperone  $\alpha$ -crystallin in vitro To better understand the mechanism of the cataract phenotype, we transgenically expressed different  $\gamma$ D-crystallin mutants in the zebrafish lens, and observed a range of lens defects that arise primarily from the aggregation of the mutant proteins. Unlike mouse models, a strong correlation was observed between the severity and penetrance of the phenotype and the level of destabilization of the mutant. We interpret this result to reflect the presence of a proteostasis network which can "sense" protein stability. In the more destabilized mutants, the capacity of this network is overwhelmed leading to the observed increase in phenotypic penetrance. Overexpression of  $\alpha$ A-crystallin had no significant effects on the penetrance of lens defects suggesting that its chaperone capacity is not limiting. While consistent with the prevailing hypothesis that a chaperone network is required for lens transparency, our results suggest that  $\alpha$ A-crystallin may not be efficient to inhibit aggregation of lens  $\gamma$ -crystallin. Our work further implicates additional inputs/factors are involved in this underlying proteostasis network and demonstrates the utility of zebrafish as a platform to delineate mechanisms of cataract.

## **The preferential heterodimerization of human small heat shock proteins HSPB1 and HSPB6 is dictated by the N-terminal domain.**

Arch Biochem Biophys. 2016 Nov 15;610:41-50.

Heirbaut M1, Lermyte F2, Martin EM3, Beelen S1, Verschueren T3, Sobott F3, Strelkov SV4, Weeks SD5.

Small heat shock proteins are ATP-independent molecular chaperones. Their function is to bind partially unfolded proteins under stress conditions. In vivo, members of this chaperone family are known to preferentially assemble together forming large, polydisperse heterooligomers. The exact molecular mechanisms that drive specific heteroassociation are currently unknown. Here we study the oligomers formed between human HSPB1 and HSPB6. Using small-angle X-ray scattering we could characterize two distinct heterooligomeric species present in solution. By employing native mass spectrometry we show that such assemblies are formed purely from heterodimeric building blocks, in line with earlier cross-linking studies. Crucially, a detailed analysis of truncation variants reveals that the preferential association between these two sHSPs is solely mediated by their disordered N-terminal domains.

## **The small heat shock protein Hsp31 cooperates with Hsp104 to modulate Sup35 prion aggregation.**

Prion. 2016 Oct 3

Aslam K1, Tsai CJ1, Hazbun TR1.

<sup>1</sup>Department of Medicinal Chemistry and Molecular Pharmacology and the Purdue University Center for Cancer Research , Purdue University , West Lafayette , IN 47907 , USA

The yeast homolog of DJ-1, Hsp31, is a multifunctional protein that is involved in several cellular pathways including detoxification of the toxic metabolite methylglyoxal and as a protein deglycase. Prior studies ascribed Hsp31 as a molecular chaperone that can inhibit  $\alpha$ -Syn aggregation in vitro and alleviate its toxicity in vivo. It was also shown that Hsp31 inhibits Sup35 aggregate formation in yeast, however, it is unknown if Hsp31 can modulate [PSI<sup>+</sup>] phenotype and Sup35 prionogenesis. Other small heat shock proteins, Hsp26 and Hsp42 are known to be a part of a synergistic proteostasis network that inhibits Sup35 prion formation and promotes its disaggregation. Here, we establish that Hsp31 inhibits Sup35 [PSI<sup>+</sup>] prion formation in collaboration with a well-known disaggregase, Hsp104. Hsp31 transiently prevents prion induction but does not suppress induction upon prolonged expression of Sup35 indicating that Hsp31 can be overcome by larger aggregates. In addition, elevated levels of Hsp31 do not cure [PSI<sup>+</sup>] strains indicating that Hsp31 cannot intervene in a pre-existing prion oligomerization cycle. However, Hsp31 can modulate prion status in cooperation with Hsp104 because it inhibits Sup35 aggregate formation and potentiates [PSI<sup>+</sup>] prion curing upon overexpression of Hsp104. The absence of Hsp31 reduces [PSI<sup>+</sup>] prion curing by Hsp104 without influencing its ability to rescue cellular thermotolerance. Hsp31 did not synergize with Hsp42 to modulate the [PSI<sup>+</sup>] phenotype suggesting that both proteins act on similar stages of the prion cycle. We also showed that Hsp31 physically interacts with Hsp104 and together they prevent Sup35 prion toxicity to greater extent than if they were expressed individually. These results elucidate a mechanism for Hsp31 on prion modulation that suggest it acts at a distinct step early in the Sup35 aggregation process that is different from Hsp104. This is the first demonstration of the modulation of [PSI<sup>+</sup>] status by the chaperone action of Hsp31. The delineation of Hsp31's role in the chaperone cycle has implications for understanding the role of the DJ-1 superfamily in controlling misfolded proteins in neurodegenerative disease and cancer.

Elizabeth

### **Oct 16**

Plant Cell Table of Contents for September 2016; Vol. 28, No. 9

Lost in Transit: Long-Distance Trafficking and Phloem Unloading of Protein Signals in Arabidopsis Homografts

Danae Simone Genevieve Paultre, Marie-Paule Gustin, Attila Molnar, and Karl J. Oparka

Plant Cell 2016 28: 2016-2025. First Published on September 6, 2016; doi:10.1105/tpc.16.00249

**OPEN**

<http://www.plantcell.org/content/28/9/2016.abstract>

In Arabidopsis homografts, proteins carrying organelle-targeting signals move from companion cells to sieve elements and traffic across the graft union to target the correct organelle in the root.

Dynamic Interactions of Arabidopsis TEN1: Stabilizing Telomeres in Response to Heat Stress

Jung Ro Lee, Xiaoyuan Xie, Kailu Yang, Junjie Zhang, Sang Yeol Lee, and Dorothy E. Shippen

Plant Cell 2016 28: 2212-2224. First Published on September 8, 2016; doi:10.1105/tpc.16.00408  
<http://www.plantcell.org/content/28/9/2212.abstract>

The Arabidopsis TEN1 telomere protein possesses a molecular chaperone activity that is responsive to heat shock and links environmental stress to genome stability.

Thylakoid Membrane Architecture in *Synechocystis* Depends on CurT, a Homolog of the Granal CURVATURE THYLAKOID1 Proteins

Steffen Heinz, Anna Rast, Lin Shao, Andrian Gutu, Irene L. Gügel, Eiri Heyno, Mathias Labs, Birgit Rengstl, Stefania Viola, Marc M. Nowaczyk, Dario Leister, and Jörg Nickelsen

Plant Cell 2016 28: 2238-2260. First Published on August 19, 2016; doi:10.1105/tpc.16.00491  
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CurT is required for shaping the thylakoid membrane architecture in *Synechocystis* and mediates osmotic stress responses.

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Nat Commun. 2016 Oct 6;7:12882. PMID: 27708256 [Unknown status]

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The small heat shock protein Hsp31 cooperates with Hsp104 to modulate Sup35 prion aggregation.

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Zhou S, Jia L, Chu H, Wu D, Peng X, Liu X, Zhang J, Zhao J, Chen K, Zhao L.  
Arabidopsis CaM1 and CaM4 Promote Nitric Oxide Production and Salt Resistance by Inhibiting S-Nitrosoglutathione Reductase via Direct Binding.  
PLoS Genet. 2016 Sep;12(9):e1006255. PMID: 27684709 [PubMed - as supplied by publisher]

The FEBS Journal Content Alert: 283, 19 (October 2016)

**To deliver or to degrade – an interplay of the ubiquitin–proteasome system, autophagy and vesicular transport in plants (pages 3534–3555)**

Katarzyna Zientara-Rytter and Agnieszka Sirko  
Version of Reco DOI: 10.1111/febs.13712rd online: 2 APR 2016 |

Autophagy is a catabolic process that involves vesicle formation. Besides the ubiquitin–proteasome system (UPS), it is main the degradation mechanism in cells. It is also an important component of vesicle transport. In this article, we summarize the knowledge on both UPS and autophagy pathways in plants, pointing the interplay between them and discussing the relationships between autophagy and vesicular transport.

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C9orf72 Dipeptide Repeats Impair the Assembly, Dynamics, and Function of Membrane-Less Organelles.  
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Stage and cell-specific expression and intracellular localization of the small heat shock protein Hsp27 during oogenesis and spermatogenesis in the Mediterranean fruit fly, *Ceratitis capitata*.  
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**Cell: Alert 14 October-21 October**

**[ALKBH1-Mediated tRNA Demethylation Regulates Translation](#) *Pages 816-828.e16***

Fange Liu, Wesley Clark, Guanzheng Luo, Xiaoyun Wang, Ye Fu, Jiangbo Wei, Xiao Wang, Ziyang Hao, Qing Dai, Guanqun Zheng, Honghui Ma, Dali Han, Molly Evans, Arne Klungland, Tao Pan, Chuan He

[PDF \(4085 K\)](#) [Supplementary content](#)

**[Toxic PR Poly-Dipeptides Encoded by the C9orf72 Repeat Expansion Target LC Domain Polymers](#) *Pages 789-802.e12***

Yi Lin, Eiichiro Mori, Masato Kato, Siheng Xiang, Leeju Wu, Ilmin Kwon, Steven L. McKnight

[PDF \(4503 K\)](#) [Supplementary content](#)

**[C9orf72 Dipeptide Repeats Impair the Assembly, Dynamics, and Function of Membrane-Less Organelles](#)**

*Pages 774-788.e17*

Kyung-Ha Lee, Peipei Zhang, Hong Joo Kim, Diana M. Mitrea, Mohona Sarkar, Brian D. Freibaum, Jaclyn Cika, Maura Coughlin, James Messing, Amandine Molliex, Brian A. Maxwell, Nam Chul Kim, Jamshid Temirov, Jennifer Moore, Regina-Maria Kolaitis, Timothy I. Shaw, Bing Bai, Junmin Peng, Richard W. Kriwacki, J. Paul Taylor  
[PDF \(14430 K\)](#) [Supplementary content](#)

Archives of Biochemistry and Biophysics: Alert 14 October-21 October

[The preferential heterodimerization of human small heat shock proteins HSPB1 and HSPB6 is dictated by the N-terminal domain](#) Pages 41-50

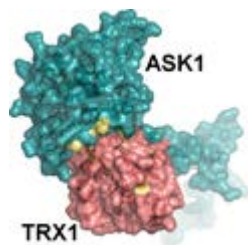
Michelle Heirbaut, Frederik Lermyte, Esther M. Martin, Steven Beelen, Tim Verschueren, Frank Sobott, Sergei V. Strelkov, Stephen D. Weeks

The FEBS Journal Content Alert: 283, 20 (October 2016)

**Cysteine residues mediate high-affinity binding of thioredoxin to ASK1 (pages 3821–3838)**

Salome Kylarova, Dalibor Kosek, Olivia Petrvalska, Katarina Psenakova, Petr Man, Jaroslav Vecer, Petr Herman, Veronika Obsilova and Tomas Obsil

Version of Record online: 18 SEP 2016 | DOI: 10.1111/febs.13893



Apoptosis signal-regulating kinase 1 (ASK1, MAP3K5) activates p38 and c-Jun kinases in response to proinflammatory and stress signals. ASK1 is inhibited by association with thioredoxin (TRX) which dissociates in response to oxidative stress, thus allowing the ASK1 activation. Here, we investigated the role of cysteine residues on the interaction between TRX1 and ASK1 in both reducing and oxidizing conditions.

The Plant Journal Content Alert (New Articles)

**CPR5 modulates salicylic acid and unfolded protein response to manage tradeoffs between plant growth and stress responses** Zhe Meng, Cristina Ruberti, Zhizhong Gong and Federica Brandizzi

Accepted manuscript online: 16 OCT 2016 10:45PM EST | DOI: 10.1111/tpj.13397

**The RRM protein CP33A is a global ligand of chloroplast mRNAs and is essential for plastid biogenesis and plant development**

Marlene Teubner, Janina Fuß, Kristina Kühn, Kirsten Krause and Christian Schmitz-Linneweber  
Accepted manuscript online: 15 OCT 2016 03:16AM EST | DOI: 10.1111/tpj.13396

Science 20 October 2016; Vol. 354, No. 6310

[Photoactivation and inactivation of \*Arabidopsis\* cryptochrome 2](#)

By Qin Wang, Zecheng Zuo, Xu Wang, Lianfeng Gu, Takeshi Yoshizumi, Zhaohe Yang, Liang Yang, Qing Liu, Wei Liu, Yun-Jeong Han, Jeong-Il Kim, Bin Liu, James A. Wohlschlegel, Minami Matsui, Yoshito Oka, Chentao Lin

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