

Minsoo

1. Mol Cell. 2017 Mar 16;65(6):975-984.e5. doi: 10.1016/j.molcel.2017.02.018.

Tardigrades Use Intrinsically Disordered Proteins to Survive Desiccation.

Boothby TC(1), Tapia H(2), Brozena AH(3), Piskiewicz S(4), Smith AE(4),
Giovannini I(5), Rebecchi L(5), Pielak GJ(4), Koshland D(2), Goldstein B(6).

Author information:

(1)Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. Electronic address: tboothby@gmail.com.
(2)Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720, USA. (3)Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC 27695, USA. (4)Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. (5)Department of Life Sciences, University of Modena and Reggio Emilia, Modena 41125, Italy. (6)Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.

Tardigrades are microscopic animals that survive a remarkable array of stresses, including desiccation. How tardigrades survive desiccation has remained a mystery for more than 250 years. Trehalose, a disaccharide essential for several organisms to survive drying, is detected at low levels or not at all in some tardigrade species, indicating that tardigrades possess potentially novel mechanisms for surviving desiccation. Here we show that tardigrade-specific intrinsically disordered proteins (TDPs) are essential for desiccation tolerance. TDP genes are constitutively expressed at high levels or induced during desiccation in multiple tardigrade species. TDPs are required for tardigrade desiccation tolerance, and these genes are sufficient to increase desiccation tolerance when expressed in heterologous systems. TDPs form non-crystalline amorphous solids (vitrify) upon desiccation, and this vitrified state mirrors their protective capabilities. Our study identifies TDPs as functional mediators of tardigrade desiccation tolerance, expanding our knowledge of the roles and diversity of disordered proteins involved in stress tolerance.

2. Mol Cell. 2017 Mar 16;65(6):1014-1028.e7. doi: 10.1016/j.molcel.2017.01.032. Epub 2017 Mar 2.

Mitochondrial Ca²⁺ Uniporter Is a Mitochondrial Luminal Redox Sensor that Augments MCU Channel Activity.

Dong Z(1), Shanmughapriya S(2), Tomar D(2), Siddiqui N(3), Lynch S(4), Nemani

N(2), Breves SL(2), Zhang X(5), Tripathi A(2), Palaniappan P(2), Riitano MF(2), Worth AM(2), Seelam A(2), Carvalho E(2), Subbiah R(2), Jaña F(2), Soboloff J(6), Peng Y(7), Cheung JY(5), Joseph SK(8), Caplan J(4), Rajan S(9), Stathopoulos PB(3), Madesh M(10).

Author information:

(1)Department of Medical Genetics and Molecular Biochemistry, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA; Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA; Institute of Burn Research, Southwest Hospital, Third Military Medical University, Chongqing 400038, PRC. (2)Department of Medical Genetics and Molecular Biochemistry, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA; Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA. (3)Department of Physiology and Pharmacology, Western University, London, ON N6A 5C1, Canada. (4)Department of Biological Sciences, Delaware Biotechnology Institute, University of Delaware, Newark, DE 19711, USA. (5)Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA. (6)Department of Medical Genetics and Molecular Biochemistry, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA. (7)Institute of Burn Research, Southwest Hospital, Third Military Medical University, Chongqing 400038, PRC. (8)MitoCare Center, Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, USA. (9)Department of Medical Genetics and Molecular Biochemistry, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA; Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA. Electronic address: sudarsan@temple.edu. (10)Department of Medical Genetics and Molecular Biochemistry, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA; Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, USA. Electronic address: madeshm@temple.edu.

Ca(2+) dynamics and oxidative signaling are fundamental mechanisms for mitochondrial bioenergetics and cell function. The MCU complex is the major pathway by which these signals are integrated in mitochondria. Whether and how these coactive elements interact with MCU have not been established. As an approach toward understanding the regulation of MCU channel by oxidative milieu, we adapted inflammatory and hypoxia models. We identified the conserved cysteine 97 (Cys-97) to be the only reactive thiol in human MCU that undergoes S-glutathionylation. Furthermore, biochemical, structural, and superresolution imaging analysis revealed that MCU oxidation promotes MCU higher order oligomer formation. Both oxidation and mutation of MCU Cys-97 exhibited persistent MCU channel activity with higher [Ca(2+)]_m uptake rate, elevated mROS, and enhanced [Ca(2+)]_m overload-induced cell death. In contrast, these effects were largely

independent of MCU interaction with its regulators. These findings reveal a distinct functional role for Cys-97 in ROS sensing and regulation of MCU activity.

3. *Front Mol Biosci.* 2017 Feb 22;4:6. doi: 10.3389/fmolb.2017.00006. eCollection 2017.

Mutant Analysis Reveals Allosteric Regulation of ClpB Disaggregase.

Franke KB(1), Bukau B(1), Mogk A(1).

Author information:

(1)Center for Molecular Biology of the Heidelberg University, German Cancer Research Center Heidelberg, Germany.

The members of the hexameric AAA+ disaggregase of *E. coli* and *S. cerevisiae*, ClpB, and Hsp104, cooperate with the Hsp70 chaperone system in the solubilization of aggregated proteins. Aggregate solubilization relies on a substrate threading activity of ClpB/Hsp104 fueled by ATP hydrolysis in both ATPase rings (AAA-1, AAA-2). ClpB/Hsp104 ATPase activity is controlled by the M-domains, which associate to the AAA-1 ring to downregulate ATP hydrolysis. Keeping M-domains displaced from the AAA-1 ring by association with Hsp70 increases ATPase activity due to enhanced communication between protomers. This communication involves conserved arginine fingers. The control of ClpB/Hsp104 activity is crucial, as hyperactive mutants with permanently dissociated M-domains exhibit cellular toxicity. Here, we analyzed AAA-1 inter-ring communication in relation to the M-domain mediated ATPase regulation, by subjecting a conserved residue of the AAA-1 domain subunit interface of ClpB (A328) to mutational analysis. While all A328X mutants have reduced disaggregation activities, their ATPase activities strongly differed. ClpB-A328I/L mutants have reduced ATPase activity and when combined with the hyperactive ClpB-K476C M-domain mutation, suppress cellular toxicity. This underlines that ClpB ATPase activation by M-domain dissociation relies on increased subunit communication. The ClpB-A328V mutant in contrast has very high ATPase activity and exhibits cellular toxicity on its own, qualifying it as novel hyperactive ClpB mutant. ClpB-A328V hyperactivity is however, different from that of M-domain mutants as M-domains stay associated with the AAA-1 ring. The high ATPase activity of ClpB-A328V primarily relies on the AAA-2 ring and correlates with distinct conformational changes in the AAA-2 catalytic site. These findings characterize the subunit interface residue A328 as crucial regulatory element to control ATP hydrolysis in both AAA rings.

4. *Nature.* 2017 Mar 16;543(7645):443-446. doi: 10.1038/nature21695. Epub 2017 Mar 1.

Cytosolic proteostasis through importing of misfolded proteins into mitochondria.

Ruan L(1,)(2), Zhou C(3), Jin E(2), Kucharavy A(1,)(2), Zhang Y(3), Wen Z(3), Florens L(3), Li R(1,)(2).

Author information:

(1)Center for Cell Dynamics, Department of Cell Biology, Johns Hopkins University School of Medicine, 855 North Wolfe Street, Baltimore, Maryland 21205, USA.

(2)Department of Chemical and Biomolecular Engineering, Whiting School of Engineering, Johns Hopkins University, Baltimore, Maryland 21218, USA. (3)Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, Missouri 64110, USA.

Loss of proteostasis underlies ageing and neurodegeneration characterized by the accumulation of protein aggregates and mitochondrial dysfunction. Although many neurodegenerative-disease-associated proteins can be found in mitochondria, it remains unclear how mitochondrial dysfunction and protein aggregation could be related. In dividing yeast cells, protein aggregates that form under stress or during ageing are preferentially retained by the mother cell, in part through tethering to mitochondria, while the disaggregase Hsp104 helps to dissociate aggregates and thereby enables refolding or degradation of misfolded proteins. Here we show that, in yeast, cytosolic proteins prone to aggregation are imported into mitochondria for degradation. Protein aggregates that form under heat shock contain both cytosolic and mitochondrial proteins and interact with the mitochondrial import complex. Many aggregation-prone proteins enter the mitochondrial intermembrane space and matrix after heat shock, and some do so even without stress. Timely dissolution of cytosolic aggregates requires the mitochondrial import machinery and proteases. Blocking mitochondrial import but not proteasome activity causes a marked delay in the degradation of aggregated proteins. Defects in cytosolic Hsp70s leads to enhanced entry of misfolded proteins into mitochondria and elevated mitochondrial stress. We term this mitochondria-mediated proteostasis mechanism MAGIC (mitochondria as guardian in cytosol) and provide evidence that it may exist in human

Thi

Protein-Remodeling Factors As Potential Therapeutics for Neurodegenerative Disease

Meredith E. Jackrel* and James Shorter*

Front Neurosci. 2017; 11: 99.

Protein misfolding is implicated in numerous neurodegenerative disorders including amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, and Huntington's disease. A unifying feature of patients with these disorders is the accumulation of deposits comprised of misfolded

protein. Aberrant protein folding can cause toxicity through a loss or gain of protein function, or both. An intriguing therapeutic approach to counter these disorders is the application of protein-remodeling factors to resolve these misfolded conformers and return the proteins to their native fold and function. Here, we describe the application of protein-remodeling factors to alleviate protein misfolding in neurodegenerative disease. We focus on Hsp104, Hsp110/Hsp70/Hsp40, NMNAT, and HtrA1, which can prevent and reverse protein aggregation. While many of these protein-remodeling systems are highly promising, their activity can be limited. Thus, engineering protein-remodeling factors to enhance their activity could be therapeutically valuable. Indeed, engineered Hsp104 variants suppress neurodegeneration in animal models, which opens the way to novel therapeutics and mechanistic probes to help understand neurodegenerative disease.

Corey

Lactuca spp. and its modulation during plant development

Tichá T1, Činčalová L1, Kopečný D2, Sedlářová M3, Kopečná M2, Luhová L1, Petřivalský M4.

1Department of Biochemistry, Faculty of Science, Palacký University, Šlechtitelů 27, CZ-783 71 Olomouc, Czech Republic.

2Department of Protein Biochemistry and Proteomics, Centre of the Region Haná for Biotechnological and Agricultural Research, Šlechtitelů 27, CZ-783 71 Olomouc, Czech Republic.

3Department of Botany, Faculty of Science, Palacký University, Šlechtitelů 27, CZ-783 71 Olomouc, Czech Republic.

4Department of Biochemistry, Faculty of Science, Palacký University, Šlechtitelů 27, CZ-783 71 Olomouc, Czech Republic. Electronic address: marek.petrivalsky@upol.cz.

Cellular homeostasis of S-nitrosoglutathione (GSNO), a major cache of nitric oxide bioactivity in plants, is controlled by the NADH-dependent S-nitrosoglutathione reductase (GSNOR) belonging to the family of class III alcohol dehydrogenases (EC 1.1.1.1). GSNOR is a key regulator of S-nitrosothiol metabolism and is involved in plant responses to abiotic and biotic stresses. This study was focused on GSNOR from two important crop plants, cauliflower (*Brassica oleracea* var. botrytis, BoGSNOR) and lettuce (*Lactuca sativa*, LsGSNOR). Both purified recombinant GSNORs were characterized in vitro and found to exist as dimers, exhibit high thermal stability and substrate preference towards GSNO, although both enzymes have dehydrogenase activity with a broad range of long-chain alcohols and ω -hydroxy fatty acids in presence of NAD⁺. Data on enzyme affinities to their cofactors NADH and NAD⁺ obtained by isothermal titration calorimetry suggest the high affinity to NADH might underline the GSNOR capacity to function in the intracellular environment. GSNOR activity and gene expression peak during early developmental stages of lettuce and cauliflower at 20 and 30 days after germination, respectively. GSNOR activity was also measured in four other *Lactuca* spp. genotypes with different degree of resistance to biotrophic pathogen *Bremia lactucae*. Higher GSNOR activities were found in non-infected plants of susceptible genotypes *L. sativa* UCDM2 and *L. serriola* as compared to resistant genotypes. GSNOR and GSNO were localized by confocal laser scanning microscopy in vascular bundles and in

epidermal and parenchymal cells of leaf cross-sections. The presented results bring new insight in the role of GSNOR in the regulation of S-nitrosothiol levels in plant growth and development.

Mary

Michael Prattes, Mathias Loibl, Gertrude Zisser, Daniel Luschnig, Lisa Kappel, Ingrid Rössler, Manuela Grassegger, Altijana Hromic, Elmar Krieger, Karl Gruber, Brigitte Pertschy & Helmut Bergler

AAA-ATPases fulfil essential roles in different cellular pathways and often act in form of hexameric complexes. Interaction with pathway-specific substrate and adaptor proteins recruits them to their targets and modulates their catalytic activity. This substrate dependent regulation of ATP hydrolysis in the AAA-domains is mediated by a non-catalytic N-terminal domain. The exact mechanisms that transmit the signal from the N-domain and coordinate the individual AAA-domains in the hexameric complex are still the topic of intensive research. Here, we present the characterization of a novel mutant variant of the eukaryotic AAA-ATPase Drg1 that shows dysregulation of ATPase activity and altered interaction with Rlp24, its substrate in ribosome biogenesis. This defective regulation is the consequence of amino acid exchanges at the interface between the regulatory N-domain and the adjacent D1 AAA-domain. The effects caused by these mutations strongly resemble those of pathological mutations of the AAA-ATPase p97 which cause the hereditary proteinopathy IBMPFD (inclusion body myopathy associated with Paget's disease of the bone and frontotemporal dementia). Our results therefore suggest well conserved mechanisms of regulation between structurally, but not functionally related members of the AAA-family.

Ian

Mioto PT, Ruiz MR, Zuccarelli R, Mot AC, Corpas FJ, Freschi L, Mercier H.

Alternative fluorimetric-based method to detect and compare total S-nitrosothiols in plants.

Nitric Oxide. 2017 Mar 5;. PMID: 28274830 [PubMed - as supplied by publisher]

Thomas R Everett. Ian B Wilkinson. Christoph C Lees.
Pre-eclampsia: the Potential of GSNO Reductase Inhibitors
Curr Hypertens Rep (2017) 19:20 DOI 10.1007/s11906-017-0717-2

Patrick

1. Alternative fluorimetric-based method to detect and compare total S-nitrosothiols in plants
Paulo Tamaso Mioto a, *, Marta Rodríguez-Ruiz b, Augustin Catalin Mot d,
Rafael Zuccarelli c, Francisco J. Corpas b, Luciano Freschi c, Helenice Mercier c


a Department of Botany, Biological Sciences Center, Universidade Federal de Santa Catarina, Campus Reitor Jo~ao David Ferreira Lima, s/n, 88040-900, Florian_opolis, Brazil b Department of Biochemistry, Cell and Molecular Biology of Plants, Estaci_ on Experimental del Zaidín, CSIC, C/Profesor Albareda 1, E-18008, Granada, Spain c Department of Botany, Institute of Biosciences, Universidade de S~ao Paulo, Rua do Mat~ao 277, 05508-090, S~ao Paulo, Brazil d Faculty of Chemistry and Chemical Engineering, Babes, -Bolyai University, 1 Mihail Kog_alniceanu, 400084, Cluj Napoca, Romania

a b s t r a c t

Nitric oxide (NO) is an important signaling molecule occurring in virtually all organisms, whose mechanism of action relies mainly on its interaction with proteins or peptides by nitrosylation, forming compounds such as S-nitrosothiols (SNO). The Saville reaction and the ozone-based chemiluminescence

method are the main techniques used for nitrosylated protein quantification. While the Saville assay is not very sensitive, the ozone-based chemiluminescence is expensive and time-consuming. Here we propose a method in which the protein-bound NO groups are exposed to UV light, cleaving the bond and allowing the quantification of the derived NO molecules by diamino-rhodamine (DAR) dyes. The DARbased method was shown to be specific in plant tissues by pre-treatment of the samples with reducing agents and parallel EPR analysis. Spike-and-recovery assays revealed 72% recovery after a GSNO spike. Moreover, the method was significantly more sensitive than the Saville reaction, and this increase in sensitivity was crucial for detecting the reduced levels of nitrosylated proteins in plant species other than Arabidopsis. The method presented here is a suitable alternative to compare plant samples, allowing simple and fast detection of nitrosylated proteins.

2. Nitro-fatty acids in plant signaling: New key mediators of nitric oxide metabolism

Capilla Mata-Pérez, Beatriz Sánchez-Calvo, María N. Padilla, Juan C. Begara-Morales, Raquel Valderrama, Francisco J. Corpas, Juan B. Barroso, 

a Group of Biochemistry and Cell Signaling in Nitric Oxide, Department of Experimental Biology, Center for Advanced Studies in Olive Grove and Olive Oils, Faculty of Experimental Sciences, Campus Universitario "Las Lagunillas" s/n, University of Jaén, E-23071 Jaén, Spain b Group of Antioxidants, Free Radicals and Nitric Oxide in Biotechnology, Food and Agriculture, Department of Biochemistry, Cellular and Molecular Biology of Plants, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Granada, Spain

A B S T R A C T

Recent studies in animal systems have shown that NO can interact with fatty acids to generate nitro-fatty acids (NO₂-FAs). They are the product of the reaction between reactive nitrogen species and unsaturated fatty acids, and are considered novel mediators of cell signaling based mainly on a proven anti-inflammatory response. Although these signaling mediators have been described widely in animal systems, NO₂-FAs have scarcely been studied in plants. Preliminary data have revealed the endogenous presence of free and protein-adducted NO₂-FAs in extra-virgin olive oil (EVOO), which appear to be contributing to the cardiovascular benefits associated

with the Mediterranean diet. Importantly, new findings have displayed the endogenous occurrence of nitrolinolenic acid (NO₂-Ln) in the model plant *Arabidopsis thaliana* and the modulation of NO₂-Ln levels throughout this plant's development. Furthermore, a transcriptomic analysis by RNA-seq technology established a clear signaling role for this molecule, demonstrating that NO₂-Ln was involved in plant-defense response against different abiotic-stress conditions, mainly by inducing the chaperone network and supporting a conserved mechanism of action in both animal and plant defense processes. Thus, NO₂-Ln levels significantly rose under several abiotic-stress conditions, highlighting the strong signaling role of these molecules in the plant-protection mechanism. Finally, the potential of NO₂-Ln as a NO donor has recently been described both *in vitro* and *in vivo*. Jointly, this ability gives NO₂-Ln the potential to act as a signaling molecule by the direct release of NO, due to its capacity to induce different changes mediated by NO or NO-related molecules such as nitration and S-nitrosylation, or by the electrophilic capacity of these molecules through a nitroalkylation mechanism. Here, we describe the current state of the art regarding the advances performed in the field of NO₂-FAs in plants and their implication in plant physiology.

Keith

Coordinated Hsp110 and Hsp104 activities power protein disaggregation in *Saccharomyces cerevisiae*.

Mol Cell Biol. 2017 Mar 13.

Kaimal JM1, Kandasamy G1, Gasser F1, Andréasson C1.

Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Sweden.

Protein aggregation is intimately associated with cellular stress and is accelerated during aging, disease and cellular dysfunction. Yeast cells rely on the ATP-consuming chaperone Hsp104 to disaggregate proteins together with Hsp70. Hsp110s are ancient and abundant chaperones that form complexes with Hsp70. Here we provide *in vivo* data showing that yeast Hsp110s Sse1 and Sse2 are essential for Hsp104-dependent protein disaggregation. Following heat shock, complexes of Hsp110 and Hsp70 are recruited to protein aggregates and functions together with Hsp104 in the disaggregation process. In the absence of Hsp110, Hsp70 and Hsp104 targeting to the aggregates is impaired and the residual Hsp104 that still reaches the aggregates fails to disaggregate. Thus, coordinated activities of both Hsp104 and Hsp110 are required to reactivate aggregated proteins. These findings have important implications for the understanding of how eukaryotic cells manage misfolded and amyloid proteins.

Elizabeth

March 14, 2017

Nature Reviews in Microbiology

Cellulosomes: bacterial nanomachines for dismantling plant polysaccharides

Lior Artzi, Edward A. Bayer & Sarah Morais

p83 | doi:10.1038/nrmicro.2016.164

Cellulosomes are sophisticated multicomponent complexes that are used by bacteria to degrade cellulose from plant cell walls. In this review, Artzi, Bayer and Morais explore the structural and functional diversity of cellulosomes and their applications; for example, in microbial biofuel production.

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Alternative fluorimetric-based method to detect and compare total S-nitrosothiols in plants.

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EMBO J

Loss of pollen-specific phospholipase NOT LIKE DAD triggers gynogenesis
in maize

Laurine M Gilles, Abdelsabour Khaled, Jean-Baptiste Laffaire, Sandrine
Chaignon, Ghislaine Gendrot, Jérôme Laplaige, H  l  ne Berg  s, Gens  ric
Beydon, Vincent Bayle, Pierre Barret, Jordi Comadran, Jean-Pierre

Martinant, Peter M Rogowsky, and Thomas Widiez

Published online 22.02.2017

The function of the patatin-like phospholipase A NOT LIKE DAD (NLD) in the sperm cells of maize pollen is necessary for successful fertilization, whereas its disruption promotes the development of haploid embryos, which represent an important plant breeding tool.

<http://EMBOJ.embopress.org/content/36/6/707?etoc>

Hsp70 displaces small heat shock proteins from aggregates to initiate protein refolding

Szymon Źwirowski, Agnieszka Kłosowska, Igor Obuchowski, Nadinath B Nillegoda, Artur Piróg, Szymon Ziętkiewicz, Bernd Bukau, Axel Mogk, and Krzysztof Liberek

Published online 20.02.2017

Small heat shock proteins initially facilitate solubilization of misfolded protein aggregates, but need to be selectively dissociated to facilitate subsequent access of ATP-dependent refolding chaperones.

<http://EMBOJ.embopress.org/content/36/6/783?etoc>

Mitochondria control store-operated Ca^{2+} entry through Na^{+} and redox signals

Tsipi Ben-Kasus Nissim, Xuexin Zhang, Assaf Elazar, Soumitra Roy, Judith A Stolwijk, Yandong Zhou, Rajender K Motiani, Maxime Gueguinou, Nadine Hempel, Michal Hershfinkel, Donald L Gill, Mohamed Trebak, and Israel Sekler

Published online 20.02.2017

The activation of mitochondrial $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger NCLX controls Orai1 redox state to ensure maintenance of store-operated calcium entry (SOCE), thus shedding light on how mitochondria are linked to regulation of SOCE.

<http://EMBOJ.embopress.org/content/36/6/797?etoc>

Cell

[mTOR Signaling in Growth, Metabolism, and Disease](#) Review Article

Pages 960-976

Robert A. Saxton, David M. Sabatini

[Stress-Triggered Phase Separation Is an Adaptive, Evolutionarily Tuned Response](#) Original Research Article

Pages 1028-1040.e19 Joshua A. Riback, Christopher D. Katanski, Jamie L. Kear-Scott, Evgeny V. Pilipenko, Alexandra E. Rojek, Tobin R. Sosnick, D. Allan Drummond

In eukaryotic cells, diverse stresses trigger coalescence of RNA-binding proteins into stress granules. In vitro, stress-granule-associated proteins can demix to form liquids, hydrogels, and other assemblies lacking fixed stoichiometry. Observing these phenomena has generally required conditions far removed from physiological stresses. We show that poly(A)-binding protein (Pab1 in yeast), a defining marker of stress granules, phase separates and forms hydrogels in vitro upon exposure to physiological stress conditions. Other RNA-binding proteins depend upon low-complexity regions (LCRs) or RNA for phase separation, whereas Pab1's LCR is not required for demixing, and RNA inhibits it. Based on unique evolutionary patterns, we create LCR mutations, which systematically tune its biophysical properties and Pab1 phase separation in vitro and in vivo. Mutations that impede phase separation reduce organism fitness during prolonged stress. Poly(A)-

binding protein thus acts as a physiological stress sensor, exploiting phase separation to precisely mark stress onset, a broadly generalizable mechanism.

SCIENCE

Perspective Synthetic Biology

Yeast genome, by design

Krishna Kannan, Daniel G. Gibson^{1,2}

Science 10 Mar 2017: Vol. 355, Issue 6329, pp. 1024-1025 DOI: 10.1126/science.aam9739

A core theme in synthetic biology, “understanding by creating,” inspired the effort to generate the first synthetic cell, JCVI-Syn1.0 (1). The project Sc2.0 is elevating this concept by attempting to create a synthetic version of a more evolved organism, *Saccharomyces cerevisiae*, a eukaryotic single-celled yeast. In a set of papers in this issue (2–8), scientists of the Sc2.0 project who previously constructed a single yeast chromosome (9) now report constructing five additional yeast chromosomes (more than one-third of the entire genome) (see the photo). Using a variety of phenotypic assays and structural and functional genomics techniques, the researchers observed that the synthetic chromosomes drive biological processes just like the natural, native chromosomes.

Plant Cell

Photodamaged Chloroplasts Are Targets of Cellular Garbage Disposal

Gregory Bertoni

Plant Cell 2017 29: 199. First Published on February 10, 2017; doi:10.1105/tpc.17.00054 **OPEN**

<http://www.plantcell.org/content/29/2/199>

Entire Photodamaged Chloroplasts Are Transported to the Central Vacuole by Autophagy

Masanori Izumi, Hiroyuki Ishida, Sakuya Nakamura, and Jun Hidema

Plant Cell 2017 29: 377-394. First Published on January 25, 2017; doi:10.1105/tpc.16.00637 **OPEN**

<http://www.plantcell.org/content/29/2/377.abstract>

An autophagy process termed chlorophagy is induced by photodamage and serves to eliminate entire damaged chloroplasts via transport to the vacuole in *Arabidopsis* leaves.

Protein Degradation Rate in *Arabidopsis thaliana* Leaf Growth and Development

Lei Li, Clark J. Nelson, Josua Trösch, Ian Castleden, Shaobai Huang, and A. Harvey Millar

Plant Cell 2017 29: 207-228. First Published on January 30, 2017; doi:10.1105/tpc.16.00768 **OPEN**

<http://www.plantcell.org/content/29/2/207.abstract>

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