Corey

A split Staphylococcus aureus Cas9 as a compact genome editing tool in plants

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Split-protein methods—where a protein is split into two inactive fragments that must re-assemble to form an active protein—can be used to regulate the activity of a given protein and reduce the size of gene transcription units. Here, we show that a Staphylococcus aureus Cas9 (SaCas9) can be split, and that split-SaCas9 expressed from Agrobacterium can induce targeted mutagenesis in Nicotiana benthamiana. Since SaCas9 is smaller than the more commonly used Cas9 derived from Streptococcus pyogenes, the split-SaCas9 provides the smallest tool yet for CRISPR/Cas9 plant genome editing. Both sets of split-SaCas9 (_430N/431C and _739N/740C) exhibited genome-editing activity, and the activity of split-SaCas9_739N/740C was almost same as that of full-length SaCas9. This result indicates that split-SaCas9_739N/740C is suitable for use in targeted mutagenesis. We also show that the split-SaCas9 fragment expressed from tomato mosaic virus could induce targeted mutagenesis together with another fragment expressed from Agrobacterium, suggesting that a split-SaCas9 system using a plant virus vector is a promising tool for integration-free plant genome editing. Split-SaCas9 has the potential to regulate CRISPR/Cas9-mediated genome editing activity in plant cells both temporally and spatially.

Alyssa

Prohibitins: mitochondrial partners in development and stress response
Olivier Van Aken, James Whelan, Frank Van Breusegem

Abstract
Twelve years after their discovery in plants, prohibitins (PHBs) have retained their status as some of the most enigmatic mitochondrial proteins. Although the original hypothesis that PHBs act as negative cell cycle regulators has lost its impetus in plants, the essential molecular function(s) PHB complexes perform in the inner mitochondrial membrane are now beginning to be understood. We review the current state of knowledge to propose a unifying model that positions the PHB complex as a universal protein scaffold for key mitochondrial processes, including protein processing, respiratory chain function and mitochondrial DNA organization. Furthermore, recent findings indicate that PHBs play an active role in stress tolerance and are involved in triggering retrograde signals in response to stress and mitochondrial dysfunction.

Thi

"Cell: Alert 17 March-24 March
The Upsides and Downsides of Organelle Interconnectivity Review Article *Pages 24-34*
Daniel E. Gottschling, Thomas Nyström
Interconnectivity and feedback control are hallmarks of biological systems. This includes communication between organelles, which allows them to function and adapt to changing cellular environments. While the specific mechanisms for all communications remain opaque, unraveling the wiring of organelle networks is critical to understand how biological systems are built and why they might collapse, as occurs in aging. A comprehensive understanding of all the routes involved in inter-organelle communication is still lacking, but important themes are beginning to emerge, primarily in budding yeast. These routes are reviewed here in the context of sub-system proteostasis and complex adaptive systems theory."

Minsoo

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MSL1 is a mechanosensitive ion channel that dissipates mitochondrial membrane potential and maintains redox homeostasis in mitochondria during abiotic stress.

Lee CP(1), Maksaev G(2), Jensen GS(2), Murcha MW(1), Wilson ME(2), Fricker M(3), Hell R(4), Haswell ES(2), Millar AH(1), Sweetlove LJ(3).

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Mitochondria must maintain tight control over the electrochemical gradient across their inner membrane to allow ATP synthesis while maintaining a redox-balanced electron transport chain and avoiding excessive reactive oxygen species production. However, there is a scarcity of knowledge about the ion transporters in the inner mitochondrial membrane that contribute to control of membrane potential. We show that loss of MSL1, a member of a family of mechanosensitive

ion channels related to the bacterial channel MscS, leads to increased membrane potential of Arabidopsis mitochondria under specific bioenergetic states. We demonstrate that MSL1 localises to the inner mitochondrial membrane. When expressed in Escherichia coli, MSL1 forms a stretch-activated ion channel with a slight preference for anions and provides protection against hypo-osmotic shock. In contrast, loss of MSL1 in Arabidopsis did not prevent swelling of isolated mitochondria in hypo-osmotic conditions. Instead, our data suggest that ion transport by MSL1 leads to dissipation of mitochondrial membrane potential when it becomes too high. The importance of MSL1 function was demonstrated by the observation of a higher oxidation state of the mitochondrial glutathione pool in msl1-1 mutants under moderate heat- and heavy-metal-stress. Furthermore, we show that MSL1 function is not directly implicated in mitochondrial membrane potential pulsing, but is complementary and appears to be important under similar conditions.

Patrick

1. **A fluorogenic probe for imaging protein S-nitrosylation in live cells**

Shiyi Shaoa,1, Bo Chenb,1, Juan Chenga, Chengkun Wanga, Yanli Zhanga, Lingxiao Shaoa, Yongzhou Hua, Yifeng Hanb, Feng Hana,□, Xin Lia,□
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b Department of Chemistry, Zhejiang Sci-Tech University, Hangzhou 310018, China

A B S T R A C T
S-nitrosylation is a posttranslational modification of protein cysteine residues leading to the formation of Snitrosothiols and its detection is crucial to understanding of redox regulation and NO-based signaling. Prototypical detection methods for S-nitrosylation are always carried out ex situ. However, the reversible nature and the tendency of transnitrosylation highlight the necessity of its probing in intact live biological contexts. Herein we provide a fluorogenic chemical probe for the detection of S-nitrosylation in live endothelial cells. The probe is weakly emissive alone and becomes highly fluorescent only after undergoing a reaction with S-nitrosothiols in live cellular environments. This probe features high degrees of specificity and desirable sensitivity. Furthermore, it has been successfully applied to image the dynamic change of protein S-nitrosylation in live endothelial cells. The applicability of the probe in complex biological systems has been additionally verified by imaging a known target of S-nitrosylation, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in live cells. Due to the versatility exemplified, this probe holds great promise for exploring the role of protein Snitrosylation in the pathophysiological process of a variety of vascular diseases.

2. **S-nitrosoglutathione reductase (GSNOR) activity is down-regulated during pepper (Capsicum annuum L.) fruit ripening**

Marta Rodríguez-Ruiz a, Paulo Mioto b, Jos_e M. Palma a, Francisco J. Corpas a, *
a Group of Antioxidants, Free Radicals and Nitric Oxide in Biotechnology, Food and Agriculture, Department of Biochemistry, Cell and Molecular Biology of

Plants, Estaci_on Experimental del Zaidín, CSIC, C/Profesor Albareda 1, E-18008 Granada, Spain
b Departamento de Bot^anica, Universidade de S~ao Paulo, Brazil

a b s t r a c t
Pepper (Capsicum annuum L.) is an annual plant species of great agronomic importance whose fruits
undergo major metabolic changes through development and ripening. These changes include emission
of volatile organic compounds associated with respiration, destruction of chlorophylls and synthesis of
new pigments (red/yellow carotenoids plus xanthophylls and anthocyans) responsible for color shift,
protein degradation/synthesis and changes in total soluble reducing equivalents. Previous data have
shown that, during the ripening of pepper fruit, an enhancement of protein tyrosine nitration takes
place. On the other hand, it is well known that S-nitrosoglutathione reductase (GSNOR) activity can
modulate the transnitrosylation equilibrium between GSNO and S-nitrosylated proteins and, consequently,
regulate cellular NO homeostasis. In this study, GSNOR activity, protein content and gene
expression were analyzed in green and red pepper fruits. The content of S-nitrosylated proteins on
diaminofluorescein (DAF) gels was also studied. The data show that, while GSNOR activity and protein
expression diminished during fruit ripening, S-nitrosylated protein content increased. Some of the
protein candidates for S-nitrosylation identified, such as cytochorme c oxidase and peroxiredoxin II E,
have previously been described as targets of this posttranslational modification in other plant species.
These findings corroborate the important role played by GSNOR activity in the NO metabolism during the
process of pepper fruit ripening.

Ian

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

Nitric Oxide. 2017 Mar 6.

Mioto PT[1], Rodríguez-Ruiz M[2], Mot AC[3], Zuccarelli R[4], Corpas FJ[2], Freschi L[4], Mercier H[4].

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2Department of Biochemistry, Cell and Molecular Biology of Plants, Estación Experimental del Zaidín, CSIC, C/Profesor Albareda 1, E-18008, Granada, Spain.
3Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, 1 Mihail Kogălniceanu, 400084, Cluj Napoca, Romania.
4Department of Botany, Institute of Biosciences, Universidade de São Paulo, Rua do Matão 277, 05508-090, São Paulo, Brazil.
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 DAR-based 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.

Keith

pH-dependent structural modulation is conserved in the human small heat shock protein HSBP1.

Cell Stress Chaperones. 2017 Mar 22

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The holdase activity and oligomeric propensity of human small heat shock proteins (sHSPs) are regulated by environmental factors. However, atomic-level details are lacking for the mechanisms by which stressors alter sHSP responses. We previously demonstrated that

regulation of HSPB5 is mediated by a single conserved histidine over a physiologically relevant pH range of 6.5-7.5. Here, we demonstrate that HSPB1 responds to pH via a similar mechanism through pH-dependent structural changes that are induced via protonation of the structurally analogous histidine. Results presented here show that acquisition of a positive charge, either by protonation of His124 or its substitution by lysine, reduces the stability of the dimer interface of the α-crystallin domain, increases oligomeric size, and modestly increases chaperone activity. Our results suggest a conserved mechanism of pH-dependent structural regulation among the human sHSPs that possess the conserved histidine, although the functional consequences of the structural modulations vary for different sHSPs.

The small heat shock proteins αB-crystallin (HSPB5) and Hsp27 (HSPB1) inhibit the intracellular aggregation of α-synuclein.

Cell Stress Chaperones. 2017 Mar 23

Cox D1,2, Ecroyd H3,4.

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Protein homeostasis, or proteostasis, is the process of maintaining the conformational and functional integrity of the proteome. Proteostasis is preserved in the face of stress by a complex network of cellular machinery, including the small heat shock molecular chaperone proteins (sHsps), which act to inhibit the aggregation and deposition of misfolded protein intermediates. Despite this, the pathogenesis of several neurodegenerative diseases has been inextricably linked with the amyloid fibrillar aggregation and deposition of α-synuclein (α-syn). The sHsps are potent inhibitors of α-syn aggregation in vitro. However, the limited availability of a robust, cell-based model of α-syn aggregation has, thus far, restricted evaluation of sHsp efficacy in the cellular context. As such, this work sought to establish a robust model of intracellular α-syn aggregation using Neuro-2a cells. Aggregation of α-syn was found to be sensitive to inhibition of autophagy and the proteasome, resulting in a significant increase in the proportion of cells containing α-syn inclusions. This model was then used to evaluate the capacity of the sHsps, αB-c and Hsp27, to prevent α-syn aggregation in cells. To do so, we used bicistronic expression plasmids to express the sHsps. Unlike traditional fluorescent fusion constructs, these bicistronic expression plasmids enable only individual transfected cells expressing the sHsps (via expression of the fluorescent reporter) to be analysed, but without the need to tag the sHsp, which can affect its oligomeric structure and chaperone activity. Overexpression of both αB-c and Hsp27 significantly reduced the intracellular aggregation of α-syn. Thus, these findings suggest that overexpressing or boosting the activity of sHsps may be a way of preventing amyloid fibrillar aggregation of α-syn in the context of neurodegenerative disease.

Elizabeth

April 4, 2017
ABNORMAL INFLORESCENCE MERISTEM1 Functions in Salicylic Acid Biosynthesis to Maintain Proper Reactive Oxygen Species Levels for Root Meristem Activity in Rice
Lei Xu, Hongyu Zhao, Wenyuan Ruan, Minjuan Deng, Fang Wang, Jinrong Peng, Jie Luo, Zhixiang Chen, and Keke Yi
Plant Cell 2017 tpc.16.00665; Advance Publication March 14, 2017; doi:10.1105/tpc.16.00665
http://www.plantcell.org/content/early/2017/03/17/tpc.16.00665.abstract

Cell: Alert 17 March-24 March
The Upsides and Downsides of Organelle Interconnectivity Review Article *Pages 24-34* Daniel E. Gottschling, Thomas Nyström
Interconnectivity and feedback control are hallmarks of biological systems. This includes communication between organelles, which allows them to function and adapt to changing cellular environments. While the specific mechanisms for all communications remain opaque, unraveling the wiring of organelle networks is critical to understand how biological systems are built and why they might collapse, as occurs in aging. A comprehensive understanding of all the routes involved in inter-organelle communication is still lacking, but important themes are beginning to emerge, primarily in budding yeast. These routes are reviewed here in the context of sub-system proteostasis and complex adaptive systems theory.
SnapShot: Subcellular mRNA Localization *Pages 178-178.e1* Mohammad Mofatteh, Simon L. Bullock
Many cells localize mRNAs to discrete locations in the cytoplasm. Coupled to local translation, this process affords precise spatial and temporal control of protein function. This SnapShot provides an overview of the key events in subcellular mRNA localization and highlights recent progress in understanding how cytoskeletal motors orchestrate mRNA trafficking.
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Pollen-mediated gene flow from glyphosate-resistant common waterhemp (Amaranthus rudis Sauer): consequences for the dispersal of resistance genes.
Sci Rep. 2017 Mar 22;7:44913. PMID: 28327669 [PubMed - in process]

Current Opinion in Structural Biology: Alert 19 March-26 March
Computational and theoretical advances in studies of intrinsically disordered proteins Review Article
Pages 147-154 Robert B Best
Physical and molecular bases of protein thermal stability and cold adaptation Review Article
Pages 117-128 Fabrizio Pucci, Marianne Rooman
PLOS Genetics Volume 13(3) March 2017
Fishing for adaptive epistasis using mitonuclear interactions
David M. Rand
This is really an evolution and population article – but an interesting perspective:
One unexpected result from these analyses is that that none of the 349 F_{st} outlier loci map to known nuclear genes encoding subunits of the OXPHOS complexes. If this study really has identified loci important in mitonuclear coadaptation, it suggests that long-range *cis*- or *trans*-regulatory factors, or downstream pathway effects, are mediating the epistatic interactions between mtDNA haplotypes and

nuclear loci other than those encoding subunits of OXPHOS complexes. Understanding the genetic basis of fitness variation in the wild is a central goal of ecological and evolutionary genetics, and the genes of central metabolism have long fascinated biologists as a logical place to search for the source of this variation [6]. Given the critical role that mitochondrial function plays in organismal performance, and the increasing knowledge of the diverse roles of mitonuclear communication in regulating homeostasis, there is a real hope that we can track down the biochemical bases of these kinds of nonneutral patterns in nature. The Baris et al. study offers a nice example of how admixed populations with divergent mtDNAs might serve as a natural genetic screen for the footprints of mitonuclear epistasis and coevolution that could point to unanticipated targets of selection on metabolic function.
The article is:

Evolved genetic and phenotypic differences due to mitochondrial-nuclear interactions

Tara Z. Baris, Dominique N. Wagner, David I. Dayan, Xiao Du, Pierre U. Blier, Nicolas Pichaud, Marjorie F. Oleksiak, Douglas L. Crawford

Don't remember if I saw this when it came out – metazoan centric, but perhaps of interest:
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Final touches and quality control on the assembly of the eukaryotic ribosome Aida Razi and Joaquin Ortega
New high-resolution cryo-EM structures capture the final stages of 60S subunit maturation, illustrating the ordered remodeling and assembly factor release steps required for the formation of a functional ribosome.
http://EMBOJ.embopress.org/content/36/7/834?etoc

Nmd3 is a structural mimic of eIF5A, and activates the cpGTPase Lsg1 during 60S ribosome biogenesis
Andrey G Malyutin, Sharmishtha Musalgaonkar, Stephanie Patchett, Joachim Frank, and Arlen W Johnson
Published online 08.02.2017
Cryo-EM structures of the yeast 60S subunit bound to assembly factors Nmd3,Lsg1, and Tif6 reveal the conformational rearrangements that take place during final ribosome maturation in the cytoplasm.
http://EMBOJ.embopress.org/content/36/7/854?etoc

GTPase ROP2 binds and promotes activation of target of rapamycin, TOR, in response to auxin
Mikhail Schepetilnikov, Joelle Makarian, Ola Srour, Angèle Geldreich, Zhenbiao Yang, Johana Chicher, Philippe Hammann, and Lyubov A Ryabova
Published online 28.02.2017
Auxin signaling activates the small GTPase ROP2, causing TOR activation and increased translation of uORF-containing mRNAs, thus making ROP2 the intervening link between auxin signaling and TOR-dependent translation control.
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PLOS Biology Volume 15(3) March 2017

Research priorities for harnessing plant microbiomes in sustainable agriculture

Posy E. Busby, Chinmay Soman, Maggie R. Wagner, Maren L. Friesen, James Kremer, Alison Bennett, Mustafa Morsy, Jonathan A. Eisen, Jan E. Leach, Jeffery L. Dangl
A better understanding of plant-associated microbiomes and ways to optimize them could enhance agricultural productivity and sustainability; this Perspective article describes five research priorities that will help achieve these goals.

Feeding a growing world population amidst climate change requires optimizing the reliability, resource use, and environmental impacts of food production. One way to assist in achieving these goals is to integrate beneficial plant microbiomes—i.e., those enhancing plant growth, nutrient use efficiency, abiotic stress tolerance, and disease resistance—into agricultural production. This integration will require a large-scale effort among academic researchers, industry researchers, and farmers to understand and manage plant-microbiome interactions in the context of modern agricultural systems. Here, we identify priorities for research in this area: (1) develop model host–microbiome systems for crop plants and non-crop plants with associated microbial culture collections and reference genomes, (2) define core microbiomes and metagenomes in these model systems, (3) elucidate the rules of synthetic, functionally programmable microbiome assembly, (4) determine functional mechanisms of plant-microbiome interactions, and (5) characterize and refine plant genotype-by-environment-by-microbiome-by-management interactions. Meeting these goals should accelerate our ability to design and implement effective agricultural microbiome manipulations and management strategies, which, in turn, will pay dividends for both the consumers and producers of the world food supply.

Science

On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacteria

By Rochelle M. Soo, James Hemp, Donovan H. Parks, Woodward W. Fischer, Philip Hugenholtz

*Science*31 Mar 2017 : 1436-1440 Full Access

Oxygen-producing photosynthesis and oxygen-consuming respiration evolved after the divergence of the main lineages of blue-green algae.

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