Keith:

Allosteric regulation points control the conformational dynamics of the molecular chaperone Hsp90.

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Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone responsible for the activation, maturation and trafficking of several hundred client proteins in the cell. It is well known that (but not understood how) residues far away from Hsp90's nucleotide binding pocket can regulate its ATPase activity, a phenomenon called allosteric regulation. Here, the computational design of allosteric mutations based was combined with in vitro and in vivo experiments to unravel nucleotide-responsive hot spots in the regulation of Hsp90. With this approach, we identified both activating and inhibiting regulation points and show that changes in those amino acids affect the conformational dynamics and ATPase activity of Hsp90 in vitro. Our observations that activating mutations loosen and inhibiting mutations ridgify the protein explain for the first time, how Hsp90 changes in response to allosteric mutations. Additionally, mutations of these allosteric regulation points can be controlled by the interplay with Hsp90 co-chaperones, thus providing cells with an efficient mechanism of modifying Hsp90's intrinsic properties via different layers of regulation. Altogether, our results show that a framework for transmitting conformational information exists in the Hsp90 structure.

Bacterial and Yeast AAA + Disaggregases ClpB and Hsp104 Operate through Conserved Mechanism Involving Cooperation with Hsp70

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Escherichia coli ClpB and *Saccharomyces cerevisiae* Hsp104 are members of the Hsp100 family of ring-forming hexameric AAA + chaperones that promote the solubilization of aggregated proteins and the propagation of prions. ClpB and Hsp104 cooperate with cognate Hsp70 chaperones for substrate targeting and activation of ATPase and substrate threading, achieved by transient Hsp70 binding to the repressing ClpB/Hsp104 M-domain. Fundamental differences in ATPase regulation and disaggregation mechanisms have been reported; however, these differences are raising doubts regarding the working principle of this AAA + chaperone. In particular, unique functional plasticity was suggested to specifically enable Hsp104 to circumvent Hsp70 requirement for derepression in protein disaggregation and prion propagation. We show here that both ClpB and Hsp104 cooperation with Hsp70 is crucial for efficient protein disaggregation and, in contrast to earlier claims, cannot be circumvented by activating M-domain mutations. Activation of ClpB and Hsp104 requires two signals, relief of M-domain repression and substrate binding, leading to increased ATPase subunit coupling. These data demonstrate that ClpB and Hsp104 operate by the same basic mechanism, underscore a dominant function of Hsp70 in regulating ClpB/Hsp104 activity, and explain a plethora of *in vivo* studies showing a crucial function of Hsp70 in proteostasis and prion propagation.

Jesse

EGRINs (Environmental Gene Regulatory Influence Networks) in Rice 4 That Function in the Response to Water Deficit, High Temperature, and 5 Agricultural Environments

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43 Environmental Gene Regulatory Influence Networks (EGRINs) coordinate the timing and rate of gene expression in response to environmental signals. EGRINs encompass many layers of regulation, which culminate in changes in accumulated transcript levels. Here, we inferred EGRINs for the response of five tropical Asian rice (Oryza sativa) cultivars to high temperatures, water deficit, and agricultural field conditions by systematically integrating time series transcriptome data, patterns of nucleosome-free

chromatin, and the occurrence of known cis- regulatory elements. First, we identified 5,447 putative target genes for 445 transcription factors (TFs) by connecting TFs with genes harboring known cis-regulatory motifs in nucleosome-free regions proximal to their transcriptional start sites. We then used network component analysis to estimate the regulatory activity for each TF based on the expression of its putative target genes. Finally, we inferred an EGRIN using the estimated TFA as the regulator. The EGRINs include regulatory interactions between 4,052 target genes regulated by 113 TFs. We resolved distinct regulatory roles for members of the heat shock factor family, including a putative regulatory connection between abiotic stress and the circadian clock. TFA estimation using network component analysis is an effective way of incorporating multiple genome-scale measurements into network inference.

Mary

A Surveillance Function of the HSPB8-BAG3-HSP70 Chaperone Complex Ensures Stress Granule Integrity and Dynamism

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Stress granules (SGs) are ribonucleoprotein complexes induced by stress. They sequester mRNAs and disassemble when the stress subsides, allowing translation restoration. In amyotrophic lateral sclerosis (ALS), aberrant SGs cannot disassemble and therefore accumulate and are degraded by autophagy. However, the molecular events causing aberrant SG formation and the molecular players regulating this transition are largely unknown. We report that defective ribosomal products (DRiPs) accumulate in SGs and promote a transition into an aberrant state that renders SGs resistant to RNase. We show that only a minor fraction of aberrant SGs is targeted by autophagy, whereas the majority disassembles in a process that requires assistance by the HSPB8-BAG3-HSP70 chaperone complex. We further demonstrate that HSPB8-BAG3-HSP70 ensures the functionality of SGs and restores proteostasis by targeting DRiPs for degradation. We propose a system of chaperone-mediated SG surveillance, or granulostasis, which regulates SG composition and dynamics and thus may play an important role in ALS.

Patrick

1. Glutaredoxin GRXS17 Associates with the Cytosolic
Iron-Sulfur Cluster Assembly Pathway
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Cytosolic monothiol glutaredoxins (GRXs) are required in iron-sulfur (Fe-S) cluster delivery and iron sensing in yeast and
mammals. In plants, it is unclear whether they have similar functions. Arabidopsis (Arabidopsis thaliana) has a sole class II
cytosolic monothiol GRX encoded by GRXS17. Here, we used tandem affinity purification to establish that Arabidopsis GRXS17
associates with most known cytosolic Fe-S assembly (CIA) components. Similar to mutant plants with defective CIA
components, grxs17 loss-of-function mutants showed some degree of hypersensitivity to DNA damage and elevated
expression of DNA damage marker genes. We also found that several putative Fe-S client proteins directly bind to GRXS17,
such as XANTHINE DEHYDROGENASE1 (XDH1), involved in the purine salvage pathway, and CYTOSOLIC THIOURIDYLASE
SUBUNIT1 and CYTOSOLIC THIOURIDYLASE SUBUNIT2, both essential for the 2-thiolation step of 5-methoxycarbonylmethyl-2-
thiouridine (mcm5s2U) modification of tRNAs. Correspondingly, profiling of the grxs17-1 mutant pointed to a perturbed flux through the purine degradation pathway and revealed that it phenocopied mutants in the elongator subunit ELO3, essential for the mcm5
tRNA modification step, although we did not find XDH1 activity or tRNA thiolation to be markedly reduced in the grxs17-1 mutant. Taken together, our data suggest that plant cytosolic monothiol GRXs associate with the CIA complex, as in other eukaryotes, and
contribute to, but are not essential for, the correct functioning of client Fe-S proteins in unchallenged conditions.

2. High CO2 Primes Plant Biotic Stress Defences through
Redox-Linked Pathways
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Industrial activities have caused tropospheric CO2 concentrations to increase over the last two centuries, a trend that is predicted to continue for at least the next several decades. Here, we report that growth of plants in a CO2-enriched environment activates
responses that are central to defense against pathogenic attack. Salicylic acid accumulation was triggered by high-growth CO2 in Arabidopsis (Arabidopsis thaliana) and other plants such as bean (Phaseolus vulgaris). A detailed analysis in Arabidopsis revealed
that elevated CO2 primes multiple defense pathways, leading to increased resistance to bacterial and fungal challenge. Analysis of gene-specific mutants provided no evidence that activation of plant defense pathways by high CO2 was caused by stomatal
closure. Rather, the activation is partly linked to metabolic effects involving redox signaling. In support of this, genetic modification of redox components (glutathione contents and NADPH-generating enzymes) prevents full priming of the
salicylic acid pathway and associated resistance by high CO2. The data point to a particularly influential role for the nonphosphorylating glyceraldehyde-3-phosphate dehydrogenase, a cytosolic enzyme whose role in plants remains unclear. Our observations add new information on relationships between high CO2 and oxidative signaling and provide novel insight into plant stress responses in conditions of increased CO2

Alyssa

Neuroendocrine Coordination of Mitochondrial Stress Signaling and Proteostasis

Abstract: During neurodegenerative disease, the toxic accumulation of aggregates and misfolded proteins is often accompanied with widespread changes in peripheral metabolism, even in cells in which the aggregating protein is not present. The mechanism by which the central nervous system elicits a distal reaction to proteotoxic stress remains unknown. We hypothesized that the endocrine communication of neuronal stress plays a causative role in the changes in mitochondrial homeostasis associated with proteotoxic disease states. We find that an aggregation-prone protein expressed in the neurons of *C. elegans* binds to mitochondria, eliciting a global induction of a mitochondrial-specific unfolded protein response (UPRmt), affecting whole-animal physiology. Importantly, dense core vesicle release and secretion of the neurotransmitter serotonin is required for the signal's propagation. Collectively, these data suggest the commandeering of a nutrient sensing network to allow for cell-to-cell

communication between mitochondria in response to protein folding stress in the nervous system.

Minsoo

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Mitochondrial Flash: Integrative Reactive Oxygen Species and pH Signals in Cell and Organelle Biology.

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SIGNIFICANCE: Recent breakthroughs in mitochondrial research have advanced, reshaped, and revolutionized our view of the role of mitochondria in health and disease. These discoveries include the development of novel tools to probe mitochondrial biology, the molecular identification of mitochondrial functional proteins, and the emergence of new concepts and mechanisms in mitochondrial function regulation. The discovery of "mitochondrial flash" activity has provided unique insights not only into real-time visualization of individual mitochondrial redox and pH dynamics in live cells but has also advanced understanding of the excitability, autonomy, and integration of mitochondrial function in vivo.

RECENT ADVANCES: The mitochondrial flash is a transient and stochastic event confined within an individual mitochondrion and is observed in a wide range of organisms from plants to Caenorhabditis elegans to mammals. As flash events involve multiple transient concurrent changes within the mitochondrion (e.g., superoxide, pH, and membrane potential), a number of different mitochondrial targeted fluorescent indicators can detect flash activity. Accumulating evidence indicates that flash events reflect integrated snapshots of an intermittent mitochondrial process arising from mitochondrial respiration chain activity associated with the transient opening of the mitochondrial permeability transition pore.

CRITICAL ISSUES: We review the history of flash discovery, summarize current understanding of flash biology, highlight controversies regarding the relative

roles of superoxide and pH signals during a flash event, and bring forth the integration of both signals in flash genesis.

FUTURE DIRECTIONS: Investigations using flash as a biomarker and establishing its role in cell signaling pathway will move the field forward. Antioxid. Redox Signal. 25, 534-549.