

Lit Lunch: 1/24/14

DAMIAN

1) Identification of endogenously S-nitrosylated proteins in Arabidopsis plantlets: Effect of cold stress on cysteine nitrosylation level

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**Plant Science, Volumes 215–216, February 2014, Pages 150-156, ISSN 0168-9452, <http://dx.doi.org/10.1016/j.plantsci.2013.10.014>.
(<http://www.sciencedirect.com/science/article/pii/S0168945213002318>)**

Multiple approaches in the past have identified S-nitrosated proteins in plants and animals, but in plants they have all generally followed treatment with an NO donor. Previous work showed NO synthesis was necessary for cold stress adaptation and induction of cold stress-activated transcription factors. Here, endogenous nitrosated proteins are identified from Arabidopsis 14-day seedlings following control or cold stress. This is in contrast to the typical identification of proteins after treatment with SNP or GSNO, which is furthermore typically performed on cell culture rather than on intact plants. The biotin switch assay and isotope-coded affinity tag (ICAT) methods were implemented to recover endogenously nitrosated proteins and compare the increase in nitrosation during cold stress, respectively. BST: After protein extraction, free thiols are blocked with MMTS, SNOs are reduced with ascorbate, and then tagged with activated biotin. Biotin is an affinity tag that reacts with streptavidin. ICAT: Similar chemical procedure, except linker between cysteine-reactive species and the biotin tag is varied (usually by 8 Daltons) such that abundance between two biological treatments can be compared. The BST identified 62 total proteins, many of them previously found to be nitrosated, but some of them not. Many of these were Calvin cycle enzymes. ICAT revealed that cold treatment enhanced the abundance of the nitrosation on the same proteins, sometimes by as much as 7-fold.

2) A comprehensive study of thiol reduction gene expression under stress conditions in Arabidopsis thaliana.

Plant, Cell & Environment. <http://dx.doi.org/10.1111/pce.12276> [10.1111/pce.12276](http://dx.doi.org/10.1111/pce.12276)

BELIN, C BASHANDY, T CELA, J DELORME-HINOUX, V RIONDET, C REICHELLED, JP.

Basically, an mRNA expression atlas for all of the GRX and TRX genes in Arabidopsis. No real big surprises here. Although, there are multiple nomenclature systems for ROXY GRXs, and this paper introduces yet another. The S-ROXYs upregulated in hot5-2 are

ROXY11-16, but not ROXY13, according to this paper's system. SROXYs of interest are expressed highest in the procambium and rosette leaves, as deduced previously from BAR Toronto. Here, however, it is explicitly quantified. The S-ROXYs of interest are not highly expressed genes in any tissue relative to other GRXs or Arabidopsis genes in general. There are few general patterns of SROXY11-16 induction, although various stresses cause moderate up- and down-regulation of individual genes. UV-B radiation causes SROXY11-16 to increase ~2.8-fold in shoots, but not roots. A similar magnitude decrease happens for these SROXYs 3 hours after Brassinosteroid treatment

3) A new role for glutathione in the regulation of root architecture linked to strigolactones.

Plant, Cell & Environment. MARQUEZ-GARCIA, BELEN NJO, MARIA BEECKMAN, TOM GOORMACHTIG, SOFIE FOYER, CHRISTINE H.

<http://dx.doi.org/10.1111/pce.12172> [10.1111/pce.12172](http://dx.doi.org/10.1111/pce.12172)

GSH essential for redox homeostasis. Knockout mutants in plants are embryo lethal, whereas plants that make little GSH (root meristemless 1 or cadmium sensitive 2), even though they make very little GSH (maybe only 5% of wild-type) are capable of survival, most likely due to partial redundancy of the thioredoxin system in maintaining cellular redox status. Lateral root formation depends on auxin, but also on strigolactones (SL), which are plant hormones derived from carotenoids (pathway not yet deciphered). SLs tend to curtail lateral root growth. The MAX2 mutation (F-box protein, leading to defect in SL signaling) leads to plants with higher tolerance to oxidative stress. max2 plants also have higher lateral root density. The aim of this study was to better understand the GSH-SL axis. GSH mutants such as pad2 and cad2 exhibited impaired lateral root formation under control conditions. Buthionine sulfoximine (BSO) inhibits GSH synthesis. When WT plants were BSO-treated, however, there was no change in lateral root density or initiation, only primary root length, and auxin responses (as assessed by a DR5::GUS line) were not different in roots either. Treatment with SL G24 caused GSH to increase in Col-0 and SL biosynthetic mutants, but not in max2, which is a SL signaling mutant. G24 also predictably caused lateral root density to be lower than in mock-treated controls, but the lateral root density drop was more pronounced in Col-0 than in GSH biosynthetic mutants. Auxin treatment typically causes LR density to go up. This still occurs in SL mutants, but the effect is less pronounced. Treatment with SLs usually causes LR density to go down. This also still occurs in SL mutants, but not as dramatically. These results, on first impression, are rather confusing. I think what we can take away from it is this: SL perception is necessary for an increase in GSH. Plants with impaired GSH biosynthesis have lower LR densities to begin with, but they are also less sensitive to G24-dependent inhibition of LR formation. There may be a link between GSH levels and LR formation, but this paper does not give us a mechanism.

KEITH

1) Cuz1/Ynl155w, a Zinc-dependent Ubiquitin-binding Protein, Protects Cells from Metalloid-induced Proteotoxicity

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL.289, NO.3, pp.1876–1885, January 17, 2014

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Protein misfolding is a universal threat to cells. The ubiquitin- proteasome system mediates a cellular stress response capable of eliminating misfolded proteins. Here we identify Cuz1/ Ynl155w as a component of the ubiquitin system, capable of interacting with both the proteasome and Cdc48. Cuz1/ Ynl155w is regulated by the transcription factor Rpn4, and is required for cells to survive exposure to the trivalent metalloids arsenic and antimony. A related protein, Yor052c, shows similar phenotypes, suggesting a multicomponent stress response path- way. Cuz1/Ynl155w functions as a zinc-dependent ubiquitin-bind- ing protein. Thus, Cuz1/Ynl155w is proposed to protect cells from metalloid-induced proteotoxicity by delivering ubiquitinated sub- strates to Cdc48 and the proteasome for destruction.

2) Conformation and dynamics of the periplasmic membrane- protein- chaperone complexes OmpX-Skp and tOmpA-Skp

Nature Structural & Molecular Biology **20**, 1265–1272 (2013)

Björn M Burmann, Congwei Wang & Sebastian Hiller

Biozentrum, University of Basel, Basel, Switzerland.

The biogenesis of integral outer-membrane proteins (OMPs) in Gram-negative bacteria requires molecular chaperones that prevent the aggregation of OMP polypeptides in the aqueous periplasmic space. How these energy-independent chaperones interact with their substrates is not well understood. We have used high-resolution NMR spectroscopy to examine the conformation and dynamics of the *Escherichia coli* periplasmic chaperone Skp and two of its complexes with OMPs. The Skp trimer constitutes a flexible architectural scaffold that becomes more rigid upon substrate binding. The OMP substrates populate a dynamic conformational ensemble with structural interconversion

rates on the submillisecond timescale. The global lifetime of the chaperone–substrate complex is seven orders of magnitude longer, emerging from the short local lifetimes by avidity. The dynamic state allows for energy-independent substrate release and provides a general paradigm for the conformation of OMP polypeptides bound to energy-independent chaperones.

FIONN

1) Nature

GM food in the public mind- facts are not what they used to be The necessity of GM food for India

2) Cell

Potentiated hsp104 variants antagonize diverse proteotoxic misfolding events. Jackrel ME1, Desantis ME2, Martinez BA3, Castellano LM4, Stewart RM1, Caldwell KA3, Caldwell GA3, Shorter J5.

Abstract

There are no therapies that reverse the proteotoxic misfolding events that underpin fatal neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). Hsp104, a conserved hexameric AAA+ protein from yeast, solubilizes disordered aggregates and amyloid but has no metazoan homolog and only limited activity against human neurodegenerative disease proteins. Here, we reprogram Hsp104 to rescue TDP-43, FUS, and α -synuclein proteotoxicity by mutating single residues in helix 1, 2, or 3 of the middle domain or the small domain of nucleotide-binding domain 1. Potentiated Hsp104 variants enhance aggregate dissolution, restore proper protein localization, suppress proteotoxicity, and in a *C. elegans* PD model attenuate dopaminergic neurodegeneration. Potentiating mutations reconfigure how Hsp104 subunits collaborate, desensitize Hsp104 to inhibition, obviate any requirement for Hsp70, and enhance ATPase, translocation, and unfoldase activity. Our work establishes that disease-associated aggregates and amyloid are tractable targets and that enhanced disaggregases can restore proteostasis and mitigate neurodegeneration.

3) Science

A spatial accommodation by neighboring cells is required for organ initiation in Arabidopsis.

Vermeer JE, von Wangenheim D, Barberon M, Lee Y, Stelzer EH, Maizel A, Geldner N.

Abstract

Lateral root formation in plants can be studied as the process of interaction between chemical signals and physical forces during development. Lateral root

primordia grow through overlying cell layers that must accommodate this incursion. Here, we analyze responses of the endodermis, the immediate neighbor to an initiating lateral root. Endodermal cells overlying lateral root primordia lose volume, change shape, and relinquish their tight junction-like diffusion barrier to make way for the emerging lateral root primordium. Endodermal feedback is absolutely required for initiation and growth of lateral roots, and we provide evidence that this is mediated by controlled volume loss in the endodermis. We propose that turgidity and rigid cell walls, typical of plants, impose constraints that are specifically modified for a given developmental process.

UMARU

1) PNAS: Human resistin, a proinflammatory cytokine, shows chaperone-like activity

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[Author Affiliations](#)

Edited by Linda L. Randall, University of Missouri, Columbia, MO, and approved November 6, 2013 (received for review April 2, 2013)

Resistin, a cysteine-rich adipocytokine, proposed as a link between obesity and diabetes in mice, was shown as a proinflammatory molecule in humans. We earlier reported that human resistin (hRes), a trimer, was resistant to heat and urea denaturation, existed in an oligomeric polydispersed state, and showed a concentration-dependent conformational change. These properties and an intimate correlation of hRes expression with cellular stress prompted us to investigate hRes as a possible chaperone. Here, we show that recombinant human resistin was able to protect the heat-labile enzymes citrate synthase and Nde1 from thermal aggregation and inactivation and was able to refold and restore their enzymatic activities after heat/guanidinium chloride denaturation. Furthermore, recombinant human resistin could bind misfolded proteins only. Molecular dynamics-based association–dissociation kinetics of hRes subunits pointed to resistin being a molecular chaperone. Bis-ANS, which blocks surface hydrophobicity, abrogated the chaperone activity of hRes, establishing the importance of surface hydrophobicity for chaperone activity. Replacement of Phe49 with Tyr (F49YhRes), a critical residue within the hydrophobic patch of hRes, although it could prevent thermal aggregation of citrate synthase and Nde1, was unable to refold and restore their activities. Treatment of U937 cells with tunicamycin/thapsigargin resulted in reduced hRes secretion and concomitant localization in the endoplasmic reticulum. *Escherichia coli* transformants expressing hRes could be rescued from thermal stress, pointing to hRes's chaperone-like function in vivo. HeLa cells transfected with hRes showed protection from thapsigargin-induced apoptosis. In conclusion, hRes, an inflammatory protein, additionally exhibited chaperone-like properties, suggesting a possible link between inflammation and cellular stress.

INDU

1. Science. 2013 Nov 22;342(6161):979-83. doi: 10.1126/science.1245321. Epub 2013 Oct 24.

Yeast reveal a "druggable" Rsp5/Nedd4 network that ameliorates α -synuclein toxicity in neurons.

Tardiff DF, Jui NT, Khurana V, Tambe MA, Thompson ML, Chung CY, Kamadurai HB, Kim HT, Lancaster AK, Caldwell KA, Caldwell GA, Rochet JC, Buchwald SL, Lindquist S.

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α -Synuclein (α -syn) is a small lipid-binding protein implicated in several neurodegenerative diseases, including Parkinson's disease, whose pathobiology is conserved from yeast to man. There are no therapies targeting these underlying cellular pathologies, or indeed those of any major neurodegenerative disease. Using unbiased phenotypic screens as an alternative to target-based approaches, we discovered an N-aryl benzimidazole (NAB) that strongly and selectively protected diverse cell types from α -syn toxicity. Three chemical genetic screens in wild-type yeast cells established that NAB promoted endosomal transport events dependent on the E3 ubiquitin ligase Rsp5/Nedd4. These same steps were perturbed by α -syn itself. Thus, NAB identifies a druggable node in the biology of α -syn that can correct multiple aspects of its underlying pathology, including dysfunctional endosomal and endoplasmic reticulum-to-Golgi vesicle trafficking.

PMID: 24158909 [PubMed - indexed for MEDLINE]

2. Science. 2013 Nov 22;342(6161):983-7. doi: 10.1126/science.1245296. Epub 2013 Oct 24.

Identification and rescue of α -synuclein toxicity in Parkinson patient-derived neurons.

Chung CY, Khurana V, Auluck PK, Tardiff DF, Mazzulli JR, Soldner F, Baru V, Lou Y, Freyzon Y, Cho S, Mungenast AE, Muffat J, Mitalipova M, Pluth MD, Jui NT, Schüle B, Lippard SJ, Tsai LH, Krainc D, Buchwald SL, Jaenisch R, Lindquist S.

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The induced pluripotent stem (iPS) cell field holds promise for in vitro disease modeling. However, identifying innate cellular pathologies, particularly for age-related neurodegenerative diseases, has been challenging. Here, we exploited mutation correction of iPS cells and conserved proteotoxic mechanisms from yeast to humans to discover and reverse phenotypic responses to α -synuclein (α -syn), a key protein involved in Parkinson's disease (PD). We generated cortical neurons from iPS cells of patients harboring α -syn mutations, who are at high risk of developing PD dementia. Genetic modifiers from unbiased screens in a yeast model of α -syn toxicity led to identification of early pathogenic phenotypes in patient neurons. These included nitrosative stress, accumulation of endoplasmic reticulum (ER)-associated degradation substrates, and ER stress. A small molecule identified in a yeast screen (NAB2), and the ubiquitin ligase Nedd4 it affects, reversed pathologic phenotypes in these neurons.

PMID: 24158904 [PubMed - indexed for MEDLINE]

3. Science. 2013 Dec 13;342(6164):1372-5. doi: 10.1126/science.1240276.

Cryptic variation in morphological evolution: HSP90 as a capacitor for loss of eyes in cavefish.

Rohner N, Jarosz DF, Kowalko JE, Yoshizawa M, Jeffery WR, Borowsky RL, Lindquist S, Tabin CJ.

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Comment in





Science. 2013 Dec 13;342(6164):1304.

In the process of morphological evolution, the extent to which cryptic, preexisting variation provides a substrate for natural selection has been controversial. We provide evidence that heat shock protein 90 (HSP90) phenotypically masks standing eye-size variation in surface populations of the cavefish *Astyanax mexicanus*. This variation is exposed by HSP90 inhibition and can be selected for, ultimately yielding a reduced-eye phenotype even in the presence of full HSP90 activity. Raising surface fish under conditions found in caves taxes the HSP90 system, unmasking the same phenotypic variation as does direct inhibition of HSP90. These results suggest that cryptic variation played a role in the evolution of eye loss in cavefish and provide the first evidence for HSP90 as a capacitor for morphological evolution in a natural setting.

PMID: 24337296 [PubMed - indexed for MEDLINE]

STEPHANIE

Asymmetric Hsp90 N Domain SUMOylation Recruits Aha1 and ATP-Competitive Inhibitors

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The stability and activity of numerous signaling proteins in both normal and cancer cells depends on the dimeric molecular chaperone heat shock protein 90 (Hsp90). Hsp90's function is coupled to ATP binding and hydrolysis and requires a series of conformational changes that are regulated by cochaperones and numerous posttranslational modifications (PTMs). SUMOylation is one of the least-understood Hsp90 PTMs. Here, we show that asymmetric SUMOylation of a conserved lysine residue in the N domain of both yeast (K178) and human (K191) Hsp90 facilitates both recruitment of the adenosine triphosphatase (ATPase)-activating cochaperone Aha1 and, unexpectedly, the binding of Hsp90 inhibitors, suggesting that these drugs associate preferentially with Hsp90 proteins that are actively engaged in the chaperone cycle. Importantly, cellular transformation is accompanied by elevated steady-state N domain SUMOylation, and increased Hsp90 SUMOylation sensitizes yeast and mammalian cells to Hsp90 inhibitors, providing a mechanism to explain the sensitivity of cancer cells to these drugs.