

Lit Lunch – 4 18 14

KEITH:

A Proposed Mechanism for the Promotion of Prion Conversion Involving a Strictly Conserved Tyrosine Residue in the β_2 - α_2 Loop of PrP^C

April 11, 2014 The Journal of Biological Chemistry, 289, 10660-10667

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The transmission of infectious prions into different host species requires compatible prion protein (PrP) primary structures, and even one heterologous residue at a pivotal position can block prion infection. Mapping the key amino acid positions that govern cross-species prion conversion has not yet been possible, although certain residue positions have been identified as restrictive, including residues in the β_2 - α_2 loop region of PrP. To further define how β_2 - α_2 residues impact conversion, we investigated residue substitutions in PrP^C using an in vitro prion conversion assay. Within the β_2 - α_2 loop, a tyrosine residue at position 169 is strictly conserved among mammals, and transgenic mice expressing mouse PrP having the Y169G, S170N, and N174T substitutions resist prion infection. To better understand the structural requirements of specific residues for conversion initiated by mouse prions, we substituted a diverse array of amino acids at position 169 of PrP. We found that the substitution of glycine, leucine, or glutamine at position 169 reduced conversion by ~75%. In contrast, replacing tyrosine 169 with either of the bulky, aromatic residues, phenylalanine or tryptophan, supported efficient prion conversion. We propose a model based on a requirement for tightly interdigitating complementary amino acid side chains within specific domains of adjacent PrP molecules, known as “steric zippers,” to explain these results. Collectively, these studies suggest that an aromatic residue at position 169 supports efficient prion conversion.

DJ-1 Is a Copper Chaperone Acting on SOD1 Activation*

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Lack of oxidative stress control is a common and often prime feature observed in many neurodegenerative diseases. Both DJ-1 and SOD1, proteins involved in familial Parkinson disease and amyotrophic lateral sclerosis, respectively, play a protective role against oxidative stress. Impaired activity and modified expression of both proteins have been observed in different neurodegenerative diseases. A potential cooperative action of DJ-1 and SOD1 in the same oxidative stress response pathway may be suggested based on a copper-mediated interaction between the two proteins reported here. To investigate the mechanisms underlying the antioxidative function of DJ-1 in relation to SOD1 activity, we investigated the ability of DJ-1 to bind copper ions. We structurally characterized a novel copper binding site involving Cys-106, and we investigated, using different techniques, the kinetics of DJ-1 binding to copper ions. The copper transfer between the two proteins was also examined using both fluorescence spectroscopy and specific biochemical assays for SOD1 activity. The structural and functional analysis of the novel DJ-1 copper binding site led us to identify a putative role for DJ-1 as a copper chaperone. Alteration of the coordination geometry of the copper ion in DJ-1 may be correlated to the physiological role of the protein, to a potential failure in metal transfer to SOD1, and to successive implications in neurodegenerative etiopathogenesis.

Molecular Basis for Preventing α -Synuclein Aggregation by a Molecular Tweezer

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Recent work on α -synuclein has shown that aggregation is controlled kinetically by the rate of reconfiguration of the unstructured chain, such that the faster the reconfiguration,

the slower the aggregation. In this work we investigate this relationship by examining α -synuclein in the presence of a small molecular tweezer, CLR01, which binds selectively to Lys side chains. We find strong binding to multiple Lys within the chain as measured by fluorescence and mass-spectrometry and a linear increase in the reconfiguration rate with concentration of the inhibitor. Top-down mass-spectrometric analysis shows that the main binding of CLR01 to α -synuclein occurs at the N-terminal Lys-10/Lys-12. Photo-induced cross-linking of unmodified proteins (PICUP) analysis shows that under the conditions used for the fluorescence analysis, α -synuclein is predominantly monomeric. The results can be successfully modeled using a kinetic scheme in which two aggregation-prone monomers can form an encounter complex that leads to further oligomerization but can also dissociate back to monomers if the reconfiguration rate is sufficiently high. Taken together, the data provide important insights into the preferred binding site of CLR01 on α -synuclein and the mechanism by which the molecular tweezer prevents self-assembly into neurotoxic aggregates by α -synuclein and presumably other amyloidogenic proteins.

INDU:

1. Science. 2014 Mar 7;343(6175):1145-8. doi: 10.1126/science.1248127. Epub 2014 Feb 6.

Mapping the epigenetic basis of complex traits.

Cortijo S(1), Wardenaar R, Colomé-Tatché M, Gilly A, Etcheverry M, Labadie K, Caillieux E, Hospital F, Aury JM, Wincker P, Roudier F, Jansen RC, Colot V, Johannes F.

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Comment in

Science. 2014 Mar 7;343(6175):1082-3.

Quantifying the impact of heritable epigenetic variation on complex traits is emerging challenge in population genetics. Here, we analyze a population of isogenic Arabidopsis lines that segregate experimentally induced DNA methylation changes at hundreds of regions across the genome. We demonstrate that several of these differentially methylated regions (DMRs) act as bona fide epigenetic quantitative trait loci (QTL(epi)), accounting for 60 to 90% of the heritability for two complex traits, flowering time and primary root length. These QTL(epi) are reproducible and can be subjected to artificial selection. Many of the experimentally induced DMRs are also variable in natural populations of this species and may thus provide an epigenetic basis for Darwinian evolution independently of DNA sequence changes.

2. Science. 2014 Feb 28;343(6174):1025-8. doi: 10.1126/science.1245125.

Cell surface ABP1-TMK auxin-sensing complex activates ROP GTPase signaling.

Xu T(1), Dai N, Chen J, Nagawa S, Cao M, Li H, Zhou Z, Chen X, De Rycke R, Rakusová H, Wang W, Jones AM, Friml J, Patterson SE, Bleecker AB, Yang Z.

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Auxin-binding protein 1 (ABP1) was discovered nearly 40 years ago and was shown to be essential for plant development and morphogenesis, but its mode of action remains unclear. Here, we report that the plasma membrane-localized transmembrane kinase (TMK) receptor-like kinases interact with ABP1 and transduce auxin signal to activate plasma membrane-associated ROPs [Rho-like guanosine triphosphatases (GTPase) from plants], leading to changes in the cytoskeleton and the shape of leaf pavement cells in Arabidopsis. The interaction between ABP1 and TMK at the cell surface is induced by auxin and requires ABP1 sensing of auxin. These findings show that TMK proteins and ABP1 form a cell surface auxin perception complex that activates ROP signaling pathways, regulating nontranscriptional cytoplasmic responses and associated fundamental processes.

DAMIAN:

1) Graham Noctor, Amna Mhamdi, and Christine H. Foyer. The Roles of Reactive Oxygen Metabolism in Drought: Not So Cut and Dried. Update on the Physiology of Reactive Oxygen Species During Drought. *Plant Physiology* 164: 1636

2) Water Permeation Drives Tumor Cell Migration in Confined Microenvironments. Kimberly M. Stroka, Hongyuan Jiang, Shih-Hsun Chen, Ziqiu Tong, Denis Wirtz, Sean X. Sun, Konstantinos Konstantopoulos. *Cell*In Press.

YICHEN:

1) *J Mol Biol.* 2014 Apr 17;426(8):1786-98. doi: 10.1016/j.jmb.2014.01.008. Epub 2014 Jan 29.

A Novel C-Terminal Homologue of Aha1 Co-Chaperone Binds to Heat Shock Protein 90 and Stimulates Its ATPase Activity in Entamoeba histolytica.

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Author information

Abstract

Cytosolic heat shock protein 90 (Hsp90) has been shown to be essential for many infectious pathogens and is considered a potential target for drug development. In this study, we have carried out biochemical characterization of Hsp90 from a poorly studied protozoan parasite of clinical importance, *Entamoeba histolytica*. We have shown that *Entamoeba* Hsp90 can bind to both ATP and its pharmacological inhibitor, 17-AAG (17-

allylamino-17-demethoxygeldanamycin), with K_d values of 365.2 and 10.77 μM , respectively, and it has a weak ATPase activity with a catalytic efficiency of $4.12 \times 10^{-4} \text{ min}^{-1} \mu\text{M}^{-1}$. Using inhibitor 17-AAG, we have shown dependence of *Entamoeba* on Hsp90 for its growth and survival. Hsp90 function is regulated by various co-chaperones. Previous studies suggest a lack of several important co-chaperones in *E. histolytica*. In this study, we describe the presence of a novel homologue of co-chaperone Aha1 (activator of Hsp90 ATPase), EhAha1c, lacking a canonical Aha1 N-terminal domain. We also show that EhAha1c is capable of binding and stimulating ATPase activity of EhHsp90. In addition to highlighting the potential of Hsp90 inhibitors as drugs against amoebiasis, our study highlights the importance of *E. histolytica* in understanding the evolution of Hsp90 and its co-chaperone repertoire.

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KEYWORDS:

17-AAG, Aha1, *Entamoeba*, Hsp90, co-chaperones

FIONN:

Frontiers in plant science.

The role of ubiquitin and the 26S proteasome in plant abiotic stress signaling

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Ubiquitin is a small, highly conserved, ubiquitously expressed eukaryotic protein with immensely important and diverse regulatory functions. A well-studied function of ubiquitin is its role in selective proteolysis by the ubiquitin-proteasome system (UPS). The UPS has emerged as an integral player in plant response and adaptation to environmental stresses such as drought, salinity, cold and nutrient deprivation. The UPS has also been shown to influence the production and signal transduction of stress-related hormones such as abscisic acid. Understanding UPS function has centered mainly on defining the role of E3 ubiquitin ligases, which are the substrate-recruiting component of the ubiquitination pathway. The recent identification of stress signaling/regulatory proteins that are the subject of ubiquitin-dependent degradation has increased our knowledge of how the UPS facilitates responses to adverse environmental conditions. A brief overview is provided on role of the UPS in modulating protein stability during abiotic stress signaling. E3 ubiquitin ligases for which stress-related substrate proteins have been identified are discussed.

Plant cell

HEAT-INDUCED TAS1 TARGET1 Mediates Thermotolerance via HEAT STRESS TRANSCRIPTION FACTOR A1a-Directed Pathways in Arabidopsis

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Many heat stress transcription factors (Hsfs) and heat shock proteins (Hsps) have been identified to play important roles in the heat tolerance of plants. However, many of the key factors mediating the heat response pathways remain unknown. Here, we report that two genes, which are targets of TAS1 (trans-acting siRNA precursor 1)-derived small interfering RNAs that we named HEAT-INDUCED TAS1 TARGET1 (HTT1) and HTT2, are involved in thermotolerance. Microarray analysis revealed that the HTT1 and HTT2 genes were highly upregulated in *Arabidopsis thaliana* seedlings in response to heat shock. Overexpression of TAS1a, whose trans-acting small interfering RNAs target the HTT genes, elevated accumulation of TAS1-siRNAs and reduced expression levels of the HTT genes, causing weaker thermotolerance. By contrast, overexpression of HTT1 and HTT2 upregulated several Hsf genes, leading to stronger thermotolerance. In heat-tolerant plants overexpressing HsfA1a, the HTT genes were upregulated, especially at high temperatures. Meanwhile, HsfA1a directly activated HTT1 and HTT2 through binding to their promoters. HTT1 interacted with the heat shock proteins Hsp70-14 and Hsp40 and NUCLEAR FACTOR Y, SUBUNIT C2. Taken together, these results suggest that HTT1 mediates thermotolerance pathways because it is targeted by TAS1a, mainly activated by HsfA1a, and acts as cofactor of Hsp70-14 complexes.

UMARU:

PROTEIN DISULFIDE ISOMERASE LIKE 5-1 is a susceptibility factor to plant viruses.

Authors

Yang P, et al. [Show all](#)

Journal

Proc Natl Acad Sci U S A. 2014 Feb 11;111(6):2104-9. doi: 10.1073/pnas.1320362111. Epub 2014 Jan 30. **Abstract**

Protein disulfide isomerases (PDIs) catalyze the correct folding of proteins and prevent the aggregation of unfolded or partially folded precursors. Whereas suppression of members of the PDI gene family can delay replication of several human and animal viruses (e.g., HIV), their role in interactions with plant viruses is largely unknown. Here, using a positional cloning strategy we identified variants of PROTEIN DISULFIDE ISOMERASE LIKE 5-1 (HvPDIL5-1) as the cause of naturally occurring resistance to multiple strains of Bymoviruses. The role of wild-type HvPDIL5-1 in conferring susceptibility was confirmed by targeting induced local lesions in genomes for induced mutant alleles, transgene-induced complementation, and allelism tests using different natural resistance alleles. The geographical distribution of natural genetic variants of HvPDIL5-1 revealed the origin of resistance conferring alleles in domesticated barley in Eastern Asia. Higher sequence diversity was correlated with areas with increased pathogen diversity suggesting adaptive selection for bymovirus resistance. HvPDIL5-1 homologs are highly conserved across species of the plant and animal kingdoms implying that orthologs of HvPDIL5-1 or other closely related members of the PDI gene family may be potential susceptibility factors to viruses in other eukaryotic species.

STEPHANIE:

[Mol Plant](#). 2014 Apr;7(4):616-25. doi: 10.1093/mp/sst142. Epub 2013 Oct 24.

PIF3 Is Involved in the Primary Root Growth Inhibition of Arabidopsis Induced by Nitric Oxide in the Light.

[Bai S¹](#), [Yao T](#), [Li M](#), [Guo X](#), [Zhang Y](#), [Zhu S](#), [He Y](#).

[Author information](#)

Abstract

PHYTOCHROME INTERACTING FACTOR3 (PIF3) is an important component in the phytochrome signaling pathway and mediates plant responses to various environmental conditions. We found that PIF3 is involved in the inhibition of root growth of *Arabidopsis thaliana* seedlings induced by nitric oxide (NO) in light. Overexpression of PIF3 partially alleviated the inhibitory effect of NO on root growth, whereas the *pif3-1* mutant displayed enhanced sensitivity to NO in terms of root growth. During phytochrome signaling, the photoreceptor PHYB mediates the degradation of PIF3. We found that the *phyB-9* mutant had a similar phenotype to that of *PIF3ox* in terms of responsiveness to NO. Furthermore, NO treatment promoted the accumulation of PHYB, and thus reduced PIF3 content. Our results further show that the activity of PIF3 is regulated by the DELLA protein RGL3[*REGULATOR OF GA1-3* LIKE 3]. Therefore, we speculate that PIF3 lies downstream of PHYB and RGL3, and plays an important role in the inhibitory effect of NO on root growth of *Arabidopsis* seedlings in light.