Lit Lunch
September 18, 2012
Vierling Lab

RPN1a, a 26S proteasome subunit, is required for innate immunity in Arabidopsis

Summary

Accumulating evidence shows that proper degradation of proteins that affect defense responses in a positive or negative manner is critical in plant immunity. However, the role of plant degradation systems such as the 26S proteasome in plant immunity is not well understood. Loss-of-function mutations in *EDR2* (*ENHANCED DISEASE RESISTANCE 2*) lead to increased resistance to the adapted biotrophic powdery mildew pathogen *Golovinomyces cichoracearum*. To study the molecular interactions between powdery mildew pathogen and Arabidopsis, we performed a screen for suppressors of *edr2* and found that mutation in the gene that encodes RPN1a, a subunit of the 26S proteasome, suppressed *edr2*-associated disease resistance phenotypes. In addition, RPN1a is required for *edr1*- and *pmr4*-mediated powdery mildew resistance and mildew-induced cell death. Furthermore, we show that *rpn1a* displayed enhanced susceptibility to the fungal pathogen *G. cichoracearum* and to virulent and avirulent bacterial *Pto* DC3000 strains, which indicated that *rpn1a* has defects in basal defense and resistance (R) protein-mediated defense. RPN1a–GFP localizes to both the nucleus and cytoplasm. Accumulation of RPN1a is affected by salicylic acid (SA) and the *rpn1a* mutant has defects in SA accumulation upon *Pto* DC3000 infection. Further analysis revealed that two other subunits of the 26S proteasome, RPT2a and RPN8a are also involved in *edr2*-mediated disease resistance. Based on these results, we conclude that RPN1a is required for basal defense and R protein-mediated defense. Our data provide evidence that some subunits of the 26S proteasome are involved in innate immunity in Arabidopsis.

Issue

The Plant Journal
Volume 71, Issue 6, pages 1015–1028, September 2012

Specialized Hsp70 Chaperone (HscA) Binds Preferentially to the Disordered Form, whereas J-protein (HscB) Binds Preferentially to the Structured Form of the Iron-Sulfur Cluster Scaffold Protein (IscU)

Abstract

The Escherichia coli protein IscU serves as the scaffold for Fe-S cluster assembly and the vehicle for Fe-S cluster transfer to acceptor proteins, such as apoferredoxin. IscU populates two conformational states in solution, a structured conformation (S) that resembles the conformation of the holoprotein IscU-[2Fe-2S] and a dynamically disordered conformation (D) that does not bind metal ions. NMR spectroscopic results presented here show that the specialized Hsp70 chaperone (HscA), alone or as the HscA-ADP complex, preferentially binds to and stabilizes the D-state of IscU. IscU is released when HscA binds ATP. By contrast, the J-protein HscB binds preferentially to the S-state of IscU. Consistent with

these findings, we propose a mechanism in which cluster transfer is coupled to hydrolysis of ATP bound to HscA, conversion of IscU to the D-state, and release of HscB.

September 7, 2012 The Journal of Biological Chemistry,

In addition a special issue on protein degradation appeared in plant phys, excellent to get a good overview.

THE PLANT CELL

Minsoo Kim, Ung Lee, Ian Small, Catherine Colas des Francs-Small, and Elizabeth Vierling

Mutations in an *Arabidopsis* Mitochondrial Transcription Termination Factor–Related Protein Enhance Thermotolerance in the Absence of the Major Molecular Chaperone HSP101

Abstract

The molecular chaperone heat shock protein101 (HSP101) is required for acquired thermotolerance in plants and other organisms. To identify factors that interact with HSP101 or that are involved in thermotolerance, we screened for extragenic suppressors of a dominant-negative allele of *Arabidopsis thaliana HSP101*, *hot1-4*. One suppressor, *shot1* (for *suppressor of hot1-4 1*), encodes a mitochondrial transcription termination factor (mTERF)–related protein, one of 35 *Arabidopsis* mTERFs about which there is limited functional data. Missense (*shot1-1*) and T-DNA insertion (*shot1-2*) mutants suppress the *hot1-4* heat-hypersensitive phenotype. Furthermore, *shot1-2* suppresses other heat-sensitive mutants, and *shot1-2* alone is more heat tolerant than the wild type. SHOT1 resides in mitochondria, indicating it functions independently of cytosolic/nuclear HSP101. Microarray analysis suggests altered mitochondrial function and/or retrograde signaling in *shot1-2* increases transcripts of other HSPs and alters expression of redox-related genes. Reduced oxidative damage is the likely cause of *shot1* thermotolerance, indicating HSP101 repairs protein oxidative damage and/or reduced oxidative damage allows recovery in the absence of HSP101. Changes in organelle-encoded transcripts in *shot1* demonstrate that SHOT1 is involved in organelle gene regulation. The heat tolerance of *shot1* emphasizes the importance of mitochondria in stress tolerance, and defining its function may provide insights into control of oxidative damage for engineering stress-resistant plants.

Andrija Finka, America Farinia Henriquez Cuendet, Frans J.M. Maathuis, Younousse Saidi, and Pierre Goloubinoff

Plasma Membrane Cyclic Nucleotide Gated Calcium Channels Control Land Plant Thermal Sensing and Acquired Thermotolerance

Abstract

Typically at dawn on a hot summer day, land plants need precise molecular thermometers to sense harmless increments in the ambient temperature to induce a timely heat shock response (HSR) and accumulate protective heat shock proteins in anticipation of harmful temperatures at mid-day. Here, we found that the cyclic nucleotide gated calcium channel (CNGC) *CNGCb* gene from *Physcomitrella patens* and its *Arabidopsis thaliana* ortholog *CNGC2*, encode a component of cyclic nucleotide gated Ca^{2+} channels that act as the primary

thermosensors of land plant cells. Disruption of *CNGCb* or *CNGC2* produced a hyper-thermosensitive phenotype, giving rise to an HSR and acquired thermotolerance at significantly milder heat-priming treatments than in wild-type plants. In an aequorin-expressing moss, CNGCb loss-of-function caused a hyper-thermoresponsive Ca^{2+} influx and altered Ca^{2+} signaling. Patch clamp recordings on moss protoplasts showed the presence of three distinct thermoresponsive Ca^{2+} channels in wild-type cells. Deletion of *CNGCb* led to a total absence of one and increased the open probability of the remaining two thermoresponsive Ca^{2+} channels. Thus, CNGC2 and CNGCb are expected to form heteromeric Ca^{2+} channels with other related CNGCs. These channels in the plasma membrane respond to increments in the ambient temperature by triggering an optimal HSR, leading to the onset of plant acquired thermotolerance.

CURRENT BIOLOGY

Unfolded protein response

Stewart Siyan Cao, Randal J. Kaufman

Summary

In eukaryotic cells, the endoplasmic reticulum (ER) is a membrane-enclosed interconnected organelle responsible for the synthesis, folding, modification, and quality control of numerous secretory and membrane proteins. The processes of protein folding and maturation are highly assisted and scrutinized but are also sensitive to changes in ER homeostasis, such as Ca^{2+} depletion, oxidative stress, hypoxia, energy deprivation, metabolic stimulation, altered glycosylation, activation of inflammation, as well as increases in protein synthesis or the expression of misfolded proteins or unassembled protein subunits. Only properly folded proteins can traffic to the Golgi apparatus, whereas those that misfold are directed to ER-associated degradation (ERAD) or to autophagy. The accumulation of unfolded/misfolded proteins in the ER activates signaling events to orchestrate adaptive cellular responses. This unfolded protein response (UPR) increases the ER protein-folding capacity, reduces global protein synthesis, and enhances ERAD of misfolded proteins.

A Novel Stress-Induced Sugarcane Gene Confers Tolerance to Drought, Salt and Oxidative Stress in Transgenic Tobacco Plants

MaKevin Begcy, Eduardo D. Mariano, Agustina Gentile, Carolina G. Lembke, Sonia Marli Zingaretti

PLOS ONE, Volume 7, Issue9, e44697

Drought is a major abiotic stress that affects crop productivity worldwide. Sugarcane can withstand periods of water scarcity during the final stage of culm maturation, during which sucrose accumulation occurs. Meanwhile, prolonged periods of drought can cause severe plant losses. In a previous study, we evaluated the transcriptome of drought-stressed plants to better understand sugarcane responses to drought. Among the up-regulated genes was Scdr1 (sugarcane drought-responsive 1). The aim of the research reported here was to characterize this gene. Scdr1 encodes a putative protein containing 248 amino acids with a large number of proline (19%) and cysteine (13%) residues. Phylogenetic analysis showed that ScDR1is in a clade with homologs from other monocotyledonous plants, separate from those of dicotyledonous plants. The expression of Scdr1 in different varieties

of sugarcane plants has not shown a clear association with drought tolerance. The overexpression of Scdr1 in transgenic tobacco plants increased their tolerance to drought,salinity and oxidative stress, as demonstrated by increased photosynthesis, water content, biomass, germination rate, chlorophyll content and reduced accumulation of ROS. Physiological parameters, such as transpiration rate (E), net photosynthesis (A), stomatal conductance (gs) and internal leaf CO2 concentration, were less affected by abiotic stresses in
transgenic Scdr1 plants compared with wild-type plants. Overall, our results indicated that Scdr1 conferred tolerance to multiple abiotic stresses, highlighting the potential of this gene for biotechnological applications.

Unique Drought Resistance Functions of the Highly ABA-Induced Clade A Protein Phosphatase 2Cs

Govinal Badiger Bhaskara, Thao Thi Nguyen, and Paul E. Verslues

Plant Physiology, Volume 160, Issue9, pp. 379－395

Six Arabidopsis (Arabidopsis thaliana) clade A protein phosphatase 2Cs (PP2Cs) have established abscisic acid (ABA) signaling roles; however, phenotypic roles of the remaining three “HAI” PP2Cs, Highly ABA-Induced1 (HAI1), AKT1-Interacting PP2C1/HAI2, and HAI3, have remained unclear. HAI PP2C mutants had enhanced proline and osmoregulatory solute accumulation at low water potential, while mutants of other clade A PP2Cs had no or lesser effect on these drought resistance traits. hai1-2 also had increased expression of abiotic stress-associated genes, including dehydrins and late embryogenesis abundant proteins, but decreased expression of several defense-related genes. Conversely, the HAI PP2Cs had relatively less impact on several ABA sensitivity phenotypes. HAI PP2C single mutants were unaffected in ABA sensitivity, while double and triple mutants were moderately hypersensitive in postgermination ABA response but ABA insensitive in germination. The HAI PP2Cs interacted most strongly with PYL5 and PYL7 to -10 of the PYL/RCAR ABA receptor family, with PYL7 to -10 interactions being relatively little affected by ABA in yeast two-hybrid assays. HAI1 had especially limited PYL interaction. Reduced expression of the main HAI1-interacting PYLs at low water potential when HAI1 expression was strongly induced also suggests limited PYL regulation and a role of HAI1 activity in negatively regulating specific drought resistance phenotypes. Overall, the HAI PP2Cs had greatest effect on ABA-independent low water potential phenotypes and lesser effect on classical ABA sensitivity phenotypes. Both this and their distinct PYL interaction demonstrate a new level of functional differentiation among the clade A PP2Cs and a point of cross talk between ABA-dependent and ABA-independent drought-associated signaling.

Molecular Cell

A Central Coupler for Recombination Initiation Linking Chromosome Architecture to S Phase Checkpoint

Molecular Cell, Volume 47, Issue 5, 722-733, 26 July 2012

Tomoichiro Miyoshi,1,5,6 Masaru Ito,1,6 Kazuto Kugou,1 Shintaro Yamada,1,2 Masaki Furuichi,3 Arisa Oda,1,2 Takatomi Yamada,1 Kouji Hirota,1,4 Hisao Masai,3 and Kunihiro Ohta1,2,*

Higher-order chromosome structure is assumed to control various DNA-templated reactions in eukary- otes. Meiotic chromosomes implement developed structures called

‘‘axes’’ and ‘‘loops’’; both are sug- gested to tether each other, activating Spo11 to catalyze meiotic DNA double-strand breaks (DSBs) at recombination hotspots. We found that the Schiz- osaccharomyces pombe Spo11 homolog Rec12 and its partners form two distinct subcomplexes, DSBC (Rec6-Rec12-Rec14) and SFT (Rec7-Rec15-Rec24). Mde2, whose expression is strictly regulated by the replication checkpoint, interacts with Rec15 to stabi- lize the SFT subcomplex and further binds Rec14 in DSBC. Rec10 provides a docking platform for SFT binding to axes and can partially interact with DSB sites located in loops depending upon Mde2, which is indicative of the formation of multiprotein-based tethered axis-loop complex. These data lead us to propose a mechanism by which Mde2 functions as a recombination initiation mediator to tether axes and loops, in liaison with the meiotic replication checkpoint.

Molecular Plant

The Arabidopsis LFR Gene Is Required for the Formation of Anther Cell Layers and Normal Expression of Key Regulatory Genes
Molecular Plant • Volume 5, Number 5, Pages 993–1000 • September 2012
Xiu-Tang Wang, Can Yuan, Ting-Ting Yuan and Su-Juan Cui1

The anther is the male reproductive organ in flowering plants. Although some genes were reported to be in- volved in anther development, the molecular mechanisms underlying the transcriptional regulation of these genes is unclear. lfr-2 (leaf and flower related-2), the null allele of Arabidopsis thaliana LFR (LEAF AND FLOWER RELATED), was male-sterile. The anthers of lfr-2 plants were defective in sporogenous cell formation, tapetum development, and pollen development. In agreement with these phenotypes, expression studies showed that LFR was expressed in all cell layers of the anther, and that expression was particularly strong in the tapetal cells and pollen grains. Quantitative RT–PCR analysis revealed that LFR is required for the normal transcription of some anther development-related genes, such as AMS, CALS5, and DYT1, MS1 and MS2, and ROXY2. Genetic analysis showed that SPL was epistatic to LFR while LFR was epistatic to DYT1. We propose that LFR may be a crucial component in the regulation of a genetic network that modulates anther development.

A gain-of-function mutation in IAA16 confers reduced responses to auxin and abscisic acid and impedes plant growth and fertility

Mauro A. Rinaldi, James Liu, Tara A. Enders, Bonnie Bartel and Lucia C. Strader

Plant Molecular Biology

Volume 79, Numbers 4-5 (2012), 359-373

Auxin regulates many aspects of plant development, in part, through degradation of the Aux/IAA family of transcriptional repressors. Consequently, stabilizing mutations in several Aux/IAA proteins confer reduced auxin responsiveness. However, of the 29 apparent Aux/IAA proteins in Arabidopsis thaliana, fewer than half have roles established through mutant analysis. We identified iaa16-1, a dominant gain-of-function mutation in IAA16 (At3g04730), in a novel screen for reduced root responsiveness to abscisic acid. The iaa16-1 mutation also

confers dramatically reduced auxin responses in a variety of assays, markedly restricts growth of adult plants, and abolishes fertility when homozygous. We compared iaa16-1 phenotypes with those of dominant mutants defective in the closely related IAA7/AXR2, IAA14/SLR, and IAA17/AXR3, along with the more distantly related IAA28, and found overlapping but distinct patterns of developmental defects. The identification and characterization of iaa16-1 provides a fuller understanding of the IAA7/IAA14/IAA16/IAA17 clade of Aux/IAA proteins and the diverse roles of these repressors in hormone response and plant development.

cry1 and GPA1 signaling genetically interact in hook opening and anthocyanin synthesis in Arabidopsis

Ana R. Fox, Gabriela C. Soto, Alan M. Jones, Jorge J. Casal, Jorge P. Muschietti and María A. Mazzella

Plant Molecular Biology

Volume 80, Number 3 (2012), 315-324

While studying blue light-independent effects of cryptochrome 1 (cry1) photoreceptor, we observed premature opening of the hook in cry1 mutants grown in complete darkness, a phenotype that resembles the one described for the heterotrimeric G-protein α subunit (GPA1) null mutant gpa1. Both cry1 and gpa1 also showed reduced accumulation of anthocyanin under blue light. These convergent gpa1 and cry1 phenotypes required the presence of sucrose in the growth media and were not additive in the cry1 gpa1 double mutant, suggesting context-dependent signaling convergence between cry1 and GPA1 signaling pathways. Both, gpa1 and cry1 mutants showed reduced GTP-binding activity. The cry1 mutant showed wild-type levels of GPA1 mRNA or GPA1 protein. However, an anti-transducin antibody (AS/7) typically used for plant Gα proteins, recognized a 54 kDa band in the wild type but not in gpa1 and cry1 mutants. We propose a model where cry1-mediated post-translational modification of GPA1 alters its GTP-binding activity.

Visualizing transient protein-folding intermediates by tryptophan-scanning mutagenesis

Alexis Vallée-Bélisle1,3 & Stephen W Michnick1,2

To understand how proteins fold, assemble and function, it is necessary to characterize the structure and dynamics of each state they adopt during their lifetime. Experimental characterization of the transient states of proteins remains a major challenge because high-resolution structural techniques, including NMR and X-ray crystallography, cannot be directly applied to study short-lived protein states. To circumvent this limitation, we show that transient states during protein folding can be characterized by measuring the fluorescence of tryptophan residues, introduced at many solvent-exposed positions to determine whether each position is native-like, denatured-like or non-native-like in the intermediate state. We use this approach to characterize a late-folding-intermediate state

of the small globular mammalian protein ubiquitin, and we show the presence of productive non-native interactions that suggest a 'flycatcher' mechanism of concerted binding and folding.

NATuRE STRuCTuRAL & mOLECuLAR bIOLOgy VOLUME 19 NUMBER 7 JULY 2012

Dynamic and static components power unfolding in topologically closed rings of a AAA+ proteolytic machine

Steven E Glynn1, Andrew R Nager1, Tania A Baker1,2 & Robert T Sauer1

In the *Escherichia coli* ClpXP protease, a hexameric ClpX ring couples ATP binding and hydrolysis to mechanical protein unfolding and translocation into the ClpP degradation chamber. Rigid-body packing between the small AAA+ domain of each ClpX subunit and the large AAA+ domain of its neighbor stabilizes the hexamer. By connecting the parts of each rigid-body unit with disulfide bonds or linkers, we created covalently closed rings that retained robust activity. A single-residue insertion in the hinge that connects the large and small AAA+ domains and forms part of the nucleotide-binding site uncoupled ATP hydrolysis from productive unfolding. We propose that ATP hydrolysis drives changes in the conformation of one hinge and its flanking domains and that the changes are propagated around the AAA+ ring through the topologically constrained set of rigid-body units and hinges to produce coupled ring motions that power substrate unfolding.

VOLUME 19 NUMBER 6 JUNE 2012 **nature structural & molecular biology**

Molecular mechanisms used by chaperones to reduce the toxicity of aberrant protein oligomers

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Edited by Susan Lindquist, Whitehead Institute for Biomedical Research, Cambridge, MA, and approved June 19, 2012 (received for review October 28, 2011)

Chaperones are the primary regulators of the proteostasis net- work and are known to facilitate protein folding, inhibit protein aggregation, and promote disaggregation and clearance of mis- folded aggregates inside cells. We have tested the effects of five chaperones on the toxicity of misfolded oligomers preformed from three different proteins added extracellularly to cultured cells. All the chaperones were found to decrease oligomer toxicity signif- icantly, even at very low chaperone/protein molar ratios, provided that they were added extracellularly rather than being overex- pressed in the cytosol. Infrared spectroscopy and site-directed labeling experiments using pyrene ruled out structural reorganiza- tions within the discrete oligomers. Rather, confocal microscopy, SDS-PAGE, and intrinsic fluorescence measurements indicated tight binding between oligomers and chaperones. Moreover, atomic force microscopy imaging indicated that larger assemblies of oligomers are formed in the presence of the chaperones. This suggests that the chaperones bind to the oligomers and promote their assembly into larger species, with consequent shielding of the reactive surfaces and a decrease in their diffusional mobility. Overall, the data indicate a generic ability of chaperones to neutralize extracellular misfolded oligomers efficiently and reveal that further assembly of protein oligomers into larger species can be an effective strategy to neutralize such extracellular species.

PNAS | July 31, 2012 | vol. 109 | no. 31 | 12479–12484

BMC Biology

Low level genome mistranslations deregulate the transcriptome and translatome and generate proteotoxic stress in yeast

João A Paredes[1], Laura Carreto[1], João Simões[1], Ana R Bezerra[1], Ana C Gomes[1], Rodrigo Santamaria[2], Misha Kapushesky[2], Gabriela R Moura[1] and Manuel AS Santos[1]

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Abstract

Background

Organisms use highly accurate molecular processes to transcribe their genes and a variety of mRNA quality control and ribosome proofreading mechanisms to maintain intact the fidelity of genetic information flow. Despite this, low level gene translational errors induced by mutations and environmental factors cause neurodegeneration and premature death in mice and mitochondrial disorders in humans. Paradoxically, such errors can generate advantageous phenotypic diversity in fungi and bacteria through poorly understood molecular processes.

Results

In order to clarify the biological relevance of gene translational errors we have engineered codon misreading in yeast and used profiling of total and polysome-associated mRNAs, molecular and biochemical tools to characterize the recombinant cells. We demonstrate here that gene translational errors, which have negligible impact on yeast growth rate down-regulate protein synthesis, activate the unfolded protein response and

environmental stress response pathways, and down-regulate chaperones linked to ribosomes.

Conclusions
We provide the first global view of transcriptional and post-transcriptional responses to global gene translational errors and we postulate that they cause gradual cell degeneration through synergistic effects of overloading protein quality control systems and deregulation of protein synthesis, but generate adaptive phenotypes in unicellular organisms through activation of stress cross-protection. We conclude that these genome wide gene translational infidelities can be degenerative or adaptive depending on cellular context and physiological condition.

UBXN7 docks on neddylated cullin complexes using its UIM motif and causes HIF1α accumulation

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Background
The proteins from the UBA-UBX family interact with ubiquitylated proteins via their UBA domain and with p97 via their UBX domain, thereby acting as substrate-binding adaptors for the p97 ATPase. In particular, human UBXN7 (also known as UBXD7) mediates p97 interaction with the transcription factor HIF1α that is actively ubiquitylated in normoxic cells by a CUL2-based E3 ligase, CRL2. Mass spectrometry analysis of UBA-UBX protein immunoprecipitates showed that they interact with a multitude of E3 ubiquitin-ligases. Conspicuously, UBXN7 was most proficient in interacting with cullin-RING ligase subunits. We therefore set out to determine whether UBXN7 interaction with cullins was direct or mediated by its ubiquitylated targets bound to the UBA domain.

Results
We show that UBXN7 interaction with cullins is independent of ubiquitin- and substrate-binding. Instead, it relies on the UIM motif in UBXN7 that directly engages the NEDD8 modification on cullins. To understand the functional consequences of UBXN7 interaction with neddylated cullins, we focused on HIF1α, a CUL2 substrate that uses UBXD7/p97 as a ubiquitin-receptor on its way to proteasome-mediated degradation. We find that UBXN7 over-expression converts CUL2 to its neddylated form and causes the accumulation of non-ubiquitylated HIF1α. Both of these effects are strictly UIM-dependent and occur only when UBXN7 contains an intact UIM motif. We also show that HIF1α carrying long ubiquitin-chains can recruit alternative ubiquitin-receptors, lacking p97's ATP-dependent segregase

activity.

Conclusions

Our study shows that independently of its function as a ubiquitin-binding adaptor for p97, UBXN7 directly interacts with neddylated cullins and causes the accumulation of the CUL2 substrate HIF1α. We propose that by sequestering CUL2 in its neddylated form, UBXN7 negatively regulates the ubiquitin-ligase activity of CRL2 and this might prevent recruitment of ubiquitin-receptors other than p97 to nuclear HIF1α.

Real-time determination of intracellular oxygen in bacteria using a genetically encoded FRET-based biosensor

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[2] AVT Biochemical Engineering, RWTH Aachen University, Worringerweg 1, D-52074 Aachen, Germany
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Abstract

Background

Molecular oxygen (O_2) is one of the key metabolites of all obligate and facultative aerobic pro- and eukaryotes. It plays a fundamental role in energy homeostasis whereas oxygen deprivation, in turn, broadly affects various physiological and pathophysiological processes. Therefore, real-time monitoring of cellular oxygen levels is basically a prerequisite for the analysis of hypoxia-induced processes in living cells and tissues.

Results

We developed a genetically encoded Förster resonance energy transfer (FRET)-based biosensor allowing the observation of changing molecular oxygen concentrations inside living cells. This biosensor named FluBO (fluorescent protein-based biosensor for oxygen) consists of the yellow fluorescent protein (YFP) that is sensitive towards oxygen depletion and the hypoxia-tolerant flavin-binding fluorescent protein (FbFP). Since O_2 is essential for the formation of the YFP chromophore, efficient FRET from the FbFP donor domain to the YFP acceptor domain only occurs in the presence but not in the absence of oxygen. The oxygen biosensor was used for continuous real-time monitoring of temporal changes of O_2 levels in the cytoplasm of *Escherichia coli* cells during batch cultivation.

Conclusions

FluBO represents a unique FRET-based oxygen biosensor which allows the non-invasive ratiometric readout of cellular oxygen. Thus, FluBO can serve as a novel and powerful probe for investigating the occurrence of hypoxia and its effects on a variety of (patho)physiological processes in living cells.

Mass Spectrometry—From Peripheral Proteins to Membrane Motors

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Abstract

That membrane protein complexes could survive in the gas phase had always seemed impossible. The lack of chargeable residues, high hydrophobicity, and poor solubility and the vast excess of detergent contributed to the view that it would not be possible to obtain mass spectra of intact membrane complexes. With the recent success in recording mass spectra of these complexes, first from recombinant sources and later from the cellular environment, many surprising properties of these gas phase membrane complexes have been revealed. The first of these was that the interactions between membrane and soluble subunits could survive in vacuum, without detergent molecules adhering to the complex. The second unexpected feature was that their hydrophobicity and, consequently, lower charge state did not preclude ionization. The final surprising finding was that these gas phase membrane complexes carry with them lipids, bound specifically in subunit interfaces. This provides us with an opportunity to distinguish annular lipids that surround the membrane complexes, from structural lipids that have a role in maintaining structure and subunit interactions. In this perspective, we track these developments and suggest explanations for the various discoveries made during this research.

Structures of Hepatitis B Virus Cores Presenting a Model Epitope and Their Complexes with Antibodies

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[3] Latvian Biomedical Research and Study Centre, 1 Ratsupites Street, LV-1067 Riga, Latvia

Abstract

The core shell of hepatitis B virus is a potent immune stimulator, giving a strong neutralizing immune response to foreign epitopes inserted at the immunodominant region, located at the tips of spikes on the exterior of the shell. Here, we analyze structures of core shells with a model epitope inserted at two alternative positions in the immunodominant region. Recombinantly expressed core protein assembles into T = 3 and T = 4

icosahedral shells, and atomic coordinates are available for the T = 4 shell. Since the modified protein assembles predominantly into T = 3 shells, a quasi-atomic model of the native T = 3 shell was made. The spikes in this T = 3 structure resemble those in T = 4 shells crystallized from expressed protein. However, the spikes in the modified shells exhibit an altered conformation, similar to the DNA containing shells in virions. Both constructs allow full access of antibodies to the foreign epitope, DPAFR from the preS1 region of hepatitis B virus surface antigen. However, one induces a 10-fold weaker immune response when injected into mice. In this construct, the epitope is less constrained by the flanking linker regions and is positioned so that the symmetry of the shell causes pairs of epitopes to come close enough to interfere with one another. In the other construct, the epitope mimics the native epitope conformation and position. The interaction of native core shells with an antibody specific to the immunodominant epitope is compared to the constructs with an antibody against the foreign epitope. Our findings have implications for the design of vaccines based on virus-like particles.

1. Science. 2012 Sep 14;337(6100):1348-52.

Structural probing of a protein phosphatase 2A network by chemical cross-linking and mass spectrometry.

Herzog F, Kahraman A, Boehringer D, Mak R, Bracher A, Walzthoeni T, Leitner A, Beck M, Hartl FU, Ban N, Malmström L, Aebersold R.

Department of Biology, Institute of Molecular Systems Biology, Eidgenössische Technische Hochschule Zürich, Wolfgang-Pauli Strasse 16, 8093 Zurich, Switzerland.

The identification of proximate amino acids by chemical cross-linking and mass spectrometry (XL-MS) facilitates the structural analysis of homogeneous protein complexes. We gained distance restraints on a modular interaction network of protein complexes affinity-purified from human cells by applying an adapted XL-MS protocol. Systematic analysis of human protein phosphatase 2A (PP2A) complexes identified 176 interprotein and 570 intraprotein cross-links that link specific trimeric PP2A complexes to a multitude of adaptor proteins that control their cellular functions. Spatial restraints guided molecular modeling of the binding interface between immunoglobulin binding protein 1 (IGBP1) and PP2A and revealed the topology of TCP1 ring complex (TRiC) chaperonin interacting with the PP2A regulatory subunit 2ABG. This study establishes XL-MS as an integral part of hybrid structural biology approaches for the analysis of endogenous protein complexes.

PMID: 22984071 [PubMed - in process]

2. Science. 2012 Sep 14;337(6100):1360-4.

Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes.

Ibarra CA, Feng X, Schoft VK, Hsieh TF, Uzawa R, Rodrigues JA, Zemach A, Chumak N, Machlicova A, Nishimura T, Rojas D, Fischer RL, Tamaru H, Zilberman D.

Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA.

The Arabidopsis thaliana central cell, the companion cell of the egg, undergoes DNA demethylation before fertilization, but the targeting preferences, mechanism, and biological significance of this process remain unclear. Here, we show that active DNA demethylation mediated by the DEMETER DNA glycosylase accounts for all of the demethylation in the central cell and preferentially targets small, AT-rich, and nucleosome-depleted euchromatic transposable elements. The vegetative cell, the companion cell of sperm, also undergoes DEMETER-dependent demethylation of similar sequences, and lack of DEMETER in vegetative cells causes reduced small RNA-directed DNA methylation of transposons in sperm. Our results demonstrate that demethylation in companion cells reinforces transposon methylation in plant gametes and likely contributes to stable silencing of transposable elements across generations.

PMID: 22984074 [PubMed - in process]

3. Science. 2012 Sep 14;337(6100):1333-6.

Initiation of cell wall pattern by a Rho- and microtubule-driven symmetry breaking.

Oda Y, Fukuda H.

Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. oda@biol.s.u-tokyo.ac.jp

A specifically patterned cell wall is a determinant of plant cell shape. Yet, the precise mechanisms that underlie initiation of cell wall patterning remain elusive. By using a reconstitution assay, we revealed that ROPGEF4 (Rho of plant guanine nucleotide exchange factor 4) and ROPGAP3 [ROP guanosine triphosphatase (GTPase)-activating protein 3] mediate local activation of the plant Rho GTPase ROP11 to initiate distinct pattern of secondary cell walls in xylem cells. The activated ROP11 recruits MIDD1 to induce local disassembly of cortical microtubules. Conversely, cortical microtubules eliminate active ROP11 from the plasma membrane through MIDD1. Such a mutual inhibitory interaction between active ROP domains and cortical microtubules establishes the distinct pattern of secondary cell walls. This Rho-based regulatory mechanism shows how plant cells initiate and control cell wall patterns to form various cell shapes.

PMID: 22984069 [PubMed - in process]

4. Science. 2012 Sep 7;337(6099):1172.

Prions: a piece of the puzzle?

Lahiri DK.

Comment on
Science. 2012 Jun 22;336(6088):1511-3.

PMID: 22955815 [PubMed - indexed for MEDLINE]

5. Science. 2012 Aug 31;337(6098):1101-4.

Network context and selection in the evolution to enzyme specificity.

Nam H, Lewis NE, Lerman JA, Lee DH, Chang RL, Kim D, Palsson BO.

Department of Bioengineering, University of California San Diego, La Jolla, CA 92093-0412, USA.

Enzymes are thought to have evolved highly specific catalytic activities from promiscuous ancestral proteins. By analyzing a genome-scale model of Escherichia coli metabolism, we found that 37% of its enzymes act on a variety of substrates and catalyze 65% of the known metabolic reactions. However, it is not apparent why these generalist enzymes remain. Here, we show that there are marked differences between generalist enzymes and specialist enzymes, known to catalyze a single chemical reaction on one particular substrate in vivo. Specialist enzymes (i) are frequently essential, (ii) maintain higher metabolic flux, and (iii) require more regulation of enzyme activity to control metabolic flux in dynamic environments than do generalist enzymes. Furthermore, these properties are conserved in Archaea and Eukarya. Thus, the metabolic network context and environmental conditions influence enzyme evolution toward high specificity.

PMID: 22936779 [PubMed - indexed for MEDLINE]

6. Science. 2012 Aug 17;337(6096):843-6. Epub 2012 Jul 26.

Identification of the Cdc48?20S proteasome as an ancient AAA+ proteolytic machine.

Barthelme D, Sauer RT.

Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

Comment in

Science. 2012 Aug 17;337(6096):813-4.

Proteasomes are the major energy-dependent proteolytic machines in the eukaryotic and archaeal domains of life. To execute protein degradation, the 20S core peptidase combines with the AAA+ ring of the 19S regulatory particle in eukarya or with the AAA+ proteasome-activating nucleotidase ring in some archaea. Here, we find that Cdc48 and 20S from the archaeon Thermoplasma acidophilum interact to form a functional proteasome. Cdc48 is an abundant and essential double-ring AAA+ molecular machine ubiquitously present in archaea, where its function has been uncertain, and in eukarya where Cdc48 participates by largely unknown mechanisms in diverse cellular processes, including multiple proteolytic pathways. Thus, proteolysis in collaboration with the 20S peptidase may represent an ancestral function of the Cdc48 family.

PMID: 22837385 [PubMed - indexed for MEDLINE]

Yichen Zhang Journal Club(irrelevant literature)

Mass spectrometry study of a transferrin-based protein

drug reveals the key role of protein aggregation for

successful oral delivery

Cedric E. Bobsta, Shunhai Wanga, Wei-Chiang Shenb, and Igor A. Kaltashova,1

aDepartment of Chemistry, University of Massachusetts, Amherst, MA 01003; and
bDepartment of Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, CA 90033

Edited* by George H. Lorimer, University of Maryland, College Park, MD, and approved July 10, 2012 (received for review April 24, 2012)

A recently designed human growth hormone/transferrin fusion protein (GHT) remains one of the very few examples of a protein capable of eliciting measurable therapeutic response after oral administration. To better understand the underlying factors that resulted in this rare success of nonparenteral protein drug delivery, we analyzed proteolytic stability and receptor binding properties of this protein, the key factors in overcoming the primary barriers to successful oral delivery. Analysis of GHT by a combination of size exclusion chromatography and mass spectrometry revealed that a significant protein population exists in an oligomeric (GHTx) state in addition to the anticipated monomer (GHT1). These states of GHT were evaluated for their survivability in stomach-like conditions, as well as their ability to bind transferrin receptor (TfR). Our results reveal an exceptional stability of GHTx, as well as the preserved ability to bind TfR, a critical first step in crossing the epithelial–in- testinal barrier through receptor-mediated transcytosis.

Molecular basis for the action of the collagen-specific chaperone Hsp47/SERPINH1 and its structure-specific

client recognition

Christine Widmera, Jan M. Gebauerb, Elena Brunsteinb, Sabrina Rosenbaumc, Frank Zauckec, Cord Drögemüllerd, Tosso Leebd, and Ulrich Baumannb,1

aDepartment of Chemistry and Biochemistry and dInstitute of Genetics, Vetsuisse Faculty University of Bern, CH-3012 Bern, Switzerland; and bInstitute of Biochemistry and cCenter for Biochemistry, University of Cologne, D-50674 Cologne, Germany

Edited by Robert Huber, Max Planck Institute Biochemistry, Planegg-Martinsried, Germany, and approved July 2, 2012 (received for review May 13, 2012)

Collagen is the most abundant protein in animals and is a major component of the extracellular matrix in tissues such as skin and bone. A distinctive structural feature of all collagen types is a unique triple-helical structure formed by tandem repeats of the consensus sequence Xaa-Yaa-Gly, in which Xaa and Yaa frequently are proline and hydroxyproline, respectively. Hsp47/SERPINH1 is a procollagen- specific molecular chaperone that, unlike other chaperones, specifi- cally recognizes the folded conformation of its client. Reduced func- tional levels of Hsp47 were reported in severe recessive forms of osteogenesis imperfecta, and homozygous knockout is lethal in mice. Here we present crystal structures of Hsp47 in its free form and in complex with homotrimeric synthetic collagen model peptides, each comprising one Hsp47-binding site represented by an arginine at the Yaa-position of a Xaa-Yaa-Gly triplet. Two of these three binding sites in the triple helix are occupied by Hsp47 molecules, which bind in a head-to-head fashion, thus making extensive contacts with the leading and trailing strands of the collagen triple helix. The important arginine residue within the Xaa-Arg-Gly triplet is recognized by a con- served aspartic acid. The structures explain the stabilization of the triple helix as well as the inhibition of collagen-bundle formation by Hsp47. In addition, we propose a pH-dependent substrate release mechanism based on a cluster of histidine residues.

Monitoring cotranslational protein folding in mammalian cells at codon resolution

Yan Hana, Alexandre Davidb, Botao Liuc, Javier G. Magadánb, Jack R. Benninkb, Jonathan W. Yewdellb, and Shu-Bing Qiana,c,1

aDivision of Nutritional Sciences, Cornell University, Ithaca, NY 14853; bLaboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; and cGraduate Field of Genetics and Development, Cornell University, Ithaca, NY 14853

Edited by Arthur L. Horwich, Yale University School of Medicine, New Haven, CT, and approved June 20, 2012 (received for review May 14, 2012)

How the ribosome-bound nascent chain folds to assume its functional tertiary structure remains a central puzzle in biology. In contrast to refolding of a denatured protein, cotranslational folding is complicated by the vectorial nature of nascent chains, the frequent ribosome pausing, and the cellular crowdedness. Here, we present a strategy called folding-associated cotransla- tional sequencing that enables monitoring of the folding compe- tency of nascent chains during elongation at codon resolution. By using an engineered multidomain fusion protein, we demonstrate an efficient cotranslational folding immediately after the emergence of the full domain sequence. We also apply folding- associated cotranslational sequencing to track cotranslational folding of hemagglutinin in influenza A virus-infected cells. In contrast to sequential formation of distinct epitopes, the receptor binding domain of hemagglutinin follows a global folding route by displaying two epitopes simultaneously when the full sequence is available. Our results provide direct evidence of domain-wise global folding that occurs cotranslationally in mammalian cells.

Nature – Sept 13

RPN-6 determines *C. elegans* longevity under proteotoxic stress conditions

David Vilchez, Ianessa Morantte, Zheng Liu, Peter M. Douglas, Carsten Merkwirth+ et al.

This study shows that nematodes without a germ line re-allocate resources to the soma, resulting in elevated proteasome activity, clearance of damaged proteins and increased longevity; this activity is associated with the increased expression of *rpn-6* mediated by the transcription factor DAF-16.

Nature – Sept 6

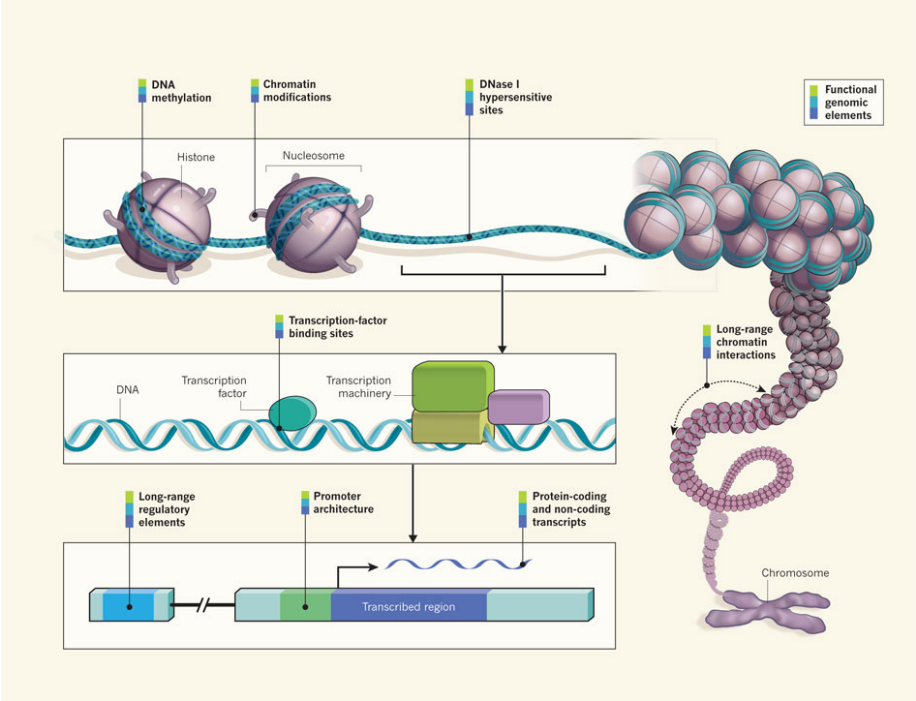
Genomics: ENCODE explained

Joseph R. Ecker, Wendy A. Bickmore, Inês Barroso, Jonathan K. Pritchard, Yoav Gilad & Eran Segal

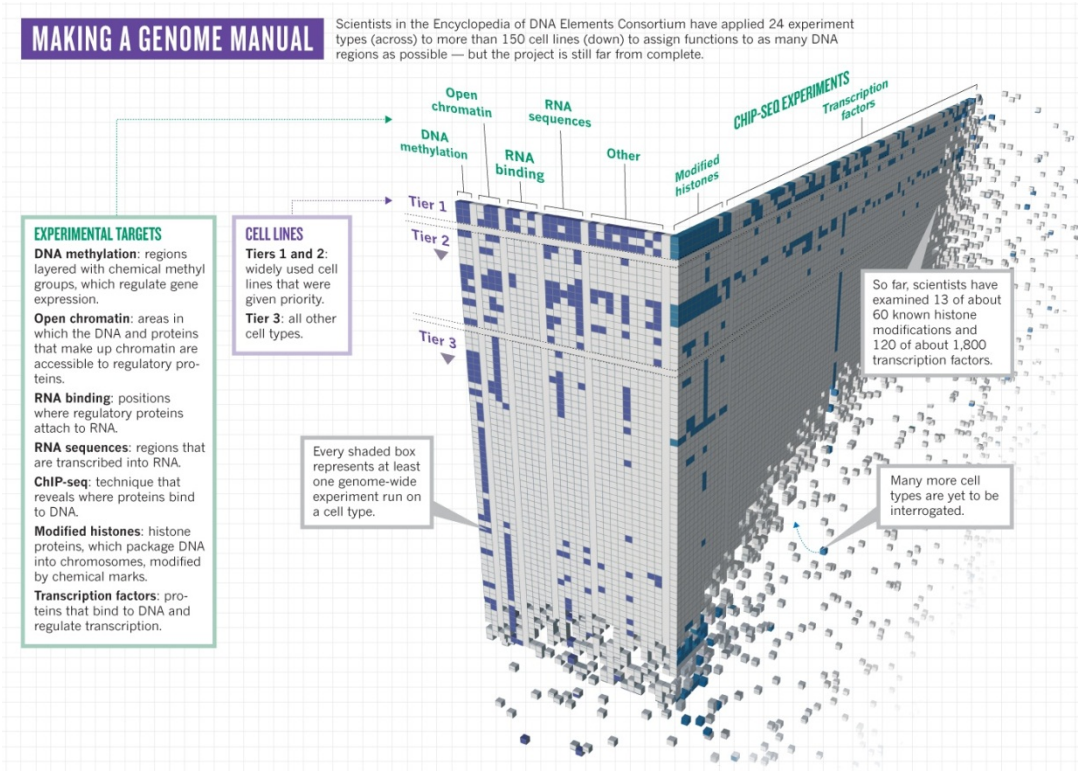
Nature 489, 52–55 (06 September 2012)

The Encyclopedia of DNA Elements (ENCODE) project dishes up a hearty banquet of data that illuminate the roles of the functional elements of the human genome. Here, six scientists describe the project and discuss how the data are influencing research directions across many fields. **See Articles** p.57, p.75, p.83, p.91, p.101 & **Letter** p.109

The 32 groups, including more than 440 scientists, focused on 24 standard types of experiment. The pilot ENCODE project cost an estimated $55 million; the scale-up was about $130 million; and the NHGRI could award up to $123 million in the next phase.



The ENCODE project[2, 3, 4, 5, 6, 7] provides information on the human genome far beyond that contained within the DNA sequence — it describes the functional genomic elements that orchestrate the development and function of a human. The project contains data about the degree of DNA methylation and chemical modifications to histones that can influence the rate of transcription of DNA into RNA molecules (histones are the proteins around which DNA is wound to form chromatin). ENCODE also examines long-range chromatin interactions, such as looping, that alter the relative proximities of different chromosomal regions in three dimensions and also affect transcription. Furthermore, the project describes the binding activity of transcription-factor proteins and the architecture (location and sequence) of gene-regulatory DNA elements, which include the promoter region upstream of the point at which transcription of an RNA molecule begins, and more distant (long-range) regulatory elements. Another section of the project was devoted to testing the accessibility of the genome to the DNA-cleavage protein DNase I. These accessible regions, called DNase I hypersensitive sites, are thought to indicate specific sequences at which the binding of transcription factors and transcription-machinery proteins has caused nucleosome displacement. In addition, ENCODE catalogues the sequences and quantities of RNA transcripts, from both non-coding and protein-coding regions.



Engineered plants can use phosphite

Many crops depend on phosphorus-based fertilizers and are threatened by herbicide-resistant weeds. Genetically modified plants that can digest an alternative phosphorus source that normally inhibits plant growth could solve both problems.

Damar López-Arredondo and Luis Herrera-Estrella at the National Polytechnic Institute's Centre for Research and Advanced Studies in Irapuato, Mexico, engineered the model plant *Arabidopsis*, and tobacco plants, to metabolize phosphite, in addition to the orthophosphate found in standard fertilizer. When phosphite was available, the transgenic plants needed 30–50% less phosphorus to generate the same amount of biomass as that produced in the presence of orthophosphate. The researchers also tested the transgenic plants against weeds. In the presence of orthophosphate, weeds dominated the transgenic plants, but with the addition of phosphite, transgenic tobacco plants easily outcompeted weeds (**pictured**).

Nature Biotechnol. http://dx.doi.org/10.1038/nbt.2346 (2012)

Miscellaneous news:
The US National Football League (NFL) is donating US$30 million to the Foundation for the National Institutes of Health to support research into brain injuries.
Lasker award The US$250,000 Albert Lasker Basic Medical Research Award has been awarded to biologist Michael Sheetz of Columbia University in New York city, biochemist James Spudich of the Stanford University School of Medicine in California and cell biologist Ronald Vale of the University of California, San Francisco, for their work on cytoskeletal motor proteins, which underlie cellular transport and muscle contraction. Winners of the award often go on to receive a Nobel prize.

Structure of a RING E3 ligase and ubiquitin-loaded E2 primed for catalysis

Anna Plechanovová, Ellis G. Jaffray, Michael H. Tatham, James H. Naismith & Ronald T. Hay

This study presents the crystal structure of a RING-type E3 ligase bound to ubiquitin-loaded E2; the structure reveals how ubiquitin binding to E2 leads to changes in the catalytic site, priming it for catalysis by the E3 enzyme.

Nature : 30 August 2012

RPN-6 determines *C. elegans* longevity under proteotoxic stress conditions ▸

David Vilchez, Ianessa Morantte, Zheng Liu, Peter M. Douglas, Carsten Merkwirth *et al.*

This study shows that nematodes without a germ line re-allocate resources to the soma, resulting in elevated proteasome activity, clearance of damaged proteins and increased longevity; this activity is associated with the increased expression of *rpn-6* mediated by the transcription factor DAF-16.

Nature: August 23

Plant nutrition: Rooting for more phosphorus

Leon V. Kochian Nature 488,466–467 (23 August 2012) The identification of an enzyme in rice that confers improved plant yields on phosphorus-deficient soils could open up new avenues for generating nutrient-efficient crops that can thrive on marginally fertile soils.

Nature: August 16

Issue has sustainable energy articles, including algal biofuels.

Nature: 09 August 2012

NRT/PTR transporters are essential for translocation of glucosinolate defence compounds to seeds ▸

Hussam Hassan Nour-Eldin, Tonni Grube Andersen, Meike Burow, Svend Roesen Madsen, Morten Egevang Jørgensen *et al.*

Two high-affinity proton-dependent transporters of glucosinolates have been identified in *Arabidopsis* and termed GTR1 and GTR2; these transporters are essential for transporting glucosinolates to seeds, offering a means to control the allocation of defence compounds in a tissue-specific manner, which may have agricultural biotechnology implications.

The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants OPEN ▸

Angélique D'Hont, France Denoeud, Jean-Marc Aury, Franc-Christophe Baurens, Françoise Carreel *et al.*

The sequencing and analysis of the banana genome is reported; these results inform plant phylogenetic relationships and genome evolution, and provide a resource for future genetic improvement of this important crop species.

Protein activity regulation by conformational entropy ▸

Shiou-Ru Tzeng & Charalampos G. Kalodimos

Some variants of the bacterial gene regulator CAP show marked differences in their affinity for DNA despite identical DNA-binding interfaces; NMR spectroscopy experiments now show that DNA binding is determined by the proteins' internal dynamics over a broad range of timescales in a manner that cannot be predicted from the proteins' ground-state structures.

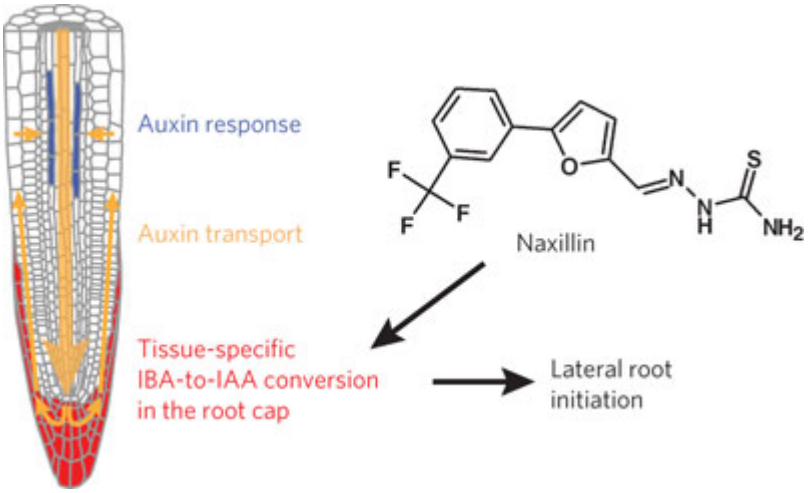
Structural biology: Dynamic binding ▸ Andrew J. Baldwin & Lewis E. Kay

Nature Chemical Biology : October – nothing in particular
Nature Chemical Biology C: September 2012 Volume 8 Number 9, pp 738 - 805

A role for the root cap in root branching revealed by the non-auxin probe naxillin pp798 - 805

Bert De Rybel, Dominique Audenaert, Wei Xuan, Paul Overvoorde, Lucia C Strader, Stefan Kepinski, Rebecca Hoye, Ronald Brisbois, Boris Parizot, Steffen Vanneste, Xing Liu, Alison Gilday, Ian A Graham, Long Nguyen, Leentje Jansen, Maria Fransiska Njo, Dirk Inzé, Bonnie Bartel and Tom Beeckman

doi:10.1038/nchembio.1044



The plant hormone auxin affects many aspects of root development, including lateral root branching. A high-throughput screen in *Arabidopsis thaliana* has led to the identification of naxillin, a non-auxin chemical probe that enhances lateral root branching and has revealed an important role of the root cap in regulating this process.

Nature Chemical Biology C: AUgust

Quality control of disulfide bond formation in pilus subunits by the chaperone FimC - pp707 - 713

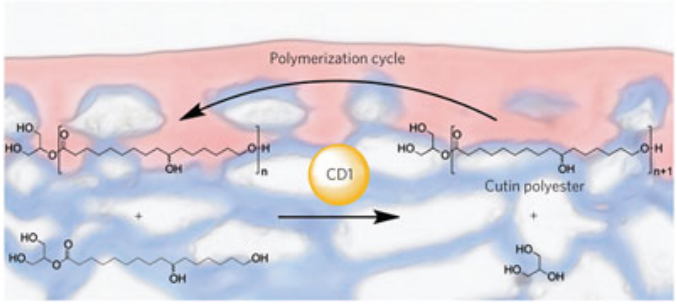
Maria D Crespo, Chasper Puorger, Martin A Schärer, Oliv Eidam, Markus G Grütter, Guido Capitani & Rudi Glockshuber

Nature Chemical Biology C: July

The identification of cutin synthase: formation of the plant polyester cutin - pp609 - 611

Trevor H Yeats, Laetitia B B Martin, Hélène M-F Viart, Tal Isaacson, Yonghua He, Lingxia Zhao, Antonio J Matas, Gregory J Buda, David S Domozych, Mads H Clausen & Jocelyn K C Rose

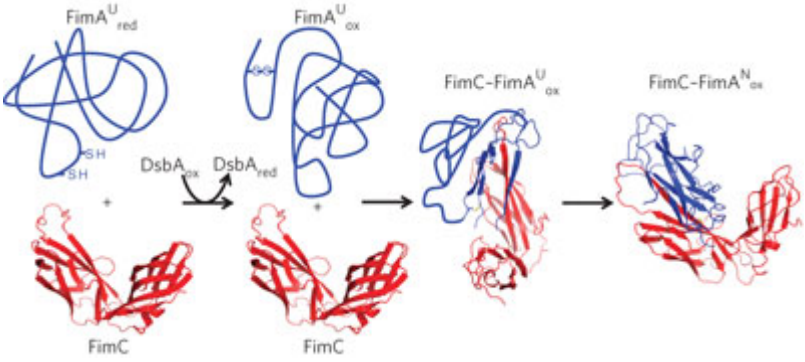
doi:10.1038/nchembio.960



Mapping of a mutation in a tomato deficient in the plant cuticle component cutin yields the first cutin synthase, as shown via accumulation of polymer precursors and *in vitro* oligomerization of synthetic substrates.

Quality control of disulfide bond formation in pilus subunits by the chaperone FimC Vol 8,707–713

Maria D Crespo, Chasper Puorger, Martin A Schärer, Oliv Eidam, Markus G Grütter, Guido Capitani & Rudi Glockshuber



The chaperone FimC only selects unfolded, disulfide-intact pilus subunits and accelerates protein folding by lowering topological complexity, thereby ensuring quality control in pilus assembly.

Small heat-shock proteins protect from heat-stroke-associated neurodegeneration

Nikos Kourtis, Vassiliki Nikoletopoulou & Nektarios Tavernarakis

Nature (2012) Published online 12 September 2012

Heat stroke is a life-threatening condition, characterized by catastrophic collapse of thermoregulation and extreme hyperthermia. In recent years, intensification of heat waves has caused a surge of heat-stroke fatalities. The mechanisms underlying heat-related pathology are poorly understood. Here we show that heat stroke triggers pervasive necrotic cell death and neurodegeneration in *Caenorhabditis elegans*. Preconditioning of animals at a mildly elevated temperature strongly protects from heat-induced necrosis. The heat-shock transcription factor HSF-1 and the small heat-shock protein HSP-16.1 mediate cytoprotection by preconditioning. HSP-16.1 localizes to the Golgi, where it functions with the Ca^{2+}- and Mn^{2+}-transporting ATPase PMR-1 to maintain Ca^{2+} homeostasis under heat stroke. Preconditioning also suppresses cell death inflicted by diverse insults, and protects mammalian neurons from heat cytotoxicity. These findings reveal an evolutionarily conserved mechanism that defends against diverse necrotic stimuli, and may be relevant to heat stroke and other pathological conditions involving necrosis in humans.