Oct 21

Analytical Biochemistry: Alert 14 October-20 October

**A Modified protein assay from microgram to low nanogram levels in dilute samples** Original Research

*Available online 14 October 2013*

Ghanshyam D. Heda, Upasana Kunwar, Rajiv P. Heda

Becker AH, Oh E, Weissman JS, Kramer G, Bukau B.
Selective ribosome profiling as a tool for studying the interaction of chaperones and targeting factors with nascent polypeptide chains and ribosomes.
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PMID: 24136347 [PubMed - as supplied by publisher]

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Î±B-crystallin: a Novel Regulator of Breast Cancer Metastasis to the Brain.
Clin Cancer Res. 2013 Oct 16;. [Epub ahead of print]
PMID: 24132917 [PubMed - as supplied by publisher]

Nahomi RB, Huang R, Nandi SK, Wang B, Padmanabha S, Santhoshkumar P, Filipek S, Biswas A, Nagaraj RH.
Acetylation of Lysine92 Improves the Chaperone and Anti-apoptotic Activities of Human Î±B-Crystallin.
Biochemistry. 2013 Oct 15;. [Epub ahead of print]
PMID: 24128140 [PubMed - as supplied by publisher]

Sherman MY, Qian SB.
Less is more: improving proteostasis by translation slow down.
Trends Biochem Sci. 2013 Oct 11;. [Epub ahead of print]
PMID: 24126073 [PubMed - as supplied by publisher]

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Small heat shock proteins are necessary for heart migration and laterality determination in zebrafish.
Dev Biol. 2013 Oct 17;. [Epub ahead of print]
PMID: 24140541 [PubMed - as supplied by publisher]

Zhang W, Chen S, Liao Y, Wang D, Ding J, Wang Y, Ran X, Lu D, Zhu H.
Expression, purification, and characterization of formaldehyde dehydrogenase from Pseudomonas aeruginosa.
Protein Expr Purif. 2013 Oct 11;. [Epub ahead of print]
PMID: 24125754 [PubMed - as supplied by publisher]

Chowdhury A, Choudhury A, Banerjee V, Banerjee R, Das KP.
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Fares A, Nespoulous C, Rossignol M, Peltier JB.
Simultaneous Identification and Quantification of Nitrosylation Sites by Combination of Biotin Switch and ICAT Labeling.
Methods Mol Biol. 2014;1072:609-20.
PMID: 24136550 [PubMed - in process]

Freschi L.
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PMID: 24130567 [PubMed - as supplied by publisher]

Boscari A, Meilhoc E, Castella C, Bruand C, Puppo A, Brouquisse R.
Which role for nitric oxide in symbiotic N<sub>2</sub>-fixing nodules: toxic by-product or useful signaling/metabolic intermediate?
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Hebelstrup KH, Shah JK, Simpson C, Schjoerring JK, Mandon J, Cristescu SM, Harren FJ, Christiansen MW, Mur LA, Igamberdiev AU.
An assessment of the biotechnological use of hemoglobin modulation in cereals.
Physiol Plant. 2013 Oct 4;. [Epub ahead of print]
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Kotani, T. *et al*. Cyclin B1 mRNA translation is temporally controlled through formation and disassembly of RNA granules. J. Cell Biol. http://dx.doi.org/10.1083/jcb.201302139 (2013)

Kmiec, B. *et al*. Organellar oligopeptidase (OOP) provides a complementary pathway for targeting peptide degradation in mitochondria and chloroplasts. Proc. Natl Acad. Sci. USA http://dx.doi.org/10.1073/pnas.1307637110 (2013)

## Nature Oct 24

### The maize Gα gene *COMPACT PLANT2* functions in CLAVATA signalling to control shoot meristem size

**Peter Bommert, Byoung Il Je, Alexander Goldshmidt & David Jackson**

The maize *COMPACT PLANT2* locus encodes a Gα subunit; its interaction with leucine-rich receptors suggests a new mode of G-protein signalling that acts through single-pass rather than seven-pass G-protein-coupled transmembrane receptors.

### Microbial production of short-chain alkanes

Microbes have already been engineered to produce diesel fuels, and now the microbial production of components of petrol (gasoline) including short-chain alkanes has been achieved using *Escherichia coli* strains metabolically engineered with components of fatty acid biosynthesis pathways.

## Journal of Agronomy and Crop Scienc... Content Alert: 199, 6 (December 2013)

**Effects of Increasing Temperatures on Spikelet Fertility in Different Rice Cultivars based on Temperature Gradient Chamber Experiments (pages 416–423)**

A. Maruyama, W. M. W. Weerakoon, Y. Wakiyama and K. Ohba
Article first published online: 17 APR 2013 | DOI: 10.1111/jac.12028

**Genetic Variation for Heat Tolerance During the Reproductive Phase in *Brassica rapa* (pages 424–435)**

Annisa, S. Chen, N. C. Turner and W. A. Cowling
Article first published online: 19 JUL 2013 | DOI: 10.1111/jac.12034

## FEBS Journal Content Alert: 280, 22 (November 2013)

**Characterizing rapid, activity-linked conformational transitions in proteins via sub-second hydrogen deuterium exchange mass spectrometry (pages 5616–5625)**

Diana Resetca and Derek J. Wilson
Article first published online: 11 JUN 2013 | DOI: 10.1111/febs.12332

This review outlines the application of time-resolved electrospray ionization mass spectrometry (TRESI-MS) and hydrogen-deuterium exchange (HDX) to study rapid, activity-linked conformational transitions in proteins. Implementation on a microfluidic chip incorporates all sample handling steps required for a 'bottom-up' HDX workflow. Combining short HDX labeling pulses with rapid

digestion enables the detailed characterization of structural transitions in diverse biochemical processes.

Proc Natl Acad Sci U S A. 2013 Feb 5;110(6):2395-400. doi: 10.1073/pnas.1213958110. Epub 2013 Jan 22.

**Small open reading frames associated with morphogenesis are hidden in plant genomes.**

Hanada K, Higuchi-Takeuchi M, Okamoto M, Yoshizumi T, Shimizu M, Nakaminami K, Nishi R, Ohashi C, Iida K, Tanaka M, Horii Y, Kawashima M, Matsui K, Toyoda T, Shinozaki K, Seki M, Matsui M.

Plant Science Center, RIKEN, Yokohama, Kanagawa 230-0045, Japan. kohanada@psc.riken.jp

It is likely that many small ORFs (sORFs; 30-100 amino acids) are missed when genomes are annotated. To overcome this limitation, we identified ~8,000 sORFs with high coding potential in intergenic regions of the Arabidopsis thaliana genome. However, the question remains as to whether these coding sORFs play functional roles. Using a designed array, we generated an expression atlas for 16 organs and 17 environmental conditions among 7,901 identified coding sORFs. A total of 2,099 coding sORFs were highly expressed under at least one experimental condition, and 571 were significantly conserved in other land plants. A total of 473 coding sORFs were overexpressed; ~10% (49/473) induced visible phenotypic effects, a proportion that is approximately seven times higher than that of randomly chosen known genes. These results indicate that many coding sORFs hidden in plant genomes are associated with morphogenesis. We believe that the expression atlas will contribute to further study of the roles of sORFs in plants.

de Pinto MC, Locato V, Sgobba A, Romero-Puertas MD, Gadadeta C, Delledonne M, De Gara L.
S-NITROSYLATION OF ASCORBATE PEROXIDASE IS PART OF THE PROGRAMMED CELL DEATH SIGNALING IN TOBACCO BY-2 CELLS.
Plant Physiol. 2013 Oct 24;. [Epub ahead of print]
PMID: 24158396 [PubMed - as supplied by publisher]

Sahara K, Kogleck L, Yashiroda H, Murata S.
The mechanism for molecular assembly of the proteasome.
Adv Biol Regul. 2013 Oct 8;. [Epub ahead of print]
PMID: 24145026 [PubMed - as supplied by publisher]

G3 (Bethesda). 2013 Oct 18. pii: g3.113.007971v1. doi: 10.1534/g3.113.007971. [Epub ahead of print]

Saccharomyces cerevisiae Genes Involved in Survival of Heat Shock.

Jarolim S, Ayer A, Pillay BA, Gee AC, Phrakaysone A, Perrone GG, Breitenbach M, Dawes IW.

The heat-shock response in cells, involving increased transcription of a specific set of genes in response to a sudden temperature increase, is a highly conserved biological response occurring in all organisms. Despite considerable attention to processes activated during heat shock, less is known about the role of genes in survival of a sudden temperature increase. Saccharomyces cerevisiae genes involved in the maintenance of heat-shock resistance in exponential and stationary phase were identified by screening the homozygous diploid deletants in non-essential genes, and the heterozygous diploid mutants in essential genes for survival after a sudden shift in temperature from 30° to 50°. More than a thousand genes were identified that led to altered sensitivity to heat shock, with little overlap between them and those previously identified to affect thermotolerance. There was also little overlap with genes that are activated or repressed during heat-shock with only 5% of them regulated by the heat shock transcription factor. The TOR and protein kinase A pathways, lipid metabolism, vacuolar $H^+$-ATPase, vacuolar protein sorting and mitochondrial genome maintenance/translation were critical to maintenance of resistance. Mutants affected in L-tryptophan metabolism were heat-shock resistant in both growth phases; those affected in cytoplasmic ribosome biogenesis and DNA double-strand break repair were resistant in stationary phase, and in mRNA catabolic processes in exponential phase. Mutations affecting

mitochondrial genome maintenance were highly represented in sensitive mutants. The cell division transcription factor Swi6p and H

Kayser J, Haslbeck M, Dempfle L, Krause M, Grashoff C, Buchner J, Herrmann H, Bausch AR.
The Small Heat Shock Protein Hsp27 Affects Assembly Dynamics andÂ Structure of Keratin Intermediate Filament Networks.
Biophys J. 2013 Oct 15;105(8):1778-85.
PMID: 24138853 [PubMed - in process]

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Hsp27 as a Therapeutic Target in Cancers.
Curr Drug Targets. 2013 Oct 13;. [Epub ahead of print]
PMID: 24138636 [PubMed - as supplied by publisher]

Bai S, Yao T, Li M, Guo X, Zhang Y, Zhu S, He Y.
PIF3 Is Involved in the Primary Root Growth Inhibition of Arabidopsis Induced by Nitric Oxide in the Light.
Mol Plant. 2013 Oct 24;. [Epub ahead of print]
PMID: 24157606 [PubMed - as supplied by publisher]

Lin Y, Yang L, Paul M, Zu Y, Tang Z.
Ethylene promotes germination of Arabidopsis seed under salinity by decreasing reactive oxygen species: Evidence for the involvement of nitric oxide simulated by sodium nitroprusside.
Plant Physiol Biochem. 2013 Oct 10;73C:211-218. [Epub ahead of print]
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Correa-Aragunde N, Foresi N, Delledonne M, Lamattina L.
Auxin induces redox regulation of ascorbate peroxidase 1 activity by S-nitrosylation/denitrosylation balance resulting in changes of root growth pattern in Arabidopsis.
J Exp Bot. 2013 Aug;64(11):3339-49.
PMID: 23918967 [PubMed - indexed for MEDLINE]

Zaffagnini M, Morisse S, Bedhomme M, Marchand CH, Festa M, Rouhier N, Lemaire SD, Trost P.
Mechanisms of nitrosylation and denitrosylation of cytoplasmic glyceraldehyde-3-phosphate dehydrogenase from Arabidopsis thaliana.
J Biol Chem. 2013 Aug 2;288(31):22777-89.
PMID: 23749990 [PubMed - indexed for MEDLINE]

Pan KT, Chen YY, Pu TH, Chao YS, Yang CY, Bomgarden RD, Rogers JC, Meng TC, Khoo KH.
Mass Spectrometry-Based Quantitative Proteomics for Dissecting Multiplexed Redox Cysteine Modifications in Nitric Oxide-Protected Cardiomyocyte Under Hypoxia.
Antioxid Redox Signal. 2013 Oct 23;. [Epub ahead of print]
PMID: 24152285 [PubMed - as supplied by publisher]

**CRISPR-Cas9 for reporter insertions in mouse Nature Methods 10, 1055 (2013)**

Nature Methods - November 2013 Volume 10 pp 1037 - 1133
**Orthogonal Cas9 proteins for RNA-guided gene regulation and editing** **pp1116 - 1121**
Kevin M Esvelt, Prashant Mali, Jonathan L Braff, Mark Moosburner, Stephanie J Yaung *et al.*
A set of Cas9 endonucleases orthogonal to the *Streptococcus pyogenes* enzyme is identified. This will enable simultaneous addressing of multiple RNA-guided activities to different genomic target sites with the CRISPR-Cas9 system.

KEITH

1) **Repetitive Protein Unfolding by the *trans* Ring of the GroEL-GroES Chaperonin Complex Stimulates Folding**
**Repetitive Protein Unfolding by the *trans* Ring of the GroEL-GroES Chaperonin Complex Stimulates Folding**

October 25, 2013 The Journal of Biological Chemistry, 288, 30944-30955

Zong Lin[‡], Jason Puchalla[§], Daniel Shoup[¶] and Hays S. Rye[¶1]

[‡]Department of Biotechnology and Biomedicine, Yangtze Delta Region Institute of Tsinghua University, Jiaxing, Zhejiang, China

[§]Department of Physics, Princeton University, Princeton, New Jersey

[¶]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas

**ABSTRACT**

A key constraint on the growth of most organisms is the slow and inefficient folding of many essential proteins. To deal with this problem, several diverse families of protein folding machines, known collectively as molecular chaperones, developed early in evolutionary history. The functional role and operational steps of these remarkably complex nanomachines remain subjects of active debate. Here we present evidence that, for the GroEL-GroES chaperonin system, the non-native substrate protein enters the folding cycle on the trans ring of the double-ring GroEL-ATP-GroES complex rather than the ADP-bound complex. The properties of this ATP complex are designed to ensure that non-native substrate protein binds first, followed by ATP and finally GroES. This binding order ensures efficient occupancy of the open GroEL ring and allows for disruption of misfolded structures through two phases of multiaxis unfolding. In this model, repeated cycles of partial unfolding, followed by confinement within the GroEL-GroES chamber, provide the most effective overall mechanism for facilitating the folding of the most stringently dependent GroEL substrate proteins.

2) **Endoplasmic Reticulum Protein Quality Control Is Determined by Cooperative Interactions between Hsp/c70 Protein and the CHIP E3 Ligase**

October 25, 2013 The Journal of Biological Chemistry, 288, 31069-31079.

Yoshihiro Matsumura[‡,§], Juro Sakai[§] and William R. Skach[‡1]

[‡]Department of Biochemistry and Molecular Biology, Oregon Health and Science University, Portland, Oregon

[§]Division of Metabolic Medicine, Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan

**ABSTRACT**

The C terminus of Hsp70 interacting protein (CHIP) E3 ligase functions as a key regulator of protein quality control by binding the C-terminal (M/I)EEVD peptide motif of Hsp/c70(90) with its N-terminal tetratricopeptide repeat (TPR) domain and facilitating polyubiquitination of misfolded client proteins via its C-terminal catalytic U-box. Using CFTR as a

model client, we recently showed that the duration of the Hsc70-client binding cycle is a primary determinant of stability. However, molecular features that control CHIP recruitment to Hsp/c70, and hence the fate of the Hsp/c70 client, remain unknown. To understand how CHIP recognizes Hsp/c70, we utilized a dominant negative mutant in which loss of a conserved proline in the U-box domain (P269A) eliminates E3 ligase activity. In a cell-free reconstituted ER-associated degradation system, P269A CHIP inhibited Hsc70-dependent CFTR ubiquitination and degradation in a dose-dependent manner. Optimal inhibition required both the TPR and the U-box, indicating cooperativity between the two domains. Neither the wild type nor the P269A mutant changed the extent of Hsc70 association with CFTR nor the dissociation rate of the Hsc70-CFTR complex. However, the U-box mutation stimulated CHIP binding to Hsc70 while promoting CHIP oligomerization. CHIP binding to Hsc70 binding was also stimulated by the presence of an Hsc70 client with a preference for the ADP-bound state. Thus, the Hsp/c70 (M/I)EEVD motif is not a simple anchor for the TPR domain. Rather CHIP recruitment involves reciprocal allosteric interactions between its TPR and U-box domains and the substrate-binding and C-terminal domains of Hsp/c70.

**3)**

October 25, 2013 The Journal of Biological Chemistry, 288, 30944-30955

Zong Lin[‡], Jason Puchalla[§], Daniel Shoup[¶] and Hays S. Rye[¶1]

[‡]Department of Biotechnology and Biomedicine, Yangtze Delta Region Institute of Tsinghua University, Jiaxing, Zhejiang, China

[§]Department of Physics, Princeton University, Princeton, New Jersey

[¶]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas

**ABSTRACT**

A key constraint on the growth of most organisms is the slow and inefficient folding of many essential proteins. To deal with this problem, several diverse families of protein folding machines, known collectively as molecular chaperones, developed early in evolutionary history. The functional role and operational steps of these remarkably complex nanomachines remain subjects of active debate. Here we present evidence that, for the GroEL-GroES chaperonin system, the non-native substrate protein enters the folding cycle on the trans ring of the double-ring GroEL-ATP-GroES complex rather than the ADP-bound complex. The properties of this ATP complex are designed to ensure that non-native substrate protein binds first, followed by ATP and finally GroES. This binding order ensures efficient occupancy of the open GroEL ring and allows for disruption of misfolded structures through two phases of multiaxis unfolding. In this model, repeated cycles of partial unfolding, followed by confinement within the GroEL-GroES chamber, provide the most effective overall mechanism for facilitating the folding of the most stringently dependent GroEL substrate proteins.

**4) Endoplasmic Reticulum Protein Quality Control Is Determined by Cooperative Interactions between Hsp/c70 Protein and the CHIP E3 Ligase**

October 25, 2013 The Journal of Biological Chemistry, 288, 31069-31079.

Yoshihiro Matsumura[‡,§], Juro Sakai[§] and William R. Skach[‡]

[‡]Department of Biochemistry and Molecular Biology, Oregon Health and Science University, Portland, Oregon

[§]Division of Metabolic Medicine, Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan

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The C terminus of Hsp70 interacting protein (CHIP) E3 ligase functions as a key regulator of protein quality control by binding the C-terminal (M/I)EEVD peptide motif of Hsp/c70(90) with its N-terminal tetratricopeptide repeat (TPR) domain and facilitating polyubiquitination of misfolded client proteins via its C-terminal catalytic U-box. Using CFTR as a model client, we recently showed that the duration of the Hsc70-client binding cycle is a primary determinant of stability. However, molecular features that control CHIP recruitment to Hsp/c70, and hence the fate of the Hsp/c70 client, remain unknown. To understand how CHIP recognizes Hsp/c70, we utilized a dominant negative mutant in which loss of a conserved proline in the U-box domain (P269A) eliminates E3 ligase activity. In a cell-free reconstituted ER-associated degradation system, P269A CHIP inhibited Hsc70-dependent CFTR ubiquitination and degradation in a dose-dependent manner. Optimal inhibition required both the TPR and the U-box, indicating cooperativity between the two domains. Neither the wild type nor the P269A mutant changed the extent of Hsc70 association with CFTR nor the dissociation rate of the Hsc70-CFTR complex. However, the U-box mutation stimulated CHIP binding to Hsc70 while promoting CHIP oligomerization. CHIP binding to Hsc70 binding was also stimulated by the presence of an Hsc70 client with a preference for the ADP-bound state. Thus, the Hsp/c70 (M/I)EEVD motif is not a simple anchor for the TPR domain. Rather CHIP recruitment involves reciprocal allosteric interactions between its TPR and U-box domains and the substrate-binding and C-terminal domains of Hsp/c70.

INDU

1. Science. 2013 Oct 4;342(6154):114-8. doi: 10.1126/science.1242113. Epub 2013 Sep

   A thylakoid-located two-pore K+ channel controls photosynthetic light utilization in plants.

   Carraretto L, Formentin E, Teardo E, Checchetto V, Tomizioli M, Morosinotto T, Giacometti GM, Finazzi G, Szabó I.

   Department of Biology, University of Padua, viale Giuseppe Colombo 3, 35121 Padua, Italy.

   Comment in Science. 2013 Oct 4;342(6154):50-1.

   The size of the light-induced proton motive force (pmf) across the thylakoid membrane of chloroplasts is regulated in response to environmental stimuli. Here, we describe a component of the thylakoid membrane, the two-pore potassium (K(+)) channel TPK3, which modulates the composition of the pmf through ion counterbalancing. Recombinant TPK3 exhibited potassium-selective channel activity sensitive to Ca(2+) and H(+). In Arabidopsis plants, the channel is found in the thylakoid stromal lamellae. Arabidopsis plants silenced for the TPK3 gene display reduced growth and altered thylakoid membrane organization. This phenotype reflects an impaired capacity to generate a normal pmf, which results in reduced CO2 assimilation and deficient nonphotochemical dissipation of excess absorbed light. Thus, the TPK3 channel manages the pmf necessary to convert photochemical energy into physiological functions.

PMID: 24009357 [PubMed - indexed for MEDLINE]

2. Science. 2013 Oct 4;342(6154):118-23. doi: 10.1126/science.1239705.

Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways.

Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, Huang HD, Jin H.

Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA.

Comment in Science. 2013 Oct 4;342(6154):45-6.

Botrytis cinerea, the causative agent of gray mold disease, is an aggressive fungal pathogen that infects more than 200 plant species. Here, we show that some B. cinerea small RNAs (Bc-sRNAs) can silence Arabidopsis and tomato genes involved in immunity. These Bc-sRNAs hijack the host RNA interference (RNAi) machinery by binding to Arabidopsis Argonaute 1 (AGO1) and selectively silencing host immunity genes. The Arabidopsis ago1 mutant exhibits reduced susceptibility to B. cinerea, and the B. cinerea dcl1 dcl2 double mutant that can no longer produce these Bc-sRNAs displays reduced pathogenicity on Arabidopsis and tomato. Thus, this fungal pathogen transfers "virulent" sRNA effectors into host plant cells to suppress host immunity and achieve infection, which demonstrates a naturally occurring cross-kingdom RNAi as an advanced virulence mechanism.

PMID: 24092744 [PubMed - indexed for MEDLINE]

3. Science. 2013 Oct 4;342(6154):85-90. doi: 10.1126/science.1238599.

Mice genetically deficient in vasopressin V1a and V1b receptors are resistant to jet lag.

Yamaguchi Y, Suzuki T, Mizoro Y, Kori H, Okada K, Chen Y, Fustin JM, Yamazaki F, Mizuguchi N, Zhang J, Dong X, Tsujimoto G, Okuno Y, Doi M, Okamura H.

Department of Systems Biology, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan.

Comment in Science. 2013 Oct 4;342(6154):52-3.

Jet-lag symptoms arise from temporal misalignment between the internal circadian clock and external solar time. We found that circadian rhythms of behavior

(locomotor activity), clock gene expression, and body temperature immediately reentrained to phase-shifted light-dark cycles in mice lacking vasopressin receptors V1a and V1b (V1a(-/-)V1b(-/-)). Nevertheless, the behavior of V1a(-/-)V1b(-/-) mice was still coupled to the internal clock, which oscillated normally under standard conditions. Experiments with suprachiasmatic nucleus (SCN) slices in culture suggested that interneuronal communication mediated by V1a and V1b confers on the SCN an intrinsic resistance to external perturbation. Pharmacological blockade of V1a and V1b in the SCN of wild-type mice resulted in accelerated recovery from jet lag, which highlights the potential of vasopressin signaling as a therapeutic target for management of circadian rhythm misalignment, such as jet lag and shift work.

PMID: 24092737 [PubMed - indexed for MEDLINE]

4. Science. 2013 Sep 27;341(6153):1483-8. doi: 10.1126/science.1240636. Epub 2013 Aug 22.

Circadian gene Bmal1 regulates diurnal oscillations of Ly6C(hi) inflammatory monocytes.

Nguyen KD, Fentress SJ, Qiu Y, Yun K, Cox JS, Chawla A.

Cardiovascular Research Institute, University of California, San Francisco, CA 94158-9001, USA.

Comment in Science. 2013 Sep 27;341(6153):1462-4.

Circadian clocks have evolved to regulate physiologic and behavioral rhythms in anticipation of changes in the environment. Although the molecular clock is present in innate immune cells, its role in monocyte homeostasis remains unknown. Here, we report that Ly6C(hi) inflammatory monocytes exhibit diurnal variation, which controls their trafficking to sites of inflammation. This cyclic pattern of trafficking confers protection against Listeria monocytogenes and is regulated by the repressive activity of the circadian gene Bmal1. Accordingly, myeloid cell-specific deletion of Bmal1 induces expression of monocyte-attracting chemokines and disrupts rhythmic cycling of Ly6C(hi) monocytes, predisposing mice to development of pathologies associated with acute and chronic inflammation. These findings have unveiled a critical role for BMAL1 in controlling the diurnal rhythms in Ly6C(hi) monocyte numbers.

PMID: 23970558 [PubMed - indexed for MEDLINE]

NATHAN

## Nature Protocols

### 1) Real-time single-molecule coimmunoprecipitation of weak protein-protein interactions

Coimmunoprecipitation (co-IP) analysis is a useful method for studying protein-protein interactions. It currently involves electrophoresis and western blotting, which are not optimized for detecting weak and transient interactions. In this protocol we describe an advanced version of co-IP analysis that uses real-time, single-molecule fluorescence imaging as its detection scheme. Bait proteins are pulled down onto the imaging plane of a total internal reflection (TIR) microscope. With unpurified cells or tissue extracts kept in reaction chambers, we observe single protein-protein interactions between the surface-immobilized bait and the fluorescent protein–labeled prey proteins in real time. Such direct recording provides an improvement of five orders of magnitude in the time resolution of co-IP analysis. With the single-molecule sensitivity and millisecond time resolution, which distinguish our method from other methods for measuring weak protein-protein interactions, it is possible to quantify the interaction kinetics and active fraction of native, unlabeled bait proteins. Real-time single-molecule co-IP analysis, which takes ~4 h to complete from lysate preparation to kinetic analysis, provides a general avenue for revealing the rich kinetic picture of target protein-protein interactions, and it can be used, for example, to investigate the molecular lesions that drive individual cancers at the level of protein-protein interactions.

STEPHANIE

PNAS

## Crystal structures of an archaeal oligosaccharyltransferase provide insights into the catalytic cycle of N-linked protein glycosylation.

Matsumoto S, Shimada A, Nyirenda J, Igura M, Kawano Y, Kohda D

Division of Structural Biology, Medical Institute of Bioregulation, and Research Center for Advanced Immunology, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan.

### Abstract

Oligosaccharyltransferase transfers an oligosaccharide chain to the asparagine residues in proteins. The archaeal and eubacterial oligosaccharyltransferases are single subunit membrane enzymes, referred to as "AglB" (archaeal glycosylation B) and "PglB" (protein glycosylation B), respectively. Only one crystal structure of a full-length PglB has been solved. Here we report the crystal structures of the full-length AglB from a hyperthermophilic archaeon, Archaeoglobus fulgidus. The AglB and PglB proteins share the common overall topology of the 13 transmembrane helices, and a characteristic long plastic loop in the transmembrane region. This is the structural basis for the formation of the catalytic center, consisting of conserved acidic residues coordinating a divalent metal ion. In one crystal form, a sulfate ion was bound next to the metal ion. This structure appears to represent a dolichol-phosphate binding state, and suggests the release mechanism for the glycosylated product. The structure in the other crystal form corresponds to the resting state conformation with the well-ordered plastic loop in the transmembrane region. The overall structural similarity between the distantly related AglB and PglB proteins strongly indicates the conserved catalytic mechanism in the eukaryotic counterpart, the STT3 (stauroporine and temperature sensitivity 3) protein. The detailed structural comparison provided the dynamic view of the N-glycosylation reaction, involving the conversion between the structured and unstructured states of the plastic loop in the transmembrane region and the formation and collapse of the Ser/Thr-binding pocket in the C-terminal globular domain.

FIONN

Nature. 2013 Oct 24;502(7472):555-8. doi: 10.1038/nature12583. Epub 2013 Sep 11.

**The maize Gα gene COMPACT PLANT2 functions in CLAVATA signalling to control shoot meristem size.**

Bommert P, Je BI, Goldshmidt A, Jackson D.

## Abstract

Shoot growth depends on meristems, pools of stem cells that are maintained by a negative feedback loop between the CLAVATA pathway and the WUSCHEL homeobox gene. CLAVATA signalling involves a secreted peptide, CLAVATA3 (CLV3), and its perception by cell surface leucine-rich repeat (LRR) receptors, including the CLV1 receptor kinase and a LRR receptor-like protein, CLV2 (ref. 4). However, the signalling mechanisms downstream of these receptors are poorly understood, especially for LRR receptor-like proteins, which lack a signalling domain. Here we show that maize COMPACT PLANT2 (CT2) encodes the predicted α-subunit (Gα) of a heterotrimeric GTP binding protein. Maize ct2 phenotypes resemble Arabidopsis thaliana clavata mutants, and genetic, biochemical and functional assays indicate that CT2/Gα transmits a stem-cell-restrictive signal from a CLAVATA LRR receptor, suggesting a new function for Gα signalling in plants. Heterotrimeric GTP-binding proteins are membrane-associated molecular switches that are commonly activated by ligand binding to an associated seven-pass transmembrane (7TM) G-protein-coupled receptor (GPCR). Recent studies have questioned the idea that plant heterotrimeric G proteins interact with canonical GPCRs, and our findings suggest that single pass transmembrane receptors act as GPCRs in plants, challenging the dogma that GPCRs are exclusively 7TM proteins.

Plant Cell

**Tissue-Specific Profiling Reveals Transcriptome Alterations in Arabidopsis Mutants Lacking Morphological Phenotypes**

Marissa Simon,a,1 Angela Bruex,a,2 Raghunandan M. Kainkaryam,b,3 Xiaohua Zheng,a Ling Huang,a Peter J. Woolf,b

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Traditional genetic analysis relies on mutants with observable phenotypes. Mutants lacking visible abnormalities may nevertheless exhibit molecular differences useful for defining gene function. To examine this, we analyzed tissue-specific transcript profiles from Arabidopsis thaliana transcription factor gene mutants with known roles in root epidermis development, but lacking a single-gene mutant phenotype due to genetic redundancy. We discovered substantial transcriptional changes in each mutant, preferentially affecting root epidermal genes in a manner consistent with the known double mutant effects. Furthermore, comparing transcript profiles of single and double mutants, we observed remarkable variation in the sensitivity of target genes to the loss of one or both paralogous genes, including preferential effects on specific branches of the epidermal gene network, likely reflecting the pathways of paralog subfunctionalization during evolution. In addition, we analyzed the root epidermal transcriptome of the transparent testa glabra2 mutant to clarify its role in the network. These findings provide insight into the molecular basis of genetic redundancy and duplicate gene diversification at the level of a specific gene regulatory network, and they demonstrate the usefulness of tissue-specific transcript profiling to define gene function in mutants lacking informative visible changes in phenotype.