Lit Lunch 2_13_15

Damian:

Rachel J. Perry, João-Paulo G. Camporez, Romy Kursawe, Paul M. Titchenell, Dongyan Zhang, Curtis J. Perry, Michael J. Jurczak, Abulizi Abudukadier, Myoung Sook Han, Xian-Man Zhang, Hai-Bin Ruan, Xiaoyong Yang, Sonia Caprio, Susan M. Kaech, Hei Sook Sul, Morris J. Birnbaum, Roger J. Davis, Gary W. Cline, Kitt Falk Petersen, Gerald I. Shulman, Hepatic Acetyl CoA Links Adipose Tissue Inflammation to Hepatic Insulin Resistance and Type 2 Diabetes, Cell, Volume 160, Issue 4, 12 February 2015, Pages 745-758, ISSN 0092-8674, http://dx.doi.org/10.1016/j.cell.2015.01.012.
(http://www.sciencedirect.com/science/article/pii/S0092867415000148)

Abstract: Summary
Impaired insulin-mediated suppression of hepatic glucose production (HGP) plays a major role in the pathogenesis of type 2 diabetes (T2D), yet the molecular mechanism by which this occurs remains unknown. Using a novel in vivo metabolomics approach, we show that the major mechanism by which insulin suppresses HGP is through reductions in hepatic acetyl CoA by suppression of lipolysis in white adipose tissue (WAT) leading to reductions in pyruvate carboxylase flux. This mechanism was confirmed in mice and rats with genetic ablation of insulin signaling and mice lacking adipose triglyceride lipase. Insulin's ability to suppress hepatic acetyl CoA, PC activity, and lipolysis was lost in high-fat-fed rats, a phenomenon reversible by IL-6 neutralization and inducible by IL-6 infusion. Taken together, these data identify WAT-derived hepatic acetyl CoA as the main regulator of HGP by insulin and link it to inflammation-induced hepatic insulin resistance associated with obesity and T2D.

Keith:

Journal of Biological Chemistry

Dimeric Structure of the Bacterial Extracellular Foldase PrsA

February 6, 2015 The Journal of Biological Chemistry, 290, 3278-3292.

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Secretion of proteins into the membrane-cell wall space is essential for cell wall biosynthesis and pathogenicity in Gram-positive bacteria. Folding and maturation of many secreted proteins depend on a single extracellular foldase, the PrsA protein. PrsA is a 30-kDa protein, lipid anchored to the outer leaflet of the cell membrane. The crystal structure of Bacillus subtilis PrsA reveals a central catalytic parvulin-type prolyl isomerase domain, which is inserted into a larger composite NC domain formed by the N- and C-terminal regions. This domain architecture resembles, despite a lack of sequence conservation, both trigger factor, a ribosome-binding bacterial chaperone, and SurA, a periplasmic chaperone in Gram-negative bacteria. Two main structural differences are observed in that the N-terminal arm of PrsA is substantially shortened relative to the trigger factor and SurA and in that PrsA is found to dimerize in a unique fashion via its NC domain. Dimerization leads to a large, bowl-shaped crevice, which might be involved in vivo in protecting substrate proteins from aggregation. NMR experiments reveal a direct, dynamic interaction of both the parvulin and the NC domain with secretion propeptides, which have been implicated in substrate targeting to PrsA.

Indu:

1. Science. 2015 Jan 23;347(6220):439-42. doi: 10.1126/science.1261197.

   Proteasomes. A molecular census of 26S proteasomes in intact neurons.

   Asano S(1), Fukuda Y(1), Beck F(1), Aufderheide A(1), Förster F(1), Danev R(1), Baumeister W(2).

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   The 26S proteasome is a key player in eukaryotic protein quality control and in the regulation of numerous cellular processes. Here, we describe quantitative in situ structural studies of this highly dynamic molecular machine in intact hippocampal neurons. We used electron cryotomography with the Volta phase plate, which allowed high fidelity and nanometer precision localization of 26S proteasomes. We undertook a molecular census of single- and double-capped proteasomes and assessed the conformational states of individual complexes. Under the conditions of the experiment?that is, in the absence of proteotoxic

stress?only 20% of the 26S proteasomes were engaged in substrate processing. The remainder was in the substrate-accepting ground state. These findings suggest that in the absence of stress, the capacity of the proteasome system is not fully used.

PMID: 25613890 [PubMed - indexed for MEDLINE]

2. Science. 2015 Jan 23;347(6220):1260419. doi: 10.1126/science.1260419.

Proteomics. Tissue-based map of the human proteome.

Uhlén M(1), Fagerberg L(2), Hallström BM(3), Lindskog C(4), Oksvold P(2), Mardinoglu A(5), Sivertsson Å(2), Kampf C(4), Sjöstedt E(6), Asplund A(4), Olsson I(4), Edlund K(7), Lundberg E(2), Navani S(8), Szigyarto CA(9), Odeberg J(2), Djureinovic D(4), Takanen JO(9), Hober S(9), Alm T(2), Edqvist PH(4), Berling H(9), Tegel H(9), Mulder J(10), Rockberg J(9), Nilsson P(2), Schwenk JM(2), Hamsten M(9), von Feilitzen K(2), Forsberg M(2), Persson L(2), Johansson F(2), Zwahlen M(2), von Heijne G(11), Nielsen J(12), Pontén F(4).

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Resolving the molecular details of proteome variation in the different tissues and organs of the human body will greatly increase our knowledge of human biology and disease. Here, we present a map of the human tissue proteome based on an

integrated omics approach that involves quantitative transcriptomics at the tissue and organ level, combined with tissue microarray-based immunohistochemistry, to achieve spatial localization of proteins down to the single-cell level. Our tissue-based analysis detected more than 90% of the putative protein-coding genes. We used this approach to explore the human secretome, the membrane proteome, the druggable proteome, the cancer proteome, and the metabolic functions in 32 different tissues and organs. All the data are integrated in an interactive Web-based database that allows exploration of individual proteins, as well as navigation of global expression patterns, in all major tissues and organs in the human body.

PMID: 25613900 [PubMed - indexed for MEDLINE]

Nathen:

Interplay between *E. coli* DnaK, ClpB and GrpE during Protein Disaggregation

Shannon M. Doyle [1] Shankar Shastry [1] Andrea N. Kravats [1] Yu-Hsuan Shih [2] Marika Miot [1,1] Joel R. Hoskins [1] George Stan [2] Sue Wickner [1]

Abstract

The DnaK/Hsp70 chaperone system and ClpB/Hsp104 collaboratively disaggregate protein aggregates and reactivate inactive proteins. The teamwork is specific:*Escherichia coli* DnaK interacts with *E. coli*ClpB and yeast Hsp70, Ssa1, interacts with yeast Hsp104. This interaction is between the middle domains of hexameric ClpB/Hsp104 and the DnaK/Hsp70 nucleotide-binding domain (NBD). To identify the site on *E. coli*DnaK that interacts with ClpB, we substituted amino acid residues throughout the DnaK NBD. We found that several variants with substitutions in subdomains IB and IIB of the DnaK NBD were defective in ClpB interaction*in vivo* in a bacterial two-hybrid assay and *in vitro* in a fluorescence anisotropy assay. The DnaK subdomain IIB mutants were also defective in the ability to disaggregate protein aggregates with ClpB, DnaJ and GrpE, although they retained some ability to reactivate proteins with DnaJ and GrpE in the absence of ClpB. We observed that GrpE, which also interacts with subdomains IB and IIB, inhibited the interaction between ClpB and DnaK *in vitro*, suggesting competition between ClpB and GrpE for binding DnaK. Computational modeling of the DnaK–ClpB hexamer complex indicated that one DnaK monomer contacts two adjacent ClpB protomers simultaneously. The model and the experiments support a common and mutually exclusive GrpE and ClpB interaction region on DnaK. Additionally, homologous substitutions in subdomains IB and IIB of Ssa1 caused defects in collaboration between Ssa1 and Hsp104. Altogether, these results provide insight into the molecular mechanism of collaboration between the DnaK/Hsp70 system and ClpB/Hsp104 for protein disaggregation

Minsoo:

1. Plant and Cell Physiology. 56(2): 311–321

Identification of mRNAs that Move Over Long Distances Using an RNA-Seq Analysis of Arabidopsis/Nicotiana benthamiana Heterografts

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Phloem is a conductive tissue that allocates nutrients from mature source leaves to sinks such as young developing tissues. Phloem also delivers proteins and RNA species, such as small RNAs and mRNAs. Intensive studies on plant systemic signaling revealed the essential roles of proteins and RNA species. However, many of their functions are still largely unknown, with the roles of transported mRNAs being particularly poorly understood. A major difficulty is the absence of an accurate and comprehensive list of mobile transcripts. In this study, **we used a hetero-graft system with Nicotiana benthamiana as the recipient scion and Arabidopsis as the donor stock, to identify transcripts that moved long distances across the graft union. We identified 138 Arabidopsis transcripts as mobile mRNAs,** which we collectively termed the mRNA mobilome. Reverse transcription–PCR, quantitative real-time PCR and droplet digital PCR analyses confirmed the mobility. The transcripts included **potential signaling factors and, unexpectedly, more general factors.** In our investigations, we found no preferred transcript length, no previously known sequence motifs in promoter or transcript sequences and no similarities between the level of the transcripts and that in the source leaves. Grafting experiments regarding the function of ERECTA, an identified transcript, showed that no function of the transcript mobilized. To our knowledge, this is the first report identifying transcripts that move over long distances using a hetero-graft system between different plant taxa.

2. Plant and Cell Physiology. 56(2): 334–345

Loss of Cytochrome cM Stimulates Cyanobacterial Heterotrophic Growth in the Dark

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Although cyanobacteria are photoautotrophs, they have the capability for heterotrophic metabolism that enables them to survive in their natural habitat. However, **cyanobacterial species that grow heterotrophically in the dark are rare.** It remains largely unknown how cyanobacteria regulate heterotrophic activity. The cyanobacterium Leptolyngbya boryana grows heterotrophically with glucose in the dark. **A dark-adapted variant dg5 isolated from the wild type (WT) exhibits enhanced heterotrophic growth in the dark.** We sequenced the genomes of dg5 and the WT to identify the mutation(s) of dg5. The WT genome consists of a circular chromosome (6,176,364 bp), a circular plasmid pLBA (77,793 bp) and two linear plasmids pLBX (504,942 bp) and pLBY (44,369 bp). Genome comparison revealed three mutation sites. **Phenotype analysis of mutants isolated from the WT by introducing these mutations individually revealed that the relevant mutation is a single adenine insertion causing a frameshift of cytM encoding Cyt cM.** The respiratory oxygen consumption of the cytM-lacking mutant grown in the dark was significantly higher than that of the WT. We isolated a cytM-lacking mutant, ΔcytM, from another cyanobacterium Synechocystis sp. PCC 6803, and ΔcytM grew in the dark with a doubling time of 33 h in contrast to no growth of the WT. The respiratory oxygen consumption of ΔcytM grown in the dark was about 2-fold higher than that of the WT. These results suggest a suppressive role(s) for Cyt cM in regulation of heterotrophic activity.

3. New Phytologist. Volume 205, Issue 4

Special Issue: Ecology and evolution of mycorrhizas

Almost all land plant species form a symbiosis with mycorrhizal fungi. **These soil fungi provide nutrients and other services to plants in return for plant carbohydrates.** The recent application of **microbial metagenomics, metatranscriptomics, and metabolomics** to plants and their immediate surroundings confirms the key role of mycorrhizal fungi, rhizosphere bacteria and fungi, and suggests a world of hitherto undiscovered interactions (van der Heijden et al., this issue, pp. 1406–1423). This novel knowledge is leading to a paradigm-shifting view: plants cannot be considered as isolated individuals any more, but as metaorganisms, or holobionts (Hacquard & Schadt, this issue, pp. 1424–1430) encompassing an active microbial community re-programming host physiology (see Pozo et al., this issue, pp. 1431–1436). This bears tremendous implications for plant ecophysiology and evolution, plant breeding, crop management and sustainable ecosystem management.

4. Nature Plants 1, Article number: 14024 (2015)

The SCARWAVE complex polarizes PAN receptors and promotes division asymmetry in maize.

Michelle R. Facette, Yeri Park, Dena Sutimantanapi, Anding Luo, Heather N. Cartwright, Bing Yang, Eric J. Bennett, Anne W. Sylvester & Laurie G. Smith.

Pre-mitotic establishment of polarity is a key event in the preparation of mother cells for asymmetric cell divisions that produce daughters of distinct fates, and ensures correct cellular patterning of tissues and eventual organ function. Previous work has shown that **two receptor-like kinases, PANGLOSS2 (PAN2) and PAN1, and the small GTPase RHO GTPASE OF PLANTS (ROP) promote mother cell polarity and subsequent division asymmetry in developing maize stomata.** PAN proteins become polarized prior to asymmetric cell division, however, the mechanism of this polarization is unknown. Here we show that the **SCAR/WAVE regulatory complex, which activates the actin-nucleating ARP2/3 complex, is the first known marker of polarity in this asymmetric division model and is required for PAN polarization.** These findings implicate actin, and specifically branched actin networks, in PAN polarization and asymmetric cell division.

Stephanie:

Plant Mol Biol. 2015 Jan 31. [Epub ahead of print]

Gene knockout of glutathione reductase 3 results in increased sensitivity to salt stress in rice.

Wu TM[1], Lin WR, Kao CH, Hong CY.
Author information

Abstract

Glutathione reductase (GR) is one of important antioxidant enzymes in plants. This enzyme catalyzes the reduction of glutathione disulfide (GSSG) to reduced glutathione (GSH) with the accompanying oxidation of NADPH. Previously, we showed that salt-stress-responsive GR3 is a functional protein localized in chloroplasts and mitochondria in rice. To learn more about the role of GR3 in salt-stress tolerance, we investigated the response to 100 mM NaCl treatment in wild-type rice (WT); GR3 knockout mutant of rice (gr3); and the functional gr3-complementation line (C1). Rice GR3 was primarily expressed in roots at the seedling stage and ubiquitously expressed in all tissues except the sheath at heading stage. GR3 promoter-GUS was expressed in the vascular cylinder and cortex of root tissues in rice seedlings, vascular tissue of nodes, embryo and aleurone layer of seeds, and young flowers. Under both normal and salt-stress conditions, total GR activity was decreased by 20 % in gr3. Oxidative stress, indicated by malondialdehyde content, was greater in gr3 than the WT under salt stress. As compared with the WT, gr3 was sensitive to salt and methyl viologen; it showed inhibited growth, decreased maximal efficiency of photosystem II, decreased GSH and GSSG contents, and the ratio of GSH to GSSG. Conversely, the gr3-complementation line C1 rescued the tolerance to methyl viologen and salinity and recovered the growth and physiological damage caused by salinity. These results reveal that GR3 plays an important role in salt stress tolerance by regulating the GSH redox state in rice.

Fionn:

Plant cell

Epigenetic Basis of Morphological Variation and Phenotypic Plasticity in Arabidopsis thaliana

Rik Kooke, Frank Johannes, René Wardenaar, Frank Becker, Mathilde Etcheverry, Vincent Colot, Dick Vreugdenhil, and Joost J.B. Keurentjes

Epigenetics is receiving growing attention in the plant science community. Epigenetic modifications are thought to play a particularly important role in fluctuating environments. It is hypothesized that epigenetics contributes to plant phenotypic plasticity because epigenetic modifications, in contrast to DNA sequence variation, are more likely to be reversible. The population of decrease in DNA methylation 1-2 (ddm1-2)-derived epigenetic recombinant inbred lines (epiRILs) in Arabidopsis thaliana is well suited for studying this hypothesis, as DNA methylation differences are maximized and DNA sequence variation is minimized. Here, we report on the extensive heritable epigenetic variation in plant growth and morphology in neutral and saline conditions detected among the epiRILs. Plant performance, in terms of branching and leaf area, was both reduced and enhanced by different quantitative trait loci (QTLs) in the ddm1-2 inherited epigenotypes. The variation in plasticity associated significantly with certain genomic regions in which the ddm1-2 inherited epigenotypes caused an increased sensitivity to environmental changes, probably due to impaired genetic regulation in the epiRILs. Many of the QTLs for morphology and plasticity overlapped, suggesting major pleiotropic effects. These findings indicate that epigenetics contributes substantially to variation in plant growth, morphology, and plasticity, especially under stress conditions.