Lit Lunch – December 6th

DAMIAN

1) Irene Merino, Angela Contreras, Zhong-Ping Jing, Fernando Gallardo, Francisco M. Canovas, and Luis Gomez.

Plantation forestry under global warming: hybrid poplars with improved thermotolerance provide new insights on the in vivo function of small-HSP chaperones.

Plant Physiol. pp.113.225730; First Published on December 4, 2013; doi:10.1104/pp.113.225730

Whether you believe in global warming or not, the role of sHSPs in thermotolerance is pretty well-established. Here, a gene encoding a class-I sHSP from chestnut was placed under the 35S promoter and transformed into poplar. 6 year-old T1 plants express the protein at levels so high that it can be detected on Coomassie-stained gels of total plant protein. Detached leaves of 6 year-old sapplings and 3-month seedlings were subjected to heat stress at 44 C. In both age groups, sHSP over-expressors looked fine, while vector control plants exhibited necrosis. The induction of HSP70 and other native sHSPs (there are quite a few in poplar) were virtually the same between the chestnut over-expressor poplars and control poplars. Glutamine synthetase solubility and the enzymatic activity of glutathione reductase were both higher in sHSP lines following heat stress. Transgenic plants also put on biomass more rapidly. Even at peak summer, naturally-growing poplars in Spain were producing the sHSP most comparable to the one from chestnut at significantly lower levels (3.8-fold less protein) than the transgenics were in the absence of heat stress.

2) David Balchin, Stoyan H. Stoychev, and Heini W. Dirr.

***S*-Nitrosation Destabilizes Glutathione Transferase P1-1.**

***Biochemistry*, Article ASAP. DOI: 10.1021/bi401414c**

The biophysical consequences of S-nitrosation are only known for a few proteins. For example, GSNO-mediated nitrosation of NPR1 facilitates its oligomerization, while SA-induced thioredoxin activation attenuates GSNO effects, leading to accumulation of monomeric NPR1 in the nucleus.

In this report, the authors decided to look at a GST (human GSTP-1, which detoxifies xenobiotics via conjugation of GSH). They chose this enzyme because it was already known to be nitrosated and that nitrosation affected its enzymatic activity. What was not known was *how*, since the nitrosated cysteine was not a catalytic or substrate-binding residue.

Principle findings: 90% of GSTP-1 activity was lost following nitrosation of Cys47 and 101, which are on the surface of the protein; nitrosation of C101 alone had little effect on activity however, and this residue is not predicted to contribute strongly to the protein's tertiary structure. Tryptophan quenching was not significantly different for the nitrosated protein compared with the native, although total alpha helical content was slightly lower as assessed by circular dichroism spectroscopy. Nitrosation also lowered the cooperativity of unfolding: rather than there being discrete FOLDED and UNFOLDED populations with a sigmoidal transition, there was instead a linear response to increasing denaturant with a more shallow slope than for the native protein. This indicated nitrosation poises the protein to be slightly unfolded in the absence of denaturant, but that even at 8 M urea, the modified protein is less denatured than the native. Re-folding is also compromised—while the native protein refolds reversibly as denaturant is removed, the nitrosated protein exhibits hysteresis and does not completely refold (as assessed by tryptophan fluorescence). The rate constant for unfolding is only slightly higher for the nitrosated protein. However,

the conformational dynamics of many residues in both domains of the protein are a lot higher (determined by hydrogen-deuterium exchange).

SIGNIFICANCE: Nitrosation at C101 may not slow down the enzyme in vitro, but given its conformational effects, it could influence its behavior toward substrates in vivo.

3) Sainz, Martha Pérez-Rontomé, Carmen Ramos, Javier Mulet, Jose Miguel James, Euan K. Bhattacharjee, Ujjal Petrich, Jacob W. Becana, Manuel (2013).

Plant hemoglobins may be maintained in functional form by reduced flavins in the nuclei, and confer differential tolerance to nitro-oxidative stress. Plant Journal. 76: 875.

This investigation is analogous to similar ones conducted for rice and Arabidopsis nonsymbiotic hemoglobins, except it was performed in the legume *Lotus japonicus*. Once again, there are three types of GLBs, numbered according to their oxygen affinities (1>2>truncated), except there are 2 1's and 2 3's in L. japonicus (as opposed to one of each, as in Arabidopsis). These proteins are speculated to play a role in NO scavenging during hypoxia. Chemically, NO is reduced by the heme, so it's not really dependent on the GLB proteins themselves—just their prosthetic groups. NO is reduced to NO3-. What is not known is how hemes might themselves be re-reduced?

Here, it is shown that flavins are present at a high enough intracellular concentration to facilitate reduction of all three GLB types (with concomitant flavin reduction via NADPH). Riboflavin and FMN were both found to work, to varying degrees, depending on the GLB being tested. GLBs were also found to be primarily nuclear in localization, suggesting that the nitrosative stress averted by GLBs could be targeting transcription factors. Both GSNO (an RNS generator) and methyl viologen (a ROS generator) effects were attenuated in a GLB-deficient yeast when complemented with L. japonicus GLBs.

INDU

1. Science. 2013 Oct 25;342(6157):475-9. doi: 10.1126/science.1241934. Epub 2013 Sep 26.

Causes and effects of N-terminal codon bias in bacterial genes.

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Most amino acids are encoded by multiple codons, and codon choice has strong effects on protein expression. Rare codons are enriched at the N terminus of genes in most organisms, although the causes and effects of this bias are unclear. Here, we measure expression from >14,000 synthetic reporters in Escherichia coli and show that using N-terminal rare codons instead of common ones increases expression by ~14-fold (median 4-fold). We quantify how individual N-terminal codons affect expression and show that these effects shape the sequence of natural genes. Finally, we demonstrate that reduced RNA structure and not codon rarity itself is responsible for expression increases. Our observations resolve controversies over the roles of N-terminal codon bias and suggest a

straightforward method for optimizing heterologous gene expression in bacteria.

PMID: 24072823 [PubMed - indexed for MEDLINE]

2. Science. 2013 Nov 1;342(6158):624-8. doi: 10.1126/science.1243825. Epub 2013 Oct 10.

Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex.

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Flagellin perception in Arabidopsis is through recognition of its highly conserved N-terminal epitope (flg22) by flagellin-sensitive 2 (FLS2). Flg22 binding induces FLS2 heteromerization with BRASSINOSTEROID INSENSITIVE 1-associated kinase 1 (BAK1) and their reciprocal activation followed by plant immunity. Here, we report the crystal structure of FLS2 and BAK1 ectodomains complexed with flg22 at 3.06 angstroms. A conserved and a nonconserved site from the inner surface of the FLS2 solenoid recognize the C- and N-terminal segment of flg22, respectively, without oligomerization or conformational changes in the FLS2 ectodomain. Besides directly interacting with FLS2, BAK1 acts as a co-receptor by recognizing the C terminus of the FLS2-bound flg22. Our data reveal the molecular mechanisms underlying FLS2-BAK1 complex recognition of flg22 and provide insight into the immune receptor complex activation.

PMID: 24114786 [PubMed - indexed for MEDLINE]

3. Science. 2013 Nov 8;342(6159):741-3. doi: 10.1126/science.1239764.

High-speed force spectroscopy unfolds titin at the velocity of molecular dynamics simulations.

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The mechanical unfolding of the muscle protein titin by atomic force microscopy was a landmark in our understanding of single-biomolecule mechanics. Molecular dynamics simulations offered atomic-level descriptions of the forced unfolding.

However, experiment and simulation could not be directly compared because they differed in pulling velocity by orders of magnitude. We have developed high-speed force spectroscopy to unfold titin at velocities reached by simulation (~4 millimeters per second). We found that a small β-strand pair of an immunoglobulin domain dynamically unfolds and refolds, buffering pulling forces up to ~100 piconewtons. The distance to the unfolding transition barrier is larger than previously estimated but is in better agreement with atomistic predictions. The ability to directly compare experiment and simulation is likely to be important in studies of biomechanical processes.

PMID: 24202172 [PubMed - in process]

KEITH

1) A Model for Small Heat Shock Protein Inhibition of Polyglutamine Aggregation

Cell Biochem Biophys. 2013 Nov 16. [Epub ahead of print]

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Polyglutamine (polyQ) repeat expansions that lead to the formation of amyloid aggregates are linked to several devastating neurodegenerative disorders. While molecular chaperones, including the small heat shock proteins (sHsp), play an important role in protection against protein misfolding, the aberrant protein folding that accompanies these polyQ diseases overwhelms the chaperone network. By generating a model structure to explain the observed suppression of spinocerebellar ataxia 3 (SCA3) by the sHsp αB-crystallin, we have identified key vulnerabilities that provide a possible mechanism to explain this heat shock response. A docking study involving a small bioactive peptide should also aid in the development of new drug targets for the prevention of polyQ-based aggregation.

2) Roles of the N domain of the AAA+ Lon protease in substrate recognition, allosteric regulation and chaperone activity

Mol Microbiol. 2013 Oct 29

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Degron binding regulates the activities of the AAA+ Lon protease in addition to targeting proteins for degradation. The sul20 degron from the cell-division inhibitor SulA is shown here to bind to the N domain of Escherichia coli Lon, and the recognition site is identified

by cross-linking and scanning for mutations that prevent sul20-peptide binding. These N-domain mutations limit the rates of proteolysis of model sul20-tagged substrates and ATP hydrolysis by an allosteric mechanism. Lon inactivation of SulA in vivo requires binding to the N domain and robust ATP hydrolysis but does not require degradation or translocation into the proteolytic chamber. Lon-mediated relief of proteotoxic stress and protein aggregation in vivo can also occur without degradation but is not dependent on robust ATP hydrolysis. In combination, these results demonstrate that Lon can function as a protease or a chaperone and reveal that some of its ATP-dependent biological activities do not require translocation.

3) Functional analysis of hsp70 inhibitors

PLoS One. 2013 Nov 12;8(11):e78443.

Schlecht R, Scholz SR, Dahmen H, Wegener A, Sirrenberg C, Musil D, Bomke J, Eggenweiler HM, Mayer MP, Bukau B.

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The molecular chaperones of the Hsp70 family have been recognized as targets for anti-cancer therapy. Since several paralogs of Hsp70 proteins exist in cytosol, endoplasmic reticulum and mitochondria, we investigated which isoform needs to be down-regulated for reducing viability of cancer cells. For two recently identified small molecule inhibitors, VER-155008 and 2-phenylethynesulfonamide (PES), which are proposed to target different sites in Hsp70s, we analyzed the molecular mode of action in vitro. We found that for significant reduction of viability of cancer cells simultaneous knockdown of heat-inducible Hsp70 (HSPA1) and constitutive Hsc70 (HSPA8) is necessary. The compound VER-155008, which binds to the nucleotide binding site of Hsp70, arrests the nucleotide binding domain (NBD) in a half-open conformation and thereby acts as ATP-competitive inhibitor that prevents allosteric control between NBD and substrate binding domain (SBD). Compound PES interacts with the SBD of Hsp70 in an unspecific, detergent-like fashion, under the conditions tested. None of the two inhibitors investigated was isoform-specific.

STEPHANIE

1) High-throughput DNA sequencing errors are reduced by orders of magnitude using circle sequencing

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2. Raul Andino[c],[2], William H. Press[b],[d],[2], and Sara L. Sawyer[a],[2]

1. 3Contributed by William H. Press, October 17, 2013 (sent for review August 31, 2013)

Abstract

A major limitation of high-throughput DNA sequencing is the high rate of erroneous base calls produced. For instance, Illumina sequencing machines produce errors at a rate of $\sim 0.1–1 \times 10^{-2}$ per base sequenced. These technologies typically produce billions of base calls per experiment, translating to millions of errors. We have developed a unique library preparation strategy, “circle sequencing,” which allows for robust downstream computational correction of these errors. In this strategy, DNA templates are circularized, copied multiple times in tandem with a rolling circle polymerase, and then sequenced on any high-throughput sequencing machine. Each read produced is computationally processed to obtain a consensus sequence of all linked copies of the original molecule. Physically linking the copies ensures that each copy is independently derived from the original molecule and allows for efficient formation of consensus sequences. The circle-sequencing protocol precedes standard library preparations and is therefore suitable for a broad range of sequencing applications. We tested our method using the Illumina MiSeq platform and obtained errors in our processed sequencing reads at a rate as low as 7.6×10^{-6} per base sequenced, dramatically improving the error rate of Illumina sequencing and putting error on par with low-throughput, but highly accurate, Sanger sequencing. Circle sequencing also had substantially higher efficiency and lower cost than existing barcode-based schemes for correcting sequencing errors.