Lit Lunch 2/6/14

KEITH:

1) Conformational Stability of Mammalian Prion Protein Amyloid Fibrils Is Dictated by a Packing Polymorphism within the Core Region

Nathan J. Cobb[‡12], Marcin I. Apostol[‡1], Shugui Chen[‡13], Vytautas Smirnovas[‡4] and Witold K. Surewicz[‡,§,¶5]

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January 31, 2014 The Journal of Biological Chemistry, 289, 2643-2650

Mammalian prion strains are believed to arise from the propagation of distinct conformations of the misfolded prion protein PrP^{Sc}. One key operational parameter used to define differences between strains has been conformational stability of PrP^{Sc} as defined by resistance to thermal and/or chemical denaturation. However, the structural basis of these stability differences is unknown. To bridge this gap, we have generated two strains of recombinant human prion protein amyloid fibrils that show dramatic differences in conformational stability and have characterized them by a number of biophysical methods. Backbone amide hydrogen/deuterium exchange experiments revealed that, in sharp contrast to previously studied strains of infectious amyloid formed from the yeast prion protein Sup35, differences in β-sheet core size do not underlie differences in conformational stability between strains of mammalian prion protein amyloid. Instead, these stability differences appear to be dictated by distinct packing arrangements (i.e. steric zipper interfaces) within the amyloid core, as indicated by distinct x-ray fiber diffraction patterns and large strain-dependent differences in hydrogen/deuterium exchange kinetics for histidine side chains within the core region. Although this study was limited to synthetic prion protein amyloid fibrils, a similar structural basis for strain-dependent conformational stability may apply to brain-derived PrP^{Sc}, especially because large strain-specific differences in PrP^{Sc} stability are often observed despite a similar size of the PrP^{Sc} core region.

2) The Molecular Chaperone Hsp70 Activates Protein Phosphatase 5 (PP5) by Binding the Tetratricopeptide Repeat (TPR) Domain

Jamie N. Connarn[‡], Victoria A. Assimon[§,¶], Rebecca A. Reed[§], Eric Tse[¶,‖], Daniel R. Southworth[§,¶,‖], Erik R. P. Zuiderweg[‖], Jason E. Gestwicki[§,¶,‖,**] and Duxin Sun[‡,§1]

Departments of [‡]Pharmaceutical Sciences,[‖]Biological Chemistry,[**]Pathology,the [§]Program in Chemical Biology, and the [¶]Life Sciences Institute, University of Michigan, Ann Arbor, Michigan

Protein phosphatase 5 (PP5) is auto-inhibited by intramolecular interactions with its tetratricopeptide repeat (TPR) domain. Hsp90 has been shown to bind PP5 to activate its phosphatase activity. However, the functional implications of binding Hsp70 to PP5 are not yet clear. In this study, we find that both Hsp90 and Hsp70 bind to PP5 using a luciferase fragment complementation assay. A fluorescence polarization assay shows that Hsp90 (MEEVD motif) binds to the TPR domain of PP5 almost 3-fold higher affinity than Hsp70 (IEEVD motif). However, Hsp70 binding to PP5 stimulates higher phosphatase activity of PP5 than the binding of Hsp90. We find that PP5 forms a stable 1:1 complex with Hsp70, but the interaction appears asymmetric with Hsp90, with one PP5 binding the dimer. Solution NMR studies reveal that Hsc70 and PP5 proteins are dynamically independent in complex, tethered by a disordered region that connects the Hsc70 core and the IEEVD-TPR contact area. This tethered binding is expected to allow PP5 to carry out multi-site dephosphorylation of Hsp70-bound clients with a range of sizes and shapes. Together, these results demonstrate that Hsp70 recruits PP5 and activates its phosphatase activity which suggests dual roles for PP5 that might link chaperone systems with signaling pathways in cancer and development.

STEPHANIE:

1) eIF5A has a function in the cotranslational translocation of proteins into the ER.

Rossi D, Galvão FC, Bellato HM, Boldrin PE, Andrews BJ, Valentini SR, Zanelli CF.
Author information **(MOLECULAR CELL)**

Abstract

The putative eukaryotic translation initiation factor 5A (eIF5A) is a highly conserved and essential protein present in all organisms except bacteria. To be activated, eIF5A requires the conversion of a specific residue of lysine into hypusine. This hypusine modification occurs posttranslationally in two enzymatic steps, and the polyamine spermidine is the substrate. Despite having an essential function in translation elongation, the critical role played by eIF5A remains unclear. In addition to demonstrating genetic interactions with translation factors, eIF5A mutants genetically interact with mutations in YPT1, which encodes an essential protein involved in endoplasmic reticulum (ER)-to-Golgi vesicle transport. In this study, we investigated the correlation between the function of eIF5A in translation and secretion in yeast. The results of in vivo translocation

assays and genetic interaction analyses suggest a specific role for eIF5A in the cotranslational translocation of proteins into the ER, but not in the posttranslational pathway. Additionally, we observed that a block in eIF5A activation up-regulates stress-induced chaperones, which also occurs when SRP function is lost. Finally, loss of eIF5A function affects binding of the ribosome-nascent chain complex to SRP. These results link eIF5A function in translation with a role of SRP in the cell and may help explain the dual effects of eIF5A in differential and general translation.

YICHEN:

Acta Biochim Biophys Sin (Shanghai). 2014 Jan 20. [Epub ahead of print]

1) Chaperone function and mechanism of small heat-shock proteins.

Fu X.

Author information

Abstract

Small heat-shock proteins (sHSPs) are ubiquitous ATP-independent molecular chaperones that play crucial roles in protein quality control in cells. They are able to prevent the aggregation and/or inactivation of various non-native substrate proteins and assist the refolding of these substrates independently or under the help of other ATP-dependent chaperones. Substrate recognition and binding by sHSPs are essential for their chaperone functions. This review focuses on what natural substrate proteins a sHSP protects and how it binds the substrates in cells under fluctuating conditions. It appears that sHSPs of prokaryotes, although being able to bind a wide range of cellular proteins, preferentially protect certain classes of functional proteins, such as translation-related proteins and metabolic enzymes, which may well explain why they could increase the resistance of host cells against various stresses. Mechanistically, the sHSPs of prokaryotes appear to possess numerous multi-type substrate-binding residues and are able to hierarchically activate these residues in a temperature-dependent manner, and thus act as temperature-regulated chaperones. The mechanism of hierarchical activation of substrate-binding residues is also discussed regarding its implication for eukaryotic sHSPs.

Mol Vis. 2014 Jan 14;20:117-24. eCollection 2014.

2) Identification of proteins that interact with alpha A-crystallin using a human proteome microarray.

Fan Q1, Huang LZ2, Zhu XJ1, Zhang KK1, Ye HF1, Luo Y1, Sun XH1, Zhou P3, Lu Y1.

Author information

Abstract

PURPOSE:

To identify proteins interacting with alpha A-crystallin (CRYAA) and to investigate the potential role that these protein interactions play in the function of CRYAA using a human proteome (HuProt) microarray.

METHODS:
The active full-length CRYAA protein corresponding to amino acids 1-173 of CRYAA was recombined. A HuProt microarray composed of 17,225 human full-length proteins with N-terminal glutathione S-transferase (GST) tags was used to identify protein-protein interactions. The probes were considered detectable when the signal to noise ratio (SNR) was over 1.2. The identified proteins were subjected to subsequent bioinformatics analysis using the DAVID database.

RESULTS:
The HuProt microarray results showed that the signals of 343 proteins were higher in the recombinant CRYAA group than in the control group. The SNR of 127 proteins was ≥ 1.2. The SNR of the following eight proteins was > 3.0: hematopoietic cell-specific Lyn substrate 1 (HCLS1), Kelch domain-containing 6 (KLHDC6), sarcoglycan delta (SGCD), KIAA1706 protein (KIAA1706), RNA guanylyltransferase and 5'-phosphatase (RNGTT), chromosome 10 open reading frame 57 (C10orf57), chromosome 9 open reading frame 52 (C9orf52), and plasminogen activator, urokinase receptor (PLAUR). The bioinformatics analysis revealed 127 proteins associated with phosphoproteins, alternative splicing, acetylation, DNA binding, the nuclear lumen, ribonucleotide binding, the cell cycle, WD40 repeats, protein transport, transcription factor activity, GTP binding, and cellular response to stress. Functional annotation clustering showed that they belong to cell cycle, organelle or nuclear lumen, protein transport, and DNA binding and repair clusters. CRYAA interacted with these proteins to maintain their solubility and decrease the accumulation of denatured target proteins. The protein-protein interactions may help CRYAA carry out multifaceted functions.

CONCLUSIONS:
One-hundred and twenty-seven of 17,225 human full-length proteins were identified that interact with CRYAA. The advent of microarray analysis enables a better understanding of the functions of CRYAA as a molecular chaperone.

DAMIAN:

1) arianne Bjordal, Nathalie Arquier, Julie Kniazeff, Jean Philippe Pin, Pierre Léopold,

Sensing of Amino Acids in a Dopaminergic Circuitry Promotes Rejection of an Incomplete Diet in Drosophila, Cell, Volume 156, Issue 3, 30 January 2014, Pages 510-521, ISSN 0092-8674,http://dx.doi.org/10.1016/j.cell.2013.12.024.
(http://www.sciencedirect.com/science/article/pii/S0092867413015936)

Animals reduce food intake when food is deficient in nutrients. If essential amino acids are missing, fruit flies and rats alike will consume less food.
Here it is shown that a specific type of brain neuron—dopaminergic neurons—control this response in Drosophila.
Knockdown of an amino acid transporter drastically reduced feeding if knockdown was limited to DA neurons, but had little effect on feeding when knocked down in other neuron types.

GCN2 is a kinase activated upon accumulation of uncharged tRNAs (a signal of amino acid shortage). Activation of GCN2 constitutively did not affect feeding unless it was specifically expressed in DA neurons, in which case feeding was drastically reduced, as was “roaming” (i.e., larvae move away from nutrient source). In contrast, GCN2 knockdown caused normal feeding behavior even in amino acid deficient media. Brains were removed from animals and analyzed in media transiently supplemented with particular nutrient mixes. Calcium release occurred in DA neurons supplemented with media deficient in essential amino acids, and this release was inhibited upon addition of the missing amino acid.

2) Caroline Heintz, William Mair,

You Are What You Host: Microbiome Modulation of the Aging Process, Cell, Volume 156, Issue 3, 30 January 2014, Pages 408-411, ISSN 0092-8674, http://dx.doi.org/10.1016/j.cell.2014.01.025. (http://www.sciencedirect.com/science/article/pii/S0092867414000774)

C. elegans longevity and B. subtilis-procured NO. Rodent longevity and decreased bacterial folate metabolism. TOR mutants and the effects of gut microbiome on longevity

FIONN:

1) Cell

Potentiated hsp104 variants antagonize diverse proteotoxic misfolding events.

Jackrel ME1, Desantis ME2, Martinez BA3, Castellano LM4, Stewart RM1, Caldwell KA3, Caldwell GA3, Shorter J5.
Author information

Abstract
There are no therapies that reverse the proteotoxic misfolding events that underpin fatal neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). Hsp104, a conserved hexameric AAA+ protein from yeast, solubilizes disordered aggregates and amyloid but has no metazoan homolog and only limited activity against human neurodegenerative disease proteins. Here, we reprogram Hsp104 to rescue TDP-43, FUS, and ?-synuclein proteotoxicity by mutating single residues in helix 1, 2, or 3 of the middle domain or the small domain of nucleotide-binding domain 1. Potentiated Hsp104 variants enhance aggregate dissolution, restore proper protein localization, suppress proteotoxicity, and in a C. elegans PD model attenuate dopaminergic neurodegeneration. Potentiating mutations reconfigure how Hsp104 subunits collaborate, desensitize Hsp104 to inhibition, obviate any requirement for Hsp70, and enhance ATPase, translocation, and unfoldase activity. Our work establishes that disease-associated aggregates and amyloid are tractable targets and that enhanced disaggregases can restore proteostasis and mitigate neurodegeneration.

2) Chemistry and biology

Distinct Prion Strains Are Defined by Amyloid Core Structure and Chaperone Binding Site Dynamics

Authors

Kendra K. Frederick, Galia T. Debelouchina, Can Kayatekin, Tea Dorminy, Angela C. Jacavone, Robert G. Griffin, Susan Lindquistsend emailSee Affiliations

Highlights

Amyloid cores of strong and weak prion fibers are structurally distinct

Chaperone binding site is more dynamic in weak prion fibers than strong prion fibers

Interaction of weak and strong prion fibers with Hsp104 are different in vivo

Both the amyloid core and the dynamics of chaperone binding define prion strains

Summary

Yeast prions are self-templating protein-based mechanisms of inheritance whose conformational changes lead to the acquisition of diverse new phenotypes. The best studied of these is the prion domain (NM) of Sup35, which forms an amyloid that can adopt several distinct conformations (strains) that produce distinct phenotypes. Using magic-angle spinning nuclear magnetic resonance spectroscopy, we provide a detailed look at the dynamic properties of these forms over a broad range of timescales. We establish that different prion strains have distinct amyloid structures, with many side chains in different chemical environments. Surprisingly, the prion strain with a larger fraction of rigid residues also has a larger fraction of highly mobile residues. Differences in mobility correlate with differences in interaction with the prion-partitioning factor Hsp104 in vivo, perhaps explaining strain-specific differences in inheritance.

3) PLoS One.

Regulation of the hsp104 middle domain activity is critical for yeast prion propagation.
Dulle JE, Stein KC, True HL.

Abstract

Molecular chaperones play a significant role in preventing protein misfolding and aggregation. Indeed, some protein conformational disorders have been linked to changes in the chaperone network. Curiously, in yeast, chaperones also play a role in promoting prion maintenance and propagation. While many amyloidogenic proteins are associated with disease in mammals, yeast prion proteins, and their ability to undergo conformational conversion into a prion state, are proposed to play a functional role in yeast biology. The chaperone Hsp104, a AAA+ ATPase, is essential for yeast prion propagation. Hsp104 fragments large prion aggregates to generate a population of smaller oligomers that can more readily convert soluble monomer and be transmitted to daughter cells. Here, we show that the middle (M) domain of Hsp104, and its mobility, plays an integral part in prion propagation. We generated and characterized mutations in the M-domain of Hsp104 that are predicted to stabilize either a repressed or de-repressed conformation of the M-domain (by analogy to ClpB in bacteria). We show that the predicted stabilization of the repressed conformation inhibits general chaperone activity. Mutation to the de-repressed conformation, however, has differential effects on ATP hydrolysis and disaggregation, suggesting that the M-domain is involved in coupling these two activities. Interestingly, we show that changes in the M-domain differentially affect the propagation of different variants of the [PSI+] and [RNQ+] prions, which indicates that some prion variants are more sensitive to changes in the M-domain mobility than others. Thus, we provide evidence that regulation of the M-domain of Hsp104 is critical for efficient prion propagation. This shows the importance of elucidating the function of the M-domain in order to understand the role of Hsp104 in the propagation of different prions and prion variants.

INDU:

1. Science. 2013 Dec 20;342(6165):1513-6. doi: 10.1126/science.1244273.

   Revealing nature's cellulase diversity: the digestion mechanism of Caldicellulosiruptor bescii CelA.

   Brunecky R, Alahuhta M, Xu Q, Donohoe BS, Crowley MF, Kataeva IA, Yang SJ, Resch MG, Adams MW, Lunin VV, Himmel ME, Bomble YJ.

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Comment in
Science. 2013 Dec 20;342(6165):1454-6.

Most fungi and bacteria degrade plant cell walls by secreting free, complementary enzymes that hydrolyze cellulose; however, some bacteria use large enzymatic assemblies called cellulosomes, which recruit complementary enzymes to protein scaffolds. The thermophilic bacterium Caldicellulosiruptor bescii uses an intermediate strategy, secreting many free cellulases that contain multiple catalytic domains. One of these, CelA, comprises a glycoside hydrolase family 9 and a family 48 catalytic domain, as well as three type III cellulose-binding modules. In the saccharification of a common cellulose standard, Avicel, CelA outperforms mixtures of commercially relevant exo- and endoglucanases. From transmission electron microscopy studies of cellulose after incubation with CelA, we report morphological features that suggest that CelA not only exploits the common surface ablation mechanism driven by general cellulase processivity, but also excavates extensive cavities into the surface of the substrate. These results suggest that nature's repertoire of cellulose digestion paradigms remain only partially discovered and understood.

PMID: 24357319 [PubMed - indexed for MEDLINE]

2. Science. 2013 Dec 20;342(6165):1241089. doi: 10.1126/science.1241089.

The Amborella genome and the evolution of flowering plants.

Amborella Genome Project.

Collaborators: Albert VA, Barbazuk WB, dePamphilis CW, Der JP, Leebens-Mack J, Ma H, Palmer JD, Rounsley S, Sankoff D, Schuster SC, Soltis DE, Soltis PS, Wessler SR, Wing RA, Albert VA, Ammiraju JS, Barbazuk WB, Chamala S, Chanderbali AS, dePamphilis CW, Der JP, Determann R, Leebens-Mack J, Ma H, Ralph P, Rounsley S, Schuster SC, Soltis DE, Soltis PS, Talag J, Tomsho L, Walts B, Wanke S, Wing RA, Albert VA, Barbazuk WB, Chamala S, Chanderbali AS, Chang TH, Determann R, Lan T, Soltis DE, Soltis PS, Arikit S, Axtell MJ, Ayyampalayam S, Barbazuk WB, Burnette JM 3rd, Chamala S, De Paoli E, dePamphilis CW, Der JP, Estill JC, Farrell NP, Harkess A, Jiao Y, Leebens-Mack J, Liu K, Mei W, Meyers BC, Shahid S, Wafula E, Walts B, Wessler SR, Zhai J, Zhang X, Albert VA, Carretero-Paulet L, dePamphilis CW, Der JP, Jiao Y, Leebens-Mack J, Lyons E, Sankoff D, Tang H, Wafula E, Zheng C, Albert VA, Altman NS, Barbazuk WB, Carretero-Paulet L, dePamphilis CW, Der JP, Estill JC, Jiao Y, Leebens-Mack J, Liu K, Mei W, Wafula E, Altman NS, Arikit S, Axtell MJ, Chamala S, Chanderbali AS, Chen F, Chen JQ, Chiang V, De Paoli E, dePamphilis CW, Der JP, Determann R, Fogliani B, Guo C, Harholt J, Harkess A, Job C, Job D, Kim S, Kong H, Leebens-Mack J, Li G, Li L, Liu J, Ma H, Meyers BC, Park J, Qi X, Rajjou L, Burtet-Sarramegna V, Sederoff R, Shahid S, Soltis DE, Soltis PS, Sun YH, Ulvskov P, Villegente M, Xue JY, Yeh TF, Yu X, Zhai J, Acosta JJ, Albert VA, Barbazuk WB, Bruenn RA, Chamala S, de Kochko A, dePamphilis CW, Der JP, Herrera-Estrella LR, Ibarra-Laclette E, Kirst M, Leebens-Mack J, Pissis SP, Poncet V, Schuster SC, Soltis DE, Soltis PS, Tomsho L.

Comment in
Science. 2013 Dec 20;342(6165):1456-7.

Amborella trichopoda is strongly supported as the single living species of the sister lineage to all other extant flowering plants, providing a unique reference for inferring the genome content and structure of the most recent common ancestor (MRCA) of living angiosperms. Sequencing the Amborella genome, we identified an

ancient genome duplication predating angiosperm diversification, without evidence of subsequent, lineage-specific genome duplications. Comparisons between Amborella and other angiosperms facilitated reconstruction of the ancestral angiosperm gene content and gene order in the MRCA of core eudicots. We identify new gene families, gene duplications, and floral protein-protein interactions that first appeared in the ancestral angiosperm. Transposable elements in Amborella are ancient and highly divergent, with no recent transposon radiations. Population genomic analysis across Amborella's native range in New Caledonia reveals a recent genetic bottleneck and geographic structure with conservation implications.

PMID: 24357323 [PubMed - indexed for MEDLINE]

Umaru:

1) Alexander Bepperling and Jeannette Winter Stefanie Mak, Andrea Steiner, Maike Krause, Adrian Drazic, Katharina M. Gebendorfer,

Factor HypT Species of the HOCl-specific Transcription Tetramers Are the Activation-competent

Protein Structure and Folding:

doi: 10.1074/jbc.M113.521401 originally published online November 25, 2013