Keith

Denaturation induced aggregation in α-crystallin: differential action of chaotropes.

J Mol Recognit. 2016 May 26

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α-Crystallin is a member of small heat shock proteins and is believed to play an exceptional role in the stability of eye lens proteins. The disruption or denaturation of the protein arrangement or solubility of the crystallin proteins can lead to vision problems including cataract. In the present study, we have examined the effect of chemical denaturants urea and guanidine hydrochloride (GdnHCl) on α-crystallin aggregation, with special emphasis on protein conformational changes, unfolding, and amyloid fibril formation. GdnHCl (4 M) induced a 16 nm red shift in the intrinsic fluorescence of α-crystallin, compared with 4 nm shift by 8 M urea suggesting a major change in α-crystallin structure. Circular dichroism analysis showed marked increase in the ellipticity of α-crystallin at 216 nm, suggesting gain in β-sheet structure in the presence of GdnHCl (0.5-1 M) followed by unfolding at higher concentration (2-6 M). However, only minor changes in the secondary structure of α-crystallin were observed in the presence of urea. Moreover, 8-anilinonaphthalene-1-sulfonic acid fluorescence measurement in the presence of GdnHCl and urea showed changes in the hydrophobicity of α-crystallin. Amyloid studies using thioflavin T fluorescence and congo red absorbance showed that GdnHCl induced amyloid formation in α-crystallin, whereas urea induced aggregation in this protein. Electron microscopy studies further confirmed amyloid formation of α-crystallin in the presence of GdnHCl, whereas only aggregate-like structures were observed in α-crystallin treated with urea. Our results suggest that α-crystallin is susceptible to unfolding in the presence of chaotropic agents like urea and GdnHCl. The destabilized protein has increased likelihood to fibrillate.

Molecular cloning and characterization of the MsHSP17.7 gene from Medicago sativa L.

Mol Biol Rep. 2016 May 19

Li ZY1, Long RC1, Zhang TJ1, Yang QC1, Kang JM2.

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Heat shock proteins (HSPs) are ubiquitous protective proteins that play crucial roles in plant development and adaptation to stress, and the aim of this study is to characterize the HSP gene in alfalfa. Here we isolated a small heat shock protein gene (MsHSP17.7) from alfalfa by homology-based cloning. MsHSP17.7 contains a 477-bp open reading frame and encodes a protein of 17.70-kDa. The amino acid sequence shares high identity with MtHSP (93.98 %), PsHSP17.1 (83.13 %), GmHSP17.9 (74.10 %) and SlHSP17.6 (79.25 %). Phylogenetic analysis revealed that MsHSP17.7 belongs to the group of cytosolic class II small heat shock proteins (sHSP), and likely localizes to the cytoplasm. Quantitative RT-PCR indicated that MsHSP17.7 was induced by heat shock, high salinity, peroxide and drought stress. Prokaryotic expression indicated that the salt and peroxide tolerance of Escherichia coli was remarkably enhanced. Transgenic Arabidopsis plants overexpressing MsHSP17.7 exhibited increased root length of transgenic Arabidopsis lines under salt stress compared to the wild-type line. The malondialdehyde (MDA) levels in the transgenic lines were significantly lower than in wild-type, although proline levels were similar between transgenic and wild-type lines. MsHSP17.7 was induced by heat shock, high salinity, oxidative stress and drought stress. Overexpression analysis suggests that MsHSP17.7 might play a key role in response to high salinity stress.

DnaK-dependent accelerated evolutionary rate in prokaryotes.

Genome Biol Evol. 2016 Apr 29

Kadibelban AS1, Bogumil D2, Landan G3, Dagan T3.

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Many proteins depend on an interaction with molecular chaperones in order to fold into a functional tertiary structure. Previous studies showed that protein interaction with the GroEL/GroES chaperonine and Hsp90 chaperone can buffer the impact of slightly deleterious mutations in the protein sequence. This capacity of GroEL/GroES to prevent protein misfolding has been shown to accelerate the evolution of its client proteins. Whether other bacterial chaperones have a similar effect on their client proteins is currently unknown. Here we study the impact of DnaK (Hsp70) chaperone on the evolution of its client proteins. Evolutionary parameters were derived from comparison of the Escherichia coli proteome to 1,808,565

orthologous proteins in 1,149 proteobacterial genomes. Our analysis reveals a significant positive correlation between protein binding frequency with DnaK and evolutionary rate. Proteins with high binding affinity to DnaK evolve on average 4.3 fold faster than proteins in the lowest binding affinity class at the genus resolution. Differences in evolutionary rates of DnaK interactor classes are still significant after adjusting for possible effects caused by protein expression level. Furthermore, we observe an additive effect of DnaK and GroEL chaperones on the evolutionary rates of their common interactors. Finally, we found pronounced similarities in the physicochemical profiles that characterize proteins belonging to DnaK and GroEL interactomes. Our results thus implicate DnaK-mediated folding as a major component in shaping protein evolutionary dynamics in bacteria and supply further evidence for the long-term manifestation of chaperone-mediated folding on genome evolution.

Crowding Modulates the Conformation, Affinity, and Activity of the Components of the Bacterial Disaggregase Machinery.

J Mol Biol. 2016 Jun 5

Celaya G1, Fernández-Higuero JA1, Martin I1, Rivas G2, Moro F1, Muga A3.

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Chaperone-mediated protein aggregate reactivation is a complex reaction that depends on the sequential association of molecular chaperones on their interaction with protein aggregates and on substrate refolding. This process could be modulated by the highly crowded intracellular environment, which is known to affect protein conformational change, enzymatic activity, and protein-protein interactions. Here, we report that molecular crowding shapes the chaperone activity of bacterial disaggregase composed of the DnaK system (DnaK, DnaJ, and GrpE) and the molecular motor ClpB. A combination of biophysical and biochemical methods shows that the excluded volume conditions modify the conformation of DnaK and DnaJ without affecting that of GrpE. These crowding-induced conformational rearrangements activate DnaK, enhance the affinity of DnaK for DnaJ, but not for GrpE, and increase the sensitivity of the chaperone activity to cochaperone concentration, explaining the tight control of their relative intracellular amounts. Furthermore, crowding-mediated disordering of the G/F domain of DnaJ facilitates the reversible formation of intermolecular DnaJ conglomerates. These assemblies could drive the formation of Hsp70 clusters at the aggregate surface with the consequent enhancement of the disaggregation efficiency through their coordinated action via entropic pulling. Finally, crowding helps ClpB to outcompete GrpE for DnaK binding, a key aspect of DnaK/ClpB

cooperation given the low affinity of the disaggregase for DnaK. Excluded volume conditions promote the formation of the bichaperone complex that disentangles aggregates, enhancing the efficiency of the disaggregation reaction.

Do nucleic acids moonlight as molecular chaperones?

Nucleic Acids Res. 2016 Apr 21

Docter BE1, Horowitz S2, Gray MJ3, Jakob U3, Bardwell JC4.

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Organisms use molecular chaperones to combat the unfolding and aggregation of proteins. While protein chaperones have been widely studied, here we demonstrate that DNA and RNA exhibit potent chaperone activity in vitro Nucleic acids suppress the aggregation of classic chaperone substrates up to 300-fold more effectively than the protein chaperone GroEL. Additionally, RNA cooperates with the DnaK chaperone system to refold purified luciferase. Our findings reveal a possible new role for nucleic acids within the cell: that nucleic acids directly participate in maintaining proteostasis by preventing protein aggregation.

Damian

Guan, M., de Bang, T., Pedersen, C., and Schjoerring, J. K. (2016) Cytosolic glutamine synthetase Gln1;2 is the main isozyme contributing to GS1 activity in Arabidopsis shoots and can be up-regulated to relieve ammonium toxicity, *Plant Physiol.*

Cytosolic glutamine synthetase (GS1) is central for ammonium assimilation in plants. High ammonium treatment enhanced the expression of the GS1 isogene Gln1;2 encoding a low-affinity high-capacity GS1 protein in Arabidopsis shoots. Under the same conditions, the expression of the high-affinity low-capacity isoform Gln1;1 was reduced. The expression of Gln1;3 did not respond to ammonium treatment while Gln1;4 and Gln1;5 in all cases were expressed at a very low level. Gln2 was highly expressed in shoots but only at a very low level in roots. To investigate the specific functions of the two isogenes Gln1;1 and Gln1;2 in shoots for ammonium detoxification, single and double knock-out mutants were grown under standard N supply or with high ammonium provision. Phenotypes of the single mutant gln1;1 were similar to the wild type, while growth of the gln1;2 single mutant and the gln1;1:gln1;2 double mutant

was significantly impaired irrespective of N regime. GS1 activity was significantly reduced in both gln1;2 and gln1;1:gln1;2. Along with this, the ammonium content increased while that of glutamine decreased, showing that Gln1;2 was essential for ammonium assimilation and amino acid synthesis. We conclude that Gln1;2 is the main isozyme contributing to shoot GS1 activity in vegetative growth stages and can be up-regulated to relieve ammonium toxicity. This reveals a novel shoot function of Gln1;2 in Arabidopsis shoots.

Piknova, B., Park, J. W., Kwan Lam, K., and Schechter, A. N. Nitrate as a source of nitrite and nitric oxide during exercise hyperemia in rat skeletal muscle, *Nitric oxide : biology and chemistry / official journal of the Nitric Oxide Society 55–56*, 54-61.
The presence of nitric oxide (NO) synthase enzymes, mainly the NOS1 isoform, in skeletal muscle had been well established; however in the last decade it has been realized that NO may also be produced by reduction of nitrate and tissue nitrite. We have recently shown that rodent skeletal muscle contains unusually high concentrations of nitrate, compared to blood and other tissues, likely produced by oxidation of NOS1-produced NO. In the present study we measured nitrate and nitrite levels in Wistar rat leg tissue before and after acute and chronic exercise of the animals on a treadmill. We found a very large decrease of muscle nitrate levels immediately after exercise accompanied by a transient increase of nitrite levels. A significant decrease in blood nitrate levels accompanied the changes in muscle levels. Using skeletal muscle tissue homogenates we established that xanthine oxidoreductase (XOR) is at least partially responsible for the generation of nitrite and/or NO from nitrate and that this effect is increased by slight lowering of pH and by other processes related to the exercise itself. We hypothesize that the skeletal muscle nitrate reservoir contributes significantly to the generation of nitrite and then, probably via formation of NO, exercise-induced functional hyperemia. A model for these metabolic interconversions in mammals is presented. These reactions could explain the muscle-generated vasodilator causing increased blood flow, with induced contraction, exercise, or hypoxia, postulated more than 100 years ago.

Bai X, Long J, He X, Yan J, Chen X, Tan Y, Li K, Chen L, Xu H.
Overexpression of spinach non-symbiotic hemoglobin in Arabidopsis resulted in decreased NO content and lowered nitrate and other abiotic stresses tolerance.
Sci Rep. 2016 May 23;6:26400.

Lafaye C, Van Molle I, Tamu Dufe V, Wahni K, Boudier A, Leroy P, Collet JF, Messens J.
Sulfur denitrosylation by an engineered Trx-like DsbG enzyme identifies nucleophilic cysteine hydrogen bonds as key functional determinant.
J Biol Chem. 2016 May 18;. [Epub ahead of print]

Exposure of bacteria to nitric oxide (NO) results in the nitrosylation of cysteine thiols in proteins

and low molecular weight thiols such as glutathione (GSH). Cells possess enzymatic systems that catalyze the denitrosylation of these modified sulfurs. An important player in these systems is thioredoxin (Trx), a ubiquitous, cytoplasmic oxidoreductase that can denitrosylate proteins in vivo and S-nitrosoglutathione (GSNO) in vitro. However, a periplasmic or extracellular denitrosylase has not been identified, raising the question as to how extracytoplasmic proteins are repaired after nitrosative damage. In this study, we tested if DsbG and DsbC, two Trx family proteins that function in reducing pathways in the *Escherichia coli* periplasm, also possess denitrosylating activity. Both DsbG and DsbC are poorly reactive towards GSNO. Moreover, DsbG is unable to denitrosylate its specific substrate protein, YbiS. Remarkably, by borrowing the CGPC active site of *E. coli* Trx-1 in combination with a T200M point mutation, we transformed DsbG into an enzyme highly reactive towards GSNO and YbiS. The *pKa* of the nucleophilic cysteine, as well as the redox and thermodynamic properties of the engineered DsbG are dramatically changed and become similar to those of *E. coli* Trx-1. X-ray structural insights suggest that this results from a loss of two direct hydrogen bonds to the nucleophilic cysteine sulfur in the DsbG mutant. Our results highlight the plasticity of the Trx structural fold and reveal that the subtle change of the number of hydrogen bonds in the active site of Trx-like proteins is the key factor that thermodynamically controls reactivity towards nitrosylated compounds.

“we present the first evidence for the importance of the CXXC motif and of the cisPro residue in determining the activity of a Trx family oxidoreductase towards nitrosylated substrates. This property had never been investigated for any of the CXXC DsbA mutants. In addition, a recent paper describing the denitrosylating properties of the human Trx related protein TRP14 does not study the role of the CXXC motif or the redox properties of this enzyme in relation to its function (26). In general, our study is a beautiful example of the plasticity of the Trx-fold.”

Minsoo

1. J Exp Bot. 2016 May;67(10):3079-93. doi: 10.1093/jxb/erw165. Epub 2016 Apr 27.

Life without complex I: proteome analyses of an Arabidopsis mutant lacking the mitochondrial NADH dehydrogenase complex.

Fromm S(1), Senkler J(2), Eubel H(2), Peterhänsel C(3), Braun HP(4).

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The mitochondrial NADH dehydrogenase complex (complex I) is of particular importance for the respiratory chain in mitochondria. It is the major electron entry site for the mitochondrial electron transport chain (mETC) and therefore of great significance for mitochondrial ATP generation. We recently described an Arabidopsis thaliana double-mutant lacking the genes encoding the carbonic anhydrases CA1 and CA2, which both form part of a plant-specific 'carbonic anhydrase domain' of mitochondrial complex I. The mutant lacks complex I completely. Here we report extended analyses for systematically characterizing the proteome of the ca1ca2 mutant. Using various proteomic tools, we show that lack of complex I causes reorganization of the cellular respiration system. Reduced electron entry into the respiratory chain at the first segment of the mETC leads to induction of complexes II and IV as well as alternative oxidase. Increased electron entry at later segments of the mETC requires an increase in oxidation of organic substrates. This is reflected by higher abundance of proteins involved in glycolysis, the tricarboxylic acid cycle and branched-chain amino acid catabolism. Proteins involved in the light reaction of photosynthesis, the Calvin cycle, tetrapyrrole biosynthesis, and photorespiration are clearly reduced, contributing to the significant delay in growth and development of the double-mutant. Finally, enzymes involved in defense against reactive oxygen species and stress symptoms are much induced. These together with previously reported insights into the function of plant complex I, which were obtained by analysing other complex I mutants, are integrated in order to comprehensively describe 'life without complex I'.

2. Mol Cell. 2016 May 19;62(4):636-48. doi: 10.1016/j.molcel.2016.04.002. Epub 2016 May 5.

Lipidomics Analyses Reveal Temporal and Spatial Lipid Organization and Uncover Daily Oscillations in Intracellular Organelles.

Aviram R(1), Manella G(1), Kopelman N(2), Neufeld-Cohen A(1), Zwighaft Z(1), Elimelech M(1), Adamovich Y(1), Golik M(1), Wang C(3), Han X(3), Asher G(4).

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Cells have evolved mechanisms to handle incompatible processes through temporal organization by circadian clocks and by spatial compartmentalization within organelles defined by lipid bilayers. Recent advances in lipidomics have led to

identification of plentiful lipid species, yet our knowledge regarding their spatiotemporal organization is lagging behind. In this study, we quantitatively characterized the nuclear and mitochondrial lipidome in mouse liver throughout the day, upon different feeding regimens, and in clock-disrupted mice. Our analyses revealed potential connections between lipid species within and between lipid classes. Remarkably, we uncovered diurnal oscillations in lipid accumulation in the nucleus and mitochondria. These oscillations exhibited opposite phases and readily responded to feeding time. Furthermore, we found that the circadian clock coordinates the phase relation between the organelles. In summary, our study provides temporal and spatial depiction of lipid organization and reveals the presence and coordination of diurnal rhythmicity in intracellular organelles.

Elizabeth

May 30 2016

Roman SG, Chebotareva NA, Kurganov BI.
Anti-aggregation activity of small heat shock proteins under crowded conditions.
Int J Biol Macromol. 2016 May 24;. PMID: 27234495 [PubMed - as supplied by publisher]

HÃ¼bscher V, Mudholkar K, Rospert S.
The yeast Hsp70 homolog Ssb: a chaperone for general de novo protein folding and a nanny for specific intrinsically disordered protein domains.
Curr Genet. 2016 May 26;. PMID: 27230907 [PubMed - as supplied by publisher]

Basu S, Bahadur RP.
A structural perspective of RNA recognition by intrinsically disordered proteins.
Cell Mol Life Sci. 2016 May 26;. PMID: 27229125 [PubMed - as supplied by publisher]

Mitzelfelt KA, Limphong P, Choi MJ, Kondrat FD, Lai S, Kolander KD, Kwok WM, Dai Q, Grzybowski MN, Zhang H, Taylor GM, Lui Q, Thao MT, Hudson JA, Barresi R, Bushby K, Jungbluth H, Wraige E, Geurts AM, Benesch JL, Riedel M, Christians ES, Minella AC, Benjamin IJ.
Human 343delT HSPB5 Chaperone associated with Early-onset Skeletal Myopathy causes Defects in Protein Solubility.
J Biol Chem. 2016 May 19 PMID: 27226619 [PubMed - as supplied by publisher]

Khan MS, Bhat SA, Tabrez S, Alama MN, Alsenaidy MA, Al-Senaidy AM.
Denaturation induced aggregation in Î±-crystallin: differential action of chaotropes.
J Mol Recognit. 2016 May 26;. PMID: 27225040 [PubMed - as supplied by publisher]

KÅ‚osowska A, Chamera T, Liberek K.
Adenosine diphosphate restricts the protein remodeling activity of Hsp104 chaperone to Hsp70 assisted disaggregation.
Elife. 2016 May 25;5. PMID: 27223323 [PubMed - as supplied by publisher]

Chandrasekhar K, Ke H, Wang N, Goodwin T, Gierasch LM, Gershenson A, Hebert DN.
Cellular folding pathway of a metastable serpin.
Proc Natl Acad Sci U S A. 2016 May 24;. PMID: 27222580 [PubMed - as supplied by publisher]

He GH, Xu JY, Wang YX, Liu JM, Li PS, Chen M, Ma YZ, Xu ZS.
Drought-responsive WRKY transcription factor genes TaWRKY1 and TaWRKY33 from wheat confer drought and/or heat resistance in Arabidopsis.
BMC Plant Biol. 2016 May 23;16(1):116. PMID: 27215938 [PubMed - in process]

Liu Y, Wang R, Zhang P, Chen Q, Luo I, Zhu Y, Xu J.
The nitrification inhibitor methyl 3-(4-hydroxyphenyl)propionate modulates root development by interfering with auxin signaling via the NO/ROS pathway in Arabidopsis.
Plant Physiol. 2016 May 23;. PMID: 27217493 [PubMed - as supplied by publisher]

Bai X, Long J, He X, Yan J, Chen X, Tan Y, Li K, Chen L, Xu H.
Overexpression of spinach non-symbiotic hemoglobin in Arabidopsis resulted in decreased NO content and lowered nitrate and other abiotic stresses tolerance.
Sci Rep. 2016 May 23;6:26400. PMID: 27211528 [PubMed - in process]

Lafaye C, Van Molle I, Tamu Dufe V, Wahni K, Boudier A, Leroy P, Collet JF, Messens J.
Sulfur denitrosylation by an engineered Trx-like DsbG enzyme identifies nucleophilic cysteine hydrogen bonds as key functional determinant.
J Biol Chem. 2016 May 18;. PMID: 27226614 [PubMed - as supplied by publisher]

Routh SB, Pawar KI, Ahmad S, Singh S, Suma K, Kumar M, Kuncha SK, Yadav K, Kruparani SP, Sankaranarayanan R.
Elongation Factor Tu Prevents Misediting of Gly-tRNA(Gly) Caused by the Design Behind the Chiral Proofreading Site of D-Aminoacyl-tRNA Deacylase. PLoS Biol. 2016 May;14(5):e1002465.
PMID: 27224426 [PubMed - in process]

Methods in Enzymology: Alert 23 May-29 May
Developing Fluorogenic Riboswitches for Imaging Metabolite Concentration Dynamics in Bacterial Cells
Pages 315-333
J.L. Litke, M. You, S.R. Jaffrey
Genetically encoded small-molecule sensors are important tools for revealing the dynamics of metabolites and other small molecules in live cells over time. We recently developed RNA-based sensors that exhibit fluorescence in proportion to a small-molecule ligand. One class of these RNA-based sensors are termed Spinach riboswitches. These are RNAs that are based on naturally occurring riboswitches, but have been fused to the Spinach aptamer. The resulting RNA is a fluorogenic riboswitch, producing fluorescence upon binding the cognate small-molecule analyte. Here, we describe how to design and optimize these sensors by adjusting critical sequence elements, guided by structural insights from the Spinach aptamer. We provide a stepwise procedure to characterize sensors in vitro and to express sensors in bacteria for live-cell imaging of metabolites. Spinach riboswitch sensors offer a simple method for fluorescence measurement of a wide range of metabolites for which riboswitches exist, including nucleotides and their derivatives, amino acids, cofactors, cations, and anions.

Current Opinion in Cell Biology: Alert 22 May-28 May
Protein quality control in the nucleus Review Article
Pages 81-89
Ramon D Jones, Richard G Gardner

Regulation of nuclear shape and size in plants Review Article
Pages 114-123
Iris Meier, Anna HN Griffis, Norman R Groves, Alecia Wagner

Nature Cell Biology
The increasing complexity of the ubiquitin code pp579 - 586
Richard Yau and Michael Rape
doi:10.1038/ncb3358
Yau and Rape discuss recent advances in our understanding of the many variations in ubiquitin chain topology and how these mediate ubiquitin-dependent signalling in the cell.

Nature Genetics
The carrot genome sequence brings colors out of the dark - pp589 - 590
Jordi Garcia-Mas & Manuel Rodriguez-Concepcion
The genome sequence of carrot (*Daucus carota* L.) is the first completed for an Apiaceae species, furthering knowledge of the evolution of the important euasterid II clade. Analyzing the whole-genome sequence allowed for the identification of a gene that may regulate the accumulation of carotenoids in the root.

India nears putting GM mustard on the table
By Priyanka Pulla
*Science*27 May 2016 : 1043
Activists hope to derail approval, citing regulators' reluctance to release safety data.

CRISPR-directed mitotic recombination enables genetic mapping without crosses
By Meru J. Sadhu, Joshua S. Bloom, Laura Day, Leonid Kruglyak
*Science*27 May 2016 : 1113-1116
CRISPR generates targeted recombination events for rapid and systematic identification of causal genetic variants in yeast.

Nature Protocols 19 May 2016
Characterization of proteins by in-cell NMR spectroscopy in cultured mammalian cells
Letizia Barbieri, Enrico Luchinat & Lucia Banci
In-cell NMR provides detailed structural information about proteins in their native environment in living cells. This in-cell NMR protocol allows the study of protein folding and maturation in the presence of cofactors in cultured mammalian cells.

Nature Protocols 12 May 2016
Comprehensive analysis of mitochondrial permeability transition pore activity in living cells using fluorescence-imaging-based techniques
Massimo Bonora, Claudia Morganti, Giampaolo Morciano, Carlotta Giorgi, Mariusz R Wieckowski & Show more authors[…] Paolo Pinton
This protocol from Bonora *et al*. describes three imaging techniques for examining mitochondrial permeability transition (mPT) in living cells.

Protocol | 28 April 2016

Six alternative proteases for mass spectrometry–based proteomics beyond trypsin
Skip authors
Piero Giansanti, Liana Tsiatsiani, Teck Yew Low & Show more authors[…] Albert J R Heck
The use of a single enzyme such as trypsin for shotgun proteomics limits the ability to cover the whole proteome and all protein post-translational modifications. This protocol describes the use of six alternative proteases that complement trypsin

White House Releases Strategic Plan for Big Data. On May 23, the White House released *The Federal Big Data Research and Development Strategic Plan* that outlines an R&D plan for big data across seven strategies. The strategies include: creating next-generation capabilities; support R&D to understand trustworthiness of data; build and enhance research cyberinfrastructure; promote sharing and management of data; understand big data in context of privacy, security, and ethics; improve the national landscape for big data education and training; and create and enhance connections in the national big data innovation ecosystem. The report is the product of a subcommittee of the interagency National Information Technology Research & Development program.

NAS Report on GMO Crops. The National Academies of Sciences, Engineering, and Medicine released a new report, *Genetically Engineered Crops: Experiences and Prospects* , that surveys the state of understanding of the issues surrounding the development, use and effects of genetically engineered characteristics in the most widely used GE commercial crops, considering effects on human health, the environment, and agriculture. In considering the regulation of crops, the report advocates that it is the product, not the process that should be regulated.

Nature contents: 26 May 2016
☐ Cell biology: Choreography of protein synthesis
Martin Ott
See alsoArticle by Couvillion et al.

☐ Synchronized mitochondrial and cytosolic translation programs
Mary T. Couvillion,Iliana C. Soto, Gergana Shipkovenska & L. Stirling Churchman
The genes encoding the subunits of oxidative phosphorylation complexes are split between the nuclear and mitochondrial genomes, but their translation is synchronized by signalling from the cytosol to the mitochondria.
See also

Ageing: The yin and yang of mitochondrial dysfunction
p331 | doi:10.1038/nrm.2016.71
Three studies provide important insights into mitochondrial function during ageing – they reveal a connection to stem cell senescence and shed light on the epigenetic mechanisms underlying UPR^{mt} activation and stress-induced longevity.

Cell

Mitochondria: Masters of Epigenetics
☐ *Pages 1052-1054*
☐ Marc Tatar, JohnM. Sedivy

PDF (354 K)Accumulating evidence argues that aging exerts a profound influence on epigenetics, and vice versa. A pair of studies by Merkwirth et al. and Tian et al now provide insights on how mitochondrial stress experienced by *C. elegans* larvae propagates a specific and persistent epigenetic response that protects adult cells and extends lifespan.

ORIGINAL ARTICLES
Zhang, H. et al. NAD+repletion improves mitochondrial and stem cell function and enhances life span in mice.
Science http://dx.doi.org/10.1126/science.aaf2693
(2016) |

Plant Breeding Content Alert (New Articles)
Breeding for increased nitrogen-use efficiency: a review for wheat (*T. aestivum* L.)
Fabien Cormier, John Foulkes, Bertrand Hirel, David Gouache, Yvan Moënne-Loccoz and Jacques Le Gouis
Version of Record online: 18 MAY 2016 | DOI: 10.1111/pbr.12371

Zhang L, Duan Z, Zhang J, Peng L.
BIOGENESIS FACTOR REQUIRED FOR ATP SYNTHASE 3 Facilitates Assembly of the Chloroplast ATP Synthase Complex in Arabidopsis.
Plant Physiol. 2016 Apr 18;. PMID: 27208269 [PubMed - as supplied by publisher]

Crombie TA, Tang L, Choe KP, Julian D.
Inhibition of the oxidative stress response by heat stress in Caenorhabditis elegans.
J Exp Biol. 2016 May 13;. PMID: 27207646 [PubMed - as supplied by publisher]

Kumar R, Kumari B, Kumar M.
PredHSP: Sequence Based Proteome-Wide Heat Shock Protein Prediction and Classification Tool to Unlock the Stress Biology.
PLoS One. 2016;11(5):e0155872.PMID: 27195495 [PubMed - in process]

Golenhofen N, Bartelt-Kirbach B.
The impact of small heat shock proteins (HspBs) in Alzheimer`s and other neurological diseases.
Curr Pharm Des. 2016 May 19 PMID: 27194440 [PubMed - as supplied by publisher]

Shen L, Qi Z, Zhu Y, Song X, Xuan C, Ben P, Lan L, Luo L, Yin Z.
Phosphorylated heat shock protein 27 promotes lipid clearance in hepatic cells through interacting with STAT3 and activating autophagy.
Cell Signal. 2016 May 13;. PMID: 27185187 [PubMed - as supplied by publisher]

Bittner LM, Arends J, Narberhaus F.
Mini review: ATP-dependent proteases in bacteria.
Biopolymers. 2016 Aug;105(8):505-17. PMID: 26971705 [PubMed - in process]

Fatma M, Masood A, Per TS, Khan NA.

Nitric Oxide Alleviates Salt Stress Inhibited Photosynthetic Performance by Interacting with Sulfur Assimilation in Mustard.
Front Plant Sci. 2016;7:521. PMID: 27200007 [PubMed]

Sun H, Bi Y, Tao J, Huang S, Hou M, Xue R, Liang Z, Gu P, Yoneyama K, Xie X, Shen Q, Xu G, Zhang Y.
Strigolactones are required for nitric oxide to induce root elongation in response to nitrogen and phosphate deficiencies in rice.
Plant Cell Environ. 2016 Jan 14;. PMID: 27194103 [PubMed - as supplied by publisher]

Vanzo E, Merl-Pham J, Velikova V, Ghirardo A, Lindermayr C, Hauck SM, Bernhardt J, Riedel K, Durner J, Schnitzler JP.
Modulation of Protein S-Nitrosylation by Isoprene Emission in Poplar.
Plant Physiol. 2016 Apr;170(4):1945-61. PMID: 26850277 [PubMed - in process]

Berger H, de Mia M, Morisse S, Marchand C, Lemaire SD, Wobbe L, Kruse O.
A light switch based on protein S-nitrosylation fine-tunes photosynthetic light-harvesting in the microalga Chlamydomonas reinhardtii.
Plant Physiol. 2016 Apr 4;. PMID: 27208221 [PubMed - as supplied by publisher]

Cell

☐ **The Structure and Dynamics of Higher-Order Assemblies: Amyloids, Signalosomes, and Granules**
Review Article ☐ *Pages 1055-1066* ☐ Hao Wu, Monika Fuxreiter
PDF (2328 K)
☐ The synergy between folded domains, linearmotifs, and intrinsically disordered regions creates a structural and dynamic continuum of higher-order assemblies.

☐ **Mechanisms and Consequences of Macromolecular Phase Separation**
Review Article ☐ *Pages 1067-1079* ☐ Louis-Philippe Bergeron-Sandoval, Nozhat Safaee, Stephen W. Michnick
PDF (1559 K)

Mitochondrial Stress Induces Chromatin Reorganization to Promote Longevity and UPR^{mt}

Original Research Article *Pages 1197-1208*
Ye Tian, Gilberto Garcia, Qian Bian, Kristan K. Steffen, Larry Joe, Suzanne Wolff, Barbara J. Meyer, Andrew Dillin
PDF (2700 K) In response to mitochondrial stress, LIN-65 accumulates in the nucleus to induce chromatin restructuring that facilitates the full activation of mitochondrial unfolded protein response and mediates stress-induced longevity.

Two Conserved Histone Demethylases Regulate Mitochondrial Stress-Induced Longevity

Original Research Article *Pages 1209-1223*
Carsten Merkwirth, Virginija Jovaisaite, Jenni Durieux, Olli Matilainen, Sabine D. Jordan, Pedro M. Quiros, Kristan K. Steffen, Evan G. Williams, Laurent Mouchiroud, Sarah U. Tronnes, Virginia Murillo, Suzanne C. Wolff, Reuben J. Shaw, Johan Auwerx, Andrew Dillin

PDF (4941 K)

Mitochondrial perturbations early in life can have beneficial effects on the lifespan of organisms. The conserved histone lysine demethylases JMJD-1.2 and JMJD-3.1 promote longevity and coordinate transcriptional outputs in response to mitochondrial dysfunction, thereby revealing an epigenetic mode for the regulation of stress signaling and lifespan downstream of mitochondrial defects.

*Science*20 May 2016 : 986-990

Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains
By Rebekka Wild, Ruta Gerasimaite, Ji-Yul Jung, Vincent Truffault, Igor Pavlovic, Andrea Schmidt, Adolfo Saiardi, Henning Jacob Jessen, Yves Poirier, Michael Hothorn, Andreas Mayer
A conserved peptide domain allows a cell to sense how much phosphate it has and regulate uptake of more phosphate if needed.

Plant Cell
The Proteasome Stress Regulon Is Controlled by a Pair of NAC Transcription Factors in Arabidopsis
Nicholas P Gladman, Richard S Marshall, Kwang-Hee Lee, and Richard D. Vierstra
Plant Cell 2016 tpc.15.01022; Advance Publication May 18, 2016; doi:10.1105/tpc.15.01022
http://www.plantcell.org/content/early/2016/05/18/tpc.15.01022.abstract