Keith

Biochemical Analyses of Sorghum Varieties Reveal Differential Responses to Drought.

PLoS One. 2016 May 6

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We have examined the biochemical responses of two sorghum cultivars of differing drought tolerance, Samsorg 17 (more drought tolerant) and Samsorg 40 (less drought tolerant), to sustained drought. Plants were exposed to different degrees of drought and then maintained at that level for five days. Responses were examined in terms of metabolic changes and the expression of drought induced proteins-Heat Shock Proteins (HSPs) and dehydrins (DHNs). Generalised phenotypic changes were studied using Fourier transform infrared (FT-IR) Spectroscopy and non-targeted Gas Chromatography Mass Spectrometry (GC-MS) was employed to detect changes in metabolites, while changes in protein expression were examined using Western blot analysis. Different response profiles of metabolites, HSPs and DHNs were observed in the two cultivars. Metabolic changes involved variation in amino acids, polysaccharides and their derivatives. A total of 188 compounds, with 142 known metabolites and 46 unknown small molecules, were detected in the two sorghum varieties. Under water deficit conditions, Samsorg 17 accumulated sugars and sugar alcohols, while in Samsorg 40 amino acids increased in concentration. This study suggest that the two Sorghum varieties adopt distinct approaches in response to drought, with Samsorg 17 being better able to maintain leaf function under severe drought conditions.

Bacterial Hsp70 (DnaK) and mammalian Hsp70 interact differently with lipid membranes.

Cell Stress Chaperones. 2016 Apr 13.

Lopez V1, Cauvi DM2, Arispe N3, De Maio A4,5,6,7.

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The cellular response to stress is orchestrated by the expression of a family of proteins termed heat shock proteins (hsp) that are involved in the stabilization of basic cellular processes to preserve cell viability and homeostasis. The bulk of hsp function occurs within the cytosol and subcellular compartments. However, some hsp have also been found outside cells released by an active mechanism independent of cell death. Extracellular hsp act as signaling molecules directed at activating a systemic response to stress. The export of hsp requires the translocation from the cytosol into the extracellular milieu across the plasma membrane. We have proposed that membrane insertion is the initial step in this export process. We investigated the interaction of the major inducible hsp from mammalian (Hsp70) and bacterial (DnaK) species with liposomes. We found that mammalian Hsp70 displayed a high specificity for negatively charged phospholipids, such as phosphatidyl serine, whereas DnaK interacted with all lipids tested regardless of the charge. Both proteins were inserted into the lipid bilayer as demonstrated by resistance to acid or basic washes that was confirmed by partial protection from proteolytic cleavage. Several regions of mammalian Hsp70 were inserted into the membrane with a small portion of the N-terminus end exposed to the outer phase of the liposome. In contrast, the N-terminus end of DnaK was inserted into the membrane, exposing the C-terminus end outside the liposome. Mammalian Hsp70 was found to make high oligomeric complexes upon insertion into the membranes whereas DnaK only formed dimers within the lipid bilayer. These observations suggest that both Hsp70s interact with lipids, but mammalian Hsp70 displays a high degree of specificity and structure as compared with the bacterial form.

Inhibition of Both Hsp70 Activity and Tau Aggregation In Vitro Best Predicts Tau Lowering Activity of Small Molecules.

ACS Chem Biol. 2016 May 13. [Epub ahead of print]

Martin MD, Baker JD, Suntharalingam A, Nordhues BA, Shelton LB, Zheng D, Sabbagh JJ, Haystead TA, Gestwicki JE, Dickey CA.

Three scaffolds with inhibitory activity against the heat shock protein 70 (Hsp70) family of chaperones have been found to enhance the degradation of the microtubule associated protein tau in cells, neurons and brain tissue. This is important because tau accumulation is linked to neurodegenerative diseases including Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE). Here we expanded upon this study to investigate the anti-tau efficacy of additional scaffolds with Hsp70 inhibitory activity. Five of the nine scaffolds tested lowered tau levels, with the rhodacyanine and phenothiazine scaffolds exhibiting the highest potency as previously described. Because phenothiazines also inhibit tau aggregation in vitro, we suspected that this activity might be a more accurate predictor of tau-lowering. Interestingly, the rhodacyanines did inhibit in vitro tau aggregation to a similar degree as phenothiazines,

correlating well with tau-lowering efficacy in cells and ex vivo slices. Moreover, other Hsp70 inhibitor scaffolds with weaker tau-lowering activity in cells inhibited tau aggregation in vitro, albeit at lower potencies. When we tested six well-characterized tau aggregation inhibitors, we determined that this mechanism of action was not a better predictor of tau-lowering than Hsp70 inhibition. Instead we found that compounds possessing both activities were the most effective at promoting tau clearance. Moreover, cytotoxicity and PAINS activity are critical factors that can lead to false-positive lead identification. Strategies designed around these principles will likely yield more efficacious tau-lowering compounds.

Molecular architecture of the inner ring scaffold of the human nuclear pore complex.

Science. 2016 Apr 15

Kosinski J1, Mosalaganti S1, von Appen A1, Teimer R2, DiGuilio AL3, Wan W1, Bui KH4, Hagen WJ1, Briggs JA5, Glavy JS3, Hurt E2, Beck M5.

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Nuclear pore complexes (NPCs) are 110-megadalton assemblies that mediate nucleocytoplasmic transport. NPCs are built from multiple copies of ~30 different nucleoporins, and understanding how these nucleoporins assemble into the NPC scaffold imposes a formidable challenge. Recently, it has been shown how the Y complex, a prominent NPC module, forms the outer rings of the nuclear pore. However, the organization of the inner ring has remained unknown until now. We used molecular modeling combined with cross-linking mass spectrometry and cryo-electron tomography to obtain a composite structure of the inner ring. This architectural map explains the vast majority of the electron density of the scaffold. We conclude that despite obvious differences in morphology and composition, the higher-order structure of the inner and outer rings is unexpectedly similar.

Hsp70 biases the folding pathways of client proteins.

Proc Natl Acad Sci U S A. 2016 May 2

Sekhar A1, Rosenzweig R2, Bouvignies G3, Kay LE4.

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The 70-kDa heat shock protein (Hsp70) family of chaperones bind cognate substrates to perform a variety of different processes that are integral to cellular homeostasis. Although detailed structural information is available on the chaperone, the structural features of folding competent substrates in the bound form have not been well characterized. Here we use paramagnetic relaxation enhancement (PRE) NMR spectroscopy to probe the existence of long-range interactions in one such folding competent substrate, human telomere repeat binding factor (hTRF1), which is bound to DnaK in a globally unfolded conformation. We show that DnaK binding modifies the energy landscape of the substrate by removing long-range interactions that are otherwise present in the unbound, unfolded conformation of hTRF1. Because the unfolded state of hTRF1 is only marginally populated and transiently formed, it is inaccessible to standard NMR approaches. We therefore developed a 1H-based CEST experiment that allows measurement of PREs in sparse states, reporting on transiently sampled conformations. Our results suggest that DnaK binding can significantly bias the folding pathway of client substrates such that secondary structure forms first, followed by the development of longer-range contacts between more distal parts of the protein.

Minsoo

1. **Convergence of light and chloroplast signals for de-etiolation through ABI4–HY5 and COP1**
Xiumei Xu, Wei Chi, Xuwu Sun, Peiqiang Feng, Hailong Guo, Jing Li, Rongcheng Lin, Congming Lu, Haiyang Wang, Dario Leister & Lixin Zhang
Published online: 09 May 2016
Abstract
Seedling de-etiolation prepares plants to switch from heterotrophic to photoautotrophic growth, a transition essential for plant survival. This delicate de-etiolation process is precisely controlled by environmental and endogenous signals. Although intracellular plastid-derived retrograde signalling is essential for the de-etiolation process, the molecular nature of these retrograde signals remains elusive1,2,3. Here we show that chloroplast and light signals antagonistically fine-tune a suite of developmental and physiological responses associated with

de-etiolation through a transcriptional module of ABA INSENSITIVE 4 (ABI4) and ELONGATED HYPOCOTYL 5 (HY5). Moreover, ABI4 and HY5 antagonistically regulate the expression of CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) and the subsequent greening process. In turn, ABI4 and HY5 are targeted for degradation by COP1 in the light and dark, respectively, to ensure a proper interplay of ABI4 and HY5 actions during seedling de-etiolation. Our study provides a new molecular mechanism for understanding how chloroplast signals converge with light signals to optimize early plant development.

2. Nucleic Acids Res. 2016 Apr 25. pii: gkw302. [Epub ahead of print]

Mitochondrial transcription termination factor 1 directs polar replication fork pausing.

Shi Y(1), Posse V(2), Zhu X(1), Hyvärinen AK(3), Jacobs HT(4), Falkenberg M(5), Gustafsson CM(5).

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During replication of nuclear ribosomal DNA (rDNA), clashes with the transcription apparatus can cause replication fork collapse and genomic instability. To avoid this problem, a replication fork barrier protein is situated downstream of rDNA, there preventing replication in the direction opposite rDNA transcription. A potential candidate for a similar function in mitochondria is the mitochondrial transcription termination factor 1 (MTERF1, also denoted mTERF), which binds to a sequence just downstream of the ribosomal transcription unit. Previous studies have shown that MTERF1 prevents antisense transcription over the ribosomal RNA genes, a process which we here show to be independent of the transcription elongation factor TEFM. Importantly, we now demonstrate that MTERF1 arrests mitochondrial DNA (mtDNA) replication with distinct polarity. The effect is explained by the ability of MTERF1 to act as a directional contrahelicase, blocking mtDNA unwinding by the mitochondrial helicase TWINKLE. This conclusion is also supported by in vivo evidence that MTERF1 stimulates TWINKLE pausing. We conclude that MTERF1 can direct polar replication fork arrest in mammalian mitochondria.

Damian

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Nitro-linolenic acid is a nitric oxide donor.
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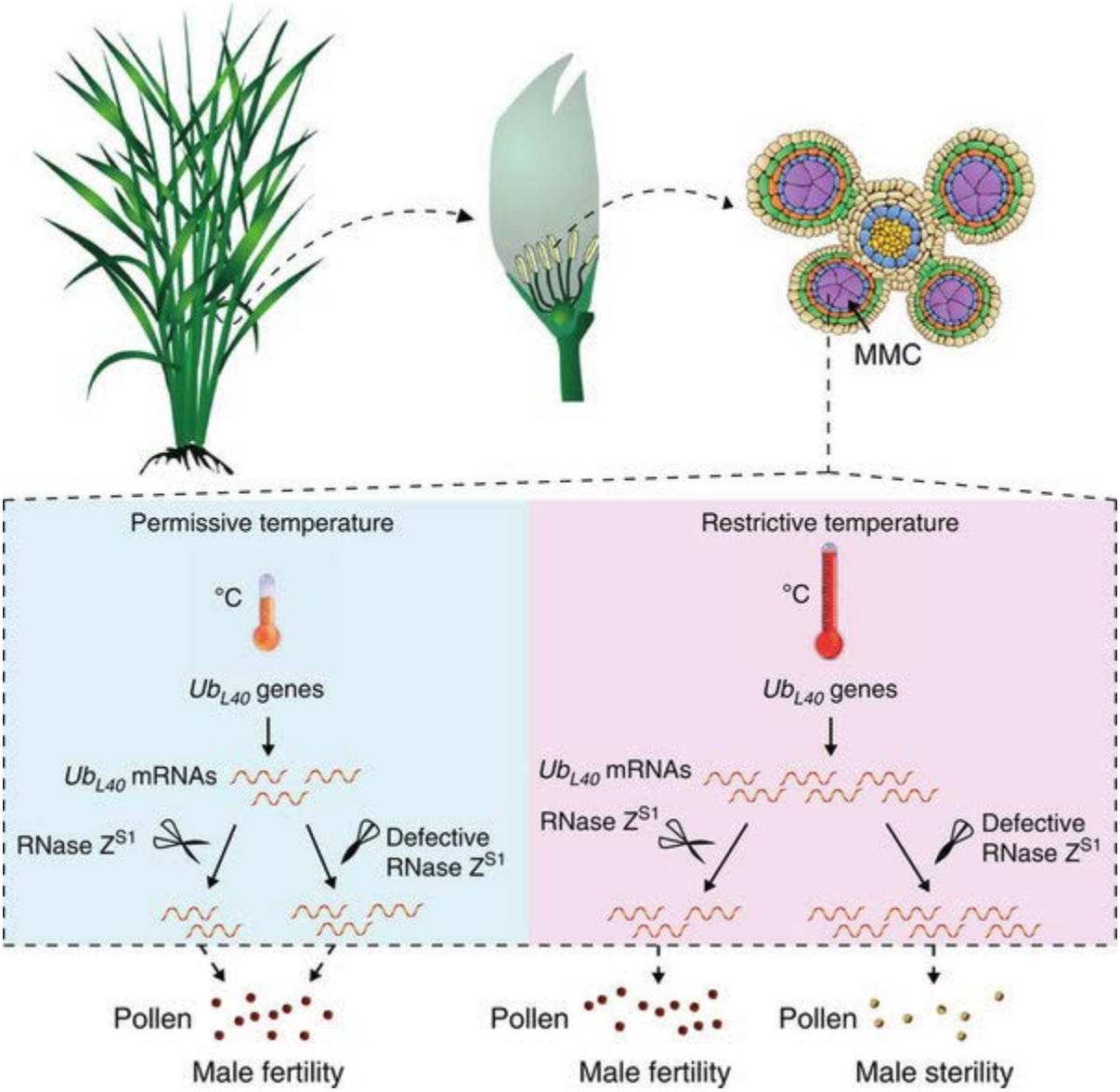
Elizabeth



In rice anther, Ub_{L40} genes preferentially express in MMCs (in purple). Ub_{L40} mRNAs are induced by high (restrictive) temperature. In wild type, RNase Z^{S1} processes mRNAs of Ub_{L40} and maintains them at normal levels. In RNase Z^{S1} defective mutant plants, excessive mRNAs of Ub_{L40} accumulate in anthers and lead to male sterility. MMC, microspore mother cells.
Nature Communications | Article

RNase Z^{S1} processes Ub_{L40} mRNAs and controls thermosensitive genic male sterility in rice
Hai Zhou, Ming Zhou, Yuanzhu Yang, Jing Li, Liya Zhu, *et al.*
Nature Communications 5, Article number: 4884 doi:10.1038/ncomms5884
Published 11 September 2014

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PLOS Biology Volume 14(4) April 2016

Farewell to the Lose–Lose Reality of Policing Plant Imports
Dani Zamir

This Perspective asks us to bid farewell to the failed strategy of policing plant imports and embark on genomics-assisted, biodiversity-based breeding to improve plant resilience to changing environments and to the pests of the future.

Freeing Crop Genetics through the Open Source Seed Initiative
Claire H. Luby, Irwin L. Goldman

This Community Page article highlights the Open Source Seed Initiative, which serves as an alternative to restrictive intellectual property rights and is working to develop an open-source release mechanism for crop plant diversity, analogous to that developed for open-source software.

Global RNA recognition patterns of post-transcriptional regulators Hfq and CsrA revealed by UV crosslinking in vivo
Erik Holmqvist, Patrick R Wright, Lei Li, Thorsten Bischler, Lars Barquist, Richard Reinhardt, Rolf Backofen, and Jörg Vogel
Published online 04.04.2016

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A new pipeline for CLIP-seq in Salmonella maps global RNA–protein interactions and offers a tool for improved understanding of post-transcriptional control in bacteria.

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Antioxidants & Redox Signaling Vol. 24, No. 13, May 2016 is now available online
Dissecting Redox Biology Using Fluorescent Protein Sensors

Markus Schwarzländer, Tobias P. Dick, Andreas J. Meyer, and Bruce Morgan

Antioxidants & Redox Signaling, Vol. 24, No. 13, May 2016: 680-712.

Abstract | Full Text HTML | Full Text PDF (1070 KB) | Full Text PDF with Links (933 KB)

Selective and Reversible Approaches Toward Imaging Redox Signaling Using Small-Molecule Probes

Jacek L. Kolanowski, Amandeep Kaur, and Elizabeth J. New

Antioxidants & Redox Signaling, Vol. 24, No. 13, May 2016: 713-730.

Abstract | Full Text HTML | Full Text PDF (779 KB) | Full Text PDF with Links (704 KB)

HyPer Family Probes: State of the Art

Dmitry S. Bilan and Vsevolod V. Belousov

Antioxidants & Redox Signaling, Vol. 24, No. 13, May 2016: 731-751.

Abstract | Full Text HTML | Full Text PDF (1681 KB) | Full Text PDF with Links (881 KB)

Quantitative Redox Imaging Software

Mark D. Fricker

Antioxidants & Redox Signaling, Vol. 24, No. 13, May 2016: 752-762.

Abstract | Full Text HTML | Full Text PDF (1431 KB) | Full Text PDF with Links (679 KB)

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Rearrangements of Î±-helical structures of FlgN chaperone control the binding affinity for its cognate substrates during flagellar type III export.
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Current Opinion in Chemical Biology: Alert 8 May-14 May
Biocatalysts from alkaloid producing plants Review Article
Pages 22-30
Hajo Kries, Sarah E O'Connor

Harvesting the biosynthetic machineries that cultivate a variety of indispensable plant natural products Review Article
Pages 66-73
Christopher R Vickery, James J La Clair, Michael D Burkart, Joseph P Noel
Recent developments in biological water oxidation Review Article
Pages 113-119
Montserrat Pérez-Navarro, Frank Neese, Wolfgang Lubitz, Dimitrios A Pantazis, Nicholas Cox
How did life survive Earth's great oxygenation? Review Article
Pages 166-178
Woodward W Fischer, James Hemp, Joan Selverstone Valentine
Plant, Cell & Environment Content Alert: 39, 6 (June 2016)

Dissecting the proteome dynamics of the early heat stress response leading to plant survival or death in Arabidopsis (pages 1264–1278)
Sira Echevarría-Zomeño, Lourdes Fernández-Calvino, Ana B. Castro-Sanz, Juan Antonio López, Jesús Vázquez and M. Mar Castellano
Version of Record online: 24 FEB 2016 | DOI: 10.1111/pce.12664

Plants launch specific molecular programs that depending on the heat stress condition can lead to either plant thermotolerance or death. In this article, we have dissected the early proteome responses, unravelled the common players and identified the specific proteins associated with these two final heat-induced fates.

The influence of alternative pathways of respiration that utilize branched-chain amino acids following water shortage in *Arabidopsis* (pages 1304–1319)
Marcel V. Pires, Adilson A. Pereira Júnior, David B. Medeiros, Danilo M. Daloso, Phuong Anh Pham, Kallyne A. Barros, Martin K. M. Engqvist, Alexandra Florian, Ina Krahnert, Veronica G. Maurino, Wagner L. Araújo and Alisdair R. Fernie
Version of Record online: 3 MAR 2016 | DOI: 10.1111/pce.12682

The data described in our work present evidence that the ETF/ETFQO pathway play a significant role in drought tolerance mechanism in Arabidopsis thaliana. The results presented here

demonstrate the enzymes IVDH and D2HGDH are important for drought tolerance mechanism. Furthermore it provides the first functional clues of a role for ETF/ETFQO pathway under stress conditions other than dark-induced senescence. Our results thus support recent findings that mitochondrial metabolism is generally highly active during drought tolerance and provides support for a role for alternative respiratory pathways within this response.

AsHSP17, a creeping bentgrass small heat shock protein modulates plant photosynthesis and ABA-dependent and independent signalling to attenuate plant response to abiotic stress (pages 1320–1337)

Xinbo Sun, Chunyu Sun, Zhigang Li, Qian Hu, Liebao Han and Hong Luo
Version of Record online: 12 FEB 2016 | DOI: 10.1111/pce.12683

Heat shock proteins (HSPs) are molecular chaperones that accumulate in response to environmental stress. A new small HSP (sHSP) gene, *AsHSP17* from creeping bentgrass (*Agrostis stolonifera*) has been cloned and shown to negatively regulate plant response to heat and salt stress through modulating photosynthesis and ABA-dependent and independent signaling pathways. Data obtained in this study provide new insight into the plant stress regulatory pathways and a better understanding of the role that sHSPs play in that response.

Nature Reviews - Microbiology

Bacterial physiology: (p)ppGpp target ribosome assembly | *PDF (129 KB)*
p266 | doi:10.1038/nrmicro.2016.44
This study shows that, as part of the stringent response in Staphylococcus aureus, (p)ppGpp target GTPases, impairing their ability to mediate ribosome assembly, which affects bacterial growth and promotes tolerance to antimicrobials.

Synthetic biology to access and expand nature's chemical diversity
Michael J. Smanski, Hui Zhou, Jan Claesen, Ben Shen, Michael A. Fischbach & Christopher A. Voigt
p135 | doi:10.1038/nrmicro.2015.24
Advances in synthetic biology have simplified the characterization and production of biologically active molecules from various organisms. In this Review, Voigt and colleagues outline the design and construction of pathways used for the synthesis of such natural products in host microorganisms.

Science

Translation dynamics of single mRNAs in live cells and neurons
Bin Wu[1,2], Carolina Eliscovich[1], Young J. Yoon[1], Robert H. Singer[1,2,3,*]
Translation is the fundamental biological process converting mRNA information into proteins. Single molecule imaging in live cells has illuminated the dynamics of RNA transcription; however, it is not yet applicable to translation. Here we report Single molecule Imaging of NAscent PeptideS (SINAPS) to assess translation in live cells. The approach provides direct readout of initiation, elongation, and location of translation. We show that mRNAs coding for endoplasmic reticulum (ER) proteins are translated when they encounter the ER membrane. Single molecule fluorescence recovery after photobleaching provides direct measurement of elongation speed (5 AA/s). In primary neurons mRNAs are translated in proximal dendrites but repressed in distal dendrites and display “bursting” translation. This technology provides a tool to address the spatiotemporal translation mechanism of single mRNAs in living cells.

Real-time quantification of single RNA translation dynamics in living cells

Tatsuya Morisaki[1,2], et al.
Although mRNA translation is a fundamental biological process, it has never been imaged in real-time with single molecule precision in vivo. To achieve this, we developed Nascent Chain Tracking (NCT), a technique that uses multi-epitope tags and antibody-based fluorescent probes to quantify single mRNA protein synthesis dynamics. NCT reveals an elongation rate of ~10 amino acids per second, with initiation occurring stochastically every ~30 s. Polysomes contain ~1 ribosome every 200-900 nucleotides and are globular rather than elongated in shape. By developing multi-color probes, we show most polysomes act independently; however, a small fraction (~5%) form complexes in which two distinct mRNAs can be translated simultaneously. The sensitivity and versatility of NCT make it a powerful new tool for quantifying mRNA translation kinetics.
Cell
Volume 165, Issue 4, 5 May 2016, Pages 990–1001
Real-Time Imaging of Translation on Single mRNA Transcripts in Live Cells
Chong Wang[1], Boran Han[1], Ruobo Zhou[1], Xiaowei Zhuang[1,,]

A new GMO! Making hornless cows
Each year, some 80% of dairy cattle in the United States have their horns removed. The horns can pose a risk both to humans and to the cattle themselves, but animal rights activists have protested the process of dehorning as cruel. Now, a team of researchers led by scientists with the St. Paul–based biotech startup Recombinetics offers a different solution: Hornless cows created with the genome-editing technique TALENs. Natural hornlessness is common in cattle breeds raised for beef, but rare in dairy cows, so the team edited into them genetic variants associated with hornlessness in beef cattle. In the end, the team reported last week in *Nature Biotechnology*, they created five live, hornless calves with no off-target effects.

Nature
Architecture of the mitochondrial calcium uniporter
Ying Dong, Chan Cao, Tanxing Cui, Yasemin Sancak + et al.

The structure of the core region of the mitochondrial calcium uniporter (MCU) is determined by NMR and electron microscopy, revealing that MCU is a homo-pentamer with a specific transmembrane helix forming a hydrophilic pore across the membrane, and representing one of the largest membrane protein structures characterized by NMR spectroscopy.

Nature Biotechnology
Ending event-based regulation of GMO crops - pp474 - 477
Steven H Strauss & Joanna K Sax
doi:10.1038/nbt.3541
Regulate genome-edited products, not genome editing itself - pp477 - 479
Dana Carroll, Alison L Van Eenennaam, Jeremy F Taylor, Jon Seger & Daniel F Voytas
doi:10.1038/nbt.3566
Full Text - Regulate genome-edited products, not genome editing itself | PDF (437 KB)
A risk-based approach to the regulation of genetically engineered organisms - pp493 - 503
Gregory Conko, Drew L Kershen, Henry Miller & Wayne A Parrott
doi:10.1038/nbt.3568

Current regulatory regimes for genetically engineered crops fail to use a scientifically defensible approach or tailor the degree of regulatory review to the level of actual hazard or risk. We describe a rational way forward.

Abstract - | Full Text - A risk-based approach to the regulation of genetically engineered organisms | PDF (1,199 KB)

Multiplexed labeling of genomic loci with dCas9 and engineered sgRNAs using CRISPRainbow - pp528 - 530
Hanhui Ma, Li-Chun Tu, Ardalan Naseri, Maximiliaan Huisman, Shaojie Zhang, David Grunwald & Thoru Pederson

doi:10.1038/nbt.3526
Multiple chromosomal sites are readily labeled using Cas9 and guide RNAs that bind fluorescent proteins, enabling visualization of chromatin dynamics.

Abstract - | Full Text - Multiplexed labeling of genomic loci with dCas9 and engineered sgRNAs using CRISPRainbow | PDF (1,548 KB) - Multiplexed labeling of genomic loci with dCas9 and engineered sgRNAs using CRISPRainbow | Supplementary information

Full Text - Ending event-based regulation of GMO crops | PDF (4,342 KB)

Molecular Cell
Molecular Mechanism of Protein Kinase Recognition and Sorting by the Hsp90 Kinome-Specific Cochaperone Cdc37 Original Research Article
Pages 260-271
Dimitra Keramisanou, Adam Aboalroub, Ziming Zhang, Wenjun Liu, Devon Marshall, Andrea Diviney, Randy W. Larsen, Ralf Landgraf, Ioannis Gelis
Cell
On the Dependency of Cellular Protein Levels on mRNA Abundance Review Article
Pages 535-550
Yansheng Liu, Andreas Beyer, Ruedi Aebersold