Isoxazole derivatives as new nitric oxide elicitors in plants

Abstract

Several 3,5-disubstituted isoxazoles were obtained in good yields by regiospecific 1,3-dipolar cycloaddition reactions between aro- matic nitrile oxides, generated in situ from the corresponding hydroxyimidoyl chlorides, with non-symmetrical activated alkynes in the presence of catalytic amounts of copper(I) iodide. Effects of 3,5-disubstituted isoxazoles on nitric oxide and reactive oxygen species generation in *Arabidopsis* tissues was studied using specific diaminofluoresceine dyes as fluorescence indicators.

lan

Regulation of protein function by S-nitrosation and S-glutathionylation: processes and targets in cardiovascular pathophysiology

Eugenia Belcastro, Caroline Gaucher, Alessandro Corti, Pierre Leroy, Isabelle Lartaud, Alfonso Pompella

Published Online: 2017-08-19 | DOI: https://doi.org/10.1515/hsz-2017-0150

Minsoo

1. Sci Adv. 2017 Aug 4;3(8):e1701726. doi: 10.1126/sciadv.1701726. eCollection 2017 Aug.

Structural pathway of regulated substrate transfer and threading through an Hsp100 disaggregase.

Deville C(1), Carroni M(1), Franke KB(2), Topf M(1), Bukau B(2), Mogk A(2), Saibil HR(1).

Author information:

(1)Department of Crystallography, Institute of Structural and Molecular Biology, Birkbeck, University of London, Malet Street, London WC1E 7HX, UK. (2)Center for Molecular Biology of the Heidelberg University, German Cancer Research Center, Heidelberg, Germany.

Refolding aggregated proteins is essential in combating cellular proteotoxic stress. Together with Hsp70, Hsp100 chaperones, including Escherichia coli ClpB, form a powerful disaggregation machine that threads aggregated polypeptides through the central pore of tandem adenosine triphosphatase (ATPase) rings. To visualize protein disaggregation, we determined cryo-electron microscopy structures of inactive and substrate-bound ClpB in the presence of adenosine 5'-O-(3-thiotriphosphate), revealing closed AAA+ rings with a pronounced seam. In the substrate-free state, a marked gradient of resolution, likely corresponding to mobility, spans across the AAA+ rings with a dynamic hotspot at the seam. On the seam side, the coiled-coil regulatory domains are locked in a horizontal, inactive orientation. On the opposite side, the regulatory domains are accessible

for Hsp70 binding, substrate targeting, and activation. In the presence of the model substrate casein, the polypeptide threads through the entire pore channel and increased nucleotide occupancy correlates with higher ATPase activity. Substrate-induced domain displacements indicate a pathway of regulated substrate transfer from Hsp70 to the ClpB pore, inside which a spiral of loops contacts the substrate. The seam pore loops undergo marked displacements, along with ordering of the regulatory domains. These asymmetric movements suggest a mechanism for ATPase activation and substrate threading during disaggregation.

Patrick

Hu et al., 2017, Molecular Cell 67, 1–9 August 17, 2017 a 2017 Elsevier Inc. http://dx.doi.org/10.1016/j.molcel.2017.06.031

Nitric Oxide Regulates Protein Methylation during Stress Responses in Plants

Jiliang Hu,1,2,5 Huanjie Yang,1,2,3,5 Jinye Mu,1 Tiancong Lu,1,2 Juli Peng,1 Xian Deng,1 Zhaosheng Kong,3 Shilai Bao,4

Xiaofeng Cao,1 and Jianru Zuo1,6,*

SUMMARY

Methylation and nitric oxide (NO)-based S-nitrosylation are highly conserved protein posttranslational modifications that regulate diverse biological processes. In higher eukaryotes, PRMT5 catalyzes Arg symmetric dimethylation, including key components of the spliceosome. The Arabidopsis prmt5 mutant shows severe developmental defects and impaired stress responses. However, little is known about the mechanisms regulating the PRMT5 activity. Here, we report that NO positively regulates the PRMT5 activity through S-nitrosylation at Cys-125 during stress responses. In prmt5-1 plants, a PRMT5c1258 transgene, carrying a non-nitrosylatable mutation at Cys-125, fully rescues the developmental defects, but not the stress hypersensitive phenotype and the responsiveness to NO during stress responses. Moreover, the salt-induced Arg symmetric dimethylation is abolished in PRMT5c125S/prmt5-1 plants, correlated to aberrant splicing of pre-mRNA derived from a stress-related gene. These findings define a mechanism by which plants transduce stress-triggered NO signal to protein methylation machinery through S-nitrosylation of PRMT5 in response to environmental alterations.

Nene et al. Journal of Biomedical Science (2017) 24:3 DOI 10.1186/s12929-016-0312-x

Aldehyde dehydrogenase 2 activation and coevolution of its EPKC-mediated

phosphorylation sites

Aishwarya Nene†, Che-Hong Chen*†, Marie-Hélène Disatnik, Leslie Cruz and Daria Mochly-Rosen

Abstract

Background: Mitochondrial aldehyde dehydrogenase 2 (ALDH2) is a key enzyme for the metabolism of many toxic aldehydes such as acetaldehyde, derived from alcohol drinking, and 4HNE, an oxidative stress-derived lipid peroxidation aldehyde. Post-translational enhancement of ALDH2 activity can be achieved by serine/threonine phosphorylation by epsilon protein kinase C (ϵ PKC). Elevated ALDH2 is beneficial in reducing injury following myocardial infarction, stroke and other oxidative stress and aldehyde toxicity-related diseases. We have previously identified three ϵ PKC phosphorylation sites, threonine 185 (T185), serine 279 (S279) and threonine 412 (T412), on ALDH2. Here we further characterized the role and contribution of each phosphorylation site to the enhancement of enzymatic activity by ϵ PKC.

Methods: Each individual phosphorylation site was mutated to a negatively charged amino acid, glutamate, to mimic a phosphorylation, or to a non-phosphorylatable amino acid, alanine. ALDH2 enzyme activities and protection against 4HNE inactivation were measured in the presence or absence of ϵ PKC phosphorylation in vitro. Coevolution of ALDH2 and its ϵ PKC phosphorylation sites was delineated by multiple sequence alignments among a diverse range of species and within the ALDH multigene family.

Results: We identified S279 as a critical εPKC phosphorylation site in the activation of ALDH2. The critical catalytic

site, cysteine 302 (C302) of ALDH2 is susceptible to adduct formation by reactive aldehyde, 4HNE, which readily renders the enzyme inactive. We show that phosphomimetic mutations of T185E, S279E and T412E confer protection of ALDH2 against 4HNE-induced inactivation, indicating that phosphorylation on these three sites by ϵ PKC likely also protects the enzyme against reactive aldehydes. Finally, we demonstrate that the three ALDH2 phosphorylation sites co-evolved with ϵ PKC over a wide range of species. Alignment of 18 human ALDH isozymes, indicates that T185 and S279 are unique ALDH2, ϵ PKC specific phosphorylation sites, while T412 is found in other ALDH isozymes. We further identified three highly conserved serine/threonine residues (T384, T433 and S471) in all

18 ALDH isozymes that may play an important phosphorylation-mediated regulatory role in this important family of detoxifying enzymes.

Conclusion: EPKC phosphorylation and its coevolution with ALDH2 play an important role in the regulation and

protection of ALDH2 enzyme activity.

Keith

Click chemistry enables preclinical evaluation of targeted epigenetic therapies.

Science. 2017 Jun 3

 $\frac{\text{Tyler DS}^{1,2}, \text{Vappiani J}^3, \text{Ca\~neque T}^{4,5,6}, \text{Lam EYN}^{1,2}, \text{Ward A}^3, \text{Gilan O}^{1,2}, \text{Chan YC}^1, \text{Hienzsch A}^{4,5,6}, \text{Rutkowska A}^3, \text{Werner T}^3, \text{Wagner AJ}^3, \text{Lugo D}^7, \text{Gregory R}^7, \text{Ramirez Molina C}^7, \text{Garton N}^7, \text{Wellaway CR}^7, \text{Jackson S}^1, \text{MacPherson L}^{1,2}, \text{Figueiredo M}^1, \text{Stolzenburg S}^1, \text{Bell CC}^{1,2}, \text{House C}^1, \text{Dawson SJ}^{1,2,8}, \text{Hawkins ED}^9, \text{Drewes G}^3, \text{Prinjha RK}^7, \text{Rodriguez R}^{4,5,6}, \text{Grandi P}^{10}, \text{Dawson MA}^{11,2,8,12}.$

- 1 Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia.
- 2 Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Victoria, Australia.
- 3 Cellzome GmbH, GlaxoSmithKline, Meyerhofstrasse 1, Heidelberg, Germany.
- 4 Chemical Cell Biology Group, Institut Curie, Paris Sciences et Lettres Research University, 26 Rue d'Ulm, 75248 Paris Cedex 05, France.
- 5 CNRS UMR3666, 75005 Paris, France.
- 6 INSERM U1143, 75005 Paris, France.
- 7 Epigenetics Discovery Performance Unit, Immuno-Inflammation Therapy Area Unit, GlaxoSmithKline, Stevenage, UK.
- 8 Centre for Cancer Research, University of Melbourne, Melbourne, Victoria, Australia.
- 9 The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.
- 10 Cellzome GmbH, GlaxoSmithKline, Meyerhofstrasse 1, Heidelberg, Germany. paola.x.grandi@gsk.com mark.dawson@petermac.org.
- 11 Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia. paola.x.grandi@gsk.com mark.dawson@petermac.org.
- 12 Department of Haematology, Peter MaCallum Cancer Centre, Melbourne, Victoria, Australia.

The success of new therapies hinges on our ability to understand their molecular and cellular mechanisms of action. We modified BET bromodomain inhibitors, an epigenetic-based therapy, to create functionally conserved compounds that are amenable to click chemistryand can be used as molecular probes in vitro and in vivo. We used click proteomics and click sequencing to explore the gene regulatory function of BRD4 (bromodomain containing protein 4) and the transcriptional changes induced by BET inhibitors. In our studies of mouse models of acute leukemia, we used high-resolution microscopy and flow cytometry to highlight the heterogeneity of drug activity within tumor cells located in different tissue compartments. We also demonstrate the differential distribution and effects of BET inhibitors in normal and malignant cells in vivo. This study provides a potential framework for the preclinical assessment of a wide range of drugs.

Artificial DnaJ Protein for protein production and conformational diseases.

Sci Rep. 2017 Aug 17

Hishiya A¹, Koya K².

- 1 Sola Biosciences, Inc., 27 Strathmore Road, Natick, MA, 01760, USA. akinori.hishiya@solabio.com.
- 2 Sola Biosciences, Inc., 27 Strathmore Road, Natick, MA, 01760, USA.

For secreted proteins, proper protein folding is essential not only for biological function but also for secretion itself. Proteins with folding problems are trapped in the endoplasmic reticulum (ER) and are eventually degraded in the cytoplasm. In this study, we exploited co-expression of an artificial fusion protein, based on the sequence of a DnaJ protein, which could interact as co-chaperones in the Hsp70-based protein-folding system, with target recombinant secreted proteins to enhance their production and secretion. The J-domain sequence or a fragment thereof was conjugated to a target protein-binding domain that was capable of binding to a portion of the target-protein sequence. Production of many of the target proteins was significantly upregulated when co-expressed with the J-domain fusion protein. Surprisingly, the enhancement of secretion was observed even when the J-domain had a mutation in the HPD motif, which is necessary for J-protein-Hsp70 interactions, suggesting the phenomenon observed is independent on functional J-protein-Hsp70 interactions. This technology has great potential for not only enhancing the production of recombinant proteins, but also to treat conformational diseases such as cystic fibrosis, and Alpha-1 antitrypsin deficiency.

Honorable Mention

Alfalfa-derived HSP70 administered intranasally improves insulin sensitivity in mice.

Cell Stress Chaperones. 2017 Aug 18.

<u>Tytell M</u>¹, <u>Davis AT</u>², <u>Giles J</u>², <u>Snider LC</u>², <u>Xiao R</u>³, <u>Dozier SG</u>³, <u>Presley TD</u>⁴, <u>Kavanagh K</u>².

- 1 Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC, 27157, USA. mtytell@wakehealth.edu.
- 2 Department of Pathology, Section on Comparative Medicine, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC, 27157, USA.
- 3 Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC, 27157, USA.
- 4 Department of Chemistry, Winston-Salem State University, 601 S. Martin Luther King, Jr Drive, Winston-Salem, NC, 27110, USA.

Heat shock protein (HSP) 70 is an abundant cytosolic chaperone protein that is deficient in insulin-sensitive tissues in diabetes and unhealthy aging, and is considered a longevity target. It is also protective in neurological disease models. Using HSP70 purified from alfalfa and administered as an intranasal solution, we tested in whether the administration of Hsp70 to diet-induced diabetic mice would improve insulin sensitivity. Both the 10 and 40 μ g given three times per week for 26 days significantly improved the response to insulin. The HSP70 was found to pass into the olfactory bulbs within 4-6 hours of a single dose. These results suggest that a relatively inexpensive, plentiful source of HSP70 administered in a simple, non-invasive manner, has therapeutic potential in diabetes.

Introgression of heat shock protein (Hsp70 and sHsp) genes into the Malaysian elite chilli variety Kulai (Capsicum annuum L.) through the application of marker-assisted backcrossing (MAB).

Cell Stress Chaperones. 2017 Aug 15

Usman MG¹, Rafii MY^{2,3}, Martini MY⁴, Yusuff OA¹, Ismail MR^{1,4}, Miah G¹.

- 1 Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
- 2 Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia. mrafii@upm.edu.my.
- 3 Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia. mrafii@upm.edu.my.
- 4 Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

Backcrossing together with simple sequence repeat marker strategy was adopted to improve popular Malaysian chilli Kulai (Capsicum annuum L.) for heat tolerance. The use of molecular markers in backcross breeding and selection contributes significantly to overcoming the main drawbacks such as increase linkage drag and time consumption, in the ancient manual breeding approach (conventional), and speeds up the genome recovery of the recurrent parent. The strategy was adopted to introgress heat shock protein gene(s) from AVPP0702 (C. annuum L.), which are heat-tolerant, into the genetic profile of Kulai, a popular high-yielding chilli but which is heat sensitive. The parents were grown on seed trays, and parental screening was carried out with 252 simple sequence repeat markers. The selected parents were crossed and backcrossed to generate F₁ hybrids and backcross generations. Sixty-eight markers appeared to be polymorphic and were used to assess the backcross generation; BC₁F₁, BC₂F₁ and BC₃F₁. The average recipient allele of the selected four BC₁F₁ plants was 80.75% which were used to produce the BC₂F₁ generation. BC₁-P₇ was the best BC₁F₁ plant because it had the highest recovery at 83.40% and was positive to Hsp-linked markers (Hsp70u2 and AGi42). After three successive generations of backcrossing, the average genome recovery of the recurrent parent in the selected plants in BC₃F₁ was 95.37%. Hsp gene expression analysis was carried out on BC₁F₁, BC₂F₁ and BC₃F₁ selected lines. The Hsp genes were found to be up-regulated when exposed to heat treatment. The pattern of Hsp expression in the backcross generations was similar to that of the donor parent. This confirms the successful introgression of a stress-responsive gene (Hsp) into a Kulai chilli pepper variety. Furthermore, the yield performance viz. plant height, number of fruits, fruit length and weight and total yield of the improved plant were similar with the recurrent parent except that the plant height was significantly lower than the Kulai (recurrent) parent.

Elizabeth - August 21

Hayashi S, Nakazaki Y, Kagii K, Imamura H, Watanabe YH.

Fusion protein analysis reveals the precise regulation between Hsp70 and Hsp100 during protein disaggregation.

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Drosophila protease ClpXP specifically degrades DmLRPPRC1 controlling mitochondrial mRNA and translation.

Sci Rep. 2017 Aug 16;7(1):8315. PMID: 28814717 [PubMed - in process]

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Introgression of heat shock protein (Hsp70 and sHsp) genes into the Malaysian elite chilli variety Kulai (Capsicum annuum L.) through the application of marker-assisted backcrossing (MAB).

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Frungillo L, Spoel SH.

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Moloculon Coll

Modulating the Modulator: Regulation of Protein Methylation by Nitric Oxide. Mol Cell. 2017 Aug 17;67(4):535-537. PMID: 28820964 [PubMed - in process]

Wiolecular Cell
Volume 67, Issue 4
Modulating the Modulator: Regulation of Protein Methylation by Nitric Oxide
☐ Pages 535-537
☐ Lucas Frungillo, Steven H. Spoel
Abstract PDF (584 K)
☐ Protein methylation is an important modulator of signal transduction pathways, but
methyltransferases themselves may also be modulated. Hu et al. (2017) demonstrate in this issue
of Molecular Cell that S-nitrosylation selectively modulates enzymatic activity of a protein
arginine methyltransferase vital to abiotic stress tolerance.
☐Nitric Oxide Regulates Protein Methylation during Stress Responses in Plants
☐ Pages 702-710.e4

☐ Jiliang Hu, Huanjie Yang, Jinye Mu, Tiancong Lu, Juli Peng, Xian Deng, Zhaosheng Kong,

Hu et al. report that nitric oxide positively regulates the methyltransferase activity of *Arabidopsis* PRMT5 through *S*-nitrosylation at Cys-125 during stress responses. *S*-nitrosylation at Cys-125 enhances the level of Arg symmetric dimethylation, leading to proper splicing-specific premRNA of stress-related genes and eventually boosting the tolerance to stresses.

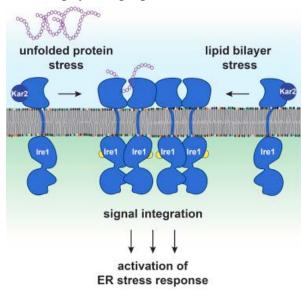
Activation of the Unfolded Protein Response by Lipid Bilayer Stress

Pages 673-684.e8

☐ Kristina Halbleib, Kristina Pesek, Roberto Covino, Harald F. Hofbauer, Dorith Wunnicke, Inga Hänelt, Gerhard Hummer, Robert Ernst

Abstract PDF (5421 K) Supplementary content

☐ The unfolded protein response (UPR) controls the secretory capacity of a cell and is activated by accumulating unfolded proteins in the lumen of the ER. More recently, it became obvious that aberrant membrane lipid compositions of the ER are equally potent in activating the UPR. Halbleib et al. identify a membrane-based mechanism of UPR activation and establish that Ire1, the most conserved transducer of ER stress, uses an amphipathic helix to sense and respond to aberrant physical properties of the ER membrane.



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Structural pathway of regulated substrate transfer and threading through an Hsp100 disaggregase.

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Drought Tolerance in <i>Pinus halepensis</i> Seed Sources As Identified by Distinctive Physiological and Molecular Markers.

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Nitric Oxide and Hydrogen Peroxide Mediate Wounding-Induced Freezing Tolerance through

Modifications in Photosystem and Antioxidant System in Wheat.

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Matsuo Y, Ikeuchi K, Saeki Y, Iwasaki S, Schmidt C, Udagawa T, Sato F, Tsuchiya H, Becker T, Tanaka K, Ingolia NT, Beckmann R, Inada T.

Ubiquitination of stalled ribosome triggers ribosome-associated quality control.

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Nature Chemical Biology Contents: September 2017, Volume 13 No 9 pp 923 - 1052

Protein engineering: Redirecting membrane machinery pp927 - 928

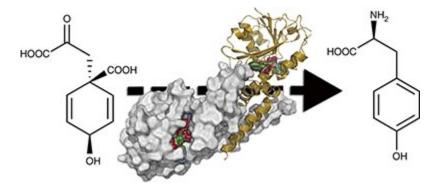
Kalistyn Burley and Celia W Goulding

doi:10.1038/nchembio.2451

The ability to solubilize membrane proteins while retaining their native function is a persistent challenge. Re-engineering of the membrane protein DsbB into a soluble cytoplasmic version maintained its activity and enabled recompartmentalization of the periplasmic DsbAB disulfide bond-forming system.

Molecular basis of the evolution of alternative tyrosine biosynthetic routes in plants pp1029 - 1035

Craig A Schenck, Cynthia K Holland, Matthew R Schneider, Yusen Men, Soon Goo Lee *et al.* doi:10.1038/nchembio.2414

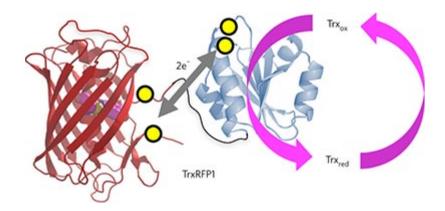


Structural, biochemical, and phylogenetic analyses of plant prephenate and arogenate

dehydrogenases reveal a single residue that defines substrate specificity and sensitivity to product inhibition in two divergent tyrosine biosynthetic pathways.

Monitoring thioredoxin redox with a genetically encoded red fluorescent biosensor pp1045 - 1052

Yichong Fan, Merna Makar, Michael X Wang and Hui-wang Ai doi:10.1038/nchembio.2417



A fluorescent biosensor to monitor thioredoxin (Trx) redox activity called TrxRFP1 was developed by genetically linking Trx1 to the redox-sensitive red fluorescent protein rxRFP1. TrxRFP1 was used to detect redox dynamics induced by various chemicals, serum, or epidermal growth factor.

Archives of Biochemistry and Biophysics: Alert 11 August-18 August\
Association between ROS production, swelling and the respirasome integrity in cardiac mitochondria Original Research

Pages 1-8

Sehwan Jang, Sabzali Javadov

The Plant Journal Content Alert (New Articles)

PALE CRESS Binds to Plastid RNAs and Facilitates the Biogenesis of the 50S Ribosomal Subunit

Jörg Meurer, Lisa-Marie Schmid, Rhea Stoppel, Dario Leister, Andreas Brachmann and Nikolay Manavski

Accepted manuscript online: 14 AUG 2017 07:05AM EST | DOI: 10.1111/tpj.13662

Physiologia Plantarum Content Alert: 161, 1 (September 2017)

<u>Structure and function of complex I in animals and plants – a comparative view (pages 6–15)</u>

Jennifer Senkler, Michael Senkler and Hans-Peter Braun

Version of Record online: 26 APR 2017 | DOI: 10.1111/ppl.12561

An update on the regulation of photosynthesis by thylakoid ion channels and transporters in *Arabidopsis* (pages 16–27)

Cornelia Spetea, Andrei Herdean, Guillaume Allorent, Luca Carraretto, Giovanni Finazzi and

Ildikò Szabo

Version of Record online: 9 JUN 2017 | DOI: 10.1111/ppl.12568

<u>Diversity of strategies for escaping reactive oxygen species production within photosystem I among land plants: P700 oxidation system is prerequisite for alleviating photoinhibition in photosystem I (pages 56–74)</u>

Daisuke Takagi, Kimitsune Ishizaki, Hitomi Hanawa, Tomohito Mabuchi, Ginga Shimakawa, Hiroshi Yamamoto and Chikahiro Miyake

Version of Record online: 24 MAY 2017 | DOI: 10.1111/ppl.12562

Comprehensive single-cell transcriptional profiling of a multicellular organism

Junyue Cao et al.

Science 18 Aug 2017: Vol. 357, Issue 6352, pp. 661-667

To resolve cellular heterogeneity, we developed a combinatorial indexing strategy to profile the transcriptomes of single cells or nuclei, termed sci-RNA-seq (single-cell combinatorial indexing RNA sequencing). We applied sci-RNA-seq to profile nearly 50,000 cells from the nematode *Caenorhabditis elegans* at the L2 larval stage, which provided >50-fold "shotgun" cellular coverage of its somatic cell composition. From these data, we defined consensus expression profiles for 27 cell types and recovered rare neuronal cell types corresponding to as few as one or two cells in the L2 worm. We integrated these profiles with whole-animal chromatin immunoprecipitation sequencing data to deconvolve the cell type—specific effects of transcription factors. The data generated by sci-RNA-seq constitute a powerful resource for nematode biology and foreshadow similar atlases for other organisms.

Current Opinion in Chemical Biology Volume 34, October 2016, Pages 1-10

Encapsulins: microbial nanocompartments with applications in biomedicine, nanobiotechnology and materials science

Encapsulins are a new class of microbial protein nanocompartments with diameters of 24 or 32 nm.

They naturally encapsulate a variety of cargo proteins related to oxidative stress response.

Cargo proteins are directed to the interior of encapsulin cages by C-terminal tags.

Encapsulins can be used as cell-specific optical probes and targeted delivery systems.

Non-native enzymes can be encapsulated within encapsulins in vivo and retain their activity.

<u>Biochemical and synthetic biology approaches to improve photosynthetic CO₂-fixation</u> Review Article

Pages 72-79 Tobias J Erb, Jan Zarzycki

Current Biology: Alert 01 November-08 November

Stomatal Function Requires Pectin De-methyl-esterification of the Guard Cell Wall

Pages 2899-2906

Sam Amsbury, Lee Hunt, Nagat Elhaddad, Alice Baillie, Marjorie Lundgren, Yves Verhertbruggen, Henrik V. Scheller, J. Paul Knox, Andrew J. Fleming, Julie E. Gray