

Lit Lunch: 11/15/13

Damian

1) Interaction between avoidance of photon absorption, excess energy dissipation and zeaxanthin synthesis against photooxidative stress in Arabidopsis

Stefano Cazzaniga^{1†}, Luca Dall'Osto^{1†}, Sam-Geun Kong², Masamitsu Wada², Roberto Bassi^{1*}

Article first published online: 3 OCT 2013

DOI: 10.1111/tpj.12314

© 2013 The Authors The Plant Journal © 2013 John Wiley & Sons Ltd

Indu

1. Science. 2013 Oct 18;342(6156):357-60. doi: 10.1126/science.1241459.

Genomically recoded organisms expand biological functions.

Lajoie MJ, Rovner AJ, Goodman DB, Aerni HR, Haimovich AD, Kuznetsov G, Mercer JA,

Wang HH, Carr PA, Mosberg JA, Rohland N, Schultz PG, Jacobson JM, Rinehart J, Church GM, Isaacs FJ.

Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.

We describe the construction and characterization of a genomically recoded organism (GRO). We replaced all known UAG stop codons in Escherichia coli MG1655 with synonymous UAA codons, which permitted the deletion of release factor 1 and reassignment of UAG translation function. This GRO exhibited improved properties for incorporation of nonstandard amino acids that expand the chemical diversity of proteins in vivo. The GRO also exhibited increased resistance to T7 bacteriophage, demonstrating that new genetic codes could enable increased viral resistance.

PMID: 24136966 [PubMed - in process]

2. Science. 2013 Oct 18;342(6156):361-3. doi: 10.1126/science.1241460.

Probing the limits of genetic recoding in essential genes.

Lajoie MJ, Kosuri S, Mosberg JA, Gregg CJ, Zhang D, Church GM.

Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.

Engineering radically altered genetic codes will allow for genomically recoded organisms that have expanded chemical capabilities and are isolated from nature. We have previously reassigned the translation function of the UAG stop codon; however, reassigning sense codons poses a greater challenge because such codons are more prevalent, and their usage regulates gene expression in ways that are difficult to predict. To assess the feasibility of radically altering the genetic code, we selected a panel of 42 highly expressed essential genes for modification. Across 80 *Escherichia coli* strains, we removed all instances of 13 rare codons from these genes and attempted to shuffle all remaining codons. Our results suggest that the genome-wide removal of 13 codons is feasible; however, several genome design constraints were apparent, underscoring the importance of a strategy that rapidly prototypes and tests many designs in small pieces.

PMID: 24136967 [PubMed - in process]

3. *Science*. 2013 Oct 18;342(6156):369-72. doi: 10.1126/science.1242369. Epub 2013 Oct 3.

Measuring chromatin interaction dynamics on the second time scale at single-copy genes.

Poorey K, Viswanathan R, Carver MN, Karpova TS, Cirimotich SM, McNally JG, Bekiranov S, Auble DT.

Department of Biochemistry and Molecular Genetics, University of Virginia Health System, Charlottesville, VA 22908, USA.

The chromatin immunoprecipitation (ChIP) assay is widely used to capture interactions between chromatin and regulatory proteins, but it is unknown how stable most native interactions are. Although live-cell imaging suggests short-lived interactions at tandem gene arrays, current methods cannot measure rapid binding dynamics at single-copy genes. We show, by using a modified ChIP assay with subsecond temporal resolution, that the time dependence of formaldehyde cross-linking can be used to extract *in vivo* on and off rates for site-specific chromatin interactions varying over a ~100-fold dynamic range. By using the method, we show that a regulatory process can shift weakly bound

TATA-binding protein to stable promoter interactions, thereby facilitating transcription complex formation. This assay provides an approach for systematic, quantitative analyses of chromatin binding dynamics in vivo.

PMID: 24091704 [PubMed - in process]

Keith

1. Science. 2013 Oct 18;342(6156):357-60. doi: 10.1126/science.1241459.

Genomically recoded organisms expand biological functions.

Lajoie MJ, Rovner AJ, Goodman DB, Aerni HR, Haimovich AD, Kuznetsov G, Mercer JA, Wang HH, Carr PA, Mosberg JA, Rohland N, Schultz PG, Jacobson JM, Rinehart J, Church GM, Isaacs FJ.

Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.

We describe the construction and characterization of a genomically recoded organism (GRO). We replaced all known UAG stop codons in *Escherichia coli* MG1655 with synonymous UAA codons, which permitted the deletion of release factor 1 and reassignment of UAG translation function. This GRO exhibited improved properties for incorporation of nonstandard amino acids that expand the chemical diversity of proteins in vivo. The GRO also exhibited increased resistance to T7 bacteriophage, demonstrating that new genetic codes could enable increased viral resistance.

PMID: 24136966 [PubMed - in process]

2. Science. 2013 Oct 18;342(6156):361-3. doi: 10.1126/science.1241460.

Probing the limits of genetic recoding in essential genes.

Lajoie MJ, Kosuri S, Mosberg JA, Gregg CJ, Zhang D, Church GM.

Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.

Engineering radically altered genetic codes will allow for genomically recoded

organisms that have expanded chemical capabilities and are isolated from nature. We have previously reassigned the translation function of the UAG stop codon; however, reassigning sense codons poses a greater challenge because such codons are more prevalent, and their usage regulates gene expression in ways that are difficult to predict. To assess the feasibility of radically altering the genetic code, we selected a panel of 42 highly expressed essential genes for modification. Across 80 *Escherichia coli* strains, we removed all instances of 13 rare codons from these genes and attempted to shuffle all remaining codons. Our results suggest that the genome-wide removal of 13 codons is feasible; however, several genome design constraints were apparent, underscoring the importance of a strategy that rapidly prototypes and tests many designs in small pieces.

PMID: 24136967 [PubMed - in process]

3. *Science*. 2013 Oct 18;342(6156):369-72. doi: 10.1126/science.1242369. Epub 2013 Oct 3.

Measuring chromatin interaction dynamics on the second time scale at single-copy genes.

Poorey K, Viswanathan R, Carver MN, Karpova TS, Cirimotich SM, McNally JG, Bekiranov S, Auble DT.

Department of Biochemistry and Molecular Genetics, University of Virginia Health System, Charlottesville, VA 22908, USA.

The chromatin immunoprecipitation (ChIP) assay is widely used to capture interactions between chromatin and regulatory proteins, but it is unknown how stable most native interactions are. Although live-cell imaging suggests short-lived interactions at tandem gene arrays, current methods cannot measure rapid binding dynamics at single-copy genes. We show, by using a modified ChIP assay with subsecond temporal resolution, that the time dependence of formaldehyde cross-linking can be used to extract *in vivo* on and off rates for site-specific chromatin interactions varying over a ~100-fold dynamic range. By using the method, we show that a regulatory process can shift weakly bound TATA-binding protein to stable promoter interactions, thereby facilitating transcription complex formation. This assay provides an approach for systematic, quantitative analyses of chromatin binding dynamics *in vivo*.

PMID: 24091704 [PubMed - in process]

Stephanie

1) Recent advances in reactive oxygen species measurement in biological systems

[J.F. Woolley](#), [J. Stanicka](#), [T.G. Cotter](#) 

- Tumour Biology Laboratory, Biochemistry Department, Bioscience Research Institute, University College Cork, Ireland

Reactive oxygen species (ROS) play an essential role in facilitating signal transduction processes within the cell. However, the precise details of the redox dynamics involved are not well understood. The generation of ROS is tightly controlled both spatially and temporally within the cell, making the study of ROS dynamics particularly difficult. In order to measure these dynamics, precise tools that can specifically examine the relevant ROS are required. Recent advancements in methodologies for ROS measurement have allowed the study of ROS biology at a level of precision previously unachievable. Here, we discuss improvements to fluorescent ROS dye technologies, genetically encoded ROS reporters, nanoparticle delivery systems, and nanotube ROS probes. These technologies improve specificity, localization and sensitivity over previously available ROS probes.

Fionn

Plant cell

Manipulation and misconduct in the handling of image data.