Principles of Systems Biology—No. 6

This month's Cell Systems Call (Cell Systems 1, 307) shows how interdisciplinary approaches can provide leverage against problems as diverse as tracing cell lineage and understanding massive cellular machines.

Tracing Lineages with Genome Editing

Aaron McKenna, Gregory M. Findlay, and Jay Shendure, University of Washington; James A. Gagnon and Alexander F. Schier, Harvard University

Principles

Determining the lineage by which an organism's cells are related to one another is essential for understanding development. In work recently published (McKenna et al., Science, published online May 26, 2016. http://dx.doi.org/10.1126/science. aaf7907), we describe genome editing of synthetic target arrays for lineage tracing (GESTALT). GESTALT uses genome editing (CRISPR/Cas9) to record a diverse pattern of mutations to a compact barcode within each cell's genome. As these accrue throughout development, we can use mutations that are shared between cells to reconstruct lineage relationships.

Applying GESTALT to zebrafish, we generated and sequenced thousands of barcodes in developing embryos and adult organs. We discovered that most cells of each organ are derived from only a few distinct progenitors. Major clades in the overall lineage are defined by a few early mutations that occur across organs, whereas subclades defined by additional mutations are increasingly restricted to specific germ layers and organs.

"GESTALT uses genome editing ... to record a diverse pattern of mutations to a compact barcode within each cell's genome."

What's Next?

We plan to perform GESTALT in additional model organisms and in disease contexts such as cancer. Optimizing barcode design and CRISPR/Cas9 delivery is essential for tailoring lineage tracing towards specific applications. We seek to unravel the interplay between a cell's lineage and its molecular identity by coupling our approach with single-cell RNA sequencing and epigenetic profiling.

CombiGEM-CRISPR for Deciphering High-Order Combinatorial Genetics

Alan S.L. Wong, University of Hong Kong; Timothy K. Lu, Massachusetts Institute of Technology

Principles

Biological systems are regulated by complex gene networks, but tools to decode these sophisticated networks are limited in scalability and throughput. Previously. we described CombiGEM, a platform for the systematic profiling of combinatorial genetic interactions (Cheng et al., PNAS 111, 12462-12467; Wong et al., Nat. Biotechnol. 33, 952-961). We now integrate CombiGEM with CRISPR gene perturbation reagents to generate barcoded combinatorial guide RNA libraries for high-throughput functional genomics (Wong, et al., PNAS 113, 2544-2549). We used CombiGEM-CRISPR to evaluate 23,409 dual guide RNA combinations targeting epigenetic gene pairs for knockout and screened for pairs that inhibit ovarian cancer cell growth and drug combinations that achieve synergistic anti-tumor effects. Our work demonstrates the feasibility of barcoded multiplexed CRISPRbased screens for functional genomics studies by combining synthetic biology and systems biology strategies.

"CombiGEM ... enables the facile construction and characterization of multiplexed [CRISPR] guide RNA libraries."

What's Next?

CombiGEM is flexible and enables the facile construction and characterization of multiplexed guide RNA libraries that can enact gene knockout, activation, repression, as well as epigenetic perturbations and more. We envision that CombiGEM-CRISPR will allow for the systematic and high-throughput mapping of complex genetic networks, ultimately leading to a deeper understanding of how these networks regulate sophisticated biological functions and the ability to modulate these networks for therapeutic applications.

Controlling Microbial Communities Using Quorum Sensing

Spencer R. Scott and Jeff Hasty, University of California, San Diego

Principles

As engineered monocultures reach their capacity for genetic modification and begin to push the limits of their productivity, synthetic biologists seek to overcome these constraints through the creation of engineered microbial consortia. Growing evidence suggests that engineered communities, through load distribution and specialization, can out-perform monocultures in their ability to produce higher yields, resist invasion, and adjust to harsh environmental fluctuations.

Recognizing the importance of regulatory processes for cooperative behavior, we recently developed quorum sensing communication systems to be used in such consortia (Scott and Hasty, ACS Synth. Biol., published online May 12, 2016. http://dx.doi.org/10.1021/acssynbio. 5b00286). Four two-component quorumsensing systems (lux, tra, las, and rpa) were multiplexed in *E. coli* to characterize all possible cross-interactions amongst their receptors, ligands, and promoters.

This technique elucidated different combinations of quorum-sensing systems that exhibit signal, promoter, or complete orthogonality—characteristics that will facilitate the development of well-controlled microbial communities.

"... orthogonality ... will facilitate the development of well-controlled microbial communities."

What's Next?

The development of microbial consortia is limited both by the genetic tools available and the number of lab-ready organisms capable of genetic modification. Adding different organisms to synthetic biologists' repertoire as well as expanding on the number of characterized quorum-sensing systems will progress this new field's ability to address issues that monocultures can't, such as metabolic overload, byproduct toxicity, and unique chemical synthesis.



Cell Systems **Cell Systems Call**

Cooperative Cross-Feeding in the Gut Microbiota

Seth Rakoff-Nahoum, Boston Children's Hospital and Harvard Medical School; Kevin Foster, Oxford University

Principles

The evolution of cooperation is fundamental to the history of life on earth, driving the origin of genomes, multicellular organisms, and societies. Cooperation is also well known in microbes, in which cells secrete enzymes and other molecules to feed others around them. We asked whether cooperation has evolved within members of the mammalian gut microbiota (Rakoff-Nahoum et al., Nature 533, 255-259). In the Bacteroidales, the most abundant order of human gut bacteria, we found that one species breaks down sugars outside the cell without sharing much with others. By contrast, a second species not only digests polysaccharides outside the cells, it also releases copious amounts of the breakdown products. Moreover, we find that it does this to feed another species of Bacteroidales for mutual gain. This looks like an example of evolved cooperation, analogous to classic examples like plant-feeding nectar to a pollinator.

"We asked whether cooperation has evolved within members of the mammalian gut microbiota."

What's Next

While we have uncovered some simple rules of interaction with the human gut microbiota, it is an extremely complex system and there is much yet to understand. The next steps are to put such pairwise interactions within the context of evolving ecological networks.

Thresholds and Ultrasensitivity from Negative Cooperativity

Sang Hoon Ha and James E. Ferrell, Jr., Stanford University

Principles

The idea of cooperativity in the interaction of multi-subunit proteins with their ligands occupied the minds of some of the greatest biochemists of the last century. In the case of positive cooperativity, the binding of one ligand makes it easier for a second ligand to bind. In the case of negative cooperativity, the binding of one ligand makes it harder for a second ligand to bind.

The standard theory on negative cooperativity says that the higher the negative cooperativity, the more graded the receptor's response. It turns out that there is a little algebraic shortcut built into the standard theory, but in many situations, especially intracellular signaling, this assumption does not hold.

If one re-derives the theory without this shortcut, the results defy expectation (Ha and Ferrell, Science 352, 990-993). Negative cooperativity can endow a receptor's response with a marked threshold, making it so that there will be no response until the ligand concentration is high enough to occupy half of the binding sites. This phenomenon was also observed in a series of synthetic biology experiments designed to test the theory.

"Negative cooperativity can endow a receptor's response with a marked threshold."

What's Next?

The literature reports that a number of receptors exhibit strong negative cooperativity in their ligand binding. It would be very nice to know whether those receptors end up with thresholds in their responses as a result.

Connecting the Proteome to the Genome in Breast Cancer

Philipp Mertins and Steven Carr, the Broad Institute of MIT and Harvard; Matthew Ellis, Baylor College of Medicine **Principles**

The Cancer Genome Atlas produced an extensive catalog of recurrent somatic mutations in cancer, but effects of these mutations on oncogenic pathways are often uncharacterized. Only a few mutated genes are true "drivers" of cancer-many more are merely "passenger" mutations with little functional consequence. Narrowing the list of candidate genes by studying their protein products and modifications could provide new biological insights and help identify therapeutic strategies. We carried out a large-scale mass-spectrometry-based proteogenomic study to define the proteomes and phosphoproteomes of breast cancer samples and integrated these data with patient-specific genomic and transcriptomic data (Mertins et al., Nature, 534, 55-62). We identified several candidate regulatory genes with copy-number alterations, including aberrantly activated kinases that are potential new therapeutic targets, and created a publically accessible resource for follow-up studies.

"We need to test the proposition that proteomics integrated with genomics will produce additional insights into metastasis and resistance to therapy."

What's Next?

The technology and informatics is now here to design much larger studies in the setting of patient samples associated with outcomes and controlled treatment. We need to test the proposition that proteomics integrated with genomics will produce additional insights into metastasis and resistance to therapy. In addition, we should characterize newly identified aberrantly activated signaling proteins, especially kinases and other enzymes such as ubiquitinases, to further validate them as therapeutic targets. Proteogenomic integration could one day prove to be a powerful clinical tool, allowing us to traverse the large knowledge gap between cancer genomics and clinical action.

Cell Systems **Cell Systems Call**

Synchronized in Translation

Mary T. Couvillion and L. Stirling Churchman, Harvard Medical School **Principles**

Within the mitochondrial matrix, a small, compact genome is expressed by a set of machinery that is entirely distinct from their nuclear/cytosolic counterparts. This genome encodes a handful of proteins that serve as the core subunits of the oxidative phosphorylation (OXPHOS) complexes that reside within the inner membrane. Nuclear-encoded OXPHOS subunits assemble with the mitochondrial-encoded cores to produce functional complexes responsible for producing much of the cell's ATP. We asked whether the two genomes coordinate their gene expression during mitochondrial biogenesis by comprehensively following transcription and translation across cellular compartments (Couvillion et al., Nature 533, 499-503).

We found that, in Saccharomyces cerevisiae, OXPHOS transcript levels from each genome increase but with vastly different kinetics. In contrast, translation regulation is synchronized so that both cytosolic and mitochondrial ribosomal pools redistribute across OXPHOS mRNAs in a coordinated fashion to initiate biogenesis. Furthermore, mitochondrial translation does not respond directly to environmental cues but is orchestrated by the cytosolic translational response. Our whole-cell genomic profiling approach establishes a foundation for global gene regulatory studies of mitochondrial biology.

nuclear-mitochondrial promotes co-regulation cellular homeostasis and function."

What's Next?

Until now, how the mitochondrial genome contributes to physiological processes has been largely ignored. A new focus on this small but critical genome will reveal how nuclear-mitochondrial coregulation promotes cellular homeostasis and function.

Genetic Interactions Illuminate RNA Structure

Grzegorz Kudla, University of Edinburgh **Principles**

A successful strategy for mapping molecular structures relies on the detection of coupling between pairs of residues, through methods as diverse as NMR, chemical crosslinking, or evolutionary analysis. We reasoned that saturation mutagenesis of a gene combined with a high-throughput functional assay could provide an independent source of structurally relevant coupling information. We measured the fitness of \sim 60,000 randomly mutated variants of a short nucleolar RNA gene in yeast (Puchta et al., Science 352, 840-844). Analysis of these data revealed the effects of all possible individual mutations and a network of genetic interactions between pairs of mutations. We used the genetic interaction data as structural constraints to improve prediction of RNA secondary structure.

"... saturation mutagenesis of a gene, combined with a high-throughput functional assay, could provide structurally relevant coupling information."

What's Next?

Future studies will generate detailed genotype to phenotype maps for medically relevant RNA- or protein-coding genes, aiding in the identification of diseasecausing mutations. The sequence and phenotype data can be integrated with structural analysis to illuminate the principles of RNA and protein folding. The combination of genetic interaction data with structure modelling software might help resolve three-dimensional structures of RNAs, proteins, and protein complexes.

Biophysical Principles of Biomachines

Rob Coalson and David Jasnow, University of Pittsburgh; Roderick Lim and Larisa Kapinos, University of Basel; Anton Zilman, University of Toronto

Principles

Nuclear pore complexes (NPCs) are multiprotein "biomachines" that control nucleocytoplasmic transport in eukaryotic cells. The centerpiece of NPC structure is the assembly of intrinsically disordered proteins, FG nucleoporins, which transiently bind cargo-carrying transport proteins and enable their selective translocation, while preventing entry of nonspecific molecules. It is hard to visualize the FG nucleoporins in vivo, and it is still unclear how their collective conformational dynamics are modulated by the transport proteins. In vitro experiments provide contradictory results, giving rise to conflicting hypotheses. We have developed a theoretical model incorporating just three basic physical properties-FG nucleoporin flexibility, inter-chain interactions, and transport protein binding. Our model explains the apparent contradictions observed in vitro-from single molecules to macroscopic assemblies-and might reconcile the conflicting hypotheses (Vovk et al., eLife 5. http://dx.doi. org/10.7554/eLife.10785).

"The biophysical principles ... can be applied to design of bio-synthetic nanopores."

What's Next?

The generality of our results might explain how NPC transport mechanism is universally conserved across different species despite variations in molecular structure and protein sequences. The biophysical principles that underpin the behaviour of NPC protein assemblies may apply to other complex disordered protein machines and can be applied to design of bio-synthetic nanopores. Further refinements to our model will include incorporating further molecular details of the interactions between the NPC constituents in realistic geometries.

Cell Systems **Cell Systems Call**

E2F and NF-κB Protein Interactions **Determine Cell Fate**

Nicholas A. Jones, David G. Spiller, and Michael R.H. White, University of Manchester; John M. Ankers, University of Liverpool

Principles

Physiological control of cell growth and survival requires accurate adaptation to the environment. Tumor necrosis factor alpha activates the dynamic response of the transcription factor nuclear factor kappa B (NF-κB) (Nelson et al., Science 306, 704-708) This response is heterogeneous between individual cells (Turner et al., JCS 123, 2834-2843). Using livecell microscopy and iterative mathematical modelling, we showed that heterogeneity is in part cell cycle dependent (Ankers et al., eLife 5, e10473). NF-κB responses were stronger at the transition between G1 and S-phase but were reduced in S-Phase, compared to other cell cycle phases. E2F-1, the key regulatory transcription factor that accumulates at G1-S phase interacts with NF-κB. These transcription factors regulate each other's transcriptional activity at key target genes. During S-phase, E2F-4 binds to NF-κB and represses its activation. These findings suggest direct interactions between E2F proteins and NF-κB prioritise cell division or inflammation.

"Using live-cell microscopy and iterative mathematical modelling, we showed that heterogeneity is in part cell cycle dependent."

What's Next?

This work describes novel mechanisms that underpin direct communication between key regulators of the cell cycle and inflammation. This is important for understanding the basis of therapeutic treatment of inflammatory disease and how chronic inflammation may contribute to other diseases such as cancer. Stress and inflammatory signals at the G1-S checkpoint may differentially regulate gene expression and other cell fate choices, including cell division and apoptosis.