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This month: Plants take center stage with fascinating insights into disease susceptibility and engineered pathways for photosynthesis. Also, a wearable stethoscope, new noncanonical miRNA targeting rules, Hsf1, and transcription.

A New Plant-Pathogen Battleground Discovered

Xiu-Fang Xin, Kinya Nomura, and Sheng Yang He, HHMI, Michigan State University

Principles

How does a plant disease happen? The answer is as complicated as attempting to answer the question of how an infectious human disease occurs. A wet environment is known to be necessary for the development of many bacterial diseases in plants, as described in the famous "disease triangle" (host-pathogen-environment) dogma more than 50 years ago, but the underlying molecular basis is not clear. In this study, we discovered that high humidity is needed for a bacterial pathogen, *Pseudomonas syringae*, to establish an aqueous living space inside infected plant leaves (in which bacteria multiply). We identified specific bacterial virulence proteins that establish the aqueous living space. In addition, we discovered that plants can mount a specific form of immune response that effectively blocks bacterial establishment of the aqueous living space, suggesting that plant and pathogen fight for controlling water as a previously unrecognized battle in plant-pathogen interactions (Xin et al., Nature *539*, 524–529).

> *"… plant and pathogen fight for controlling water as a previously unrecognized battle …."*

What's Next?

This foundational study could lead to several future research directions. One direction would be to understand how bacterial virulence proteins establish the aqueous living space in the plant leaf. Another would be to answer how plant immune response inhibits bacteria from creating the aqueous living space. Finally, one can think about whether this basic knowledge can be used to design new strategies for controlling plant diseases.

CETCH Me if You Can: Constructing Artificial Metabolic Networks for the Fixation of CO_2

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Principles

Can we beat the serendipity of evolution? Can we reconstruct fundamental new metabolism based on rational considerations? To answer these questions we sought to realize an artificial pathway for the conversion of CO_2 in a reductionist approach from principal components (Schwander et al., Science *354*, 900–904).

During evolution, Nature has evolved at least six different pathways for the fixation of CO_2. Inspired by this natural diversity, we designed several non-natural, hypothetical CO_2 fixation cycles following fundamental chemical rules. In a next step, we then reconstituted one of these theoretical pathways, the CETCH cycle, as a proof of principle.

The CETCH cycle is an in vitro-metabolic network of 17 reactions that was established with enzymes originating from nine different organisms. The cycle was optimized in several rounds by enzyme engineering and metabolic proofreading. CETCH version 5.4 is slightly faster and requires 20% less energy per CO_2 fixed than the Calvin cycle.

> *"The CETCH cycle is an in vitro-metabolic network of 17 reactions that was established with enzymes from nine different organisms."*

What's Next?

How important are metabolite channeling, compartmentalization, and allosteric control in metabolic networks, and how do we realize such features synthetically? With the CETCH cycle, we will be able to address these questions in a defined in vitro-model system. The grand challenge, however, will be to transplant our artificial CO_2 fixation cycle into living cells. Only this will prove whether we have understood the design principles to realize "metabolism 2.0."

Tinkering with Photosynthesis

Johannes Kromdijk, Katarzyna Głowacka, and Stephen P. Long, University of Illinois; Laurie-beth Leonelli, Stéphane Gabilly, Masakazu Iwai, and Krishna K. Niyogi, University of California Berkeley

Principles

Photosynthesis is well known as the process which converts sunlight energy, through CO_2 assimilation and reduction, directly or indirectly into our entire food supply. However, the process is also notoriously inefficient (1%–2% efficiency in our best crops). Yet evidence suggests that the theoretical efficiency is close to 10%. Computer simulation of the complete process from metabolism at the leaf level to the crop canopy suggested several potential routes to improvement (Long et al., Cell *161*, 56–66). This analysis identified the speed at which photoprotection adapts to shade (as a cloud obscures the sun or one leaf moves into the shade of another, for example) as critical for photosynthetic efficiency, costing a crop up to 40% of potential productivity (Zhu et al., J. Exp. Bot. *55*, 1167–1175).

Mutant analysis identified several genes involved in dynamic adjustment of photoprotection (Li et al., Nature *403*, 391–395; Niyogi et al., Plant Cell *10* 1121–1134). Recently, we overexpressed three of these genes in parallel (photosystem II subunit S, violaxanthin de-epoxidase, zeaxanthin epoxidase) in tobacco plants. The increased expression accelerated the rate of adjustment of photoprotection and improved photosynthetic efficiency by 15%. Most importantly, in three independent transformants, this was shown to result in increased productivity of 14%–20% in replicated plot field trials (Kromdijk et al., Science *354*, 857–861).

> *"This analysis identified the speed at which photoprotection adapts to shade … as critical for photosynthetic efficiency, costing a crop up to 40% of potential productivity."*

What's Next?

To show that this also works in important food crops, we are transforming and testing it in cowpea, cassava, rice, maize, and soybean.

Cell Systems Call

Wearable Stethoscope that Can Listen to the Body Sounds

Yuhao Liu, University of Illinois at Urbana-Champaign; Jae-Woong Jeong, University of Colorado Boulder

Principles

The stethoscope is the conventional tool that can be used to listen to the body sounds made by organs, such as the hearts and lungs, for the purpose of medical examination. However, typical bulky and rigid mechanical or electrical stethoscopes are limited in their ability to capture continuous, low-amplitude body acoustics.

We recently described an ultralight, soft, and stretchable form of mechano-acoustic sensors that can be attached on almost any part of the skin for body sound sensing (Liu et al., Sci. Adv. *2*, e1601185). It uses an accelerometer chip embedded on stretchable metal interconnects and encapsulated in soft silicone to capture physiological acoustic signatures through vibrations of the skin surface. The low areal mass density and low modulus of the device allow robust mechanical coupling between the skin and the sensor, enabling high-precision measurement. Our wearable sensor is capable of monitoring heart sounds, detecting cardiovascular defects, and enabling speech recognition and voice-activated machine controls. Combined with wireless technology, we envision our sensor can potentially enable telemedicine to provide ubiquitous healthcare and monitoring.

"… we envision our sensor can potentially enable telemedicine to provide ubiquitous healthcare and monitoring."

What's Next?

Future research and development should focus on seamless connectivity, wireless powering, and on-board data processing to improve user experience and device performance. More clinical studies are needed to expand use cases beyond cardiology and to establish acoustic databases for diagnosing disease states.

Deciphering the General Rules for Functional MicroRNA Targeting

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Principles

MicroRNAs (miRNAs) are short, ~22nt RNA molecules that form a complex with the Argonaute protein and target mRNAs to regulate their gene expression as a fine tuner. Previous studies have demonstrated that miRNAs target mRNAs primarily through four canonical interactions involving 6–7 nt Watson-Crick pairings. However, these previous efforts have examined only a fraction of the numerous interactions that can potentially occur between miRNAs and target mRNAs. We used large-scale microarray data to systematically evaluate over two billion miRNA-mRNA interactions to determine the functional rules for which miRNAs govern the transcriptome. Accordingly, we discovered seven non-canonical interactions in addition to the four canonical interactions, while ruling out numerous false positives from previous studies. The non-canonical interactions were functionally validated by additional experiments and by analyzing independent microarrays. Overall, we found that the estimated impact on the transcriptome exerted by the non-canonical interactions may be comparable to that of the canonical interactions (Kim, D. et al., Nat. Genet. *48*, 1517–1526).

"… the intricate nature by which miRNAs impact the gene regulatory network may be far more complex than currently perceived."

What's Next?

Our massive-scale, systematic evaluation of functional miRNA targeting expands not only the functional targeting repertoire of the miRNAs but also demonstrates that the intricate nature by which miRNAs impact the gene regulatory network may be far more complex than currently perceived. These findings offer a step closer to unraveling the comprehensive gene regulatory network and provide aid to future improvements for miRNA target prediction.

Heat Shock Signaling, Fast and Slow

David Pincus, Whitehead Institute; Ahmad S. Khalil, Boston University

Principles

Heat shock factor 1 (Hsf1) activates genes that encode molecular chaperones to maintain protein homeostasis (proteostasis). Despite putative roles in cancer and neurodegenerative diseases and its conservation in all eukaryotes, the mechanisms that regulate Hsf1 activity have remained unresolved. Recently, we combined experimental and theoretical approaches to arrive at a quantitative model of Hsf1 regulatory dynamics during heat shock in budding yeast (Zheng et al., eLife *5*, e18638). We showed that Hsf1 is controlled by two orthogonal mechanisms: a fast-acting chaperone feedback loop and a delayed phosphorylation tuner. Under basal conditions, the Hsp70 chaperone binds to and represses Hsf1. During heat shock, unfolded proteins titrate Hsp70 away to "switch on" Hsf1; free Hsf1 then induces more Hsp70, establishing the negative feedback loop. Hsf1 also becomes phosphorylated, which is dispensable for the activation switch. This occurs with delayed kinetics relative to Hsp70 dissociation and sustains Hsf1 activity over longer timescales. Together, these dual regulatory modes allow Hsf1 to flexibly control the proteostasis machinery according to need.

"Hsf1 is controlled by two orthogonal mechanisms: a fast-acting chaperone feedback loop and a delayed phosphorylation tuner."

What's Next?

Next steps will be to probe the Hsp70-mediated regulatory model by breaking the transcriptional feedback loop and mapping/disrupting Hsp70 binding sites on Hsf1. Additionally, if phosphorylation is not necessary to activate Hsf1, what other roles is it playing? We will investigate the functional roles of Hsf1 phosphorylation during other stresses and in controlling cell-to-cell variation in the heat shock response.

Combining Cancer Genomics with Single-Cell Transcriptomics

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Principles

Recent studies have used single-cell RNA sequencing (scRNA-seq) to profile gene expression dynamics at fine resolution. In nonmalignant tissues, scRNA-seq produces a transcriptional "snapshot" of different cellular states that can be used to infer cell lineage relationships, and the sequence of gene expression changes that occur during lineage commitment.

However, the application of this approach for cancer tissues depends on reconstructing lineage relationships that respect a clonal ordering of the mutations in single cells. Successful integration of genomics information provides novel opportunities to address this challenge. The ability to precisely specify the contribution of infiltrating stromal cells to gene expression, or the ability to map gene expression signatures to tumor subclones and phylogenies, are only two of many examples. We developed an approach combining scRNA-seq with exome-sequencing, to triage individual cells by inferred clone of origin. We applied this approach to compare the transcriptional kinetics of tumor evolution, between EGF and PDGF driven gliomas. In so doing, we identified a high-frequency family of gain-of-function deletions in the PDGF-receptor (Müller et al., Mol. Sys. Bio. *12*, 889).

"… the ability to map gene expression signatures to tumor subclones and phylogenies …."

What's Next?

We are applying this approach to map cell types and states in the glioma microenvironment, and to understand the effect of treatment on glioma evolution.

Transcription Can Stress Out

Stuart Sevier and Herbert Levine, Center for Theoretical Biological Physics, Rice University

Principles

An understanding of transcription is of paramount importance for determining cellular behavior. Several decades of work has revealed that stochastic processes play a central role in transcription. Yet, the source of transcriptional bursting, one of the most well-known and universal aspects of this stochasticity, has continued to evade understanding.

Recent experiments have pointed to mechanical feedback as a possible source of transcriptional bursting. This feedback arises because of DNA helicity; as the RNA polymerase attached to the growing mRNA chain moves, it either must turn or twist the DNA. Rotating is hard for a large object and twisting becomes progressively more difficult as the DNA becomes supercoiled ahead of the polymerase. In our tractable model (Sevier et al., PNAS, published online on November 22, 2016. http://dx.doi.org/10.1073/pnas.1612651113), these effects cause stalling of transcriptional events until the twist is relieved by the action of a topoisomerase, giving rise to the aforementioned transcriptional bursts.

The interplay between stress-induced arrest and relaxation very naturally explains data on the inevitable rise of the Fano factor (a measure of non-Poissonian noise) with mean. And surprisingly, the solution of our tractable model predicts universal curves for intrinsic noise versus mean expression level.

"The interplay between stress-induced arrest and relaxation very naturally explains data on the inevitable rise of the Fano factor …."

What's Next?

To study how these mechanical aspects give rise to gene-gene interactions and contribute to the formation of various kinds of chromatin structure.

Minimizing the Cost of Living

Idan Frumkin, Dvir Schirman, and Yitzhak Pilpel, Weizmann Institute of Science

Principles

Natural selection dictates the expression level of genes according to the required cellular concentration of each protein. A fundamental question is how cells can achieve a specific expression level of a gene while minimizing its expression costs.

While genes differ in functions, their production cost is probably governed by similar mechanisms, giving hope for a study of universal costs components. To uncover molecular processes that determine expression cost, we measured fitness of ~14,000 *E. coli* strains, each expressing a reporter gene with a unique 5′ architecture (Frumkin et al., Mol. Cell, published online on December 15, 2016. http://dx.doi.org/10.1016/j.molcel.2016.11.007). By comparing cost-effective and cost-ineffective architectures, we found elements that reduce cost per protein molecule. These mechanisms include lowering transcription levels, regulating translation speeds, and utilizing amino acids that are less hydrophobic and cheap-to-synthesize. We then constructed a model that predicts the cost-effectiveness of a given 5′ sequence, which revealed that natural selection prefers the cost-minimization of highly expressed genes.

"To uncover molecular processes that determine expression cost, we measured fitness of ~14,000 E. coli strains, each expressing a reporter gene with a unique 5′ architecture."

What's Next?

To fully decipher the cost of living, we should uncover more mechanisms that govern expression cost. A more detailed investigation into the rate of protein and mRNA production and degradation should provide an even better model for cost. Our work has focused on protein expression cost. Yet, not all expressed genes are protein coding and many function as non-coding RNAs. This calls for further investigations regarding mechanisms that specifically minimize RNA production cost.