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MEETING REPORT

Biology by the numbers on the Hawaiian Islands

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One could imagine it might be hard to focus on science at a beautiful location such as the famous Waikiki Beach on Oahu Island, Hawaii. Amazingly, however, that's just what more than 330 participants did at the First Annual Winter Q-Bio Meeting at the Hilton Hawaiian Village resort February 18–21, 2013.

The interdisciplinary field of quantitative biology (q-bio) has expanded tremendously in the last several years, especially with the growth of systems and synthetic biology as integral components of q-bio. The organizers for this first Winter Q-Bio meeting were led by Jeff Hasty (University of California, San Diego; San Diego, USA) and included Bill Ditto (University of Hawaii, Manoa, USA), William Hlavacek (University of New Mexico, Albuquerque, USA), Alex Hoffmann (University of California, San Diego; San Diego, USA), Brian Munsky (New Mexico Consortium, Los Alamos, USA) and Lev Tsimring (University of California, San Diego; San Diego, USA). The idea was to capitalize on the growing interest in the intersection of the physical and biological sciences by bringing together scientists, from all different backgrounds and expertise, interested in quantitative principles of biological systems. In this report, we have highlighted a few themes that we took home from the meeting.

SYNTHETIC BIOLOGY 2.0: MAKING SYNTHETIC BIOLOGY MORE ROBUST

Even for those attendees who may have spent more time on their surfboards than attending the lectures, it would be hard not to highlight synthetic biology as one of the themes of the meeting (it was, after all, the focus of this year's conference). Speakers illustrated how quantitative approaches could make synthetic biology more efficient, more robust, and facilitate scaling-up for industrial applications.

J. Craig Venter (J. Craig Venter Institute, Rockville, USA) provided an overview of the intersection of DNA sequencing and synthesis with synthetic biology. His talk spanned from the past (such as sequencing the first genomes) to future aspirations in synthetic biology (e.g., emailing and printing personal vaccines on demand). Techniques (including Gibson assembly), improvements in automation, and use of combinatorial approaches now make it possible to construct very large DNA sequences without introducing errors. Venter compared life itself to a computer; if we change the software (e.g., the DNA), we can also change the species. Of course, many challenges remain in trying to achieve such lofty goals – if we introduce a synthetic chromosome, how do we ensure it will "boot up"? And how can we achieve ultimate digital to biological conversion, analogous to what we now have in the material world with the advent of 3D printers?

Similarly, Chris Voigt (Massachusetts Institute of Technology, Boston, USA) took us through the development of a programming language for cells, focusing on simple circuit design. He discussed the challenges in going beyond simple circuits to more complex systems in which cells utilize nuanced signal processing. These synthetic regulatory systems require a precise balance of biochemical parameters and are limited by orthogonality

– components are mixed together within the cell, and crosstalk can cause these systems to fail (see the "burrito problem" in the conclusions below). To address these issues, Voigt argued for a need for more computer-aided design (CAD) – followed by cycles of synthesis, quantitative measurements, and further CAD – in building more robust circuits.

Timothy Gardner (Amyris Inc., Emeryville, USA), one of the few representatives from industry at the conference, advocated for applying "Six Sigma" approaches to synthetic biology. Amyris started out as a company focused on the development and production of artemisinic acid, a precursor to a potent antimalarial drug artemisinin normally derived from sweet woodworm but which now can be produced in engineered yeast using synthetic biology approaches. Scaling-up this reengineered biological production system initially proved challenging. The solution turned out to be improved precision and quality control, revealing quantitative differences between synthetic strains that had originally been lost in the noise. Gardner noted that if similar improvements to experimental reproducibility were applied to the development of new therapeutics, companies could save time and millions of dollars.

Wendell Lim (University of California, San Francisco, San Francisco, USA) discussed the use of synthetic biology to elucidate the design principles of cell polarization. He and his colleagues have identified simple networks (positive feedback, mutual inhibition and inhibitor with positive feedback) that are capable of achieving polarization [1]. These minimal motifs could achieve polarization in a coarse–grained computational model, but circuits combining two or more of the minimal motifs were much more robust. Furthermore, when these motifs were tested experimentally, the cells were able to form poles. In the second half of his talk, he discussed collaborative work with Lei Stanley Qi (University of California, San Francisco; San Francisco, USA) on a promising technology platform for future synthetic biology and bioengineering approaches, which repurposes the bacterial immunity system clustered regularly interspaced palindromic repeats (CRISPR) for controlling gene expression [2]. A catalytically dead endonuclease, dCas9, from the CRISPR system, along with a short guide RNA, was used to inhibit gene expression in both bacteria and mammalian cells. The system could reversibly suppress multiple target genes at the same time, and thus is likely be extremely valuable for synthetic biology approaches on a genome-wide scale.

LIMITED RESOURCES: IMPLICATIONS OF SUBCELLULAR CONSTRAINTS FOR CELLULAR BEHAVIORS

The myriad constraints and tradeoffs that physics imposes are essential to successfully describe subcellular properties (see Berg and Purcell [3] and Strong et al. [4] for classical work in this area and Lan et al. [5] for a more recent perspective). Thanks to high-throughput technologies, biologists have an abundance of information on subcellular processes, ranging from metabolism to genetics to molecular interactions. A central goal of systems biology is to infer macroscopic physiological properties of cells from these mechanistic details; the related field of synthetic biology searches for design principles that underlie cellular behaviors and would aid in their reengineering. Work presented at the Winter Q-Bio conference addressed whether the tradeoffs imposed at the subcellular level inform constraints at the level of the whole cell.

Terry Hwa (University of California, San Diego; San Diego, USA) emphasized the central role of growth laws in extrapolating physiological constraints. Briefly stated, growth laws explain how ratios of protein concentrations constrain the growth rate of microorganisms [6]. These constraints can be naturally incorporated into flux balance analysis, which has traditionally optimized metabolic fluxes to maximize growth rate. Hwa used a combination of traditional experiments and growth rate measurements to show that cells make simple and direct tradeoffs in protein expression in response to nutrient deprivation. Hwa explained how this type of analysis is the first step toward a realistic and quantitative understanding of how proteins constrain macroscopic properties such as growth rate.

Ned Wingreen (Princeton University, Princeton, USA) discussed how enzymatic networks may spatially reorganize within a cell to deal with a crucial constraint such as toxic intermediates. He hypothesized that enzymes within a cell colocalize to form clusters; the optimal cluster radius is determined by a tradeoff between the benefits of the pathway output and the toxicity of the intermediate. Theoretical studies suggest multiple medium-sized enzyme clusters throughout the cytosol are optimal for conditions commonly found in microorganisms. If true in practice, this suggests chemical constraints have far reaching consequences.

Strongly correlated outputs of biochemical pathways are often used as evidence of direct physical interactions or shared transcription. Ruth Williams (University of California, San Diego; San Diego, USA) emphasized that such correlations may also arise indirectly, through competition for limited resources. For instance, if the number of ribosomes in a cell is limited, then protein concentrations from vastly different pathways may become correlated over time as proteins are produced. Or, if many distinct protein species are degraded by a common scarce protease, over time their concentrations may become strongly correlated. In a beautiful connection

between theory and experiment, Williams applied techniques from queuing theory (commonly used in engineering to model propagation delay in traffic jams and computer networks [7]) to analyze a synthetic model system [8].

CONTROL AND COORDINATION OF TEMPORAL AND SPATIAL EVENTS IN CELLS

A popular topic in systems biology is the control and coordination of various events in the cell cycle. In this context, John Tyson (Virginia Polytechnic Institute and State University, Blacksburg, USA) gave an overview of five irreversible transitions in terms of nonlinear dynamics. Transitions between critical events in the cell cycle, such as initiation of DNA replication, are made irreversible by feedback loops that serve as bistable switches. Tyson examined bistability and irreversibility in a common double-negative feedback motif between a kinase (Cdc28) and an inhibitor that is degraded in response to phosphorylation by that kinase (Sic1). Tyson observed that multisite phosphorylation provides a robust mechanism for irreversible switches, and could play a role in other decision-making events in the cell.

START, known as restriction point in mammalian cells, is the transition during which yeast commits to complete the cell cycle. It has long been thought that passage through START was triggered by cell size. Chao Tang (Peking University, Beijing, China) reported that the trigger is actually an unstable kinase, Cln3-Cdk1, a sensitive indicator of internal and external conditions. By placing Cln3 under the control of an inducible promoter, Tang and colleagues demonstrated that the activity of Cln3-Cdk1 is simply recorded through multi-site phosphorylation of Whi5. In other words, phosphorylated Whi5 level is proportional to the integral of the Cln3-Cdk1 signal. Phosphorylated Whi5 is then excluded from the nucleus, flipping the state of Whi5-Cln1/2-Cdk1 double-negative feedback loop. Lower Cln3-Cdk1 activity generates lower levels of phosphorylated Whi5, which in turn, lengthens the G1 phase. We learned from this talk that the cell size control is a by-product of the slow integration of Cln3-Cdk1 activity by Whi5.

There were also several contributed talks and posters on the same theme. For example, Sylvia Santos (Stanford University, Stanford, USA) spoke about a positive feedback that cyclin B1-Cdk1 forms with itself. Phosphorylation loop of Cdk1-cyclin B promotes its translocation from cytoplasm to nucleus, and increasing nuclear concentration of cyclin B1-Cdk1 further promotes its auto-phosphorylation. Essentially, Santos reported that the redistribution process acts as a spatial positive feedback loop, contributing to the irreversibility of the mitotic entry [9]. Cyclin B1-Cdk1 was also mentioned in a contributed talk by Qiong Yang (Stanford University, Stanford, USA). It is known that Cyclin B1-Cdk1 forms a negative feedback with APC-Cdc20, generating robust oscillations. Yang reported that the high degree of ultrasensitivity in this feedback loop is responsible for the robustness of the oscillations.

James Ferrell (Stanford University, Stanford, USA) spoke on the spatial coordination of mitosis in cells. Cells in frog eggs divide in exquisite synchrony, and the timing of each round is extremely precise. However, due to the large size of the frog cells, such coordination can not occur simply via diffusion of a signaling molecule. Ferrell explained how a bistable medium can accelerate information transfer. Once a nucleating event has activated enough cyclin B1-Cdk1, the kinase not only sustains its own activity, but also generates a wave that ripples through the bistable medium as regions with high and low concentrations of active cyclin B1-Cdk1 mix, and flip to the high state. Such "trigger waves" are able to spatially coordinate the cell's cleavage because they do not slow down with distance unlike diffusive signals. Ferrell's talk provided another example of how simple mechanisms can give rise to robust biologic processes.

DYNAMICS MATTER: LEARNING HOW CELLS READ AND WRITE DYNAMIC SIGNALS

It has become clear that biology can encode information, not merely through the presence or absence of a particular molecule, but also in that molecule's temporal behavior. This idea has truly come of age as technologies for following signaling dynamics in single cells [10] have revealed the power and ubiquity of dynamic signaling [11]. Several of the presenters at the Winter Q-Bio conference (including Galit Lahav, Harvard Medical School, Boston, USA; Mariko Okada, RIKEN Research Center for Allergy and Immunology, Yokohama City, Japan; and Michael Elowitz, California Institute of Technology, Pasadena, USA) are working at the forefront of this nascent field, and showcased model systems in which critical cell fate decisions are gated by upstream regulatory dynamics. Their work begins to answer three central questions: (i) How does the cellular regulatory machinery generate these complex signals? (ii) What mechanisms enable cells to read out a time-varying input? and (iii) What is the functional utility of dynamic signaling?

One of the first concepts to emerge from this line of inquiry was the idea that different upstream signals can lead to different dynamical patterns of the same molecule.

The tumor suppressor p53 is a master regulator of two mutually incompatible cellular behaviors: cell cycle arrest (in response to gamma-radiation) and apoptosis (following UV-radiation). Galit Lahav explained how dynamic signaling enables a single protein to play this dual role: gamma-radiation triggers a series of p53 pulses with fixed amplitude and duration, whereas UV-radiation initiates a single sustained pulse. Importantly, if p53 dynamics are perturbed, such that gamma-radiation results in sustained p53 activation, the downstream phenotype is also switched (from arrest to apoptosis). Through a combination of live cell microscopy, computational modeling, targeted perturbations, and RNA sequencing, Lahav is elucidating the molecular mechanisms for pulse formation (Wip1 regulation of interlinked feedback loops) and pulse detection (feedforward loops converging on p21 and E2F1). These results suggest that it may ultimately be possible to develop therapeutic strategies based on the understanding and precise control of protein dynamics.

Depending on the timescale on which cells are observed, the same protein can exhibit very different dynamics, such that even in a well-studied model system surprises can still emerge. Mariko Okada presented recent work on NF-kappaB signaling during B cell maturation, in which fast-timescale sampling of upstream regulator TAK1 revealed a second peak of activation just minutes after the initial stimulus. This second pulse, generated by rapid post-translational feedback loop, underlies NF-kappaB's digital response to higher stimulant concentrations. This work highlights the central role that post-translational regulation can play in rapidly processing dynamic inputs upstream of a slow-timescale cell fate decision. Notably, she also studied the mechanism of persistence detection in MCF-7 cells, where growth factors EGF and HRG trigger different ERK signaling dynamics (transient and sustained, respectively) that mediate differential activation of c-Fos, and, in turn, direct cells to either proliferate or differentiate. In a direct parallel to Lahav's work, Okada presented computational models of the regulatory network in which multiple feedforward loops mediate persistence detection. As more cellular systems that read out dynamic information are characterized, it will be interesting to see if a distinct set of network motifs emerge as convergent solutions.

The average dynamical behavior of a population often represents a distorted version of the heterogeneous responses of individual cells. However, bulk measurement completely masks the presence of stochastic pulses-prompting Michael Elowitz to call them "the dark matter of cellular dynamics". While studying timing of sporulation in *Bacillus subtillus*, he discovered that the master regulator, sigma factor B, is activated in short, erratic pulses by an ultrasensitive phosphor-switch [12]. Observing that only a few sigma factors were active in the cell at any given time, Elowitz concluded that stochastic pulses may afford functional advantages over static inputs, including the ability to efficiently share a common resource (the RNA polymerase) and to flexibly coordinate the expression of multiple downstream proteins. In work that was just published, Elowitz showed that the amplitude of the sigma B pulses is regulated by slow negative feedback, enabling cells to match their stress response to the rate of environmental change [13]. These mechanisms may begin to explain why stochastic signaling appears to be a common feature of biological regulatory systems.

CONCLUSIONS

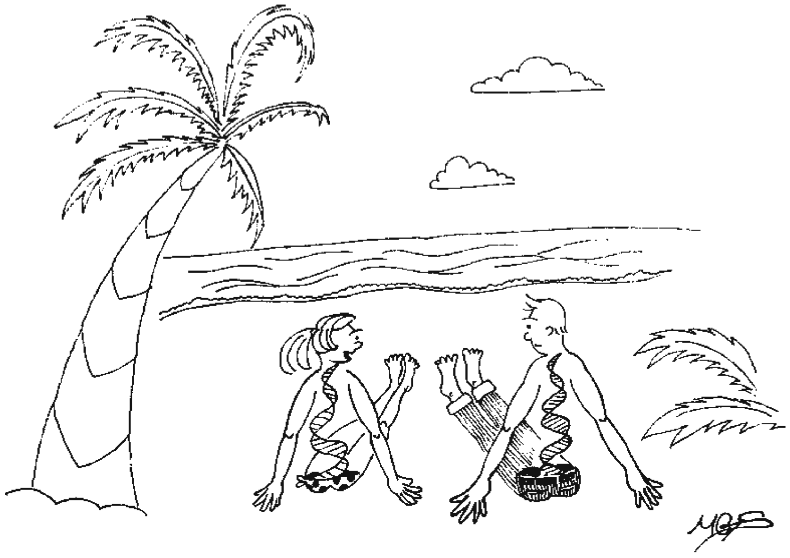
By focusing on a few selected themes, we have not attempted to convey the entire spectrum of work exhibited at the first Winter Q-Bio conference. For instance, another interesting topic discussed at the meeting was the use of time-varying inputs as a new way to probe and characterize biological systems (Timothy Elston, University of North Carolina, Chapel Hill, USA; Andre Levchenko, Johns Hopkins University, Baltimore, USA). In addition, therapeutic applications of q-bio were highlighted in talks discussing antibiotics, evolution of resistance, and bacterial populations (James Collins, Boston University, Boston, USA; Roy Kishony, Harvard Medical School, Boston, USA; Gurol Suel, University of California, San Diego; San Diego, USA; Kit Pogliano, University of California, San Diego; San Diego, USA) and the use of prosthetic networks for potential treatment of diabetes (Martin Fussenegger, Technische Hochschule Zürich, Zürich).

After a stimulating conference such as this one, questions naturally arise. We would like to conclude with four emergent challenges for the future of q-bio.

(i) How do we resolve the "burrito" problem alluded to by Chris Voigt? In an effort to scale up and build a large synthetic circuit within a cell, we are lacking good strategies for spatially separating components from each other and from the rest of the cell. Thus, the synthetic parts – analogous to the ingredients in a burrito – get all mashed up together.

(ii) Can we use dynamics in biological systems to our advantage? Can we foresee "dynamic therapies", where one is not treating a steady-state condition but rather able to take into account the role of signaling dynamics in human disease?

(iii) As pointed out by Bernhard Palsson (University of California, San Diego; San Diego, USA), microbial metabolic systems biology has become a genome-scale science. What steps can be taken to facilitate the



Nice genes. Are they designers?

Illustration by Mischa Stephens

incorporation of this powerful new resource into the design of the next generation of synthetic biology?

(iv) How do we address the continuing challenge of quantitatively determining fundamental tradeoffs in biology? For instance, *E. coli* are observed to double every 30 min. This is not a fundamental law, but doubling at a faster rate damages the metabolism of the organism in ways that perhaps can be quantitatively determined. Understanding such a penalty would certainly be useful to future synthetic biologists.

Next year's Winter Q-Bio meeting will take place February 17–20, 2014, on the Big Island of Hawaii, at the Hilton Waikoloa Village. The organizers will include Kevin Bennett (University of Hawaii, Manoa, USA), Bill Ditto (University of Hawaii, Manoa, USA), Hana El-Samad (University of California, San Francisco; San Francisco), Jeff Hasty (University of California, San Diego; San Diego, USA), Alex Hoffmann (University of California, San Diego; San Diego, USA), Galit Lahav (Harvard Medical School, Boston, USA), Eva-Maria SchoetzCollins (University of California, San Diego; San Diego, USA), Chao Tang (Peking University, Beijing, China), and Lev Tsimring (University of California, San Diego; San Diego, USA). The goal is to make the Winter Q-Bio conference an annual gathering, with meetings rotating between Hawaiian Islands and with a different thematic focus within Q-Bio each year. For this, we say *mahalo* to the organizers.

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