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Keystone Symposia Conference on Precision Genome Engineering and Synthetic Biology Brings Together Players from Both Disciplines

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Scientists from at least 21 different countries, representing both academic and private sector interests, convened at the Beaver Run Resort in snowy Breckenridge, Colorado, March 17−22, 2013, to attend the "Precision Genome Engineering and Synthetic Biology: Designing Genomes and Pathways" conference. This Keystone Symposia conference was organized by Dana Carroll (University of Utah) and Jef D. Boeke (Johns Hopkins) and sponsored by Life Technologies and Sangamo BioSciences. Dr. Carroll described that the original proposal was for a conference on precision genome engineering, but that this was later expanded to include synthetic genomes and synthetic biology in hopes that bringing these communities together would spur collaboration and cross-pollination.

Following preliminary remarks from the organizers, the conference began with a keynote address delivered by Frances H. Arnold of Caltech, wherein she described using directed evolution techniques to improve enzymatic catalysis or evolve enzymes with novel catalytic abilities. In her approach, diversity is generated by recombination between divergent parental enzymes[1] and/or random or site-directed mutagenesis, followed by screening of the resulting enzymes to discover variants with new or improved activities. As examples she discussed the discovery of cellobiohydrolases with improved thermostability[2] and an evolved cytochrome P450 that can catalyze a cyclopropanation reaction,[3] a wholly new chemistry for this enzyme. The ensuing conference comprised of 52 speakers spread over the various themed plenary sessions and workshops, as well as two poster sessions with around 87 entries.

SCIENTIFIC MEETING OVERVIEW

Tools for Genome Engineering. A major aspect of the conference was the discussion of tools for small- or large-scale genome engineering. One important group of tools discussed were designer nucleases, reagents that allow precision chromosome editing. These tools function via the introduction of targeted double-strand breaks into the genome, which can lead to either gene inactivation through non-homologous end joining (NHEJ) or, if an appropriate donor DNA sequence is provided, gene modification/transgene insertion through homology directed repair (HDR). This category of gene-targeting reagents includes zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), CRISPR/Cas systems, and homing endonucleases. Discussing the use, improvement, and/or comparison of ZFNs and TALENs were Dana Carroll (University of Utah), Thomas Lahaye[4,5] (Ludwig Maximilians University Munich, Germany), Toni Cathomen (University Medical Center, Freiburg, Germany), Marcus B. Noyes (Princeton), Shengdar Q. Tsai[6] (Massachusetts General Hospital), Gregory D. Davis[7] (Sigma-Aldrich), and Jin-Soo Kim[8] (Seoul National University), who discussed an online database of human gene-editing TALENs (http://www.talenlibrary.net) hosted by his institution. Several presentations focused on the use of the Cas9 RNA-guided endonuclease from the bacterial CRISPR/Cas system as a gene-editing tool, with Jin-Soo Kim, Rachel E. Haurwitz (Caribou Biosciences), and Prashant Mali discussing Cas9-mediated genome engineering in human cells,[9−11] and Shengdar Tsai demonstrating the use of this system in zebra fish.[12] Barry L. Stoddard (Fred Hutchinson Cancer Research Center) presented on "MegaTALs", designer nucleases that combine both a TALE array and a homing endonuclease in order to attain superior target specificity. Dr. Stoddard also discussed the engineering of homing endonucleases to act at new target sites. David R. Edgell (University of Western Ontario) described the fusion of the I-TevI nuclease domain to a DNA binding domain (either zinc-fingers, TALE repeats, or inactive homing endonucleases) allowing the design of modular, monomeric gene-targeting reagents.[13] Transposons and recombinases also made an appearance at the conference, with Nancy L. Craig (John Hopkins) presenting on the use of *piggyBac* transposons in genome engineering in mammalian cells[14] and Andrew C. Mercer (Scripps Research Institute) discussing the design of chimeric zinc-finger and TALE recombinases that can effect targeted integration at user-defined sites.[15,16]

Farren Isaacs (Yale) provided an update on the use of Multiplex Automated Genome Engineering (MAGE) and Conjugative Assembly Genome Engineering (CAGE) to engineer the *E. coli* genome.[17] He considered the promise of this technology for rapid pathway optimization as well as for recoding the genome,[18] discussing how recoding could be a means of generating a genetic firewall (preventing horizontal gene transfer or viral infection) or freeing up codons for potential reprogramming with unnatural amino acids (e.g., see Jason Chin's work below).

At the other end of the genome engineering spectrum were Daniel G. Gibson (J. Craig Venter Institute and Synthetic Genomics) and Jef D. Boeke, who both presented on entirely synthetic genomes. Dr. Gibson provided a nice overview of the work performed at JCVI on the synthesis, assembly (in yeast), and transplantation of genomes, work that culminated in the creation of a *Mycoplasma mycoides* strain with a completely synthetic genome.[19] He proceeded to discuss current applications of the technology such as the cloning and genetic engineering of genomes from genetically intractable organisms in yeast.[20] He also provided an update on the quest for a minimal *Mycoplasma* genome, one that encodes only the

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minimal set of machinery necessary to support life. Jef Boeke (John Hopkins) recounted progress on the international effort to assemble a synthetic *Saccharomyces cerevisiae* genome.[21] He discussed the design principles being employed and how the work will allow exploration of the structure/organization and plasticity of the genome as well as the essentiality of introns/RNA splicing and repetitive elements. They also aim to explore the universe of minimal genome sets. To aid in this endeavor they have developed a mutagenesis system dubbed "Synthetic chromosome rearrangement and modification by loxP-mediated evolution" (SCRaMbLE), where a loxP site is inserted in the 3′ UTR of nonessential genes and at other landmarks. Cre recombinase is used to induce genomic instability, generating inversions, deletions, and duplications, resulting in a population of diverse genomes. Leslie A. Mitchell (Boeke lab) further elaborated by presenting chromosome engineering tools that aid the pursuit of these goals.

Applications of Precision Genome Engineering. Exciting applications of these precision genome engineering tools were also presented. Scott C. Fahrenkrug (U. of Minnesota and CEO of Recombinetics) described applications of TALEN-mediated gene modifications in livestock (pigs and cows) leading to improvements in agriculture and superior biomedical models of human disease.[22,23] Angelo Lombardo (San Raffaele Telethon Institute for Gene Therapy, Milan, Italy) reported on the possible use of ZFN technology to treat X-linked severe combined immune deficiency via the repair of a defective *IL2RG* locus,[24] to edit T cell specificity for cancer immunotherapy,[25] and to insert transgene expression cassettes into a safe insertion site in the human genome.[26] Julien Valton (R&D project leader at Cellectis) discussed the use of TALENs to correct mutations in the *XPC* locus that cause xeroderma pigmentosum, which required the development of TALENs insensitive to cytosine methylations present at the target site.[27] Michele P. Calos (Stanford) outlined an interesting strategy for the treatment of muscular dystrophy. One iteration of this approach relies on the sequential use of three different site-specific recombinases (phiC31, Bxb1, and Cre). First, phiC31 is used to insert reprogramming genes at a pseudo attP site in the genome, allowing the reprogramming of fibroblasts to iPS cells.[28] A functional copy of the dystrophin gene is then inserted next to the reprogramming cassette by Bxb1. Finally, Cre is used to remove the reprogramming cassette, leaving the new dystrophin gene intact. These cells can then be differentiated and grafted back into the host.

Daniel Voytas (U. of Minnesota) and Voytas lab graduate student Nicholas Baltes discussed various delivery methods for and applications of gene targeting reagents in plants,[29] describing the promise of these techniques for improving agricultural productivity. Sangamo BioScience's Philip D. Gregory provided an update on the most advanced human application of gene targeting reagents: a clinical trial for SB-728-T, a ZFN-based HIV-1 therapy.[30] In this approach engineered ZFNs are used to disrupt *CCR5* (a chemokine receptor required for infection by R5 tropic HIV-1) in patient-derived CD4+ T cells. These cells are expanded and infused into the patient, thus providing a population of CD4+ cells now resistant to infection. Dr. Gregory also discussed the possibility of moving the treatment from CD4+ T cells to hematopoietic stem/progenitor cells (HSPCs),[31] a strategy that will likely provide a more persistent effect and will yield *CCR5*-null lymphoid and myeloid lineages. This strategy could mimic the curative *CCR5*Δ*32* stem cell transplantation in the "Berlin Patient",[32] but with autologous cells.

Engineering Biomolecules, Circuits, Modules, Systems, and Pathways. Following the goals of synthetic biology to engineer at all levels (from parts to modules to systems), presentations on biological design spanned a broad range of biological scales, from engineering individual biomolecules/biomolecular interactions, through simple biological circuits, to complex systems and pathways. A number of speakers covered engineering at the scale of individual proteins, investing them with new or improved functionalities. Jason W. Chin (MRC) highlighted recent advances in the use of orthogonal tRNA/aminoacyl-tRNA synthase pairs to direct the incorporation of nonnatural amino acids into ribosomally encoded proteins *in vivo*. He demonstrated the incorporation of amino acids with bioorthogonal reactive groups for site-specific protein labeling,[33] generation of photoactivated kinases for the study of signaling networks in mammalian cells,[34] and genetic code expansion in an animal.[35] Graduate student Lauren Polstein (Gersbach lab, Duke) reported the design of a light-inducible gene regulatory system based on the light-induced heterodimerization of the GIGANTEA protein and LOV domain of FKF1 from *Arabidopsis thaliana*. This system enables spatiotemporal control over gene expression in a reversible and tunable manner.[36] Tanja Kortemme (UCSF) described the use of computational approaches to design proteins with novel behaviors, one example being the design of a protein/peptide interaction that behaves in a switchable manner depending on phosphorylation state of the protein.[37]

Virginia W. Cornish (Columbia) discussed an ingenious directed evolution strategy relying on a yeast three-hybrid assay (wherein enzyme chemistry is linked to cell survival) to discover enzymes (e.g., cellulases[38]) with improved catalytic activities. Capitalizing on yeast's ability to sexually reproduce, Dr. Cornish demonstrated how her directed evolution methodology could be coupled with a heritable recombination system to make mutations directly in the cell and effectively increase library diversity.[39] Rounding out the discussion on protein engineering, Eddy Rubin (JGI, Lawrence Berkeley National Laboratory) described the utilization of metagenomic data for the discovery of novel cellulases from microbes inhabiting the cow rumen,[40] an approach that enabled the discovery of enzymes with desirable properties for industrial applications (e.g., thermostability, acid/salt tolerance).

There were also those trying to understand complex biological processes by employing synthetic biology approaches. Petra Schwille (Max Planck Institute of Biochemistry), embracing a bottom-up approach to understanding biological systems, discussed the *in vitro* reconstitution of a protein oscillator (composed of minD and minE) in micrometer-scale reaction compartments.[41] In *E. coli* the oscillatory behavior of MinD and MinE is involved in the proper positioning of the machinery for cell division. The *in vitro* system allows exploration of the influence of compartment geometry and size on system behavior, the results suggesting that these features of *E. coli* play an important role in determining the oscillatory behavior of the Min proteins. Sriram Kosuri (Wyss institute) presented on a study of cis-regulatory elements, wherein he assembled a large library of cis-regulatory element combinations and systematically interrogated the effect of different combinations of elements on transcription and translation, work aimed to understand the synergistic effects of these elements on gene expression.

Moving up in biological complexity, a number of presentations focused on the design of biological circuits and pathways. Ahmad 'Mo' Khalil (Boston U.) reported on the design of modular, synthetic eukaryotic transcription factors based on engineered zinc-finger proteins, demonstrating their use in synthetic transcriptional circuits to generate a range of programmable information processing behaviors.[42] Timothy K. Lu (MIT) related the design of recombinase-based genetic circuits that allow the implementation of memory and all 16 two-input Boolean logic functions in *E. coli*.[43] Jeff Hasty (UCSD) provided an update on his work on modeling and designing synthetic biological oscillators, demonstrating the synchronization of oscillations across cellular populations at centimeter-length scales.[44] There were also several interesting examples of biological circuits used to perform diagnostic computations. Yaakov Benenson (ETH Zurich, Switzerland) described the design of RNAi-based circuits that can sense and integrate multiple endogenous disease biomarkers in cells. If the circuit determines that the cell is diseased, it directs the production of an apoptosis-inducing protein.[45] Along the same lines, June I. Medford (Colorado State U.) presented on the design of "plant sentinels", plants with engineered circuitry that can detect inputs (e.g., pollutants or chemical/biological threats) and provide an alert through a detectable output (e.g., degreening of leaves).[46,47]

A huge challenge in synthetic biology is the development of effective design strategies for working with large-scale circuits/systems. In an effort to address part of this challenge, Ron Weiss (MIT) and Douglas Densmore (Boston U.) both discussed the use of CAD technologies for synthetic biology, software analogous to the Electronic Design Automation (EDA) tools currently used by electrical engineers for electronic circuit design.[48−50] These software tools aid in the rapid design and implementation of synthetic biological systems by automating the design process (and in some cases the assembly) with user-defined design criteria and specifications. A key feature of these applications is the ability to "learn" from experimental successes and failures reported by users. In a very impressive example of synthetic biological manipulation of a complex system, Christopher Voigt (MIT) reported on the "refactoring" of the nitrogen fixation gene cluster from *Klebsiella oxytoca*, aiming to break all native regulation and facilitate interspecies transferability of the pathway.[51] The gene cluster was built from the ground up by first making the DNA sequence of the essential genes as different as possible from WT through the selection of alternative codons and then putting these recoded genes under the control of characterized parts (e.g., ribosome binding sites, promoters, terminators). He also described how Doug Densmore's software can be utilized to generate many potential "refactored" gene clusters in a massively parallel fashion.

There were also a large number of presentations focused on the metabolic engineering of organisms for the production of chemicals of interest, with a big focus on biofuels. Sang Yup Lee (KAIST, South Korea) introduced many of the tools available for systems metabolic engineering, highlighting the use of synthetic bacterial small regulatory RNAs as a means to rapidly generate a variety of metabolic modifications in *E. coli* without making permanent changes to the genome.[52] Stephen B. del Cardayre (LS9) and Jack Newman (Amyris) shared strategies used at their respective companies to optimize strains for the production of a range of chemicals such as alkanes in *E. coli* (LS9)[53] or farnesene in yeast (Amyris). Klavs Riishede Hansen (Evolva Biotech) introduced the use of expressible Yeast Artificial Chromosomes (eYACs)[54] as a powerful tool for metabolic engineering, demonstrating how they can aid in the identification of a missing gene from a pathway, identify the best combinations of analogous genes, or optimize expression levels of pathway genes. Xiaoxia Nina Lin (U. of Michigan) outlined the use of synthetic microbial consortia rather than a single "superbug" for the production of biofuels from cellulosic feedstocks. Also discussed were bioethanol production in *E. coli*[55] (Neha Munjal, ICGEB, India) and plastics and aromatics production in *Pseudomonas putida* (Vitor Martins dos Santos, Wageningen U.).

At the other end of the biological scale (that of whole organisms) Jonathan R. Karr (Covert lab, Stanford) described the fabrication of a virtual organism, a whole-cell *in silico* model of *Mycoplasma genitalium* that models the behavior of all cellular processes for a complete cell replication cycle.[56] The model, based on thousands of experimentally observed parameters, successfully recapitulates experimental data and provides valuable insight into several biological processes that can be used to guide future experimentation/engineering. Such models hold great promise for aiding basic and applied science projects by facilitating rapid analysis of genomic/metabolic perturbations *in silico*.

IN CONCLUSION

Synthetic biology and genome engineering hold great promise for both advancing basic knowledge of the biological world and providing green solutions to looming challenges facing an ever-increasing human population (e.g., healthcare, growing scarcity of material and energy resources). With the growing acknowledgment of the potential of these disciplines, the time was ripe for a conference to bring these fields together, a fact attested by the observation that the meeting drew about twice the number of attendees than was initially projected. The conference brought together a broad spectrum of established players as well as excited newcomers from both fields and provided a venue for the free exchange of ideas and the cultivation of new collaborative efforts.

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Notes

The authors declare no competing financial interest.

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