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Preview

Rise of synthetic yeast: Charting courses to new applications

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Microbes have long provided us with important capabilities, and the genome engineering of microbes has greatly empowered research and applications in biotechnology. This is especially true with the emergence of synthetic biology and recent advances in genome engineering to control microbial behavior. A fully synthetic, rationally designed genome promises opportunities for unprecedented control of cellular function. As a eukaryotic workhorse for research and industrial use, yeast is an organism at the forefront of synthetic biology; the tools and engineered cellular platform being delivered by the Sc2.0 consortium are enabling a new era of bespoke biology. This issue highlights recent advances delivered by this consortium, but hurdles remain to maximize the impact of engineered eukaryotic cells more broadly.

Thousands of years before humans first viewed cells under a microscope, genome modification already underpinned many of our working relationships with microbes, from fermenting foodstuffs to producing medicines to brewing alcohol. For example, propagation in different beverages led to hundreds of varieties of brewer's yeast (*Saccharomyces cerevisiae*) with unique fermentation and flavor profiles. Today, our partnership with yeast extends far beyond the beverage industry, as *S. cerevisiae* is the origin of numerous foundational discoveries in cell biology and arguably the most well-studied eukaryote. In recent decades, the genetic tractability of yeast supported their widespread use as microbial cellular factories for chemical bioproduction, tools for medical research, and a growing array of bioengineering platforms.[1] Now, we have moved beyond small genetic changes to redesign yeast genomically from the bottom up. In the process, we continue to learn a great deal about conserved biomolecular mechanisms in eukaryotes and gain traction in engineering both yeast and other organisms. Engineered eukaryotes promise to offer an invaluable and essential platform to address current and future pressing societal needs and challenges in biomanufacturing, biomeasurement, healthcare, and beyond.

The Synthetic Yeast Genome Project (Sc2.0) consortium labors at the frontier of our human-yeast partnership, as researchers endeavor to fully rewrite the yeast genome to optimize downstream utility. The benefits of the project fall into two categories: (1) tools to assemble and manipulate genomes and (2) the synthetic yeast cell itself as a platform. Together, these are poised to help researchers interrogate foundational questions, such as synthetic genome design and assembly, dependencies between genes, and cellular replication, as well as perform industrial tasks. The synthetic chromosomes contain many stability upgrades and useful features for further projects, for example, revised telomeres, deleted transposons and introns, PCR-tagged open reading frames (ORFs), and inducible gene shuffling for whole-genome optimization. The outputs of the Sc2.0 consortium will provide a template for building new eukaryotes in the future, previewed by the powerful genome engineering and analysis schemata showcased in this special issue.

Much like repairing Theseus's famous ship, the general approach to rebuild yeast is to replace the entire genome *in situ* by transformation, piecewise. The most recent protocols allow for replacement of an entire native chromosome at once.[2] After genome modification, strains are compared to wild-type yeast to discover unforeseen phenotypic consequences of the rewriting process. CRISPR-based techniques enable pinpointing of deleterious synthetic loci by making use of the Sc2.0 genomic PCR tags.[3] Optimizing chromosomal parameters, such as location and context of the centromere and three-dimensional structure, underscores the difficulty of rewriting entire cellular genomes.[4,5] As a core optimization technique, the Sc2.0 genome contains sites for synthetic chromosome rearrangement and modification by loxP-mediated evolution (SCRaMbLE), enabling inducible shuffling genome-wide. SCRaMbLE is remarkably versatile, applied across the Sc2.0 consortium to form stable extrachromosomal circular DNAs, investigate aneuploidy, and even utilize tetraploid strains.[6–8] SCRaMbLE is also compelling for end users in industry, medicine, and synthetic biology—offering the ability to quickly generate useful phenotypes through evolution.[9]

Despite the considerable progress in tools for engineering yeast described in this special issue, challenges and limitations remain: over half of the genome awaits rewriting, with significant sections of yeast chromosomes still unannotated, biocontainment has yet to be to be installed, and current software tools are a barrier to future adaptability. First, more than half of the genome remains to be synthesized, and approximately one-fifth of the yeast genome lacked functional annotation as recently as 2019.[10] Knowing which of these sequences may be removed without functional consequences is arguably the greatest snarl of building synthetic yeast and will pose a challenge more broadly in synthetic

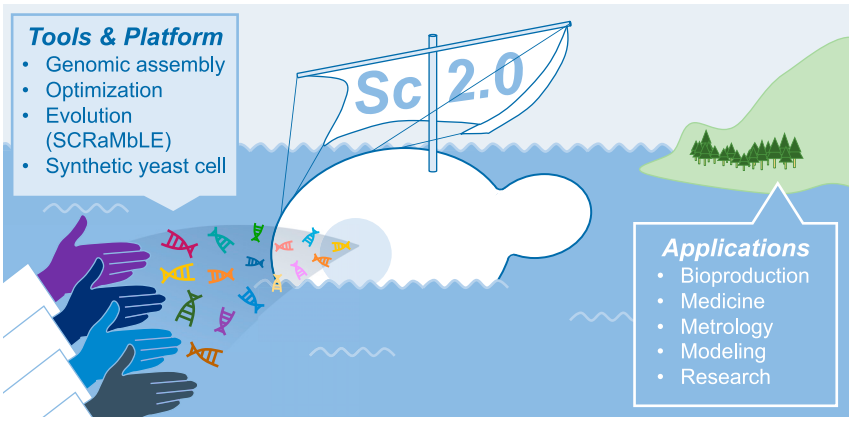

Figure 1. Sailing into the era of bespoke biology

The Sc2.0 consortium is an international effort, focused on yeast, to engineer and synthesize the whole genome of a eukaryotic cell. This special issue reports progress to build and deliver the tools and cell platform necessary to chart new courses, advancing foundational knowledge and applications in biotechnology.

biology as the field grows. Second, due in part to the growth advantage of wild yeast as compared to the lab-derived parent of the Sc2.0 platform, installing a nutrient-dependent biocontainment "safeguard"is not currently planned.[11] This is arguably wise to include before completion of the project. PCR tags should offer an identification method to discriminate the Sc2.0 platform from wild yeasts in the event of release. Third, software tools for designing DNA have become ubiquitous. The Sc2.0 consortium relies on an open-source tool, BioStudio, to design its genomic elements. BioStudio is ostensibly provided to the public as either an Amazon Web Services image or a software repository,[12] but our attempts to trial the tool failed, because neither cited installation method had current information. As the completed Sc2.0 platform finds widespread use, an accessible software workflow for designing further modifications will be critical to apply the platform to its full potential.

The ambitious Sc2.0 consortium exists in a broader ecosystem of collaborations in engineering and synthetic biology, and many opportunities exist to exchange ideas and protocols to speed progress. After an extensive shakedown of cellular engineering protocols in yeast, the lessons learned and methods published will provide critical guidance for building future synthetic eukaryotes. Using yeast as a toolkit for assembly of genomes is currently feasible; however, it remains to be demonstrated how these advanced protocols will generalize to direct *in vivo* usage within other eukaryotes. The Sc2.0 platform and future synthetic cells promise to revolutionize bioinformatics, given that genomic variables may be defined and modified by the experimenter both *in silico* and *in vivo*. Exciting possibilities arise to improve the descriptive and predictive accuracy of computational whole-cell modeling, as well as to discover new phenotypes via simulation to test *in vivo* by modifying living cells accordingly. From a metrology perspective, the tools and platform delivered by the Sc2.0 consortium should speed development of living measurement systems, engineered biosensors motivated by broad measurement needs in biotechnology. The Sc2.0 consortium serves as a model for international and interlaboratory cooperation, and robust communication with other large biology collaborations, such as other efforts to achieve minimal and synthetic cells, will undoubtedly lead to transformative opportunities.

This special issue greets us at the heady burgeoning of our next (bio)technological revolution. The rewards of the Sc2.0 tools and platform will be far reaching, spurring innovation in safe, accessible, and open-source biotechnological breakthroughs for a vibrant global bioeconomy. Just as scientists and engineers miniaturized electronic circuits and transformed the world at the start of the computer revolution, the Sc2.0 consortium encapsulates a paradigm shift from discovered biology to bespoke biology. Like the ship of Theseus, the Sc2.0 consortium offers a means to travel forward: the former across the sea, the latter into the newest currents of our research and industrial partnerships with living cells (Figure 1). Although we can and have sailed to many destinations aboard yeast, the Sc2.0 platform offers the chance to jump from an acquired vessel of partially understood construction to a sleek craft built for purpose. As long as there are biotechnological applications to chart, there are engineered cellular ships to build; by combining the knowledge, experience, and resources from the Sc2.0 consortium and similar synthetic cell efforts, we are eminently more equipped to build them.

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DECLARATION OF INTERESTS

The authors declare no conflicts of interest.

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