EDITORIAL

Synthetic yeast genome project and beyond

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Saccharomyces cerevisiae, commonly known as budding yeast, is a unicellular eukaryotic organism used as a model to study a wide range of biological processes, owing to its simplicity, rapid growth, and genetic manipulability. Additionally, it is also an invaluable industrial microorganism for production of bread, beer and pharmaceuticals. To further make this organism suitable for various applications, a group of scientists worldwide launched the Synthetic Yeast Genome Project (Sc2.0 project) to provide it with a genomic overhaul through designer chromosomes.[1] Through the implementation of numerous deliberate modifications, the Sc2.0 project seeks to investigate numerous otherwise challenging and fundamental questions related to chromosome properties, genome organization, genome function, and evolution.

The Sc2.0 genome stands out as the most extensively modified synthetic genomes to date, featuring several major design features: 1) Removal of DNA elements. Retrotransposons and their derived sequences, known for triggering genome instability due to their repetitive nature in the *S. cerevisiae* genome, were considered nonessential and thus eliminated. Similarly, the redundant tRNA genes (tDNA), notorious for being genome instability hotspots, were systematically excised from their original positions and transferred to a specific chromosome. Furthermore, Y′ elements, a type of telomeric repeat found at certain telomeric ends but with unclear functionality, were also excised from the genome. 2) Elimination of introns in pre-mRNAs and pre-tRNAs. In the yeast genome, there are approximately 285 introns, which have been shown to be nonessential when deleted individually. Creating a eukaryotic genome without introns offers the opportunity to investigate the feasibility of eliminating the splicing machinery in eukaryotes. This approach enables the investigation of potential evolutionary mechanisms underlying intron generation; 3) Introduction of PCRTags. In order to differentiate synthetic chromosomes from the native ones, stretches of amino acids within each open-reading frame are recoded synonymously, i.e., changing the DNA sequences without altering the amino acid sequences. These PCRTags serve as PCR primers, facilitating the rapid identification of strains containing synthetic sequences during the construction of synthetic chromosomes. They also enable the tracking of synthetic DNA presence in the future. 4) Stop codon replacement. All TAG stop codons are replaced by TAA, which will lead to a genome containing only 63 codons. This vacant codon can be utilized to introduce artificial amino acids into specific proteins of interest. Alternatively, it can be harnessed to integrate innovative traits like virus resistance into the genetic makeup. 5) Insertion of loxPsym sites. To enhance the genetic flexibility of synthetic chromosomes and enable the investigation of desired genome compositions under particular conditions, symmetrical loxP sites are introduced into the 3′ UTR of all non-essential genes and synthetic landmarks. The incorporation of these loxPsym sites serves as the foundation for the SCRaMbLE (Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution) system, which will be explained in detail below. Collectively, the resulting Sc2.0 genome is roughly 8% smaller, with 1.1 megabases of sequence modifications, and is expected to achieve a fitness level close to that of the wild type.[2]

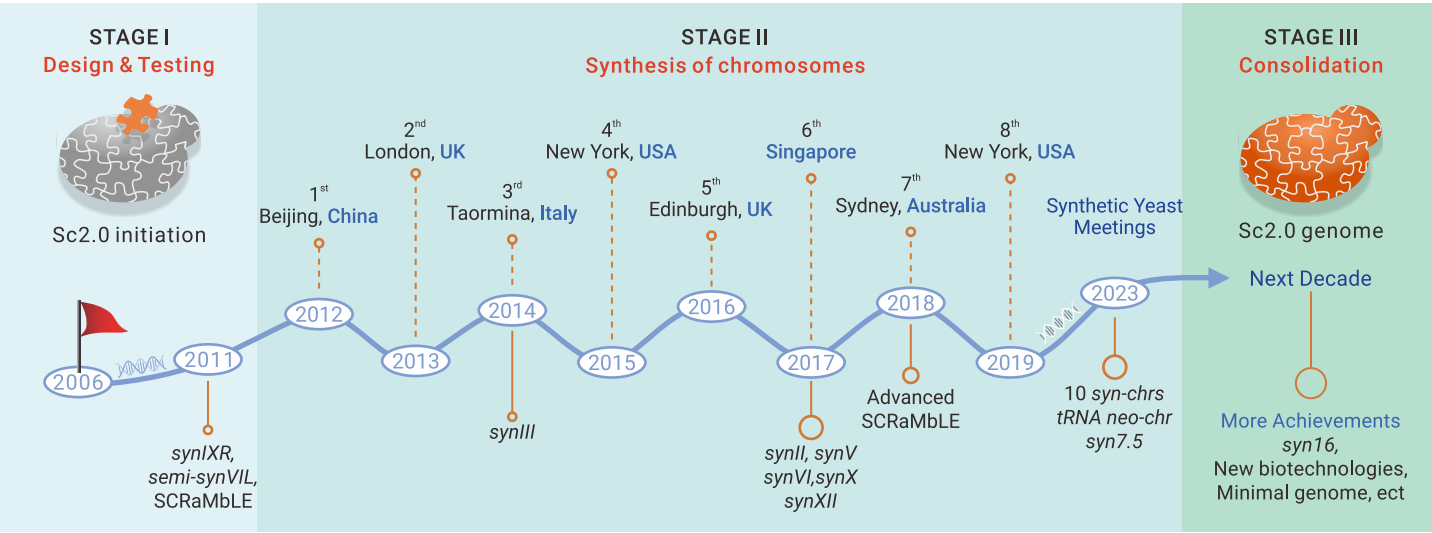

Figure 1. The timeline of the Synthetic Yeast Genome Project.

A key aspect of the Sc2.0 genome is the integrated conditional genome instability system known as SCRaMbLE.[1] The symmetrical loxP sites lack the directionality of canonical loxP sites, enabling the production of inversions and deletions with equal probability. Moreover, they can also create other genomic rearrangements such as translocations and duplications. However, the design principles mandate that SCRaMbLE remains inactive until intentionally activated by Cre recombinase induction, facilitating the generation of genetic diversity as required. To prevent any leaky expression of Cre recombinase, an engineered Cre recombinase fused with the murine estrogen binding domain (EBD) was introduced. This fusion construct is under the control of the daughter cell-specific promoter *SCW11*, ensuring precise and pulsatile expression of Cre recombinase. Since its initial demonstration in 2011, SCRaMbLE has been utilized to create strains for product production, explore how structural variations can result in phenotypic changes, minimize synthetic chromosomes, and more. Looking ahead, SCRaMbLE holds the promise of being leveraged for refining, prototyping, or even uncovering novel

complex pathways across a diverse array of biotechnological applications.

The Sc2.0 project has gone through three different stages (Figure 1). The initial phase, from 2006 to 2011, focused on genome design and proof-of-concept tests conducted at Dr. Jef D. Boeke's lab at Johns Hopkins University. This phase concluded with the successful construction of the first synthetic chromosome arms, *synIXR* and *semi-synVIL*.[1] During this stage, the primary limitation lies in the availability of large DNA fragments, as gene synthesis costs approximately $1 per base pair even for smaller fragments around 1kb in size. To address this cost challenge, the Build-a-Genome class was created to synthesize 750bp fragments within undergraduate courses, proving to be an excellent training opportunity. Additionally, several technical hurdles were overcome, including methods for manipulating large chromosomal arms, laying the groundwork for chromosome synthesis. In 2012, the inaugural synthetic yeast genome meeting took place in Beijing, uniting scientists and funders from various countries like the USA, China, UK, Australia, Singapore and India. At this meeting, the Sc2.0 international consortium was officially established, with each chromosome assigned to a different group, signifying the commencement of the second stage of Sc2.0. Subsequently, the synthetic yeast genome meeting became an annual event. In 2014, the construction of the first synthetic yeast chromosome (*synIII*) was completed. In 2017, five more chromosomes (*synII*, *synV*, *synVI*, *synX* and *synXII*) were successfully build, representing a pivotal milestone of this international collaboration. Recently, the consortium achieved another significant feat by synthesizing the remaining 10 synthetic chromosomes, along with a novel tRNA neo-chromosome and the creation of a yeast strain with a half-synthetic genome.[3] At this stage, the primary challenge arises from coordinating synthesis progress across various teams. Given the complexity of chromosomes, distinct technical or biological issues were faced by different groups. To address these difficulties, diverse strategies were devised, such as the pooled PCRTag mapping (PoPM) method to swiftly pinpoint potential problematic regions. These methodologies hold promise for adoption in future genome synthesis initiatives of other organisms. Presently, the project enters its third stage, known as "the beginning of the end", with the objective of assembling a strain containing all 16 synthetic chromosomes.[4]

During the synthesis of these chromosomes, numerous scientific problems have been identified and solved. Unexpected issues, or "bugs", such as defects caused by the loxPsym insertion, intron removal, PCRtag insertion and combinational bugs across chromosomes have been observed. Many of these issues stemmed from seemingly harmless designs, like synonymous codon replacements. In fact, a significant portion of the project's timeline was dedicated to the debugging process. To expedite the debugging procedures, the consortium has developed a series of strategies that could be applied in future genome synthesis endeavors. Additionally, with the efficient methods developed for chromosome consolidation, it is foreseeable that in the near future, a yeast strain with a fully functional synthetic genome will be successfully constructed. This achievement will mark the first instance of a eukaryotic cell being entirely sustained by a tailored synthetic genome, promoting the paradigm shift from discovered biology to bespoke biology.

Yeast cells harboring a fully synthetic genome offer an invaluable opportunity to explore fundamental biological questions on a genome-wide scale, such as probing the minimal eukaryotic genome. In subsequent studies, based on the synthetic version of the chromosome arm, we investigated the necessity of sequences using the SCRaMbLE-based genome compaction (SGC) method, along with additional bottom-up strategy to simplify yeast chromosomes. We found that as few as 20% of the sequences were capable of sustaining cell viability.[5]Leveraging yeast cells with the Sc2.0 genome and high fitness will enable us to effectively explore these questions on a genome-wide level. Simultaneously, synthetic yeast cells, in conjunction with advanced DNA assembly technologies, will expedite progress in biotechnologies. These advancements encompass the creation of new chassis with improved environmental adaptability, the integration and optimization of biosynthesis pathways for valuable products such as biofuels, antibiotics, and vaccines.

Valuable lessons have been learned from the Sc2.0 international collaboration.[4] Firstly, it emphasizes the importance of establishing well-defined "smart" goals for an international project, ensuring they are specific, measurable, attainable, relevant, and time-bound. Setting consensus-driven research goals, along with achievable and time-bound milestones, is crucial for attracting top-tier researchers. Secondly, effective communication within the consortium is vital for coordinating the progress of different groups to align with a defined timeline. The success of the Sc2.0 project has demonstrated that mutual trust and regular meetings, including annual gatherings and frequent online calls, greatly facilitate communication despite geographical distances. Thirdly, separating design from synthesis enables participants to develop unique synthesis technologies based on their individual research backgrounds, ultimately leading the consortium to produce a series of diverse yet strongly related articles. Lastly, each participant is required to secure their own funding to initiate the project. The achievements of the consortium enhance participants' reputation within the scientific community and support them in securing additional external funding. In summary, the Sc2.0 project fosters a collaborative, inclusive, innovative, and responsible culture, which can be extended to future global research communities.

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DECLARATION OF INTERESTS

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