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RESEARCH HIGHLIGHT

Enabling whole pathway reconstruction using artificial chromosomes

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In synthetic biology it is desirable to transfer megabase-scale DNA as this enables introduction of complete biological pathways into a new host organism. A new study in *Cell Research* reports HAnDy (Haploidization-based DNA Assembly and Delivery in yeast) that enables efficient assembly of a 1.024 Mb artificial chromosome and chromosomal transfer between *Saccharomyces* species.

Our understanding of model organisms, such as yeast, has been significantly advanced through development of new systems and synthetic biology techniques. However, systemically engineering cells by large-scale genetic manipulation remains challenging.[1,2] One of the key obstacles is the lack of convenient tools for assembly and delivery of large-scale DNAs,[3] as existing methods often require complex steps.[4,5] By overcoming the traditional hurdles associated with large DNA manipulation, Ma et al.[6] developed a state-of-the-art method called HAnDY (Haploidization-based DNA Assembly and Delivery in yeast) for the assembly and delivery of megabase-scale DNA by spontaneous mating and programmed haploidization in *Saccharomyces cerevisiae*.

The ability of megabase-scale DNA manipulation and chromosomal transfer endows future analysis of nature's evolution as well as heterologous synthesis of products that requires long pathways. In this study, during the mating process of two yeast cells, the CRISPR/Cas9 system was initiated to promote the assembly of DNA fragments from individual cells, and meanwhile, a set of genomes (16 chromosomes) was selectively eliminated to achieve haploidization. The programmed haploidization method that bypasses the natural process of meiosis could be a very useful tool for microbial breeding. With the programmed haploidization, the assembled strains remained haploid and could readily mate with another donor strain, initiating the next round of assembly easily. Compared with other assembly strategies, HAnDy is an easy-to-use, size-independent method with no need for any fussy in vitro manipulations. Additionally, the method can also serve as a direct cell–cell large DNA transfer approach by selectively eliminating the genome of the donor strain during mating. The approach was employed to successfully deliver assembled megabase-scale synthetic DNAs from the yeast strain BY4742 into six phylogenetically diverse yeasts. These methods significantly enhanced synthetic biology opportunities of whole biosynthetic gene cluster expression in yeast for eukaryotic natural product identifications, as well as heterologous expression of whole central carbon metabolism pathways.[7] Moreover, these methods hold potentials for the manipulation of ~10 Mb DNA, enabling studies of synthetic genomes in higher organisms.

The constructed synthetic accessory chromosome (synAC) containing 542 accessory genes from a collection of 1011 *S. cerevisiae* isolates[8,9] exhibited great potential for the analysis of cell growth and tolerance, and more importantly, yeast evolution passages and mechanisms, which could also potentially enlighten the evolution analysis in general. Briefly, Ma et al. designed and synthesized a 1.024 Mb brand-new chromosome from scratch, which is the largest episomal synthetic functional chromosome reported. Different from synthetic chromosomes in Sc2.0,[10] synAC does not use a native genome as a template for its design. Therefore, it is a de novo designed chromosome comprised of numerous exogenous genes and regulatory elements. As a functional expansion chromosome, synAC could rapidly promote the host's adaptations, such as tolerance to high and low temperatures. Future scramble of synAC could be one of the ways to further understand the above-mentioned cellular mechanisms.

Another core finding of this paper is that synAC significantly expanded the host's metabolic network and enhanced the host's biosynthetic capacity. Several compounds that were commonly thought not to be produced in yeast have been identified in yeasts harboring synAC. It is of particular interest that, not all yeast strains and species achieved the same traits when transformed with synAC. Genes in the artificial chromosome acted differently in cells of different origins, indicating that not all phenotypic traits are conferred by the artificial chromosome, and those accessory genes in the chromosome may not be completely responsible for the exhibited additional traits, such as increased tolerance, utilization of additional substrates, and production of novel compounds. This result can be explained by the need for close interplay between the accessory genes and the endogenous network of the different yeast species. These findings may, however, through future analysis of the yeast strains lead to new insight into how different yeasts have evolved to accommodate different environments, and how different compounds have been evolved to be produced.

In summary, Ma et al. presented an efficient large-scale DNA assembly and delivery method, and demonstrated its application through design, assembly, and delivery of a 1.024 Mb synAC to various yeast species. This breakthrough in the field of synthetic genomics overcomes the bottleneck of megabase-scale DNA

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manipulation, paving the way for larger DNA manipulation (> 10 Mb). Moreover, their novel strategy for large-scale introduction of exogenous genes into the host represents an extraordinary approach to diversifying host properties and systematically investigating complex traits acquired when a large number of additional genes are transferred to a host cell.

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