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Review

Plant synthetic genomics: Big lessons from the little yeast

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SUMMARY

Yeast has been extensively studied and engineered due to its genetic amenability. Projects like Sc2.0 and Sc3.0 have demonstrated the feasibility of constructing synthetic yeast genomes, yielding promising results in both research and industrial applications. In contrast, plant synthetic genomics has faced challenges due to the complexity of plant genomes. However, recent advancements of the project SynMoss, utilizing the model moss plant *Physcomitrium patens*, offer opportunities for plant synthetic genomics. The shared characteristics between *P. patens* and yeast, such as high homologous recombination rates and dominant haploid life cycle, enable researchers to manipulate *P. patens* genomes similarly, opening promising avenues for research and application in plant synthetic biology. In conclusion, harnessing insights from yeast synthetic genomics and applying them to plants, with *P. patens* as a breakthrough, shows great potential for revolutionizing plant synthetic genomics.

INTRODUCTION

Synthetic genomics is a nascent field of synthetic biology, seeking to chemically synthesize whole chromosomes and genomes and replace their natural counterparts. This field aims to create new life forms for innovative applications and to deepen our understanding of fundamental biological processes. Historically, genetic modifications were primarily limited to isolated gene mutations or insertions at specific sites within the genome. However, the field of synthetic genomics is expanding rapidly due to significant advancements in DNA synthesis and assembly technologies. This emerging field is characterized by its ability to enact larger-scale modifications across entire genomes, marking a shift toward more comprehensive genome-level interventions. Especially in recent decades, synthetic genomics has undergone a remarkable evolution, starting with the synthesis of viral genomes, such as the poliovirus and ϕX174 bacteriophage.[1,2] These early successes paved the way for more ambitious projects, including the synthesis of bacterial genomes like *Mycoplasma genitalium* and *Escherichia coli*.[3,4] Recently, yeast achieved the milestone of becoming the first man-made eukaryote with synthetic chromosomes created from scratch. This endeavor is known as the Synthetic Yeast Genome Project (Sc2.0) and represents the collaborative effort of a global team of scientists.[5–7] The synthetic yeast genome, equipped with built-in diversity generators and markers, serves as a pioneering platform for unraveling the intricate organization of yeast genomes.[7] This initiative not only provides insights into the fundamental principles governing genome architecture but also opens avenues for enhancing genomes to cultivate more resilient organisms.[7,8]

However, the realm of plant synthetic genomics has lagged behind, as it is considered one of the most intricate domains within synthetic biology.[9] The importance of extending synthetic biology principles to plants cannot be overstated, as they offer distinct advantages in synthetic biology[10] (Figure 1). Plants serve as a vast repository of over 200,000 unique specialized metabolites, including therapeutic phytochemicals and essential nutrients for humans.[11] Furthermore, plants naturally produce these compounds in various tissues and organelles, allowing for compartmentalization of complex metabolic pathways. While microbes struggle to replicate certain intricate plant pathways, plants are inherently equipped for the production of valuable chemicals using light and water.[12] This makes cultivation of plants as a production platform cost-effective, eliminating the need for expensive fermenters and extraction processes.[13,14] Additionally, plants hold a pivotal role particularly in the context of enhancing photosynthetic efficiency in the field of synthetic biology, potentially addressing the challenges posed by global population growth and climate change.[15,16] In addition to these applications, synthetic genomics aids in addressing fundamental questions in plant biology by allowing the systematic manipulation of biological systems.[17,18] However, our understanding of plant systems remains limited. One major challenge is comprehending the interactions among multiple cells, which exhibits remarkable genomic diversity and complexity comparing to the yeast.[19] Synthetic genomics thus opens opportunities for addressing fundamental biological

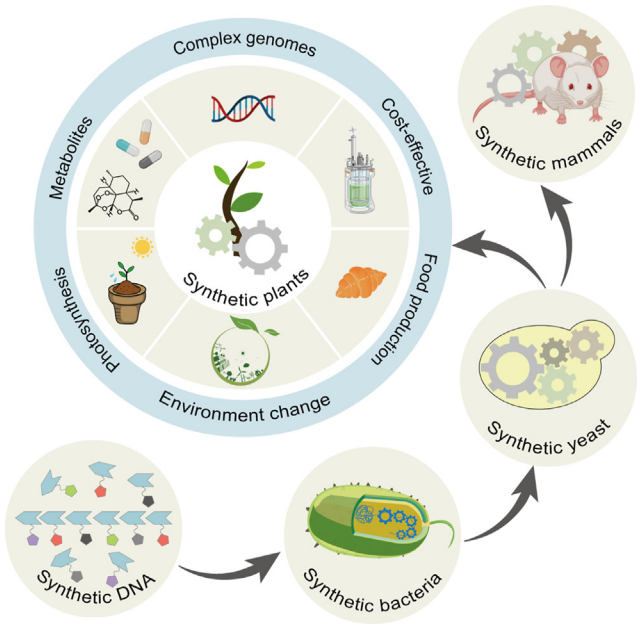

Figure 1. Advantages of plant synthetic genomics in application
Evolutionary trajectory of synthetic genomics. Plant synthetic biology promises distinctive opportunities for innovation and practical application.

questions through genetic manipulation and *de novo* synthesis[18] (Figure 1).

In this review, our journey extends beyond yeast-centric studies to the realm of plant synthetic genomics, with a focus on *Physcomitrium patens*—a moss species occupying a crucial phylogenetic position in land plant evolution. The central idea guiding our exploration is to harness the power of yeast synthetic genomics and apply them to engineer plant genomes, unlocking avenues for exploration and application in plant synthetic biology.

INSIGHTS FROM YEAST SYNTHETIC GENOMICS

Yeast, particularly *Saccharomyces cerevisiae*, has become the go-to organism in synthetic biology endeavors such as pathway engineering and protein production.[20] Our exploration extends beyond these conventional realms of synthetic biology, focusing on the genetic and genomic feasibility in synthetic genomics, exemplifying yeast's pivotal role in advancing the field.

The yeast artificial chromosome (YAC) represents a significant milestone in synthetic biology, serving as a versatile platform for dissecting genome fundamentals and engineering new functions in a context that closely mimics the native chromosomal environment. YACs provide a robust system for delivering genes of up to 3 Mbp into mammalian cells, overcoming limitations associated with viral vectors.[21] Unlike viral vectors, YACs are episomal, non-integrative, and can carry large genomic loci with all regulatory elements, faithfully mimicking natural gene expression patterns. Moreover, YACs have played a crucial role in fundamental studies, including investigations into chromosome instability, recombination, and chromosomal transfer technologies. Their ability to carry entire genomic loci makes YACs invaluable tools in exploring questions related to chromosome behavior and entire genome analysis, contributing significantly to the field of synthetic genomics research[22,23] and laying the groundwork for more ambitious projects like the Sc2.0.

The Sc2.0 project aims to construct the first synthetic eukaryote genome and catalyze further breakthroughs in synthetic genomics. This collaborative effort involves research laboratories worldwide and centers on the complete redesign and construction of the yeast genome[24] (Figure 2A). The challenge posed by this project is significant, given that the yeast genome is typically much larger and more complicated compared to that of prokaryotic cells. By the end of the year 2023, this challenging project, undertaken by a consortium of over 250 researchers worldwide, is approaching completion after more than a decade of dedicated work.[7,25] The ambitious goal is to create synthetic yeast with heavily modified versions of all 16 chromosomes with an additional unnatural neochromosome that harbors the genes encoding all 275 tRNAs in yeast.[26]

The genomic modifications involve eliminating retrotransposons, subtelomeric repeats, and introns, as well as introducing diverse genetic engineering elements such as incorporating multiple PCR watermarks and numerous LoxPSym sites. The LoxPSym sites facilitate controlled evolution and modification of the synthetic yeast genome. When activated by Cre recombinase, they trigger recombination between two LoxPSym sites, resulting in random genetic changes like deletion, inversion, translocation, and duplication of DNA sequences flanked by LoxPSym[27] (Figure 2B). Consequently, a previously single isogenic strain transforms into a highly diverse population, facilitating screening for candidates with enhanced properties. This innovative system is dubbed "synthetic chromosome rearrangement and modification by LoxP-mediated evolution" (SCRaMbLE),[6] creating a diverse array of genome variants for selection and study. Complementing this, adaptive laboratory evolution (ALE) is employed to improve the fitness and functionality of synthetic strains by subjecting them to iterative rounds of selection under specific conditions.[28] This technology has already been employed to develop *S. cerevisiae* strains with increased alkali tolerance and heightened ethanol and heat tolerance, as well as antibiotic resistance.[29–31]

Besides creating more robust and versatile yeast strains, the Sc2.0 project also addresses scientific inquiries including genome plasticity and chromosome packaging. In recent studies, the Sc2.0 teams demonstrated the yeast genome's remarkable tolerance to 3D structural alterations. For instance, the engineered synthetic fusion chromosome (synI-synIII) showed continued yeast growth despite significant 3D structural changes.[32] Furthermore, a technology utilizing designer proteins was developed to manipulate the 3D conformation of synIV, resulting in the silencing of most synIV genes without altering the DNA code.[33] This result represents a significant advancement in predictable 3D genome engineering within synthetic genomics. Along with the successful transfer of synIV, a cell finally incorporates 6.5 synthetic chromosomes, representing over 50% of the genetic information in synthetic form.[25] This achievement underscores the significant progress made in synthetic genomics. Additionally, the team introduced innovative techniques such as "CRISPR D-BUGS" (CRISPR directed bbiallelic URA3-assisted genome scan) for identifying synthetic DNA sequence

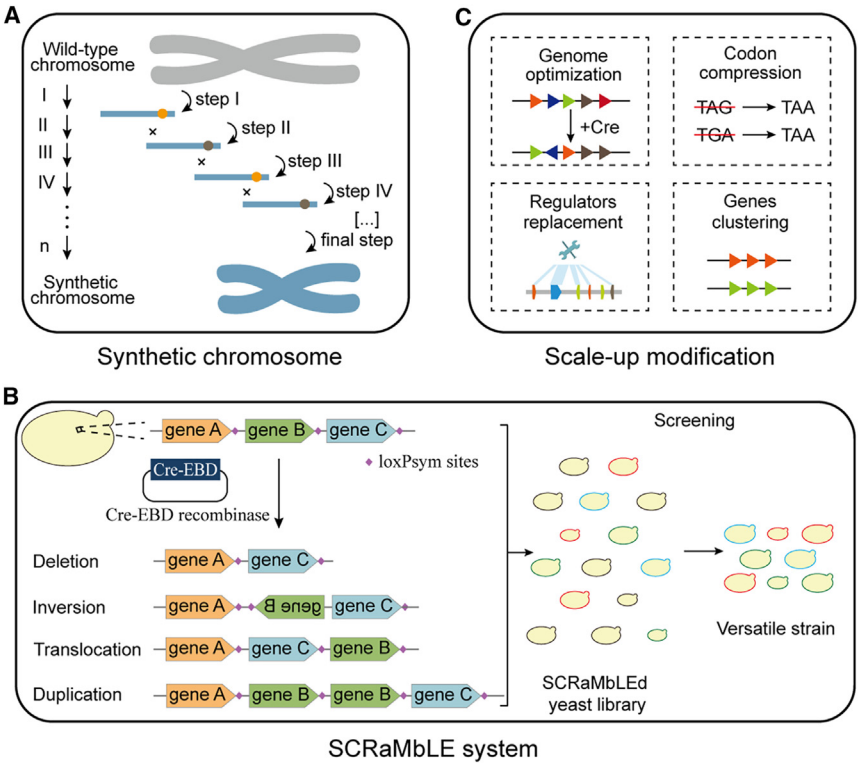

Figure 2. Evolutionary pathways in synthetic yeast genomics

(A) Step-by-step replacement of yeast wild-type chromosomes with synthetic ones via homologous recombination in project Sc2.0.

(B) The SCRaMbLE system involves inducing Cre recombinase activity on loxPsym sites within synthetic genomes, resulting in various genomic rearrangements such as deletion, inversion, duplication, or translocation. This generates a highly diverse population of cells with different genetic configurations, which can be screened for desired phenotypes.

(C) Sc3.0 aims for more extensive modification of the whole Sc2.0 genome, involving genome optimization, codon compression, regulator replacement, and gene clustering to enhance the yeast genome's functionality and adaptability for diverse biotechnological applications. SCRaMbLE, synthetic chromosome rearrangement and modification by LoxP-mediated evolution.

errors and utilized SCRaMbLE to quickly produce variant strains with potentially advantageous properties.[25] In summary, the ongoing Sc2.0 is meaningful as it not only pushes the boundaries of synthetic genomics but also holds promise for practical applications in diverse fields.

In summary, Sc2.0 leverages a suite of cutting-edge technologies to design, synthesize, and manipulate entire genomes from scratch. Key technologies include high-throughput chemical synthesis of DNA sequences, enabling the creation of long DNA strands that can form the basis of synthetic genomes. Techniques such as polymerase cycling assembly (PCA),[34] transformation-associated recombination (TAR),[35] and Gibson assembly are used to join smaller DNA fragments into larger constructs, eventually forming complete genomes.[36] CRISPR-Cas9 and other gene editing tools are employed to precisely edit and rearrange genomic sequences, facilitating the removal of non-essential genes and the introduction of desired genetic modifications.[37] Synthetic genome transplantation replaces the host's native genome and allows the expression of the synthetic genome's traits.[38] Advanced algorithms and bioinformatics tools are used to design and model synthetic genomes, predicting the functionality and viability of the designed sequences.[39] Additionally, SCRaMbLE and ALE facilitate yeast evolution for specific functional applications by enabling rearrangement of synthetic chromosomes under specific conditions.[29]

As an extension of the Sc2.0, Sc3.0 is proposed aiming to take synthetic genomics to the next level by redesigning and optimizing the yeast genome.[8,40] The Sc3.0 strategy involves restructuring essential genes with regulatory elements, validating their functionality, and assembling them into dedicated chromosomes. One key innovation is the construction of essential gene arrays (eArray), relocating vital genes to a non-SCRaMbLEable circular centromeric DNA, ensuring their stability.[40] The SCRaMbLE system enables controlled genome minimization by inducing deletions, with eArray enhancing the variety of deletions. To achieve a more compact and synthetic yeast genome, Sc3.0 proposes reprogramming remaining genes by recoding open reading frames and replacing regulatory elements with artificial or validated sequences[41,42] (Figure 2C). As a part of this pioneering work, a recent study introduces SparLox83, a yeast strain with 83 strategically placed loxPsym sites across all 16 chromosomes, enabling versatile genome-wide genomic evolutions upon Cre recombinase induction.[43] Analysis of evolved strains reveals the impact of genomic rearrangements on the transcriptome, 3D genome structure, and phenotypes, highlighting SparLox83 as a potent tool for studying and accelerating strain engineering in yeast.[43] Ultimately, building on the achievements of the Sc2.0 project, Sc3.0 offers an exciting opportunity to explore fundamental questions about genome redundancy, compactness, and organization, paving the way for the creation of synthetic organisms with even greater capabilities and versatility, revolutionizing diverse fields including plant synthetic genomics.

PLANT SYNTHETIC GENOMICS

Despite the clear advantages of plant synthetic genomics (Figure 1), progress has been hindered due to the inherent complexity of plant genomes. However, through *de novo* synthesis, synthetic genomics offers a pathway to overcome these obstacles, enabling a better understanding of genome complexities for various applications.

Plant artificial chromosomes

Artificial chromosomes serve as essential components in the toolkit of synthetic biologists, enabling them to engineer living systems with unprecedented precision and complexity.[44] Similar

Table 1. Plant artificial chromosomes

Species	Construction method	Reference
Arabidopsis thaliana	Top-down; TMCT	Teo et al.[51]
Arabidopsis thaliana	Top-down; Cre/LoxP	Murata et al.[52]
Arabidopsis thaliana	Top-down; LacO/LacI	Teo et al.[53]
Brassica napus	Top-down; TMCT	Yan et al.[54]
Brassica napus	Top-down; TMCT	Yin et al.[55]
Diploid barley	Top-down; TMCT	Kapusi et al.[56]
Oryza sativa	Top-down; TMCT	Xu et al.[57]
Oryza sativa	Top-down; TMCT	Yang et al.[58]
Triticum aestivum	Top-down; TMCT	Yuan et al.[59]
Zea mays	Bottom-up	Carlson et al.[60]
Zea mays	Top-down; TMCT	Yu et al.[49]
Zea mays	Bottom-up	Ananiev et al.[61]
Zea mays	Top-down; TMCT	Swyers et al.[62]

TMCT, Telomere-mediated chromosome truncation; Cre/LoxP, cyclization recombinase/Locus of crossing-over on phage P1; LacO/LacI, Lactose Operator/Lactose Repressor.

to YACs, Plant artificial chromosomes (PACs) offer a promising array of advantages in genetic engineering in plants. These engineered carrier systems provide a solution to the limitations of conventional transgenes by independently introducing exogenous DNA in abundance, thereby circumventing issues such as insertional inactivation and positional effects.[45,46] Furthermore, PACs represent a powerful tool for advancing genetic research. For example, PACs could offer a non-disruptive platform for functional genomics studies, enabling researchers to decipher the roles of specific genes in plant development, physiology, and responses to environmental stimuli. In the field of synthetic genomics, PACs also enable researchers to investigate fundamental principles of chromosome organization, dynamics, and regulation.[47]

PAC construction involves two approaches: top-down and bottom-up strategies.[48] Top-down approach is a widely used method involves telomere-mediated chromosome truncation (TMCT). Telomeric DNA sequences are integrated into the plant genome via Agrobacterium or biotic-mediated transformation, leading to chromosomal truncation, resulting in the production of artificial minichromosomes.[49] Minichromosomes generated through the TMCT retain the genetic content of the native plant chromosomes, rendering them inherently stable and more likely to be activated and functional within cells.[50] To date, minichromosomes have been constructed in many plant species (Table 1). However, minichromosomes constructed using the TMCT method may result in large segment deletions, potentially leading to the loss of functional genes in the plant genome, affecting normal plant growth and even viability.[48,63] Conversely, the bottom-up approach involves assembling artificial chromosomes based on fully synthesized functional chromosome elements. The bottom-up approach represents a promising strategy for engineering artificial chromosomes with enhanced customization, precision, and functionality, potentially overcoming the limitations associated with top-down methods.[64] However, the bottom-up strategy is not as effective largely due to the complexity of plant genomes, including the abundance of repetitive sequences and intricate epigenetic modifications, which makes it challenging to synthesize and assemble functionally in a controlled manner. Although pioneering work remains limited, some notable advancements have been made in maize. Using a bottom-up approach, a circular plasmid containing a 19 kb centromere sequence was constructed and segments were introduced into maize embryonic tissues through particle bombardment. Positive transformation materials demonstrated stable inheritance across four generations, with genes carried on the vector exhibiting consistent expression.[60,61] However, controversy arises from concerns regarding the efficacy of the biolistic method, which may result in the integration of exogenous DNA into artificial chromosomes.[45] Nevertheless, much effort lies ahead to overcome challenges and fully leverage the potential of PACs in plant synthetic biology.

From genome engineering to synthetic genome

Plant genome engineering initially focused on the effects of individual genes on plant growth and development, advancements in technology have led to a shift toward understanding the role of multiple genes and pathways engineering.[65] Concurrently, various gene editing techniques for plant genomes have been improved, leading to significant progress in genome engineering technology. For instance, researchers employed a multigene metabolic engineering approach and developed an enhanced multigene stacking system named TGSII-UNiE.[66] This system facilitated the efficient cloning of long DNA fragments of varying sizes and the assembly of multiple gene cassettes. Using the TGSII system, they successfully transferred 8 anthocyanin-related genes into rice, leading to the specific synthesis of anthocyanins in rice endosperm, producing what is known as "purple endosperm rice[67]". Despite these advancements, the increasing demand for crop improvement has prompted the emergence of genome engineering in large scale. For instance, broad-spectrum resistance (BSR) involves resistance against multiple pathogens, achieved by stacking multiple R genes with different resistance spectra.[68,69] Elite rice varieties with stacked *Xa4*, *Xa21*, *Xa7*, *Xa23*, and *Xa27* genes exhibit broader resistance spectra and higher resistance levels compared to single-gene lines.[70,71] Similarly, integrating combinations of powdery mildew R genes *Pm2*, *Pm4a*, and *Pm21* into wheat cultivar Yang 158 results in broad-spectrum powdery mildew resistance.[72] This approach can also be extended to integrate resistance genes against both diseases and insects, offering dual-resistant materials. Nonetheless, traditional breeding methods are time consuming, and many R gene analogs' functions remain unknown. Plant synthetic genomics presents a solution by enabling the integration of R gene clusters on a large scale, facilitating the development of plants with the broadest spectrum of resistance. This is where synthetic genomics comes into play, offering the potential to streamline and amplify the process of engineering plants with desirable traits on a large scale. However, the advancement of plant synthetic genomics is hindered due to challenges such as the complexity of plant genomes and the lack of standardized tools and methodologies.

To date, the field of plant synthetic genomics has not experienced major developments, as the complexity of plant genomes continues to pose substantial challenges. Plant genomes exhibit considerable variation in size and ploidy, ranging from small

genomes like *Arabidopsis thaliana* (135 Mbp, significantly larger than that of yeast) to extremely large genomes, such as *Tmesipteris oblanceolata*, which reaches up to 160 Gbp.[73] A notable feature of plant genomic DNA is the abundance of repeated sequences, including transposable elements and tandem repeat arrays. For instance, repetitive elements can constitute up to 80% of the maize genome. Additionally, extensive gene copy number variations are frequently observed in plants.[74] Consequently, much of the progress in this area has been incremental rather than revolutionary. However, the breakthrough came with ongoing research on *P. patens*, a model plant that offers an opportunity to overcome these challenges. *P. patens*, also known as "green yeast",[75] is emerging as a prominent figure in plant synthetic biology. Similar to yeast, it features a dominant haploid nature and the highest rate of homologous recombination among plants.[76,77] This renders it an exemplary model for functional genomics in multicellular eukaryotes, facilitating manipulation akin to working with yeast.[78] The unique evolutionary status of *P. patens* provides crucial insights into early plant development.[79,80] With a straightforward anatomical structure and a predominantly haploid life history, *P. patens* allows for efficient extraction of biomolecules and simplified developmental patterns, facilitating experimental manipulation. The moss's totipotent cells enable direct regeneration, supporting genetic manipulation and ensuring the purity of obtained mutants for accurate phenotypic analysis.[81] Despite its simple anatomy, *P. patens* exhibits complex morphological structures and responses to growth factors, resembling those of other terrestrial plants. Its capacity to fold and glycosylate expressed exogenous genes makes it an ideal model for studying plant development and metabolism.[82]

Recent advancements in nucleic acid sequencing further underscore *P. patens* as a superior model plant, emphasizing its significance in molecular biology and genetics research.[83] In plant synthetic genomics, advancements have reached a significant milestone with the synthesis of part of the genome of *P. patens*. This ambitious project is dubbed "SynMoss", a research initiative focused on synthesizing the genome of *P. patens*, aiming to explore the potential of creating artificial genomes for multicellular organisms and leveraging the moss for various applications. In the project of SynMoss, scientists began by synthesizing a portion of the short arm of chromosome 18, successfully replaced a 155,181 bp region with a 68,530 bp redesigned sequence, resulting in an approximately 55.8% size reduction.[84] The design principle focuses on the strategic simplification of the moss genome while preserving its gene functions. This is achieved by retaining essential coding and regulatory sequences, eliminating repetitive and non-essential DNA, substituting stop codons to facilitate future genetic manipulations, and introducing PCRTag for rapid detection of successful genome replacements. Additionally, the project adjusts gene positions for optimal assembly and incorporates LoxPsym sites for potential genetic rearrangements.[43,84,85] The project ingeniously combined plant and yeast recombination systems to assemble and replace synthetic DNA fragments in the moss *P. patens*. Mini-chunks of DNA, synthesized chemically and amplified in *E. coli*, were pieced together into larger mid-chunks within yeast, capitalizing on yeast's efficient homologous recombination. These mid-chunks were then transformed into *P. patens* protoplasts, where the plant's own recombination machinery facilitated the *in vivo* replacement of endogenous sequences. The project also successfully implemented a single mega-chunk replacement strategy, demonstrating the potential of large-scale genome engineering in plants.[84,85] This cross-kingdom approach to synthetic genomics holds promise for future genetic manipulation in plants. The resulting plants exhibited normal growth, reproduction, and stress resistance, demonstrating the feasibility of engineering a multicellular organism.[84] The preliminary progress of SynMoss and the yeast projects have significantly advanced the field of synthetic genomics by targeting different organisms and providing unique insights into genome organization, function, and engineering. The moss results demonstrated the feasibility of removing a substantial portion of a plant genome, including repetitive sequences and transposable elements, without affecting the organism's viability, thereby challenging the indispensability of such elements in plants. In contrast, Sc2.0 has synthesized and assembled designer yeast chromosomes, eliminating non-essential sequences and introducing novel genetic features in single-cell organisms.[40,86] Both projects have explored the impact of genome design on epigenetic regulation, with SynMoss showing the re-establishment of the epigenetic landscape in the synthetic region and Sc2.0 investigating the effects of gene density and chromosome structure on gene expression.[86] Furthermore, the progress of SynMoss challenges the conventional belief that synthetic genomes are only feasible in microbial organisms and underscores the potential of plant synthetic genomics to revolutionize synthetic biology applications.[87] Looking ahead, researchers aim to complete the synthesis of the entire moss genome within the next decade, further advancing the field of plant synthetic genomics.

The advancements in understanding the basic principles of plant biology through synthetic genomics in *P. patens* pave the way for broader applications across other plant species (Figure 3A). By reconstructing the whole genome, researchers can gain a deeper understanding of the evolutionary processes that have shaped plant genomes over time. Synthetic genomics enables the systematic study of the function of various genomic elements, such as transposons, non-coding RNA, and intergenic regions, providing insights into their roles in plant biology. Additionally, synthetic genomics allows for precise modifications to the moss genome, facilitating the engineering of desired traits for applications in agriculture, medicine, and environmental sustainability. *P. patens* serves as a model organism for studying various aspects of plant biology, and by synthetically reconstructing its genome, researchers can develop valuable tools and resources for further investigations in plant science. Furthermore, the process of synthesizing the moss genome pushes the boundaries of synthetic biology and genome engineering, driving technological advancements that can be applied to other organisms and research areas (Figure 3A). For example, studying the mechanisms underlying homologous recombination in *P. patens* provides valuable insights that can be applied to other plants and even animals, expanding the scope of genetic manipulation beyond microbes. This understanding of genetic processes enables the development of innovative strategies for synthetic biology in multicellular organisms, facilitating the design of custom genomes tailored to specific purposes (Figure 3B).

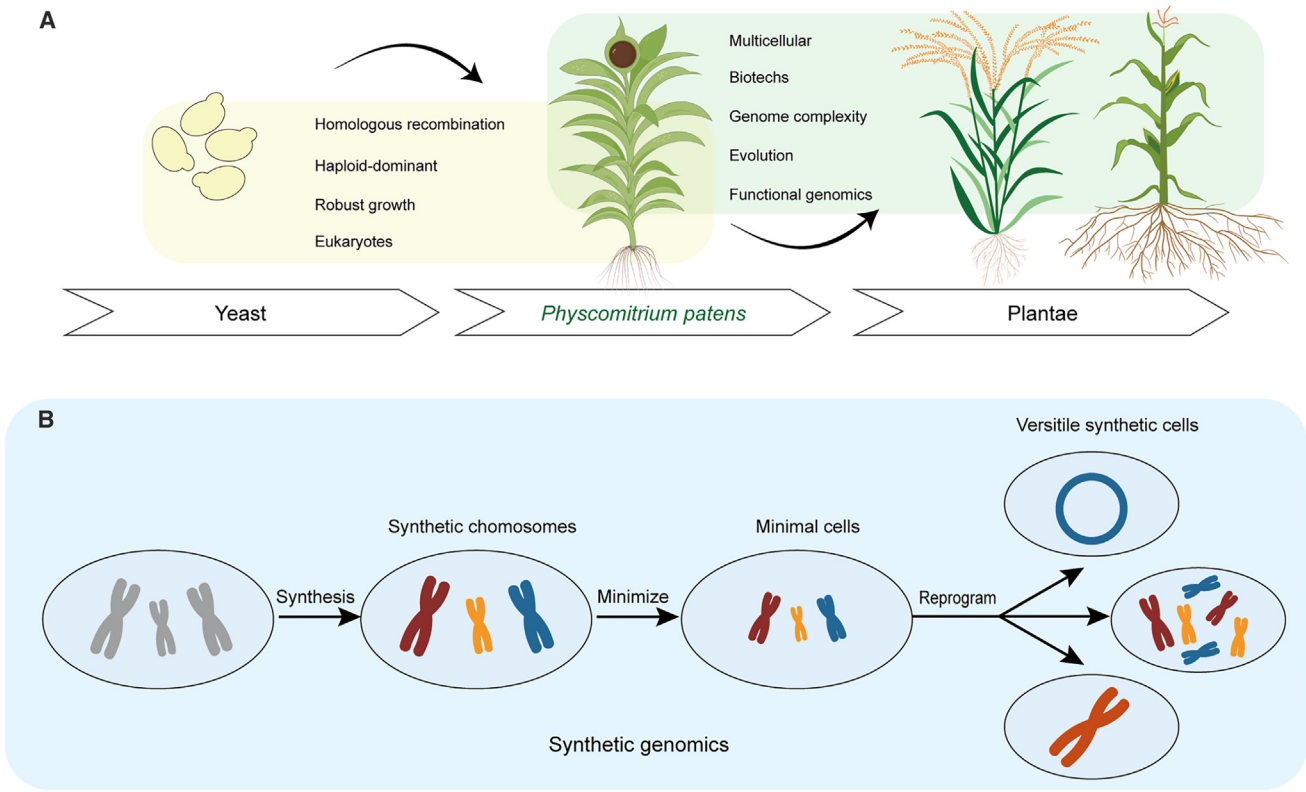

Figure 3. Translating synthetic genomics from yeast to plants via *Physcomitrium patens*

(A) *P. patens* shares fundamental genetic mechanisms with yeast, including homologous recombination and haploid-dominant traits, robust growth, and eukaryotic characteristics. These shared traits make *P. patens* an ideal candidate to leverage insights from yeast synthetic genomics studies like Sc2.0 and Sc3.0. In addition, *P. patens* possesses a unique evolutionary position and shares genome complexity and multicellularity with other higher plants, positioning it as a versatile platform for developing biotechnologies applicable to broader plant species.

(B) Drawing insights and expertise from yeast synthetic genomics to explore the creation of more versatile life forms through plant synthetic genomics. These designer life forms including higher plants with minimal genome, specific numbers of chromosomes, or a single circular chromosome without telomeres.

Through continued research and technological innovation, the insights gained from studying *P. patens* hold immense potential to revolutionize plant synthetic biology and drive transformative advancements in various fields, offering practical solutions for addressing global challenges.

CHALLENGES AND PERSPECTIVES

Plant synthetic genomics encounters numerous challenges stemming from the complex nature of plant genomes. These challenges include intricate genetic regulation, complex metabolic pathways, and the presence of numerous non-coding regions, alongside epigenetic modifications, among others.[88] To address some of these challenges, we can employ the "build-to-learn" principle, enabling us to adapt our methods promptly based on the insights gained during the process. Foremost among the challenges in synthesizing plant genomes are centromere obstacles, transmission barriers, controllable replication, and transformation efficiency.

The centromere presents a formidable challenge in the synthesis of functional chromosomes. In human artificial chromosomes (HACs), centromere sequences may remain inactive or bypassed during the formation of new centromeres.[89] Notably, the DNA sequences commonly found at human centromeres is neither necessary nor sufficient for centromere identity and function, suggesting complicated epigenetic modifications.[90,91] Plant centromeres exhibit even greater diversity, comprising tandem repeats, retrotransposons, and low-copy sequences that are vital for chromosome stability and gene regulation.[92,93] While plant centromeres primarily consist of tandem repeats, the length and composition of these repeats vary among different species. For instance, maize and *Arabidopsis thaliana* have shorter repeat units (156 bp and 178 bp, respectively), while potatoes have longer ones (~5,390 bp).[94] Research indicates that the repetitive sequences in centromere regions form highly structured complexes with nucleosomes, crucial for the formation of functional centromeres.[95] Moreover, retrotransposons and functional genes are present in centromere regions, where their products regulate chromatin conformation, thereby impacting the localization of the centromere-specific histone 3 (CENH3).[96] The binding of CENH3 is essential for proper chromosome segregation during cell division. Recent developments in maize artificial centromere construction using the LexA-LexO system offer promising avenues for overcoming these challenges.[97] This method involves recombining LexO repeat sequences onto chromosome arms and recruiting the natural centromere protein H3 (CENH3) to these positions via a fusion protein, LexA-CENH3, resulting in stable and functional

neocentromeres that can be inherited without an activated conventional centromeric sequences.[64,97]

The transmission barrier poses another challenge, primarily due to the generalized failure of pairing and sister cohesion.[98,99] This means that synthetic chromosomes may not segregate properly during cell division, leading to potential issues in genetic transmission. However, these barriers could be overcome through various approaches. One strategy involves using truncated natural chromosomes such as B chromosomes, which exhibit homolog pairing and faithful sister chromatid cohesion, mitigating transmission problems. Another approach is to introduce gametophyte selection on synthetic chromosomes, ensuring their transmission through generations by placing genes that confer viability specifically in pollen grains.[63] While the underlying mechanisms of transmission barrier are not yet fully understood, we can gain insights from model plants. For example, *P. patens*' capability to sustain growth in the haploid phase and undergo induced meiosis provides a valuable experimental platform for studying the mechanisms governing small chromosomes pairing during meiosis, thus addressing the transmission barrier issue across plant species.

Replication of a synthetic chromosome presents another challenge. For most eukaryotic organisms including plants, origin of replication (ORI) lacks common DNA sequences.[100] In plants, studies have distinguished between the ORI and non-ORI regions of *Arabidopsis thaliana* through computational modeling, with an overall prediction accuracy of 69.5%.[101] However, DNA sequence alone does not play a decisive role in replication initiation, it requires integration of various factors including nucleosome positioning, DNA methylation, and histone modification for more precise localization of replication origins. Furthermore, studies on the strength of ORI during development have identified several Arabidopsis ORI sites, revealing associations with various chromatin signals, including transcription start sites, as well as proximal and distal regulatory and heterochromatin regions. Additionally, quantitative analysis of ORI activity has shown that active ORI sites have higher GC content and GGN trinucleotide clusters. Among the studied Arabidopsis ORI sites, ORI strength is more correlated with plant developmental stages rather than the sequence. However, we can still leverage sequences from other species to possibly address this issue. For example, studies have utilized the sequence of the mild strain of the geminivirus Bean yellow dwarf virus (BeYDV-m) to enhance vector replication and passage through generations. Nonetheless, this approach introduces a challenge of uncontrollable number of copy number. In contrast, a recent study demonstrated a method for efficiently forming single-copy HACs by utilizing a large construct containing distinct chromatin types at the inner and outer centromere regions. This strategy avoids rampant multimerization of the initial DNA molecule by employing a ~750-kb construct, and delivery to mammalian cells is facilitated through yeast spheroplast fusion.[102]

Other challenges include the technical barriers associated with plant transformation. While biolistic bombardment permits the transfer of DNA fragments exceeding 1 Mbp, concerns arise regarding DNA integrity and the potential for chromosomal and cellular damage during the intense process.[103–105] To achieve chromosome-level delivery, the development of large-scale transformation methods is crucial, such as nanoparticle delivery, yeast spheroplast fusion, and microinjection, along with the subsequent regeneration of transformed plant cells. While *P. patens* shows promise by enabling large DNA transformation of up to approximately 100 kb, surpassing other plant species, further advancements are required to realize fully synthetic chromosomes. A current strategy involves replacing chromosome segments incrementally. Understanding the transformation and homologous recombination mechanisms inherent in *P. patens* could make a paradigm shift and pave the way for assembling DNA fragments *in vivo* in plant cells.

CONCLUDING REMARKS

Plant synthetic biology is indispensable for addressing global challenges related to food, health, environment, and sustainable development. Within this field, plant synthetic genomics shows immense promise for pioneering applications and advancing our understanding of plant biology. Enhanced crop traits, such as improved yield, stress tolerance, and nutritional value, can be achieved through redesigned genomes, leading to crops that are more resilient to climate change. Sustainable agricultural practices can be advanced through artificial chromosomes with complex metabolic pathways for nitrogen fixation, nutrient uptake, and pests resistance, thereby reducing reliance on chemical fertilizers and pesticides. Moreover, plants can be designed and synthesized as biosynthesis platforms for valuable compounds such as pharmaceuticals and biofuels, offering a renewable source of these substances. Customized synthetic chromosomes designed for specific functions could revolutionize plant breeding and genetic research, facilitating the study of gene interactions and regulatory networks under controlled conditions. Integrating synthetic genomics with precision agriculture technologies will optimize crop performance tailored to distinct farming practices and environmental conditions, thereby enhancing resource efficiency (Figure 1). By exploring these opportunities, synthetic genomics has the potential to significantly transform not only plant science and agriculture but also to offer innovative solutions to a wide array of global challenges. However, our advancements in this field have faced considerable setbacks, primarily attributed to the intricate nature of plants. Yet, within the vast diversity of plant species, there exists a strategic opportunity to overcome these challenges by identifying model plants that can serve as pivotal breakthroughs for the development of both plant synthetic biology and plant science. A notable example is the moss species *P. patens*. By drawing parallels with successful methods and ideas proven effective in yeast, we can readily apply and adapt these strategies to enhance our understanding and manipulation of this model plant. Leveraging the unique attributes of such model plants allows us to pioneer a comprehensive understanding. Subsequently, the methodologies and knowledge derived from these model plants can be extrapolated and applied to a broader spectrum, encompassing all organisms.[9] By strategically employing this approach, we aim to tackle profound questions central to biology, including the complexities of genomes, the minimal forms of multicellular organisms, and the functions of seemingly non-coding regions and intergenic regions, among others. Ultimately, unlocking these mysteries provides crucial insights into the fundamental nature of life itself, as we explore the realm of plant synthetic

biology, drawing inspiration from the principles established in yeast studies.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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