特约评述

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植物人工染色体的研究现状与应用前景

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摘要： 由于生物科学与技术的持续革新，传统基因工程的局限性日益凸显。随着合成生物学的迅猛发展，植物人工染色体（plant artificial chromosome，PAC）应运而生。PAC不仅加深了我们对染色体结构与功能的理解，还可作为人为设计与构建的相对独立的工具型染色体，能够避免位置效应与不利连锁现象，为多基因协同表达、复杂性状组合以及完整代谢通路的构建提供高效平台。本文总结了PAC的研究历史和现状，包括截短改造内源染色体与组装染色体元件构建PAC的策略、从头设计与合成染色体片段以实现基因组的改写、功能性着丝粒的构建以及DNA大片段转移技术。讨论了该领域发展所面临的挑战，包括着丝粒合成的复杂性、DNA大片段转移困难、PAC的不稳定性。展望了PAC在染色体基础研究、合成生物技术及农业基因工程中的广阔应用前景。

关键词： 植物人工染色体；微型染色体；合成基因组；人工着丝粒；DNA大片段转移

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Plant artificial chromosomes: current research progress and future application perspectives

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Abstract: Continuous and remarkable innovation in biology and biotechnology has increasingly exposed the limitations of traditional genetic engineering, including random integration of transgenes and challenges in regulating multiple genes, in both basic research and practical applications. With the rapid advancement of synthetic biology, which emphasizes the design and construction of novel biological systems with predefined functions, plant artificial chromosomes (PACs) have emerged as a pivotal development. PACs not only deepen our understanding of chromosome structure and function at the molecular level but also serve as precisely engineered chromosomal vectors. These constructs effectively avoid position effects and linkage drag, providing a robust platform for the co-expression of multiple genes, stacking of complex traits, and engineering of metabolic pathways. This review summarizes the

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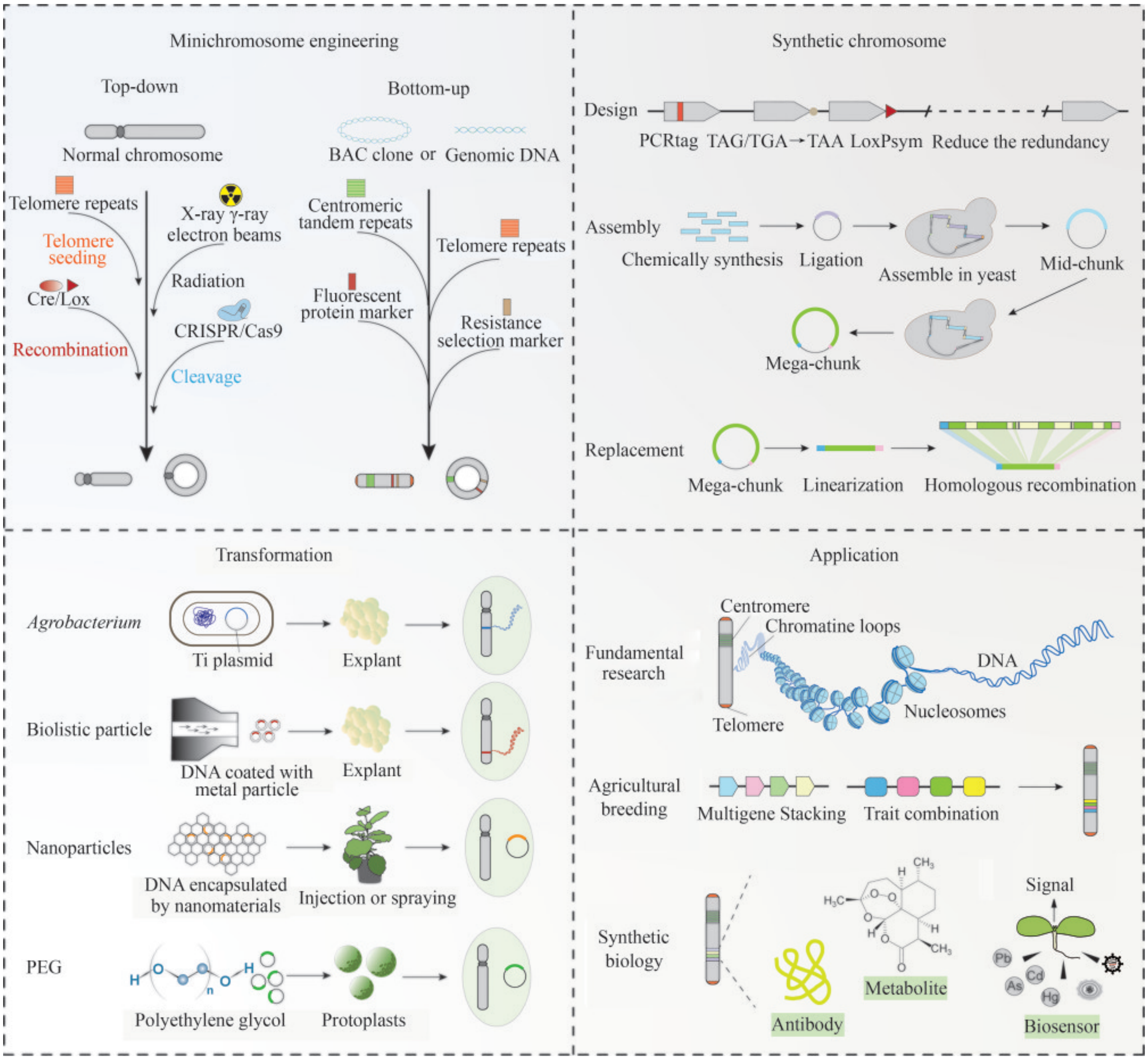
history, progress, and current status of PACs research, highlighting various construction strategies. This includes truncating and modifying endogenous chromosomes, assembling chromosomal elements, such as telomeres, centromeres, and replication origins, to construct PACs, as well as *de novo* designing and synthesizing chromosomal fragments for genome rewriting. The latter approach involves creating entirely new DNA sequences tailored to specific research or application needs. In addition, the review also addresses the critical challenges of constructing functional centromeres, which are essential for accurate chromosome segregation during cell division, and explores techniques for delivering large DNA fragments into plant cells — a crucial step for the efficient introduction of PACs. Furthermore, this paper highlights persistent challenges in the field, such as difficulties in synthesizing centromere, technical bottlenecks in delivering large DNA fragments, and the instability of artificial chromosomes. Finally, it highlights the extensive application potential of PACs in basic chromosome research, synthetic biotechnology, and agricultural genetic engineering. By integrating emerging technologies such as gene editing and AI-driven design, PACs are poised to become core tools for elucidating chromosomal mechanisms, enabling precise crop improvement, and advancing green biomanufacturing. This integration will drive sustainable agricultural development and breakthroughs in plant science.



Keywords: plant artificial chromosome; minichromosome; synthetic genome; artificial centromere; large DNA fragment transfer

染色体是真核生物遗传信息的核心载体，通过其在细胞核内的高度组织化结构，绘制出生命的遗传蓝图。染色体精密的空间结构与动态行为对基因表达调控、细胞分裂以及遗传稳定性具有至关重要的作用。随着生物技术的发展，科学家们已逐步开发出人工染色体技术，实现了从“读取”遗传信息向“编写”生命密码的跨越。人工染色体不仅是对染色体结构与功能认知的终极检验，还可以通过人为设计并组装染色体必要元件，构建出可承载目标DNA序列的可编程遗传平台[1-3]。

在酿酒酵母（*Saccharomyces cerevisiae*）、细菌和哺乳动物中，独立人工染色体的构建已相继实现[4-6]。植物人工染色体（PAC）的研究起步并不晚，早期尝试主要通过简化天然染色体以构建独立的小型人工染色体，这类“自上而下”的策略虽然取得了一定进展，但在微小染色体上进行遗传操作及实现其个体间转移仍存在显著技术障碍。另一方面，通过改造细菌人工染色体，发展出可转化的细菌人工染色体（transformable BAC，TAC，又称binary BAC），实现了约100 kb级别外源片段在植物染色体中的随机整合[7-8]。随着DNA合成、大片段组装、大片段转移及定向整合等技术的突破与综合应用，科学家开始通过“自下而上”的策略，尝试在植物中构建更完整且具生物功能的人工染色体。PAC除了具备作为克隆工具的潜力，还可作为最小化的染色体平台，充当多基因叠加、多性状模块整合与复杂合成代谢通路组装的工程底盘。此外，PAC在解析染色体结构特性、复制与分离规律以及表观遗传重塑机理方面也展现出重要价值。

在本综述中，我们系统梳理了PAC的构建策略，重点探讨了染色体从头设计与合成、功能性着丝粒在PAC构建中的核心作用以及大片段DNA转移技术的当前进展，并总结了该领域未来发展所面临的关键挑战。最后，我们展望了PAC在染色体基础研究、合成生物技术及农业基因工程中的潜在应用。

1 植物微型染色体（minichromosome）工程

微型染色体是独立于正常基因组以外的小型染色体，具有端粒、复制起点和着丝粒等基本元件，几乎不包含额外的遗传信息[9]。这类染色体若能够在细胞有丝分裂和减数分裂过程中保持稳定，则可以作为遗传载体用于堆叠复杂性状所需的多基因，从而为加速植物育种提供平台。此外，微型染色体结构更为简化，可塑性更强，操作上也有望相对简便，因此可以作为研究染色体结构和功能的重要工具[10]。

1.1 “自上而下”的植物微型染色体工程

“自上而下”的染色体工程以宿主植物的内源染色体为起点，通过尽可能去除含基因的染色体臂并保留一个有功能的着丝粒，实现对现有染色体的改造。目前已有多种方法可用于缩短内源染色体，从而产生新型的微型染色体，包括端粒介导的染色体截断［图1(a)］[5, 11-13]、电离辐射诱导染色体断裂［图1(b)］[14-15]、位点特异性重组酶介导的染色体截断［图1(c)］[16-18]，以及规律成簇间隔短回文重复序列(CRISPR)/CRISPR相关蛋白（Cas）介导的染色体敲除［图1(d)］[19-21]等。

1.1.1 端粒介导的染色体截断

端粒是位于染色体末端的DNA-蛋白质复合体，防止染色体末端融合或降解。其DNA重复序列在进化上高度保守，大多数被子植物具有拟南芥型端粒重复序列(TTTAGGG)$_n$[22]。端粒介导的染色体截断在植物中的首次应用，是通过农杆菌（*Agrobacterium*）将2.6 kb的拟南芥（*Arabidopsis thaliana*）端粒重复序列转化到玉米（*Zea mays*）中，成功实现了A（必需）和B（非必需）染色体的截断[23]。其中，来源于B染色体的小型染色体出现频率高于A染色体[24]，原因在于B染色体对玉米而言是非必需的[25]，其截断不会对植物产生不利影响，而某些A染色体发生截断的植物可能在组织培养和再生过程中被选择性地淘汰。此外，B染色体能够在寄主植物群体中长期稳定维持[26]，并能够在不同物种间转移[27-29]。截断后的小型B染色体还能够通过减数分裂进行传递[24]。这些特征使B染色体成为植物中构建微型染色体的理想平台[3, 30]。“端粒播种”在玉米中被证实了有效性后，又陆续被应用于拟南芥[31-33]、水稻（*Oryza sativa*）[34-35]、大麦（*Hordeum vulgare*）[36]、小麦（*Triticum aestivum*）[37]

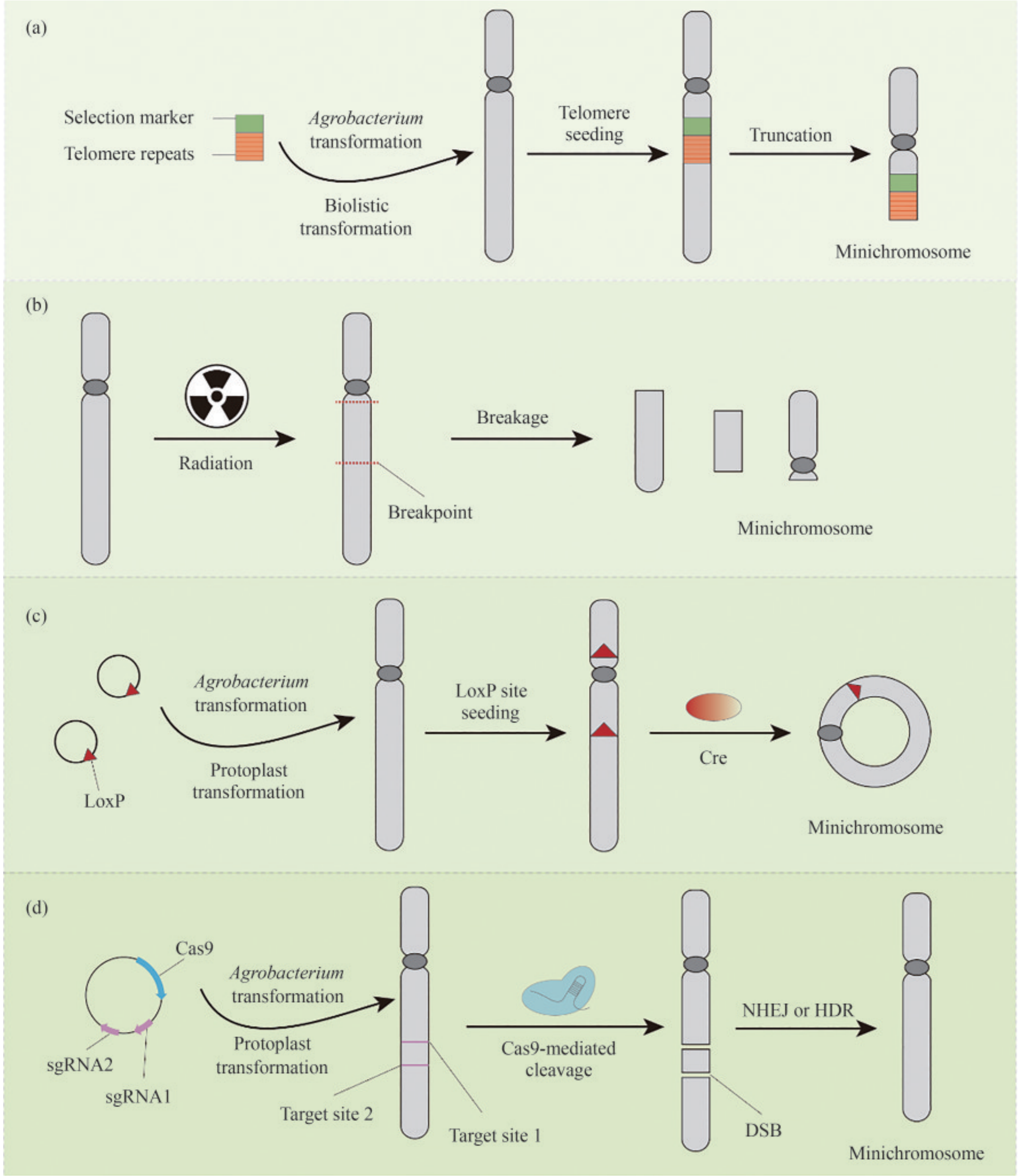


图1　“自上而下”的植物微型染色体构建策略

（a）端粒介导的染色体截断；（b）电离辐射诱导的染色体断裂；（c）位点特异性重组酶介导的染色体截断；（d）CRISPR/Cas9介导的染色体敲除

NHEJ—非同源末端连接；HDR—同源定向修复

Fig. 1　Top-down approach for minichromosome construction in plant

(a) Telomere-mediated chromosome truncation; (b) Ionizing radiation-induced chromosome breakage; (c) Site-specific recombinase-mediated chromosome truncation; (d) CRISPR/Cas9-mediated chromosome deletion

NHEJ—the non-homologous DNA end joining; HDR—the homology-directed recombination

以及甘蓝型油菜（*Brassica napus*）[38-39]等植物中。

1.1.2　电离辐射诱导染色体断裂

电离辐射是诱导染色体结构变异的有效手段。植物经电离辐射处理后，染色体会发生随机断裂，断裂片段在重新连接过程中可能发生缺失、倒位、易位等结构变异[40]。早在1956年，Sears就开展了

植物染色体工程研究，首次利用X射线处理小麦-小伞山羊草（*Aegilops umbellulata*）单体附加系，诱导染色体片段易位，成功将小伞山羊草中的叶锈病抗病基因导入小麦[41]。在此后的小麦育种中，电离辐射持续发挥重要作用[42-43]。通过电离辐射诱导染色体断裂也可形成微型染色体，例如使用X射线和γ射线照射玉米花粉，并利用9号染色体与B染色体的易位事件产生玉米微型染色体[44]。此外，通过电子束辐照小球藻也获得了800 kb、550 kb和450 kb长且源自1号染色体的3条微型染色体[45]。这些微型染色体不仅可用于分离天然染色体的基本构架元件，还有潜力被改造成携带目标基因的人工染色体。

1.1.3 位点特异性重组酶介导的染色体截断

在植物中，插入较长的端粒序列更容易导致染色体截断[2, 31]，然而农杆菌介导的“端粒播种”存在长片段重复序列不稳定以及端粒序列抑制转化效率的问题。同时，由于插入位点的随机性，微型染色体的形成过程难以精确控制。重组酶介导的位点特异性重组系统在植物中的成功应用，提高了“自上而下”构建微型染色体的精确性，也为操作微型染色体提供了有效工具。多种酪氨酸类重组系统（如Cre/Lox、Flp/FRT）和丝氨酸类重组系统（如φC31/att、Bxb1/att）已在植物中被验证有效[46-47]。在拟南芥中，通过Cre/LoxP和Activator（Ac）/Dissociation（Ds）转座元件系统相结合，成功构建了环状微型染色体*At*ARC1。该染色体源自2号染色体，包含一个由180 bp重复序列组成的约250 kb的着丝粒区域，总大小为2.85 Mb，远小于原本26.3 Mb的2号染色体[48]。尽管*At*ARC1不参与减数分裂配对，但能够通过有丝分裂和减数分裂稳定遗传，表现出比线性微型染色体更高的稳定性[48-49]。在水稻、玉米和小麦中，也借助Cre/Lox和Flp/FRT系统实现了微型染色体和正常染色体的重组[24, 34, 37]，以及对微型染色体的改造[50]。

1.1.4 CRISPR/Cas系统介导的染色体敲除

CRISPR/Cas技术因其简单、通用性强且具有高特异性，已成为植物染色体工程中的关键工具。该系统通过单链引导RNA（sgRNA）识别目标序列，并引导Cas核酸酶实现精准切割[51-52]，可在染色体上引入两个或多个双链断裂（DSB），进而引发大规模染色体重排。目前已在植物中实现了大片段染色体的删除[53-56]，利用该技术进一步缩短常规染色体即可构建微型染色体。此外，CRISPR/Cas系统还可用于产生大型倒位和易位，打破基因连锁，实现基因组重排[57-59]，这将显著增强微型染色体工程的灵活性，实现精准的染色体操纵与改造。

1.2 “自下而上”的PAC构建策略

“自下而上”的人工染色体构建是指从头开始组装新的染色体。该方法主要通过整合着丝粒、端粒、自主复制序列等基本元件和选择标记、基因组DNA，从头合成带端粒的线性染色体或不带端粒的环状染色体[60]。这种策略不涉及内源染色体片段的删除，因此可避免染色体片段丢失导致的植物生长障碍；同时也不会整合到宿主基因组中，从而规避了插入突变和位置效应等问题[61]。

1.2.1 人工染色体载体系统的构建

目前，已在多个物种构建了人工染色体载体系统，包括酵母人工染色体（YAC）、细菌人工染色体（BAC）、哺乳动物人工染色体（MAC）、人类人工染色体（HAC）[60]。研究者最早在酵母中构建了长度为55 kb的YAC，包含复制起点、着丝粒、端粒以及目标基因，表现出许多天然酵母染色体的特性[4]。随后，通过改造大肠杆菌中的F质粒，开发出BAC载体，并成功插入了300 kb的外源DNA大片段[6]。BAC因其高稳定性、低嵌合率和高转化效率，被广泛应用于基因组学、合成生物学等领域[62-65]。

在植物中，也有研究尝试构建人工染色体。例如，Carlson等将玉米染色体元件在体外进行拼接，形成微型染色体。通过将标记基因*DsRed*、筛选基因*npt Ⅱ*与包含卫星序列、逆转录转座子及其他重复序列的着丝粒区段相结合，并利用粒子轰击将这段DNA传递到玉米胚性组织中，成功构建了环状微型染色体[66]。这类微型染色体能够在分裂旺盛的愈伤组织长期留存，并在再生植株的根尖中被检测到，表明其可在有丝分裂和器官发生过程中的正常复制和传递[67]。

1.2.2 BAC在植物基因组研究和大片段转化中的应用

BAC已被应用于植物大片段转化。早在1996年，科学家就在BAC基础上加入农杆菌复制起始

位点和转化植物所需的T-DNA元件，开发出双元细菌人工染色体（BiBAC），能够将长达150 kb的外源DNA转移到植物基因组中[7，68]。类似的，刘耀光等在1999年开发出TAC[8]。BiBAC/TAC以及其他类型的BAC和YAC被用于拟南芥和其他物种的基因组测序项目，构建了覆盖大多数染色体区域的基因组文库[69-73]。不同于YAC、BAC等载体，TAC可用于植物转化，因此在植物基因组文库构建中得到广泛应用，包括番茄（*Solanum lycopersicum*）[74]、桃（*Prunus persica*）[75]、菰（*Zizania latifolia*）[76]等物种。

2　植物染色体的从头合成

设计、合成和组装基因组是“自下而上”构建人工染色体的基本路径，并已应用于对天然基因组序列的重新设计与合成。随着DNA测序技术的持续进步，越来越多物种的全基因组信息被获取。从头合成基因组以创造新的生物体，将有助于我们探索基本生物学问题并解析生物系统的复杂性。基因组合成是利用化学方法从零开始合成DNA片段，随后逐步将小片段组装成更大的结构直到形成完整的染色体[60]。此方式可实现对最终序列的完全控制，在基因组各区域实现大规模改写[77]。近年来，基因组合成已从病毒、原核生物拓展至真核生物[78-81]，甚至在植物中取得突破[82]。

2.1　合成基因组的设计原则

合成基因组设计遵循的基本原则包括简化、扩展和重建[60，83]。多数真核生物的基因组中存在大量的非编码区及一些非必需基因，其中包含影响基因组稳定性的串联重复序列和转座子。人工设计基因组时可在确保基因组稳定性和功能性的前提下大幅简化。扩展指将异源基因整合到原始基因组中以实现新的生物学功能。为实现预期功能，需根据宿主的密码子偏好对编码序列进行优化，此举可显著提高异源基因表达效率[77，84]。重建则涉及基因组多维度的改造，涵盖密码子替换、基因表达元件模块化设计以及染色体结构重塑等[85-86]。

酵母基因组合成项目（Sc2.0）提出合成全部16条酿酒酵母染色体的计划并在2023年完成[87-93]，成为染色体合成研究的重要里程碑。Sc2.0项目为植物基因组合成带来启示，研究者选择模式植物小立碗藓（*Physcomitrium patens*）作为底盘，启动首个植物基因组合成项目（SynMoss），提出小立碗藓基因组设计原则并验证其可行性，为推进其他植物基因组合成研究提供了关键参考[82，94-96]。

具体设计原则包括：第一，移除转座子这一不稳定元件。小立碗藓基因组中转座子约占57.2%，主要由长末端重复反转录转座子组成，且多数远离蛋白质编码基因[97-98]，这为基因组简化提供了前提条件，并可探究转座子消除对基因组稳定性和植物的适应性的影响。第二，删除大部分基因间区域。通过分析确定基因编码序列后，保留注释基因上游3 kb和下游2 kb的序列，删除其余序列以进一步简化基因组。第三，统一终止密码子。将合成基因组中所有终止密码子替换为TAA[80，99-100]，TAG和TGA则可分配用于编码非标准氨基酸[101]。第四，引入PCR标签。为区分合成与天然基因组，需对开放阅读框进行同义密码子替换以形成短的新编码序列，称为PCR标签[80]。在设计小立碗藓基因组时，PCR标签优先置于内含子中并避免干扰mRNA剪接。第五，引入SCRaMbLE系统。在每个基因终止密码子下游3 bp处插入LoxPsym序列，在Cre重组酶作用下实现合成染色体快速大规模重排，从而加速基因组的进化[80，102]。在酵母中，该系统已经成为优化宿主和提高合成产物产量的有效工具[103-105]。

2.2　大片段DNA的组装技术

从头合成人工染色体需依赖超大DNA片段组装技术，包括DNA高通量化学合成、酶依赖型的聚合酶循环组装（PCA）、Gibson组装、依赖生物体内同源重组的组装等技术，这些技术已广泛应用于合成基因组学研究。小立碗藓自身具备较强的同源重组能力，转化三段带重叠区域的3 kb小片段进入细胞，成功替换了内源8.7 kb的染色体区域[82]。然而，直接利用植物系统进行重组难以实现更大片段的替换，而在大肠杆菌或酵母等微生物中能够将中小片段拼接成大片段[106-107]。因此，

SynMoss项目研究者巧妙结合植物和酵母的重组系统，将设计好的基因组片段拆分成小片段进行化学合成，随后在酵母中将小片段组装成中型片段，再利用酵母进行二轮组装将中型片段拼接成大片段[94-96]。最终，使用68.53 kb人工合成的片段替换小立碗藓155.18 kb的内源染色体[82]，生成了首个含部分合成染色体的多细胞真核植物（semi-syn18L）。

2.3 合成染色体的表观遗传修饰

合成基因组一方面旨在重现天然基因组的表观遗传景观，另一方面能够通过建立新的表观遗传修饰模式重塑染色体三维结构，为探索新表型与新物种的创制提供新路径。表观遗传修饰涵盖DNA甲基化、组蛋白修饰、染色质重塑及三维空间构象等维度。在小立碗藓合成系semi-syn18L与天然株系的对比研究中，二者表观遗传景观高度相似，表明表观遗传标记能够在合成片段上完全从头建立。值得注意的是，由于重复序列的去除，合成片段中抑制性标记H3K9me2和DNA甲基化水平显著降低；同时，合成区段及邻近区域的染色质可及性较野生型也有所提高[82]。

目前，染色体从头设计主要聚焦于基因组序列，但染色体在细胞内的空间组织密切影响生命活动。在酿酒酵母中，通过CRISPR-Cas9介导的染色体融合产生了染色体数目为2～16条不等的菌株[104]，甚至实现了将全部16条染色体融合为1条染色体[108]。类似地，裂殖酵母（*Schizosaccharomyces pombe*）中也成功将3条染色体融合成1条[109]。这些菌株尽管染色体三维结构上发生显著变化，但仍然能维持细胞活性。在哺乳动物研究中，利用小鼠单倍体胚胎干细胞对两条最大染色体（1号和2号）及两条中等大小染色体（4号和5号）进行融合，其中由4号和5号染色体构成的较小融合染色体能够被传递给纯合子后代[110]。这表明部分原核与真核生物的基因组具有高度可塑性，能够耐受染色体三维结构的大规模改变。人工合成染色体相较于天然染色体在构象上已呈现显著差异[111]，在酵母中，通过LoxPsym位点调控从头合成染色体synⅣ的三维结构，实现了合成基因组的高阶结构的设计[112]。在植物研究中，对比小立碗藓人工合成染色体与野生型，发现重新设计区域及其邻近染色体序列中的染色质环发生明显变化，重新设计区域和远端序列间的相互作用丢失，而与邻近区域建立了新连接[82]，值得注意的是，这些变化并未显著影响小立碗藓的原丝体和茎叶体表型，以及孢子的正常产生。

3 植物人工着丝粒

着丝粒一直被视为基因组中最神秘的区域，位于染色体的主缢痕处，作为唯一介导动粒复合体形成的染色质结构［图2(a)］，其对染色体分离和基因组稳定性具有至关重要的作用[113-114]。功能性着丝粒特指染色体上能与动粒相互作用的区段[115-116]，主要由着丝粒DNA和着丝粒相关蛋白复合物组成。着丝粒与动粒结合的决定性因素是一种表观遗传标记，即着丝粒特异性组蛋白H3变体（酵母中称为Cse4/Cnp1，哺乳动物中为CENP-A，果蝇中为CID，植物中为CENH3）。根据动粒蛋白在染色体上的结合位点数量和分布特征，着丝粒可分为点着丝粒、区域着丝粒和全着丝粒[117-118]。

3.1 着丝粒特异性DNA

尽管着丝粒在真核生物中功能高度保守，但其相关DNA序列的长度和结构在物种内及物种间均表现出显著多样性[119-121]。着丝粒DNA序列主要包括特异性串联重复序列（tandem repeat，TR）和反转录转座子（centromeric retrotransposon，CR），部分物种的着丝粒区还鉴定到具有转录活性的基因[122-123]。典型的着丝粒卫星重复序列单元长度为120～180 bp，目前已在多种植物中完成着丝粒特异串联重复序列的鉴定，其物种间呈现丰富多样性[117]。植物着丝粒DNA中通常还分布着长末端重复反转录转座子（long terminal-repeat retrotransposon，LTR-RT）。在拟南芥中，*ATHILA*反转录转座子整合于*CEN178*重复序列内，而CENH3与*ATHILA*共定位的概率显著低于其与*CEN178*共定位的概率[120，124]。然而在许多单子叶植物中，CENH3富集区域虽部分与串联重复序列重叠，但更多对应于反转录转座子[125-129]。

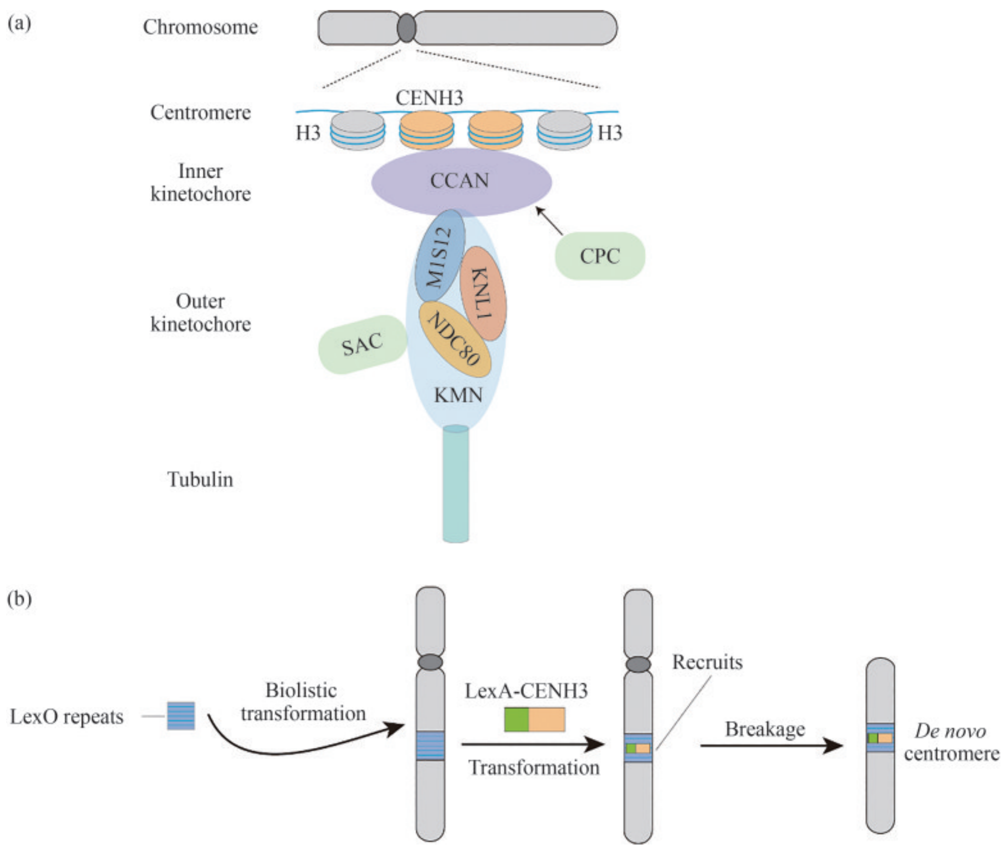


图2 动粒模型以及着丝粒的合成策略

（a）着丝粒具有特异性的CENH3核小体，CENH3负责招募动粒蛋白。动粒复合体包括内部动粒亚复合体（constitutive centromere-associated network，CCAN）和外部动粒亚复合体（KNL1-MIS12-NDC80，KMN）。动粒组装及其与微管连接还涉及动粒调节因子，如纺锤体装配检查点（spindle assembly checkpoint，SAC）和染色体乘客复合体（chromosome passenger complex，CPC）。（b）通过向染色体中插入LexO重复序列，利用该序列招募LexA-CENH3，从而形成新的着丝粒

Fig. 2 An organization of the kinetochore and the synthesis strategy of the centromere

(a) Centromere is defined by specific CENH3 nucleosomes, which are responsible for recruiting kinetochore proteins. The kinetochore comprises an inner constitutive centromere-associated network (CCAN) and an outer KMN network (KNL1-MIS12-NDC80). Kinetochore assembly and its attachment to microtubule also involve kinetochore regulators, such as spindle assembly checkpoint (SAC) and chromosome passenger complex (CPC). (b) *De novo* centromere formation is achieved by recruiting LexA-CENH3 to the LexO repeat array inserted into the genome.

酵母的着丝粒DNA最早被成功分离，含有该片段的染色体能在有丝分裂和减数分裂中保持稳定[130]。Kouprina等虽分离出人类着丝粒DNA，但该序列未能形成有丝分裂稳定的HAC，表明内源染色体功能性着丝粒的形成不简单依赖DNA序列[131]。与哺乳动物类似，植物着丝粒的形成同样是由表观遗传机制决定[132]，功能性着丝粒的建立未必依赖于DNA序列[133]。有研究人员将天然着丝粒DNA序列引入植物细胞，发现动粒蛋白无法与之结合，不能形成有效着丝粒[134]。目前植物着丝粒区域的建立机制和结合蛋白仍远未解析清晰，功能性着丝粒的构建成为PAC合成中最困难的环节。

3.2 人工着丝粒的构建策略

尽管着丝粒DNA在着丝粒形成和功能中或许起作用，但对多数真核生物而言，仅依赖特异性DNA序列无法构建新着丝粒。着丝粒区存在由H2A、H2B、CENH3和H4组成的着丝粒特异性核小体，其与典型的H3核小体交替排列，其中CENH3是招募动粒蛋白的核心因子[118]。CENH3具有保守的C端组蛋白折叠域（histone fold domain，HFD）以及序列和长度高度变异的N端[135-136]。在拟南芥中，CENH3的C端HFD即使缺失N端仍能引导其靶向加载至着丝粒并支持有丝分裂过程[137-138]；然而若C端HFD的第130位赖氨

酸发生突变（L→I或L→F），则会严重破坏CENH3在着丝粒的沉积[139]。此外，通过RNAi敲低*CENH3*基因会显著抑制有丝分裂，并干扰减数分裂导致不育[138]，*cenh3-1*（-/-）突变体表现为胚胎致死[140]，而这些缺陷可通过转*GFP-CENH3*或*GFP-tailswap*进行弥补[140-141]。GFP-tailswap具有完整的CENH3 C端结构域，其N端则被H3.3的N端融合GFP取代。有趣的是，GFP-tailswap融合蛋白在有丝分裂过程中能够加载到染色体上，而在减数分裂时无法加载[142]。当GFP-tailswap植株与野生型杂交时，子代的着丝粒大小不平衡，来自GFP-tailswap植株的染色体被清除，从而形成单倍体[142-143]。以上这些研究充分证实了CENH3在形成功能性着丝粒中的核心作用。

构建植物着丝粒的关键在于实现CENH3在特定DNA序列上的从头加载，并确保其在DNA复制和细胞分裂过程中稳定维持。科学家将细菌乳糖阻遏蛋白/乳糖操纵子（lactose repressor/lactose operator）系统引入果蝇和人类细胞系，利用LacO重复序列招募CID-GFP-LacI或LacI-HJURP融合蛋白，其中HJURP能够驱动CENP-A异位加载和动粒形成[144-145]，成功构建了功能性人工着丝粒[146-148]。通过类似策略，Teo等在含有LacO重复序列的拟南芥株系中将CENH3-GFP-LacI-NLS招募至LacO位点，实现了着丝粒蛋白的从头组装[149]。在玉米中，LexA-CENH3融合蛋白能够将内源CENH3蛋白招募至染色体臂上的LexO重复序列，形成足以引发染色体断裂的功能性着丝粒［图2(b)］，释放的染色体片段能够通过减数分裂稳定遗传[150]。断裂后形成的携带内源着丝粒的片段4a与携带合成着丝粒的片段4b构成互补染色体对，当4a和4b染色体均纯合时，其减数分裂分离行为与野生型近乎一致，并能产生结实率正常的健康植株，表明合成着丝粒功能完全[151]。植物人工着丝粒的成功构建将为未来开发可稳定传代的人工染色体奠定关键基础。

4 染色体大片段递送

将合成的DNA大片段完整转移至细胞内并维持其结构完整性是构建人工染色体的核心环节。尽管目前已有能力人工构建兆碱基级别的复杂DNA片段，但将这些大片段完整递送至植物细胞内部仍面临重大挑战。哺乳动物细胞中已发展出多种物理、化学和生物技术以促进DNA大片段的递送[60, 152]，这些方法为植物染色体大片段递送研究提供了重要借鉴。

4.1 染色体大片段递送方法

物理递送方法中应用最广泛的是电穿孔技术，其利用高强度电脉冲瞬间增加膜通透性并形成孔隙，促使离子与大分子通过磷脂双分子层结构缺陷进入细胞[153]，通过此方法，100～200 kb的DNA大片段已成功导入小鼠胚胎干细胞[154-155]。化学转移法则主要依赖化学试剂［如Ca^{2+}、Li^{+}、聚乙二醇（PEG）］改变细胞膜通透性，或利用脂质体与细胞膜融合实现大片段递送。例如，以脂质体为载体，2.3 Mb YAC和404 kb HAC被成功转移至HT1080细胞[156-157]。

前述物理和化学方法都需预先扩增和纯化DNA，但此过程易导致DNA大片段断裂。细胞间直接转移技术可规避体外DNA操作，从而保障DNA完整性，常见策略包括接合转移[158-160]和诱导细胞融合。兆碱基级别的YAC可通过酵母细胞与哺乳动物细胞融合实现转移[161-162]。Gambogi等在酵母中组装750 kb的人工染色体后，诱导酵母原生质体与人类细胞融合，成功在人类细胞中构建单拷贝HAC[148]。此外，微细胞介导的染色体转移（MMCT）技术通过产生含单个染色体的微细胞，再与受体细胞融合实现染色体的高效转移。该技术自1974年建立[163]后持续优化，沿用至今[164-167]。

4.2 植物中DNA大片段递送的研究现状

植物系统中已探索了多种大片段递送方法（表1）。传统策略是依赖农杆菌介导的转化，其中BiBAC/TAC兼具BAC的大片段克隆能力与农杆菌转化优势，可转移超过100 kb的外源DNA片段。1996年，Hamilton等通过BiBAC系统将30 kb酵母DNA和150 kb人类DNA片段导入烟草（*Nicotiana tabacum*）基因组[7]，后续该技术扩展至番茄[173]、

表1 植物中DNA大片段的递送方法

Table 1 Summary of different delivery methods of large DNA fragments in plants

转化方法 Transformation method	物种 Species	靶向组织 Targeted tissue	递送的最大片段 The maximum size of the delivered fragment	局限性 Limitations
农杆菌介导转化	水稻[168]	种子来源的愈伤组织	164 kb	宿主限制，片段随机插入，周期较长
基因枪粒子递送	烟草[169]	悬浮细胞	150 kb	大片段易断裂，转化效率较低，设备昂贵
纳米材料递送	棉花[170]	花粉	15 kb	普适性待定，递送效率较低
PEG介导转化	烟草[171]	原生质体（叶片来源）	300 kb	细胞毒性，单细胞再生效率低
电穿孔法	灵芝[172]	原生质体（菌丝来源）	100 kb	大片段易断裂，组织损伤

拟南芥[174]、玉米[175]及水稻[176-177]的转化中。Wang等将长度达164 kb（重复序列占比88.1%）的复杂玉米BiBAC转化至水稻基因组，实现了农杆菌介导递送片段长度的突破[168]。然而，农杆菌转化法存在整合位点随机性、片段限制、片段长度与转化效率负相关等局限，且存在一定物种限制。

基因枪递送系统几乎无物种限制，其通过高压氦气脉冲加速包裹DNA的金属颗粒穿透细胞壁和膜[178-180]。在烟草中，80 kb和150 kb的YAC通过基因枪轰击成功递送至悬浮细胞[174]。马铃薯（*Solanum tuberosum*）中也借助该技术导入了106 kb的BAC质粒[181]。Chang等将BiBAC文库与基因枪技术结合，将平均长度108 kb的拟南芥BiBAC文库导入烟草，获得的转基因植株不仅携带完整BiBAC DNA，且能稳定遗传并表达基因[182]。但该方法存在DNA断裂风险。

纳米材料的兴起推动了生物纳米技术在递送领域的应用。金属纳米颗粒、脂质体纳米复合物、聚合物基纳米颗粒以及硅基和碳基纳米颗粒等可传递核酸、蛋白质及药物[183-184]。纳米颗粒递送无需去除细胞壁，并能保护DNA免遭降解。Demirer等在本氏烟草（*N. benthamiana*）、芝麻菜（*Eruca sativa*）、小麦以及棉花（*Gossypium hirsutum*）中通过纳米材料实现无转基因整合的DNA传递与蛋白表达[185]。类似结果在拟南芥、烟草中亦有报道[186-188]。尽管当前纳米递送存在可重复性差、可递送DNA长度短、效率低、机理不明等问题，尚未广泛应用，但随着纳米传递系统的持续优化，大片段DNA高效递送前景可期。

聚乙二醇（PEG）介导的原生质体转化是外源DNA递送的常用方法。PEG作为水溶性聚合物，其烷基链通过疏水作用锚定于细胞膜磷脂双分子层，引起膜结构重排并增强通透性[189]。同时，PEG能促进DNA与细胞表面结合，从而提升DNA内化效率[152，190]。1997年，van Wordragen等尝试利用PEG向烟草细胞递送YAC大片段[171]。近期的研究中，69 kb人工合成染色体片段通过PEG法成功导入小立碗藓原生质体[82]。尽管PEG介导的递送潜力显著，但DNA大片段纯化过程仍烦琐。PEG介导的酵母-植物细胞融合技术有望规避体外DNA操作，实现兆碱基级染色体递送。该技术在哺乳动物中已成熟应用[161-162]，植物中仅进行过初步尝试[191-192]。这一方向仍是PAC研究领域亟待突破的关键生物技术。

5 PAC研究面临的挑战

PAC作为染色体工程领域极具潜力的技术创新，为精准操控基因组及解决复杂农业问题提供了新平台。然而，受限于植物内在的生物复杂性与亟待突破的技术瓶颈，PAC研究仍处于初级阶段，其发展面临多重挑战，亟需创新性的解决方案。

5.1 着丝粒的合成极具挑战性

着丝粒是确保染色体在分裂过程中精准分离的核心元件。然而，天然植物着丝粒区通常由大量卫星重复序列和反转录转座子组成，受当前测序与组装技术限制，多数植物着丝粒区序列仍处于缺失或碎片化状态，难以获得完整准确的参考序列。更重要的是，植物着丝粒功能的发挥未必依赖特定DNA序列，而是主要受到CENH3这一表

观遗传标记的影响。组蛋白需通过伴侣蛋白加载到染色质中[193]，例如哺乳动物中的HJURP[144, 194]、酵母中的Scm3和NASP[195-198]以及果蝇中的CAL1[199]均被鉴定为CENH3的加载分子伴侣。尽管拟南芥中已发现KNL2能与CENH3的N端相互作用，并在促进CENH3沉积至着丝粒DNA过程中起关键作用[200-201]，但CENH3在植物体内如何被精准招募与调控的机制仍不明确。这导致构建的人工染色体难以在宿主细胞中稳定招募CENH3。科学家虽利用“乳糖阻遏蛋白/乳糖操纵子系统”或“自上而下截断内源染色体”等策略规避此问题，但此类方法构建的人工着丝粒的在物种普适性与代际稳定性等方面仍需进一步验证。

此外，着丝粒功能与DNA甲基化密切相关。拟南芥中编码保守E3泛素连接酶的*VMI*基因发生突变时，CEN178重复序列的甲基化水平降低，此类表观遗传变化导致着丝粒功能异常，引发严重的染色体分离缺陷[202-203]。除DNA甲基化外，着丝粒还具有异染色质的其他特征，而这些表观遗传环境对着丝粒身份确立与功能行使的影响尚未充分解析。人工合成着丝粒是否需要重建内源性表观遗传修饰以优化功能，仍是构建稳定功能性着丝粒需解决的核心问题。

5.2 染色体大片段递送困难

将DNA大片段或整个染色体完整递送至植物细胞仍是PAC领域的艰巨挑战。农杆菌转化所用的常规双元载体容量有限，BiBAC系统虽然能够转移超过100 kb的片段，但无法定点插入。基因枪、电穿孔、脂质体融合及PEG转化等方式需在体外收集大量DNA，而提取和纯化过程难以维持DNA大片段的完整性。

当前超大片段拼接主要依赖转化关联重组（transformation-associated recombination，TAR）技术，该技术利用酿酒酵母强大的同源重组能力高效精准完成DNA大片段的拼接，已广泛应用于动植物人工染色体构建[82, 148]。此外，在大肠杆菌中基于λ-Red重组体系的组装技术也能获得兆碱基级DNA大片段[204-205]。向植物递送合成DNA大片段的典型流程包括：①在酵母中经多轮组装获得DNA大片段；②将完整DNA大片段转移至大肠杆菌扩增并纯化；③通过PEG转化将DNA大片段递送至植物原生质体中[82]。然而，从酵母中提取兆碱基级质粒的操作烦琐且难度高，同时PEG介导的大片段转化植物原生质体效率极低[206]。诱导酵母与植物原生质体融合，或将微细胞介导的染色体转移技术引入植物系统，有望实现DNA大片段从酵母到植物的直接递送，突破物理传递的上限。

5.3 人工染色体的不稳定性

当前合成的PAC存在结构和遗传不稳定性问题。由于对染色体架构（尤其是着丝粒、端粒、复制起始位点及调控元件等核心结构）的认知不完整，人工染色体的精确设计面临困难。着丝粒功能缺陷会导致人工染色体在细胞分裂中出现异常，随细胞传代而丢失。线性人工染色体末端需端粒结构保护以防止降解或融合，但构成端粒的重复序列容易丢失，导致端粒缩短而丧失功能[207]，进而破坏人工染色体结构稳定性。人为设计的端粒序列需与宿主细胞的端粒酶系统兼容，才能有效维持端粒稳态[208]。此外，人工染色体需要整合有效的复制起点以实现精准复制，其数量与分布的平衡直接影响人工染色体在细胞分裂中的稳定传递。然而，植物的复制起始位点没有明确的特异性序列[209-211]，拟南芥的复制起始区域具有AT富集的序列，两侧由GC含量更高的区域包围[212-213]。基于这些特征，在设计PAC时，可以考虑添加AT富集区和侧翼GC岛以增强复制起始信号，但这种方法的有效性和普适性还有待检验。此外，DNA的复制在很大程度上受到表观遗传修饰的调控[214-216]，这也是保证PAC稳定复制的重要因素。

PAC通过减数分裂实现世代间稳定传递同样面临挑战。缺乏同源染色体可能导致人工染色体在减数分裂中配对失败或形成非整倍体配子而无法稳定遗传。若人工着丝粒功能不全，可能引发纺锤体附着失败，造成染色体分离滞后和丢失。

6 应用与展望

PAC作为基因组工程与合成生物学的核心工

具，近年来在技术研发领域取得了一系列突破性进展。这些进展不仅深化了我们对染色体结构和功能调控机制的认知，同时为作物精准改良及绿色生物制造等方向开辟了广阔的应用前景。

6.1 染色体结构与功能的研究工具

人工染色体已成为解析染色体复杂结构和功能的宝贵工具，尤其适用于探究染色体的未知特征。通过逐步重构染色体，研究者得以深化对染色体生物学的认知。在酵母和哺乳动物中，YAC、MAC和HAC被用于研究着丝粒、端粒等染色体核心元件的组成和功能[111, 217-222]，深入解析了着丝粒表观遗传学、端粒长度调控及染色体三维构象等基础科学问题。在植物研究中，科学家通过将人工设计的重复序列插入内源染色体形成新的CENH3招募位点，构建出双着丝粒染色体，并基于其在有丝分裂和减数分裂中的行为验证了新着丝粒的功能完整性[149-151]。这些研究明确了决定着丝粒身份和功能的核心因素，对理解染色体结构具有深远意义。

未来可通过CENH3招募序列替换内源着丝粒区域，追踪其动粒蛋白结合过程与表观遗传模式重建机制，以直观确认其功能完备性。此外，植物领域尚未实现“自下而上”构建含功能性着丝粒的人工染色体。通过整合人工着丝粒构建策略并组合其他染色体部件，结合位点特异性重组酶系统及基因编辑系统，有望创制能够稳定遗传且可定向改造的PAC。

转座子和异染色质等基因组“暗物质”在合成植物基因组设计时被人为删除，为系统性研究这些结构的功能提供了独特机会[82, 206]。未来通过对合成基因组进行靶向组蛋白修饰及三维结构重塑，并利用SCRaMbLE系统挖掘新功能基因与加速基因组定向进化，将深化对植物基因组的认知，为构建更复杂的基因组蓝图铺就道路。

6.2 多基因和优良性状的堆叠平台

PAC为精准操控基因及解决生物制造、复杂作物改良问题提供了新平台。传统基因工程需将目的基因插入宿主基因组，多基因聚合依赖不断杂交和回交，易导致连锁累赘、位置效应及表达沉默。人工染色体可容纳超大DNA片段并独立于内源基因组外，有望彻底改变多基因性状导入、多性状聚合以及代谢通路重构等关键育种环节。

目前植物中已开发多种基因堆叠策略[223-225]，其中位点特异性重组酶介导的整合系统广泛应用于多基因插入和删除[226-228]。随着CRISPR及其衍生技术的发展，植物领域已涌现新型引导编辑系统[229]、转座元件介导的DNA精准插入系统[230]以及基于Cre-Lox系统的可编程染色体编辑技术[231]，能够实现千碱基到兆碱基级别DNA片段的精准操作。这些技术将加速PAC的创新和应用，为植物合成生物学与作物改良提供有力支持。

以人工染色体为载体的代谢通路工程可提升作物营养价值并赋予新功能。例如将花青素合成途径[232]、维生素A合成途径[233-234]整合至人工染色体，可实现大宗作物的营养改良。今后将C_4植物的碳代谢途径[235-236]设计导入到C_3植物，可提高光合效率；将固氮途径[237-238]借助人工染色体转移至谷类作物，可赋予其自主固氮能力。随着代谢等通路的持续解析与完善，进而通过人工染色体技术工程化，农业育种有望实现突破性创新。

6.3 合成生物学的操作底盘

植物凭借生产成本低、易规模化、生物量大、环境适应能力强及完备的翻译后修饰能力等优势，正成为合成生物学的理想反应器。PAC可作为合成通路的操作底盘，助力植物细胞工厂构建。YAC、MAC及HAC已用于异源蛋白或代谢产物合成[239-241]，而PAC在此领域的应用尚处于起步阶段。当前以烟草等模式植物为平台可生产抗体、疫苗等[242-243]，但仍无法规避传统遗传转化的位置效应与多基因组合难题。将抗体、疫苗、工业酶及青蒿素、紫杉醇等药用产物的完整表达模块嵌入PAC，可根据产物特性灵活选择最适物种底盘实现规模化生产。此外，PAC凭借其独立性、可遗传性和大容量特性，可用于设计复杂基因环路与生物传感器，构建包含“信号感知-信号转换处理-信号输出”完整逻辑的工具型染色体，使植物具备对重金属、病原体或逆境胁迫的实时监测能力。

综上，PAC领域正在蓬勃发展并展现出广阔的应用前景。尽管当前面临诸多挑战，这些问题亦为未来创新指明方向。通过与基因编辑及人工智能设计等新兴技术融合，PAC有望成为解析染色体机制、精准改良作物及实现绿色制造的核心工具，持续推动农业可持续发展与植物科学突破。

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