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·综 述·

植物环状 RNA 研究进展

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摘 要：随着高通量测序技术的发展，环状 RNA (circular RNA, circRNA) 逐渐成为非编码 RNA 研究领域的热点。CircRNA 是由 3′端下游供体和 5′端上游受体经反向剪接形成的共价闭合环状分子，普遍存在于真核生物中。CircRNA 过去被认为是错误剪接的副产物，近年来相关研究爆炸式增长，才将这种错误概念推翻。相较于动物中的大量研究，植物 circRNA 的研究还处于起步阶段。文中从植物 circRNA 的发现引入，总结了植物 circRNA 的环化特征、表达特异性、保守性和稳定性等特征；关注了 circRNA 的鉴定工具、主要类型和生成机制；归纳了植物 circRNA 作为 microRNA (miRNA) 海绵和翻译模板的潜在功能，以及在生物/非生物胁迫应答中的重要作用；简单概括了植物 circRNA 的降解与定位。最后讨论了植物 circRNA 研究存在的问题并对进一步开展植物 circRNA 研究进行了展望。

关键词：环状 RNA；生成机制；反向剪接；miRNA 海绵

Progress in circular RNAs of plants

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Abstract: With the development of high-throughput sequencing technology, circular RNAs (circRNAs) have gradually become a hotspot in the research on non-coding RNA. CircRNAs are produced by the covalent circularization of a downstream 3′ splice donor and an upstream 5′ splice acceptor through backsplicing, and they are pervasive in eukaryotic cells. CircRNAs used to be considered byproducts of

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false splicing, whereas an explosion of related studies in recent years has disproved this misconception. Compared with the rich studies of circRNAs in animals, the study of circRNAs in plants is still in its infancy. In this review, we introduced the discovery of plant circRNAs, the discovery of plant circRNAs, the circularization feature, expression specificity, conservation, and stability of plant circRNAs and expounded the identification tools, main types, and biogenesis mechanisms of circRNAs. Furthermore, we summarized the potential roles of plant circRNAs as microRNA (miRNA) sponges and translation templates and in response to biotic/abiotic stress, and briefed the degradation and localization of plant circRNAs. Finally, we discussed the challenges and proposed the future directions in the research on plant circRNAs.

Keywords: circular RNAs; biogenesis mechanism; backsplicing; miRNA sponge

非编码 RNA (non-coding RNAs, ncRNAs)，包括 microRNA (miRNA)、小干扰 RNA (small interfering RNA, siRNA)、环状 RNA (circular RNA, circRNA) 和长链非编码 RNA (long non-coding RNA, lncRNA)，在真核生物的转录组序列中占很大比例，参与蛋白编码基因在转录和转录后水平上的表达调控[1-3]。circRNA 是近年来加入非编码 RNA 家族的新成员，具有共价闭合的环状结构，区别于大多数线性 RNA。研究表明，circRNA 结构稳定、种类丰富、序列保守、在细胞和组织中特异性表达，并具备很多潜在功能，在调控基因表达和各种生物学功能方面发挥着重要作用[4]。高通量测序结果显示，circRNA 广泛存在于多种植物中，参与花的发育、果实的成熟和胁迫响应等生长发育进程[5-6]。本文从 circRNA 的发现引入，总结了植物 circRNA 的鉴定工具、特征、主要类型和生成机制；结合动物中的研究，概述了植物 circRNA 的潜在功能、降解和定位，对植物 circRNA 研究存在的问题进行了讨论，并对进一步研究作了展望，期望能为植物 circRNA 的研究提供参考。

1 circRNA 的发现

1976 年，Sanger 等首次在植物类病毒中发现了闭合环状的 RNA 分子[7]，紧接着，1980 年，Arnberg 等在酵母 (*Saccharomyces cerevisiae* H.) 线粒体中发现类似的环状转录本[8]，1986 年 Kos 等发现了第一个含有 circRNA 的动物病毒-丁型肝炎病毒 (hepatitis delta virus, HDV)[9]，1990 年 Matsumoto 等首次在真核细胞中发现 circRNA 的身影[10]。1991 年 Nigro 等在对人类结肠癌缺失 (delete in colorectal carcinoma, DCC) 细胞转录本的研究中，首次发现 circRNA 来源于内源 RNA[11]。此后，研究人员在多种生物中均发现了 circRNA，包括小鼠 (*Mus musculus*)[12]、斑马鱼 (*Danio rerio*)[13]、秀丽隐杆线虫 (*Caenorhabditis elegans*, *C. elegans*)[14] 等，甚至古菌 (archaea)[15]中也发现了 circRNA 的身影。目前已经在近 30 种植物中发现了大量 circRNA，包括拟南芥 (*Arabidopsis thaliana*)[5]、水稻(*Oryza sativa* L.)[6]、大麦 (*Hordeum vulgare* L.)[16]、番茄(*Lycopersicon esculentum* Miller)[17]、大豆 (*Glycine max* (Linn.) Merr.)[18]、小麦 (*Triticum aestivum* L.)[19]、玉米 (*Zea mays* L.)[20]、猕猴桃 (*Actinidia*)[21]、葡萄(*Vitis vinifera* Linn.)[22]和杜梨 (*Pyrus betulifolia Bunge*)[23]以及中草药[24]等。

虽然早在几十年前就已发现 circRNA，但由于 circRNA 不具有游离的 3′和 5′末端，无法通过快速扩增 cDNA 末端 (rapid amplification of cDNA ends, RACE) 或基于 poly(A) RNA 建

库的二代测序技术实现检测[25-26]；同时，可环化外显子是经反向剪接连接的，异于经典的线性剪接，早期转录组分析的映射算法无法直接将测序得到的片段匹配到基因组，使得人们一度认为circRNA只是错误剪接的副产物[27]。随着高通量测序技术和生物信息学工具的发展，2012年Salzman等首次发现circRNA是由前体mRNA (pre-mRNA)经反向剪接产生的环状分子，并且大量存在于人类不同类型的细胞中[28]；2015年，Ye等在水稻和拟南芥中进行了植物circRNA的全基因组鉴定，在水稻根茎组织中鉴定到12 037个circRNA，拟南芥叶片中鉴定到6 012个circRNA[29]，引发了植物circRNA研究的热潮。

2 植物circRNA的鉴定工具

目前研究人员已经开发出许多计算工具来预测circRNA (表1，在刘旭庆等[60]、Chen等[61]基础上补充)，例如pcircRNA_finder[57]是唯一为植物circRNA预测而设计的软件，它结合了多个算法来检测反向剪接读数，为植物circRNA提供了一种更全面、更灵敏、更精确的预测方法，但是它仅能鉴定外显子circRNA；CircPlant[58]考虑植物和哺乳动物基因组的差异，应用几种特定于植物的标准从RNA-seq数据中准确地检测植物circRNA并预测其功能，特异性、准确度和预测效率更优。但是这些生物信息学工具在识别circRNA时，精确度、灵敏度和计算成本方面表现不同，因此使用不同的软件进行同时预测分析可以提高circRNA鉴定的效率。

随着大量植物circRNA被鉴定出来，一系列储存和可视化植物circRNA的数据库被建立(表2，在刘旭庆等[60]基础上补充)，这些数据库具有不同功能，不仅包含大量植物circRNA的序列、基因注释和功能预测等信息，同时也提供互作分析、可视化和miRNA靶点预测等工具。例如PlantCircBase[64]拥有超过115 000个来自16种不同植物的circRNA，还提供了特定circRNA结构的可视化、相应物种中涉及circRNA-miRNA-mRNA的潜在相互作用网络、Sanger测序的验证信息以及功能注释、组织表达、保守性验证等更多信息，PlantCircBase有望成为研究植物circRNA最全面的数据库资源；GreenCircRNA[68]是第一个预测植物circRNA可以作为miRNA诱饵的数据库，收录了69种植物的21万多条circRNA，预测了38种植物的circRNA作为miRNA诱饵的可能性。这些数据库的建立为阐明植物circRNA的各种机制方面提供了巨大的帮助。

3 植物circRNA的特征

3.1 环化特征

2012年，Salzman等首次发现pre-mRNA通过反向剪接将3′下游剪接供体连接到5′上游剪接受体上形成circRNA[28]，催化pre-mRNA剪接的U2依赖型剪接体(U2-dependent spliceosome)剪接5′端供体位点“GU”和3′端受体位点“AG”信号[69]。已有研究证明典型的剪接机制和剪接体信号都是反向剪接环化所必需的[70-71]。大多数高表达circRNA通常由pre-mRNA的内部外显子加工而成，包含多个外显子，说明反向剪接通常与典型剪接结合[72]。在拟南芥和水稻的研究中发现分别有13%和34%的circRNA通过反向剪接形成[29]。拟南芥中99%的circRNA均具有典型的GU/AG剪接信号[73]，而在水稻circRNA中仅有7.3%具有典型GU/AG剪接信号，其余为不同的非典型剪接信号[39]，此外葡萄[22]、棉花(*Gossypium* spp.)[74]等植物circRNA形成过程中也依赖非典型剪接信号，表明植物circRNA中存在特定的剪接信号模式，但是还需要在更多的植物中进一步验证。

表 1 circRNA 预测工具

Table 1 The tools for circRNA prediction

Tool names	Uniform resource locator	Description	References
CircPro	http://bis.zju.edu.cn/CircPro	Detection of circRNAs with protein-coding potential.	[30]
CIRCfinder	https://github.com/YangLab/CIRCfinder	Using junction reads to identify circular intronic RNAs.	[31]
Acfs	https://code.google.com/p/acfs/	Using single- and paired-ended RNA-Seq data to identify and quantify circRNAs.	[32]
CircRNAFisher	https://github.com/duolinwang/CircRNAFisher	Using different back splicing junction reads to identify circRNAs.	[33]
Circtools	https://github.com/dieterich-lab/circtools	Providing a complete workflow for circRNAs from prediction to functional insights.	[34]
FUCHS	https://github.com/dieterich-lab/FUCHS	Long reads data based to learn more about the exon coverage, the number of double break point fragments and the alternatively spliced exons.	[35]
PRAPI	http://www.bioinfor.org/bioinfor/tool/PRAPI/	ISO-seq data based to analyze alternative transcription initiation, alternative splicing and circRNAs.	[36]
CircView	https://github.com/GeneFeng/CircView	Help to understand potential functions of circRNAs and design the experiments.	[37]
CircPrimer	http://www.bioinf.com.cn/	Allow to search, annotate, and visualize circRNAs, help to design primers and to determine the specificity of the primers.	[38]
Circseq_cup	https://github.com/bioinplant/circseq-cup/	Using the back splicing RNA-Seq and paired-end reads to assemble the full-length sequences of circRNAs.	[39]
CIRI-full	https://sourceforge.net/projects/ciri-full/	An approach for reconstruction of full-length circRNAs and isoform-level quantification from the transcription.	[40]
CircAST	https://github.com/xiaofengsong/CircAST	A tool to assemble full-length circRNA transcripts and estimate their expression by using multiple splice graphs.	[41]
MapSplice	http://www.netlab.uky.edu/p/bioinfo/MapSplice	A second algorithm for the alignment of RNA-seq reads to splice junctions.	[42]
circRNA_finder	https://github.com/orzechoj/circRNA_finder.git	A *de novo* circRNA forecasting tool without the need of gene annotations.	[43]
segemehl	www.bioinf.uni-leipzig.de/Software/segemehl/	A novel, unbiased algorithm to detect splice junctions from single-end cDNA sequences.	[44]
KNIFE	https://github.com/lindaszabo/KNIFE	Using a statistical approach to discover and quantify circular and linear RNA splicing events.	[45]
DCC	https://github.com/dieterich-lab/	Detection of back-splice junctions and circRNA versus host gene expression.	[46]
UROBORUS	http://uroborus.openbioinformatics.org/	Detection of circRNAs with low expression levels in total RNA-seq.	[47]
NCLcomparator	https://github.com/TreesLab/NCLcomparator	Non-co-linear (NCL, circular, intragenic trans-spliced or fusion RNAs) detecte tool.	[48]
CircMarker	https://github.com/lxwgcool/CircMarker	Take advantage of transcription annotation files to create k-mer table for circular RNA detection.	[49]
CircDBG	https://github.com/lxwgcool/CircDBG	A new method for circular RNA detection with De Bruijn graph.	[50]
find_circ	https://github.com/marvin-jens/find_circ	The first RNA-Seq-based circRNA prediction tool.	[51]
CircExplorer2	https://github.com/YangLab/CIRCexplorer2	Annotating different types of alternative splicing events in circRNAs.	[52]
CIRI2	https://sourceforge.net/projects/ciri/files/CIRI2	Using an adapted maximum likelihood estimation to identify back splicing junction reads and to filter mapping errors.	[53]
CircSplice	https://github.com/GeneFeng/CircSplice	Identify internal alternative splicing in circRNA and compare differential circRNA splicing events.	[54]
CirComPara	http://github.com/egaffo/CirComPara	An bioinformatics pipeline to detect, quantify and annotate circRNAs from RNA-seq data.	[55]
CircRNAwrap	https://github.com/liaoscience/circRNAwrap	Measuring the effectiveness of existing tools on collected and simulated data.	[56]
PcircRNA_finder	https://github.com/bioinplant/PcircRNA_finder/	The frist circRNA prediction software for plants.	[57]
CircPlant	http://bis.zju.edu.cn/circplant	With the incorporation of several plant-specific criteria detect plant circRNAs and predict function.	[58]
PCirc	https ://github.com/Lilab -SNNU/Pcirc	Using a machine learning method to predict plant circRNAs from RNA-seq data.	[59]

表 2 植物 circRNA 数据库

Table 2 Databases of plant circRNAs

Tool names	Uniform resource locator	Description	References
AtCircDB	http://genome.sdau.edu.cn/circRNA	A comprehensive tissue-specific database to help store, retrieve, visualize and download *Arabidopsis* circular RNAs.	[62]
PlantCircNet	http://bis.zju.edu.cn/plantcircnet/index.php	An integrated database that provides visualized plant circRNA miRNA mRNA regulatory networks containing identified circRNAs in eight model plants.	[63]
PlantcircBase	http://ibi.zju.edu.cn/plantcircbase/	Providing the most comprehensive information about plant circRNAs.	[64]
CircFunBase	http://bis.zju.edu.cn/CircFunBase	A web-accessible functionally annotated circRNA database.	[65]
CropCircDB	http://deepbiology.cn/crop/	A comprehensive collection of circRNAs in crop response to abiotic stress.	[66]
ASmiR	http://forestry.fafu.edu.cn/bioinfor/db/ASmiR	A database of miRNA targets in alternatively spliced linear and circRNAs for plant.	[67]
GreenCircRNA	http://greencirc.cn	The first database for the prediction of plant circRNAs that act as miRNA decoys.	[68]

3.2 表达特异性

circRNA 在植物中通常表现出不同组织、细胞和发育阶段的特定表达模式，既有表达有无的差异，也有表达量的差异。其在拟南芥和水稻的根、茎、叶和种子等组织中均有表达，但是表达量不同，且有很大差异[6,29]；大豆和猕猴桃根、茎、叶组织中具有不同表达量的circRNA，而且在不同组织中特异性表达[18,21]。在拟南芥中叶绿体 circRNA 数量多于线粒体，在不同细胞中特异性表达，暗示 circRNA 可能参与植物光合作用和呼吸作用[73]。在早花枳壳(*Poncirus trifoliata* L. Raf.) 突变体及其野生型转录组数据分析中发现，176 个差异表达的 circRNA 可能在早花过程中起重要作用[75]；circRNA *circbHLH93* 在毛竹 (*Phyllostachys edulis* (Carr.) Mitford cv. Pubescens) 8 个不同发育阶段的竹笋中存在表达差异[76]；来源于八氢番茄红素合成酶 1 (PSY1)和八氢番茄红素脱氢酶(PDS)的 circRNA 被发现在番茄果实成熟的不同阶段有不同的表达[77]；在水稻从正常发育到衰老的剑叶鉴定到的 6 612 个 circRNA 中发现其中 113 个 circRNA 在叶片衰老过程中有差异表达[78]。同时，植物 circRNA 也在不同逆境下特异性表达，例如在水稻磷酸盐失衡[29]、小麦干旱[19]和葡萄低温[22]等不同胁迫条件下 circRNA 的表达量均发生差异变化。

3.3 保守性

各种 RNA-seq 数据显示了植物不同物种 circRNA 的保守性质。水稻和拟南芥中有超过 700 个 circRNA 的来源基因存在同源性，并有 300 多个同源基因从相似的位置产生 circRNA[29]；对拟南芥、水稻和大豆可以产生 circRNA 的 8 362、9 385 和 1 995 个来源基因进行保守性分析发现，大豆和拟南芥之间有 685 个同源，大豆与水稻之间有 1 095 个同源，3 个物种中共有 551 个来源基因同源[18]。杨树(*Populus tomentosa* Carr.) 响应干旱的 circRNA 来源基因中有一半与拟南芥和玉米同源，表明参与干旱响应转录调控的 circRNA 在单子叶和双子叶植物中是保守的[79]。CircRNA 的序列保守性表明，这些 circRNA 可能具有相似的潜在生物学功能，这些保守的 circRNA 在植物中的功能还需

要进一步的研究和验证。

3.4 稳定性

与线性 RNA 相比，两端共价闭合和二级结构的存在使 circRNA 更稳定，具有核糖核酸外切酶 (如 RNase R) 抗性，因此不易降解[80]。RNase R 能降解线性 RNA 但不能降解 circRNA，用其可以提高检测 circRNA 的灵敏度，减少假阳性的数量。Zeng 等[75]通过实时荧光定量 PCR (real-time PCR) 实验对 11 个已证实的枳壳 circRNA 进行了抗 RNase R 测验，证实了植物 circRNA 的 RNase R 抗性。且 circRNA 半衰期的中位数超过 48 h，而它对应的线性 RNA 半衰期的中位数不到 20 h，证明了 circRNA 在细胞内具有高度稳定性[81]。

4 植物 circRNA 的类型

随着研究的深入，circRNA 的类型不断积累，是研究的重点关注领域之一[82]，主要的 3 种类型分别是外显子 circRNA、内含子 circRNA 和外显子-内含子 circRNA[83]。Chu 等[25]根据 circRNA 在基因组中两个剪接位点的位置，将 circRNA 分为 10 种类型 (图 1，在 Chu 等[25]基础上修改)。此外，还有一些其他的 circRNA，例如融合基因来源的 circRNA (fusion-circRNA, f-circRNA)[84-85]、聚合酶II转录通读形成的通读 circRNA (read-through circRNA, rt-circRNA)[86]、具有重叠区域和不同位点的 circRNA，称作相互包容的 circRNA (mutually inclusive circular RNAs)[76]等，不同的研究方法和分类方式以及可变剪接的存在使我们更大程度上认识到了 circRNA 的多样性，因此能开展更深入地研究。

5 植物 circRNA 的生成机制

CircRNA 来源于 pre-mRNA，由 RNA 聚合酶Ⅱ(Pol Ⅱ) 转录[70-72]。CircRNA 的生成调控依赖于控制剪接的顺式调控元件和反式作用因子。除此之外，通过核酶自剪切也可以调控植物 circRNA 的生成。

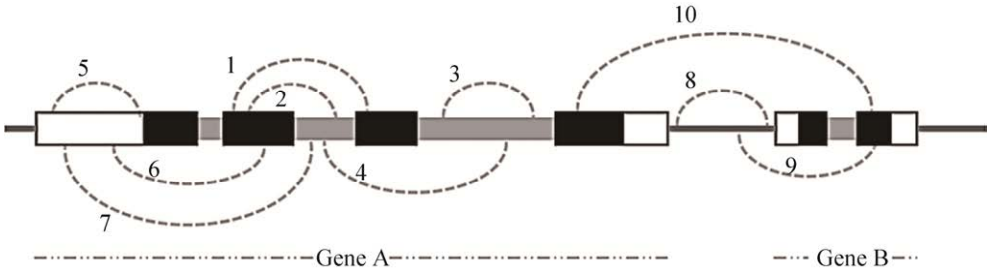

图 1 circRNA 的类型[25]

Figure 1 Types of circRNAs[25]. 1: e-circRNA, two back-splicing sites of a circRNA are both at exons; 2: ei-circRNA, one back-splicing site of a circRNA is at exon while the other is at intron; 3: i-circRNA, two back-splicing sites of a circRNA are both at a single intron; 4: ie-circRNA, two back-splicing sites of a circRNA are at two different introns across one or several exons; 5: u-circRNA, two back-splicing sites of a circRNA are both at UTRs; 6: ue-circRNA, one back-splicing site of a circRNA is at UTR while the other is at exon; 7: ui-circRNA, one back-splicing site of a circRNA is at UTR while the other is at intron; 8: ig-circRNA, two back-splicing sites of a circRNA are both at a single intergenic region; 9: igg-circRNA, one back-splicing site of a circRNA is at intergenic region while the other is at genic region; 10: ag-circRNA, two back-splicing sites of a circRNA are at two different genes. The black, gray and blank bars represent exons, introns and UTRs, respectively. The black lines represent intergenic region of the genomes.

5.1 顺式作用元件调控

5.1.1 内含子互补

研究发现侧翼内含子序列互补有助于大多数外显子环化，Gao 等通过烟草瞬时表达葡萄 circRNA 发现侧翼内含子的长度会影响成环效率，且长度越长，成环效率越高，短至 40 nt 的侧翼内含子重复序列足以实现外显子环化[22,87-88]。在动物环化外显子的侧翼内含子中富集反向互补序列 (reverse complementary pairs, RCPs)，通常来源于重复元件，例如 RCMs (reverse complementary matches)[88] 、ICSs (intronic complementary sequences)[89]及 Alu 元件[88]。然而，植物外显子 circRNA 的侧翼内含子中具有有限的 RCPs，例如，在水稻、拟南芥和大豆中，RCPs 的比例分别为 6.2%、2.7%和 0.3%[6,18,29]，在 13 个经过验证的水稻 circRNA 中，只有两个在其侧翼内含子中包含>15 bp 的 RCPs[29]，另一项研究在水稻外显子 circRNA 侧翼内含子序列中发现微型反向重复转座元件 (miniature inverted repeat transposable elements, MITEs)[6]；在毛竹 circRNA 侧翼内含子中富集长终端重复转座元件 (long terminal repeat transposable elements, LTRTEs)[90]；在玉米 circRNA 侧翼区域发现 LINE1-like 元件 (long interspersed nuclear element 1-like elements, LLEs) 及其 RCPs (LLERCPs)[20]，随着 LLERCPs 数量增加，circRNA 积累量上升；在杨树 circRNA 侧翼内含子区域发现的完整 MITE 元件及其 RCPs，对杨树 *Circ_0003418* 的生成极为重要[79]，证明了转座元件对植物 circRNA 的生成可能起到重要作用，为内含子互补促进环化的机制提供了充足的证据。

5.1.2 外显子跳读

当 pre-mRNA 进行经典的 GU/AG 剪接时，可以发生跨外显子的剪接方式，即外显子跳读，产生包含内含子-外显子的套索中间体，随后该中间体发生反向剪接形成 circRNA[91]。内含子 circRNA 是套索驱动模型中的特殊方式，其依赖于 5′剪接位点的 7 nt 富含 GU 基序和靠近分支位点的 11 nt 富含 C 基序，通过聚合酶II的转录形成一个套索内含子，最终通过 2′-5′磷酸二酯键共价连接而环化，接着从内含子 3′端到分支位点的多余序列被降解[31]。目前在拟南芥、番茄、水稻和玉米中的研究表明，这种套索结构在植物中是广泛存在的，并识别到了大量由套索结构驱动环化的 circRNA[92]。在拟南芥中，*SEPALLATA3* (SEP3) 的第六个外显子跳读生成的 circRNA 与宿主基因 DNA 杂交形成 RNA:DNA-环，从而延缓转录延伸，以增强外显子跳读引起的环状转录本生成，此研究为 circRNA 生成与外显子跳读之间的关系提供了证据[93]。

5.2 反式作用因子调控

除了顺式元件外，反式作用因子，如 RNA 结合蛋白 (RNA binding protein, RBP) 在某些条件下，也可作为 circRNA 生成的激活剂或抑制剂，如 musclebling (MBL)[70] 、quaking (QKI)[94-95]、adenosine deaminase 1 (ADAR1)[96]、fused in sarcoma (FUS)[97]和 DEAH-box helicase 9 (DHX9)[98]等，虽然已在拟南芥中鉴定出这些 RBP 的同源蛋白，但它们在 circRNA 生成过程中的潜在功能仍有待研究。在人类上皮-间质细胞转换中，QKI 通过结合到宿主 RNA 侧翼内含子上形成二聚体，促进内含子-内含子之间相互作用使 3′端和 5′端接近以进行环化[94-95]。拟南芥中的 QKI 同源蛋白包含 26 个 KH 结构域，其中有 5 个与 QKI 高度相似，被证明参与 pre-mRNA 加工[99]，在植物的开花调节[100-101]、应激反应[102-104]和激素信号转导[105]中起重要作用，同时也可能与 QKI 蛋白具有相似功能，例

如参与 circRNA 生成[106]，但这只是一个推测，并未有更多报道来证实或推翻。大多数 RBP 促进 circRNA 生成，但也有部分抑制环化。例如 DHX9 是一个有 RNA 结合功能域和解旋酶功能域的核 RNA 解旋酶，通过与反向互补 Alu 元件相结合，之后行使 RNA 解旋酶功能，导致 Alu 元件的解旋进而抑制 circRNA 的形成[98]。目前关于植物 RBP 调控促进或抑制 circRNA 生成的报道仍很少。

5.3 核酶自剪切

植物 circRNA 还可能通过核酶自剪切来协助产生，最早报道的 circRNA 含有小的自切割 RNA 基序，如锤头状核酶 (hammerhead ribozymes, HHR) 和发夹核酶 (hairpin ribozymes, HR)[7,107]，HDV 也编码自切割基序，称为 HDV 核酶[108-109]。这些核酶的参与会有效促进 circRNA 的积累。例如对几种植物如麻风树 (*Jatropha curcas* L.)、草莓 (*Fragaria* × *ananassa* Duch.)、桉树 (*Eucalyptus robusta* Smith)或柑橘 (*Citrus* L.) 的不同体细胞和生殖组织进行 Northern blotting 分析和 RT-PCR 实验表明，植物基因组中存在两侧串联有III型 HHR 基序并能精确表达为环状 RNA 的序列[110]。另一项研究预测了夏威夷群岛无花果树 (*Ficus carica* Linn.) 相关的类病毒 RNA，其中一个 RNA 被鉴定为大小在 357–360 个核苷酸之间的 circRNA，这个类病毒 circRNA 在每条极性链上都含有 HHR 基序，在转录过程中，HHR 被证明在预测的位点上进行自切割[111]。除反向剪接外，通过 HHR 的参与来促进 circRNA 的表达被认为是第二种 circRNA 生成的途径，具有作为基因调控新形式的潜力[112]。

6 植物 circRNA 的功能

由于 circRNA 的低水平存在导致人们的研究受到极大阻碍，到目前为止，大多数 circRNA 功能不够明确。最近的研究发现，植物 circRNA 可以作为 miRNA 海绵，通过竞争性内源 RNA 机制来调节基因表达，同时也具有蛋白质编码潜力，此外植物 circRNA 在激素刺激和逆境胁迫等条件下也呈现差异表达模式，影响植物生长发育和参与细胞间信号传递，初步体现了其功能的多样性，相信在未来植物 circRNA 在功能发挥和应用潜力上的研究会越来越深入和多样化。

6.1 作为 miRNA 海绵

circRNA 已证实的功能之一是通过发挥 miRNA 海绵吸附作用直接或间接结合靶标 miRNA，阻断 miRNA 对其靶基因的调控[113]、建立 circRNA-miRNA-mRNA 网络和影响基因表达和转录调控。目前鉴定到的不同植物 circRNA 中都预测到一定比例的 circRNA 是潜在的 miRNA 靶标，拟南芥中有 5.0%的 circRNA 具有作为 miRNA 海绵的潜在能力[29]；水稻 1 356 个外显子 circRNA 中发现 235 个推测的 miRNA 结合位点，只有 31 个 circRNA 含有两个或两个以上的 miRNA 结合位点[6]；玉米 2 804 个 circRNA 中有 15 个 miRNA 结合位点[20]；枳壳 29 个 circRNA 可以作为 16 个 miRNA 的潜在靶标[75]；*circRNA45* 和 *circRNA447* 可能作为 *miR477-3P* 海绵来调节靶标抗性基因 *SpRLK1/2* 的表达，在番茄对晚疫病的免疫中起到正调节作用[114]；另一项研究比较野生型和乙烯信号转录因子 (ethylene-responsive transcription factors, LeERF1) 转基因番茄果实发现 61 个 circRNA 可能具有吸附 miRNA 的作用，其中一些 miRNA 已被发现参与乙烯信号转导途径[115]；在棉花差异表达的 circRNA 中发现，其中有 7 个可以与 17 个 miRNA 相结合参与棉花纤维发育的调控[116]；在水稻中，circRNA *Os08Circ16564*

被预测为 *miR172* 的目标模拟物，但是 RT-PCR 结果表明，在 *Os08Circ16564* 过表达植株中 *miR172* 的表达水平与野生型没有显著差异，表明 *Os08circ16564* 可能不是 *miR172* 的真正海绵[6]。一个 miRNA 可以靶向多个 circRNA，同时一个 circRNA 也可以被几个不同的 miRNA 作为靶标，但是到目前为止，在各种生物体中发现的大多数 circRNA 表达量较低，很少包含相同 miRNA 的多个结合位点，因此许多 circRNA 似乎不可能作为 miRNA 海绵发挥作用。此外即使预测到 miRNA 结合位点，也仅停留在预测阶段或者出现预测错误的情况，因此需要使用更多候选 circRNA 来进行实验验证。

6.2 具有翻译潜力

circRNA 被归入非编码 RNA 就是由于其翻译潜力未得到太多关注。近年来有研究证明了 circRNA 的蛋白质编码潜力[117-119]。例如存在于果蝇头部的 *circMbl*，可以产生 37.04 kDa 的蛋白质[118]；人类 *Circ-ZNF609* 包含一个完整的开放读码阅读框 (open reading frame, ORF)，具有起始和终止密码子，可以被内部核糖体进入位点 (internal ribosome entry site, IRES) 翻译成蛋白质[117]；除 IRES 外，在存在修饰位点的情况下翻译带有 ORF 的 circRNA，翻译效率与 N^6-甲基腺苷 (N^6-methylation of adenosine, m^6A) 位点的数量呈正相关[119]。目前，在大豆响应低温胁迫的 3 个 circRNA 中预测到其至少包含一个 IRES 元件和一个 ORF[120]；Han 等在玉米中预测到 229 个 circRNA 具有编码潜力，发现较长的 circRNA 具有更高的编码潜力[121]；在拟南芥中发现 m^6A 的修饰位点通常位于 mRNA 的起始和终止密码子附近[122-123]，暗示植物 circRNA 也具有翻译潜力。

6.3 响应生物/非生物胁迫

多项研究表明，circRNA 响应生物/非生物胁迫，在植物发育过程中发挥重要作用。Ye 等发现水稻中有 27 个外显子 circRNA 在磷酸盐充足和饥饿条件下差异表达[29]；番茄和拟南芥中分别有 163 个和 1 583 个 circRNA 在冷热处理条件下差异表达[17,124]；小麦有 62 个 circRNA 在脱水胁迫条件下的幼苗中有差异表达[19]；冷胁迫处理不同时间段，共有 475 个葡萄 circRNA 差异表达[22]；黄瓜 (*Cucumis sativus* Linn.) circRNA 在盐胁迫中差异表达[125]；在拟南芥中过表达来源于外向整流型钾离子通道基因 *circGORK*，发现转基因植株的种子萌发对脱落酸 (ABA) 超敏感，且抗旱性增强，为 circRNA 直接调控干旱胁迫提供了有力证据[126]。

除了非生物胁迫，circRNA 也被报道对生物胁迫有反应。例如，大豆中 199 个 circRNA 在棉铃虫取食叶片损伤胁迫下的抗病和感病样本之间存在差异表达[127]；猕猴桃中 584 个差异表达的 circRNA 响应丁香假单胞猕猴桃致病变种 (*Pseudomonas syringae* pv. *actinidiae*, PSA) 的感染[21]；马铃薯 (*Solanum tuberosum* L.) 中 429 个差异表达的 circRNA 响应胡萝卜软腐果胶杆菌巴西亚种 (*Pectobacterium carotovorum* subsp. *brasiliense*, PCB) 的感染[128]，在 PCB 感染时，circRNA 在感病品种中表达下调，抗病品种中表达上调；棉花感染黄萎病菌 (*Verticillium*) 后共发现 280 个差异表达的 circRNA，且易感病株系中差异表达的 circRNA 数约为抗病株系的两倍[129]；番茄感染黄化曲叶病毒 (tomato yellow leaf curl virus, TYLCV) 病叶与对照之间分别有 32 个和 83 个 circRNA 特异表达，且感染病毒后 circRNA 的表达量低于对照[130]；过量表达 *CircR5g05160* 的转基因水稻可提高对稻瘟病菌 (*Magnaporthe oryzae*) 的抗病性[131]。这些结果表明，circRNA 可能在植物对生物/非生物胁迫的应答中发挥着重要而

多样的功能，另外这些差异表达的 circRNA 也可能作为植物中有效的生物标记。

除此之外，植物 circRNA 还有其他功能，例如过表达拟南芥 *AT5G37720* 的第一个内含子产生的套索 circRNA (lariat41)发现能调节基因表达并影响拟南芥发育[132]，过表达植株 (*lariat41-OE*) 表现出叶片卷曲和簇生、开花晚和育性低的特点，并伴随 800 多个基因表达量的改变[133]。其中关键的开花时间调节因子 FT 在 *lariat41-OE* 植株中的表达水平显著降低，可能是导致出现晚花表型的原因。此项研究表明，circRNA 通过调控基因的表达量从而影响植株的生长发育，但只是少数 circRNA 在功能上得到了验证，具体如何调控还需进一步了解。植物 circRNA 还可能作为信号分子参与细胞之间信号传递和木质部韧皮部的长距离运输。例如马铃薯纺锤块茎类病毒 (potato spindle tuber viroid, PSTVd) 通过韧皮部的长距离运输强调了 circRNA 通过植物维管系统传递分子的可能性[134-135]，推测植物内源 circRNA 可能采取类似的方式参与信号传递，因此在这个方向上需要更广泛地研究。植物 circRNA 的功能在很大程度上仍未被探索，但越来越多的证据开始显示，植物 circRNA 在许多生物过程中发挥着重要作用。

7 circRNA 的降解与定位

circRNA 没有自由末端，因此并不能沿用大多数 RNA 降解途径。MiRNA 介导的 circRNA 降解是目前得到比较明确证明的 circRNA 降解途径，例如 circRNA *CDR1as* 被 *miR-671* 介导的核酸内切酶 (argonaute-2, Ago2) 切割[136]。*miR-671* 在 *CDR1as* 的结合位点与 miRNA 完全互补，导致 Ago2 切割[113]，但是其他 circRNA 是否能通过 miRNA 介导的途径降解尚不清楚。m^6A 可促进潜在可降解 circRNA 的核酸内切酶的招募[137]，其修饰的 circRNA 被人类 YTH 结构域家族 2 (YTH domain containing family 2, YTHDF2) 蛋白识别，结合的环状转录本被选择性降解[138]，另一项研究发现 HeLa 细胞经 poly (I:C) 刺激或病毒感染即会激活核酸内切酶 RNase L，从而导致整体 circRNA 的降解[139]。此外，果蝇 DL1/S2 细胞中 *GW182* 缺失后，circRNA 水平升高[140]，*GW182* 通常被认为是 miRNA 介导的基因沉默关键因子，且其同源物在调节 HeLa 细胞 circRNA 降解方面显示出类似的作用[141]，因此表明 *GW182* 对 circRNA 的降解起特异性作用[142]。Guria 等通过计算预测了大约 85%的 miRNA 与植物 circRNA 的完美互补结合位点[143]，这些位点可能使 circRNA 作为 miRNA 海绵导致 miRNA 活性被抑制，也可能是 miRNA 在植物中切割 circRNA 的一种调控机制，miRNA 中第 10 和第 11 核苷酸的序列可能决定了 circRNA 是作为 miRNA 的海绵，还是被 miRNA 切割。如果 miRNA 中的第 10 和第 11 个核苷酸与 circRNA 完全匹配，miRNA 可能会有更多的机会切割和降解 circRNA[144]。目前我们对于植物 circRNA 的降解仍知之甚少，因此需要更多的研究来探索。

一些报告证实细胞质中存在外显子 circRNA[50,80,145]，而保留内含子的 circRNA，如外显子-内含子 circRNA 和内含子 circRNA，仅在细胞核中发现[31,146-147]。但是，在 Neuro2a (N2a) 细胞中，发现外显子 circRNA 也定位在细胞核中[98]，表明外显子 circRNA 的定位仍不清楚。此外发现，circRNA 一旦在细胞核中产生，通常被运输到细胞质中，以实现其正常的功能或降解[28,148]。Sun 等发现 circRNA 中存在多个内部互补碱基配对序列 (internal complementary base-pairing sequences, ICBPS)，

推测其可能不是简单的环状结构，还含有双链结构，这种结构的形成有助于 circRNA 与 RBP 牢固的结合，从而便于它们从细胞核输出到细胞质中，并且双链结构可能使 circRNA 更容易被相关的酶降解[149]。

8 讨论与展望

随着测序技术和生物信息学方法的发展，越来越多的 circRNA 被鉴定出来，然而使用不同算法在同一物种中鉴定的 circRNA 的数量不同，假阳性占比也很高，因此需要不同测序技术、测序深度、预测工具和算法以及处理方法等同时结合来提高 circRNA 鉴定的准确性和精度。同时得益于测序技术的不断改进，circRNA 的神秘面纱逐渐被揭开。目前检测 circRNA 常用的二代测序根据短片段进行 RNA 组装获得全长 circRNA，易出现线性转录本污染问题且很难准确定量，三代测序的出现解决了这个问题，它能够实现>10 kb 长度 circRNA 的全序列组装，使人们真正看到 circRNA 的完整序列，可识别 circRNA 隐藏的复杂结构，并且其不需要扩增，实现了对基因组的均匀覆盖，能检测到更多低水平表达和新的 circRNA，准确度、检出率和灵敏度大大提升[150-152]。大规模获得全长 circRNA 有助于人们理解其内部结构及可变剪切方式，进而揭示更多 circRNA 的功能和调控过程。

在鉴定出大量 circRNA 的同时人们对于 circRNA 的认识也达到了新的层面，circRNA 的生成机制和功能应用已成为一个相关的研究课题。在动物中，circRNA 两侧内含子的长度明显大于所有内含子的平均长度，这与在植物中的发现一致[29]，这些侧翼内含子还包含介导 circRNA 生成的 RCPs[153]。植物侧翼内含子含有与动物相比较少的重复序列，表明植物与动物 circRNA 的生成机制可能不同。植物侧翼内含子中包含的转座子元件和 RCPs 对 circRNA 生成的影响还有待进一步研究。同时植物和动物之间 circRNA 生成的差异也将是一个有趣的研究方向。

在 circRNA 的功能方面，circRNA 在生物体中动态表达，与 mRNA、miRNA 及 lncRNA 等形成互作网络，参与调控宿主基因的转录及转录后表达。植物 circRNA 目前的研究主要集中在预测及鉴定工作，对于它们的功能认知还不是很明朗，由于 circRNA 来源于与线性 RNA 相同的 pre-RNA[80]，所以很难独立于线性 RNA 来研究 circRNA，这使得用传统的 RNAi 很难沉默 circRNA[154]。随着 CRISPR-Cas 技术的发展[155-158]，为研究 circRNA 的功能提供了新的思路，其在分析非编码基因功能方面特别强大，可以通过靶向缺失产生空等位基因，这是传统诱变工具难以实现的。Zhou 等基于 CRISPR-Cas9 介导的基因组长片段缺失策略实现了 4 个水稻 circRNA 的敲除与功能鉴定。该研究不仅提供了植物 circRNA 位点通过海绵吸附响应来负调控 miRNA 的明显证据，而且还为进一步通过编辑植物 circRNA 位点改良农艺性状提供了新思路[158]。杨树 MITE 元件及其反向互补序列的存在为利用 CRISPR-Cas9 进行杨树遗传改良提供了理想的大片段缺失靶位点[79]。最近出现的 CRISPR-Cas13 允许通过靶向跨越反向剪接接头的序列来识别 circRNA，有效而特异地区分了 circRNA 和 mRNA，同时能够高效地降低 circRNA 表达而不影响其来源线性 mRNA 表达，使 CRISPR-Cas13 成为在单个和大规模水平上发现和研究 circRNA 功能的有用工具[157,159-160]。以 circRNA 为靶点的基因编辑在植物 circRNA 功能探究中的应用正处于新兴阶段，是研究的热点之一。

此外，circRNA 在不同植物物种中的鉴定尚不全面，如柑橘、油菜、大麦和小麦等的 circRNA 鉴定数量仍然较少。对于植物 circRNA 在细胞中的定位、输出和降解机制等尚不清楚。此外植物 circRNA 特异性环化的原因、以何种机制启动环化、环化后是否影响来源基因及临近基因表达，会产生什么样的生物学效应等具体问题仍未可知，解决好具体问题将有助于研究者开展未来的探索，同时也有助于拓宽植物 circRNA 的研究领域。虽然在过去几年植物 circRNA 的研究进展迅猛，但是同动物相比，仍处于起步阶段，随着人们的广泛关注，相信在将来会对植物 circRNA 有一个全面的认知。

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