综 述 Reviews

MicroRNA调控被子植物花发育的研究进展

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摘要: MicroRNA (miRNA)是一类由内源基因编码的长度为21~23 nt的非编码单链小RNA分子, 通过与靶基因的互补位点结合而降解或抑制靶mRNA的翻译, 从而在转录后水平上调控基因的活性。miRNA在调控植物发育方面发挥着广泛的作用。从成花诱导到花器官特征属性的形成, miRNA在整个花发育过程均发挥着关键作用。*miR172*和*miR156/157*参与由营养生长向生殖生长转换的调控, *miR172*和*miR169*在花发育的早期阶段通过界定靶基因的表达区域而调控花器官的属性, *miR319*、*miR159*、*miR164*以及*miR167*在花发育的晚期阶段决定细胞的特化。文章综述了miRNA调控被子植物花发育的研究进展, 为深入了解miRNA的作用机制奠定基础。

关键词: microRNA (miRNA); 花发育; 转录后调控

Progress in Research of MicroRNA Regulation on Angiosperm Flower Development

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Abstract: MicroRNA (miRNA) are approximately 21–23 nucleotides (nt) long, small single strand non-coding RNAs which are encoded by endogenous genes. miRNA plays a key role in regulating gene activity at post-transcriptional levels by binding to complementary sites in target genes causing degradation and/or translational repression of the target mRNAs. miRNA function broadly to regulate many aspects of plant development. miRNA function throughout flower development, from the floral induction to floral organ specification. *miR172* and *miR156/157* play a critical role in the vegetative to reproductive transition. *miR172* and *miR169* control floral organ identity fate during the early stages of flower development by defining the spatial boundaries of expression of target genes. *miR319*, *miR159*, *miR164*, and *miR167* specify particular cell types during later stages of flower development. This review focuses on research progress in roles of miRNA in angiosperm flower development and provides a basis for better understanding the function mechanism of miRNA.

Key words: microRNA (miRNA); flower development; post-transcriptional regulation

miRNA是由内源基因编码、长度为21~23 nt的非编码小RNA分子, 作为负调控因子主要在转录后水平上调节基因的活性(Hüttenhofer等2002)。在植物中, miRNA发挥作用的基本过程如下: (1)内源miRNA基因在RNA聚合酶II的作用下形成pri-miRNA; (2) pri-miRNA在DDL (DWADLE)蛋白作用下形成较为稳定的初级茎环结构(Yu等2008); (3)这个初级结构在类似RNase-III的DCL1 (DICER-LIKE1)蛋白(Kurihara和Watanabe 2004)、锌指蛋白SE (SERRATE) (Grigg等2005; Kurihara等2006; Yang等2006a; Laubinger等2008)以及双链DNA结合蛋白HYL1 (HYPONASTIC LEAVES 1) (Han等2004)的共同作用下形成发夹结构的pre-miRNA; (4) pre-miRNA被DCL1再次处理后形成miRNA-miRNA*复合物(Dong等2008), 该复合物的3'端被HEN1 (HUA ENHANCER 1)甲基化(Park等2005; Yang等2006b; Yu等2005); (5)甲基化修饰的miRNA-miRNA*复合物在Exportin5的同系物——HASTY协助下从细胞核进入细胞质(Park等2005); (6)在细胞质中, miRNA*被降解而miRNA与细胞质内的一些酶形成RISC复合物, 该复合物通过与靶

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mRNA的相互作用最终使得靶mRNA降解或者抑制mRNA翻译(Nag和Jack 2010)。miRNA在植物发育中起关键作用的证据分别来自无效等位基因*dcl1*和*ago1* (*ARGONAUTE1*)突变体的胚胎致死和幼苗致死表型(Schauer等2002; Vaucheret等2004)。等位基因*dcl1*功能部分丧失突变体表现出与*dcl1*突变体一样的缺陷表型效应, 该效应主要表现在叶片形态、叶腋分生组织维持、开花时间、花器官样式及胚珠发育(Park等2005); 等位基因*ago1*功能部分丧失突变体表现为侧生器官极性、叶与花形态表型缺陷(Kidner和Martienssen 2004; Vaucheret等2004)。*ago1*与*dcl1*突变体的miRNA积累总量降低, 而miRNA靶基因的表达水平升高。miRNA生物合成系统缺陷突变体表现出的多效性发育表型证实miRNA在植物发育过程中发挥了关键调控作用。

花发育是有花植物从营养生长向生殖生长的转换、实现世代交替的关键环节, 涉及不同发育方式的转换。植物感应环境因子(如光周期、温度等)和自身内部因子(赤霉素)的诱导, 经过一系列信号转导过程, 包括光周期途径、春化途径、自主途径、赤霉素途径和温敏途径, 启动成花决定过程中的调控基因, 引起花芽分化, 进而导致花器官发生。5条调控植物开花时间的途径既彼此独立又互相交联, 构成一个复杂的网络实现对植物开花时间的精密调节。在拟南芥中, 这5条途径激活开花途径整合子*FLC* (*FLOWERING LOCUS C*)、*FT* (*FLOWERING LOCUS T*)和*SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*), 进而激活花分生组织特征基因*LFY* (*LEAFY*)和*AP1* (*APETALA1*), 花分生组织特征基因激活了花器官特征基因, 如A功能基因*AP1*、*AP2*、*LUG* (*LEUNIG*); B功能基因*AP3*、*PI* (*PISTILLTA*); C功能基因*AG* (*AGAMOUS*); D功能基因*STK* (*SEEDSTICK*)、*SHP1/2* (*SHATTERPROOF1/2*)以及E功能基因*SEP1/2/3/4* (*SEPALLA1/2/3/4*)。基于对拟南芥和金鱼草花同源异型突变体的分析, 研究者提出了调控被子植物4轮花器官发育的“ABC”模型(Coen和Meyerowitz 1991)。随后发现了控制胚珠发育的D功能基因(Colombo等1995; Angenent等1995)以及控制花萼、花瓣、雄蕊和雌蕊发育的E功能基因(Pelaz等2000), “ABC”模型被拓展为“ABCDE”模型(Theiβen 2001): A＋E功能基因调控萼片的发育, A+B+E功能基因调控花瓣的发育, B+C+E功能基因调控雄蕊的发育, C+E功能基因调控心皮的发育, C+D+E功能基因调控胚珠的发育。最后, 花器官特征基因激活下游决定组织和细胞类型的基因, 从而形成各轮花器官(Simpson和Dean 2002; Lee和Lee 2010; Srikanth和Schmid 2011)。控制被子植物花发育的分子机理已成为植物发育生物学关注的焦点, 研究表明, miRNA在被子植物花发育过程中发挥着至关重要的作用(Jones-Rhoades等2006)。miRNA通过与靶基因mRNA的3'或5'端(大多数在3'端)的miRNA结合位点发生特异结合而调控花发育并影响花器官性状。本文重点综述了*miR172*、*miR156*、*miR157*、*miR164*、*miR165/166*、*miR167*等miRNA家族基因在花发育中的生物功能及其作用机制。

1 *miR172*调控拟南芥开花时间和花器官特性相关的基因*AP2*的表达并调控玉米花序的发育

拟南芥中存在5个编码*miR172*家族的基因, *miR172*通过调控*AP2*-like基因的表达而影响开花时间(Aukerman和Sakai 2003)、花器官属性(Chen 2004)和花的确定性(Zhao等2007)。在由营养生长向生殖生长转换的过程中, 含有*miR172*结合位点的AP2转录因子基因*TOE1* (*TARGET OF EAT1*)、*TOE2*、*SNZ* (*SCHNARCHZAPFEN*)和*SMZ* (*SCHLAFMUTZE*)受*miR172*的调控(Jung等2007), *miR172*通过降解靶mRNA和抑制其翻译两种方式发挥功能(Aukerman和Sakai 2003; Chen 2004; Jung等2007; Schwab等2005)。这4个基因中任何一个组成型表达都会出现开花延迟的表型。然而, *miR172*过表达或*toe1*功能缺失突变体表现为早花表型(Aukerman和Sakai 2003; Jung等2007)。完成营养生长向生殖生长转变后, 拟南芥的*miR172*表达水平升高, 致使*TOE*基因活性降低, 这些证据表明, *TOE*基因表达水平下调是诱导拟南芥启动开花程序所必需的。

*miR172*在长日条件下表达量增加以及在*co*和*ft*突变体中日长对*miR172*影响作用被扰乱的事实表明, *miR172*参与了诱导拟南芥开花的光周期途径(Schmid等2003)。光周期途径中, 其表达受节律钟调控的*GI* (*GIGANTEA*)基因在*CO* (*CONSTANS*)上游起作用, *CO*通过激活*FT*基因转录而促进开花(Mizoguchi等2005), 而*miR172*却以独立于*CO*的途

径诱导*FT*基因表达, 从而参与到光周期成花途径中(Jung等2007)。在*miR172*促进开花途径中, 光周期调控*miR172*的丰度是通过*GI*介导的*miR172*加工成熟而不是*GI*促进*miR172*基因的转录。成熟的*miR172*会逐渐降低*TOE1*和*TOE2*基因的转录水平, 相应地会提升*FT*基因的转录水平。此外, *GI*还可以通过与*FT*抑制子SVP (SHORT VEGETATIVE PHASE)、TEM1 (TEMPRANILLO)、TEM2或*FT*启动子结合而直接激活*FT* (Sawa和Kay 2011)。

*miR172*在花器官特征属性决定方面也发挥着关键作用。编码参与miRNA加工成熟的甲基转移酶的基因*HEN1* (*HUA ENHANCER 1*)与*ag-4*突变体的异常表型相关, 表明miRNA可能参与调控花器官特征属性(Boutet等2003)。ABCDE模型指出, 调控花器官特征属性基因*AP2*和*AG*之间通过相互抑制限制彼此的表达区域而发挥各自特化花被器官和生殖器官的作用。*AP2*对*AG*的表达抑制需要*LUG* (*LEUNIG*)、*SEU* (*SUESS*)、*SAP* (*STERILE APETALA*)和*ANT* (*AINTEGUMENTA*)基因的参与(Liu和Meyerowitz 1995; Elliott等1996; Klucher等1996; Byzova等1999; Conner和Liu 2000; Franks等2002)。在金鱼草中, *AP2*的同源基因*LIP1*和*LIP2*却不能抑制C功能基因在第1和2轮花中异位表达, 而是由*FIS* (*FISTULATA*)、*STY* (*STYLOSA*)和*CHO* (*CHORIPETALA*)介导了对C功能基因的表达抑制(Jack 2004)。此外, 在AP2蛋白的协助下, LUG/SEU结合到*miR172*启动子上共同抑制*miR172*在花的第1和2轮表达, 说明AP2可抑制*miR172*的表达(Grigorova等2011)。生物信息学分析发现*AP2*基因具有1个*miR172*结合位点(Jones-Rhoades等2006)。而且, 组成型表达*miR172*的转基因株系表现出与*ap2*突变体相似的花器官表型, 说明*miR172*在花器官特征属性决定过程中下调*AP2*的活性(Chen 2004; Zhao等2007)。在花发育过程中, *miR172*下调*AP2*活性对生殖器官的适当发育和花干细胞生长的及时终止是非常关键的; *miR172*对于*AP2*的抑制作用可能通过依赖*AG*基因和独立于*AG*基因[可能为*CLV* (*CLAVATA*)途径]的两条路径并最终整合于*WUS* (*WUSCHEL*)途径来影响花分生组织形状维持和干细胞生长, 同时也定义了B功能基因(*AP3*和*PI*)表达域的内边界, 可能是通过依赖于*AG*基因的途径完成的(Zhao等2007)。在开花早期*miR172*对于*AP2*基因的抑制作用主要被限制在花原基中心将形成雄蕊和心皮的3、4轮区域(图1-A; Nag和

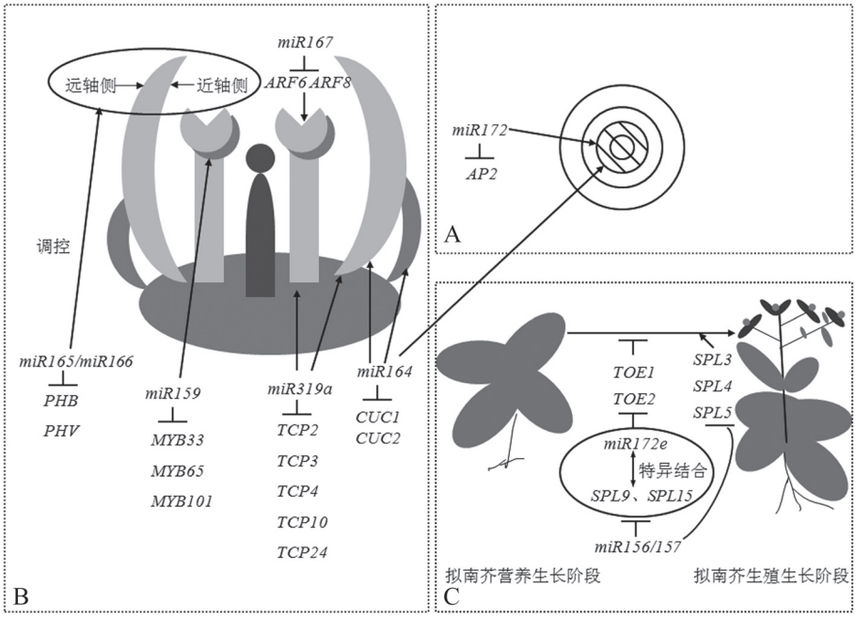

图1 miRNA调控拟南芥花发育的机制

Fig.1 Regulation of *Arabidopsis thaliana* flower development by miRNA

A: *miR172*调控拟南芥花发育的机制; B: *miR159/164/165/166/167/319*调控拟南芥花发育的机制; C: *miR156/157/172e*调控拟南芥花发育的机制。A和B中从外到里各层依次为萼片、花瓣、雄蕊和雌蕊; ⊥表示抑制作用, →表示促进作用。

Jack 2010)。在第3和4轮, *AP2*作为调控靶位, *miR172*可降解*AP2* mRNA (Schwab等2005; Jung等2007)或抑制其翻译(Chen 2004)。

*miR172*还是玉米花序发育的重要调控因子。由*TS4* (*TASSELSEED4*)编码的*miR172e*与AP2类转录因子IDS1 (INDETERMINATE SPIKELET1)和SID1 (SISTER OF IDS1)结合而控制花的性别以及分生组织细胞的命运(Nag和Jack 2010; Chuck等2007; Chuck等2008)。*IDS1*和*SID1*促进小穗分生组织(spikelet meristems, SM)发育为花分生组织(floral meristem, FM), 而*miR172*负调控*IDS1*和*SID1*, 且*IDS1*负调控*SID1*。

研究发现, *miR172*还是一类应答环境温度信号的miRNA (*miR156*, *miR163*, *miR169*, *miR172*, *miR398*, *miR399*)之一(Lee等2010)。在23 ℃或16 ℃条件下, 过量表达*miR172a*的植株均表现为早花, 说明应答环境温度信号的*miR172*活性的改变会影响拟南芥对环境温度信号的应答。那么, *miR172*如何将环境温度信号整合到开花遗传途径中? 拟南芥的温敏开花途径中的信号传递有赖于*FCA* (*FLOWERING CONTROL LOCUS A*)和*FVE*的参与(Blázquez等2003), FCA通过调控非编码翻译RNA对邻近多聚腺苷化位点的选择而在表观遗传学水平上调控开花抑制子*FLC*的表达(Liu等2010; Hornyik等2010)。23 ℃条件下*miR172*的积累是FCA促进pri-miR172加工成熟的结果。FCA与FY协同调控pri-miR172的3′-末端加工过程(Simpson等2003), 形成有利于pri-miR172加工成熟的二级结构, 通过RNA识别基序, FCA与pri-miR172茎环结构的侧翼序列结合, 招募DCL1复合体的核心组分(Jung等2012)。FCA-miR172可能是由*FLC*、*FVE*和*SVP*介导的多条温敏开花信号通路中的一条, 并不是关键通路, 仅对环境温度启动开花机制发挥微调作用。

MADS-box蛋白SVP在FCA和FVE的下游起作用, 遗传学分析表明, SVP不能调控*FLC*的表达(Lee等2007), 然而二者免疫共沉淀的事实表明SVP与FLC可能在温敏途径中协同起作用, SVP优先与*FT*启动子的vCArG基序结合、FLC优先与*FT*第一个内含子的CArGVII基序结合, 因此SVP可能以FLC依赖的方式调控*FT*等基因的表达(Lee等2007; Li等2008)。在23 ℃或16 ℃条件下, *svp-32*突变体的*miR172a*表达量升高, 说明*miR172*在*SVP*的下游起作用, *miR172*下调具有开花抑制活性的靶基因(如*TOE1*、*SMZ*等), *SVP-miR172*信号最终汇集于*FT*, 从而使*svp-32*表现为早花(Lee等2010)。过量表达*miR172*不仅上调*FT*的表达, 而且还降低*FLC*的表达。但是, 过量表达*TOE1*和*SMZ*并不能改变*FLC*的表达水平, 表明在温敏途径中, *miR172*的靶基因可能以间接方式调控*FLC*的表达。*FLC*在*miR172*介导的开花信号通路中的作用还有待于进一步研究。

2 *miR156*对生长周期转变和开花诱导的调控

对于有花植物来说, 在其发育的每个阶段, 茎尖分生组织的周围会形成形态和功能不尽相同的特定器官。拟南芥发育过程有3个重要转变: 胚胎发育阶段向营养生长阶段的转变(vegetative phase change)、营养生长阶段向生殖生长阶段的转变(reproductive or floral transition)和分生组织特征的转变(meristem identity transition, MI转变) (Yamaguchi等2009; Poethig 2003)。在整合内外开花诱导途径的信号后, 有花植物经历MI转变, 茎尖分生组织周围的花原基形成生殖结构——花, 这一重要过程是由植物特异性转录因子LFY和MADS-box家族成员AP1/FUL (FRUITFULL)调控的(Yamaguchi等2009; Benlloch等2007; Litt和Irish 2003)。事实表明, *LFY*、*AP1*和*FUL*基因中任何一个表达水平上调都会引起早熟的分生组织特异性转变(Yamaguchi等2009; Ferrandiz等2000; Mandel和Yanofsky 1995; Weigel和Nilsson 1995)。然而, 仅仅依赖这3类基因还不足以解释开花转变这一重要过程, 其中还涉及金鱼草中能与*SQUA*启动子结合并调控其表达的*SQUA*启动子结合蛋白(SQUAMOSA PROMOTER BINDING PROTEIN, SBP) (Klein等1996), 连同拟南芥及苔藓中的SBP统称为SPL (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE)。SPL蛋白均含有一个结合DNA所必需的高度保守结构域SBP-box (Birkenbihl等2005), 其中部分SPL蛋白拥有*miR156*的作用靶点。

*miR156*是拟南芥、玉米和水稻等生育周期转变的关键调控因子(Poethig 2009)。拟南芥基因组共编码6个*miR156*基因, 这些基因在不同物种间很保守(Yamaguchi等2009; Xie等2005), 上调表达这些基因都会延迟营养阶段向生殖阶段的转变。拟

南芥miRNA核输出蛋白基因*HASTY* (*HST*)功能缺失突变体能加速幼年期向成年期的转变(Telfer和Poethig 1998), *hst*突变体中*miR156*表达水平显著高于野生型, 且幼年期显著高于成年期; 组成型表达*miR156*能显著延迟植株生长周期转变和开花(Wu和Poethig 2006); 过表达*miR156*模拟序列(mimic sequence)则使植株提前开花(Wu等2009)。这些研究结果表明, *miR156*对拟南芥幼年期的发育是必要的。

拟南芥具有16个SBP家族转录因子, 其中11个转录因子受*miR156*家族miRNA的转录后调控(Gandikota等2007; Schwab等2005; Wu和Poethig 2006)。*SPL3*、*SPL4*和*SPL5*功能冗余, 主要调控开花时间, 上调其表达水平会使MI转变提前; 而SPL9和SPL15主要调节叶片生长速率(Cardon等1997; Gandikota等2007; Schwab等2005; Schwarz等2008; Wang等2008; Wu和Poethig 2006)。在启动开花和花分生组织决定方面, SPL9启动*FUL*、*SOC1*和*AGL24* (*AGAMOUS-LIKE 24*)的转录(Wang等2009); SPL3启动*FUL*、*LFY*和*AP1*的转录(Wang等2009; Wu和Poethig 2006; Yamaguchi等2009); SPL4/5启动*FUL*、*SOC1*、*LFY*、*AP1*和*FT*的转录(Wu和Poethig 2006)。因此, *SPL*基因可能是启动并控制拟南芥开花的一个新的通路。在玉米和拟南芥中, 营养生长早期*miR156*高水平表达, 随着发育进程表达水平下降。当*miR156*的含量逐渐降低时, 其靶基因*SPL*的表达量逐渐上升, 进而激活下游基因的表达, 诱导植物开花。

*SPL9*和*SPL15*由于具有结合*miR172e*的调控位点而介导*miR156*对于*miR172*的调控, 而*miR172e*可以通过抑制*TOE1*和*TOE2*基因的活性(图1-C; Nag和Jack 2010; Wu等2009)来介导*miR156*对于*miR172*的调控。玉米中, 拥有*miR172*结合位点的*GL15*活性提高可增加幼态特征的叶片数目并延迟开花。在营养生长的早期阶段表达量很低的*miR172*随着发育进程其表达水平不断升高, 进而使得*GL15*表达下调, 促进植株由幼苗期向成年期转变。营养生长早期高水平表达的*miR156*, 在发育后期表达水平逐渐降低, 通过靶基因*SPL*而间接调控*miR172*, *miR156*与*miR172*表现拮抗效应(Wu和Poethig 2006)。

最近研究表明, 作为对环境温度的应答, *miR156-SPL3*相互作用模块和*FT*基因共同调控温敏植物的开花时间(Kim等2012)。因此, *miR156*对于开花植物的阶段转变、开花时机、生殖器官花的形成起着重要的调节作用, 同时也可以调控其他*miRNA* (如*miR172*)的活性。

3 *miR157*调节拟南芥阶段转变和开花诱导

拟南芥*SPL3*、*SPL4*和*SPL5*的3'-UTR上以及其他受*miR156*家族miRNA调控的*SPL*基因的编码区上还具有*miR157*的结合位点, 表明这些*SPL*基因可能同时受*miR156*和*miR157*调控(图1-C)。Gandikota等(2007)利用实时定量PCR和Western杂交对不同类型转基因植株中幼苗和花序中*SPL3*的mRNA和蛋白水平进行检测的结果表明, *miR156*和*miR157*在拟南芥发育早期主要通过抑制*SPL3* (Kasschau等2003) mRNA的翻译来防止提前开花, 而在成年叶片和花序生长时期*miR156*和*miR157*水平达到最低, 使得*SPL3*等基因得以积累来调控花的形成。*miR156*和*miR157*对*SPL3*的调控方式与*miR172*对*AP2*的调节方式相类似, 很可能是通过同一个或者类似的机制来完成(Gandikota等2007), 值得进一步讨论。转基因实验证明许多植物中的*miR156*/*157*都具有诱导浓密叶片的形成和延迟营养阶段向生殖阶段转变的功能。而且*miR156*/*157*对SBP-box基因的调控方式在物种间保守, 例如玄参科植物夏堇中的*miR156*/*157*能通过抑制*SPL3* mRNA的翻译来防止过早开花。过量表达*miR157b*基因的夏堇形成浓密叶片的现象, 既可应用于增加生物量和作物生产, 也可应用于园艺植物的分子育种(Shikata等2012)。

4 *miR164*通过控制*CUC1*、*CUC2*来调节拟南芥花瓣数量、萼片和花瓣的器官发生及花器官边界的形成

*miR164*家族包括3个成员: *miR164a*、*miR164b*和*miR164c*, 在茎尖分生组织两侧器官的分化、器官边界的形成和器官的增殖过程中发挥着重要的作用。*miR164*特异调控的靶基因是NAC家族基因*CUC1* (*CUP-SHAPED COTYLEDON1*)和*CUC2* (*CUP-SHAPED COTYLEDON2*), 这两个基因在分生组织维持和侧生器官分离过程中起作用(Aida等1997), 其中*CUC2*的功能可能是抑制细胞分化、促进分离花器官的发育(Takada等2001)。*miR164c*功能缺失突变体*eep1* (*early extra petals 1*)在开花初

期的花中出现多余的花瓣, 表明*miR164c*是一个重要的花瓣数量调节因子(Baker等2005)。*miR164c*/*miR164a*/*miR164b*三突变体的心皮融合、花瓣与萼片数增加、雄蕊数减少、花器官大小和叶序发生变化(Sieber等2007), 在*CUC1*和*CUC2*不受miRNA调控的株系中也出现相似的表型。表明这类紧密相关的miRNA尽管功能相互冗余, 但可能是由于其表达模式的差异而在植物发育过程中发挥着不同的作用。

*miR164*以及*CUC1*和*CUC2*基因的RNA在花器官轮间和轮内的边界区域表达并存在部分重叠, 在*miR164c*/*miR164a*/*miR164b*三突变体中, *CUC1*和*CUC2*基因的表达区域稍有扩展, 但是其表达水平却显著增加(Sieber等2007), 这说明*miR164*主要调控*CUC1*和*CUC2* mRNA的表达水平, 而不是界定这些mRNA的表达区域。*miR164*主要通过降解靶基因*CUC1*和*CUC2*的mRNA发挥功能(Mallory等2004a)。Huang等(2012)提出由*RBE* (*RABBIT EARS*)编码的C_2H_2锌指结构转录抑制因子RBE直接作用于*miR164c*的启动子区域并使其表达量下调, 从而控制其靶基因*CUC1*和*CUC2*的积累, 最终调控萼片和花瓣的器官发生这些重要的发育事件。因此*miR164c*主要在开花早期通过控制*CUC1*和*CUC2* mRNA的积累而不是限定它们的表达区域来调节花瓣的数量和花器官边界的形成以及萼片和花瓣的器官发生(图1-B), 然而*miR164a*和*miR164b*在花芽时期对于花瓣数量的调节作用微乎其微(Baker等2005)。

5 *miR165/miR166*调控花分生组织发育和花器官近远轴极性

*miR165/miR166*与其靶基因协同调控植物的系列发育过程, 包括茎尖和侧面的分生组织形成、叶片的极性、花发育和导管发育。*miR165/miR166*的靶基因包括HD-ZIPIII转录因子基因*PHB* (*PHABULOSA*)、*PHV* (*PHAVOLUTA*)、*REV* (*REVOLUTA*)、*CNA* (*CORONA*)和*ATHB-8*。*miR165/miR166*主要通过调控HD-ZIPIII类转录因子PHB的分布来实现花器官近轴-远轴的极性建成(Husbands等2009; Mallory等2004b)。*miR166*通过对靶基因*ATHB15*的直接切割而影响拟南芥花序中维管组织的发育, 从而影响花器官极性(Kim等2005); *miR165/miR166*动态调控茎尖分生组织SAM (Shoot apical meristem)和花发育的途径与*WUS-CLV*途径类似, 主要是通过结合并降解编码HD-ZIPIII类转录因子的mRNA从而对茎尖分生组织和花分生组织的发育进行微细调控(Jung和Park 2007; McConnell等2001; Otsuga等2001; Emery等2003; Prigge等2005)。

6 *miR167*调控开花植物的繁殖能力(包括雄蕊的可育性)

植物生长激素在生长和发育过程中发挥着关键性作用。在拟南芥中, Aux/IAA和生长素应答因子ARF (AUXIN RESPONSE FACTORS)是介导生长素响应的2个重要蛋白家族, 属于生长素早期反应基因的转录调控因子(Hagen和Guilfoyle 2002), 在转录水平激活或抑制调节生长素反应的基因。ARF通过结合生长素应答家族基因成员*GH3*启动子上的顺式元件而完成对生长素信号的应答(Ulmasov等1997)。组成型表达*miR167*的植株出现与*arf6*/*arf8*双突变体相似的表型, 即雄蕊花丝变短、花药不能适时散粉、雌性不育(Nagpal等2005; Wu等2006), 说明生长素应答因子*ARF6*和*ARF8*基因是*miR167*的直接靶点。在内源*ARF6*和*ARF8*基因调控序列的控制下, 抗miRNA的*ARF6*和*ARF8*基因的表达使植株产生雌雄均不育的花。在胚珠和花药中*miR167*和*ARF6/ARF8*的表达模式是互相排斥的(Wu等2006)。因此, *miR167*主要通过降解*ARF8* mRNA和抑制*ARF6* mRNA的翻译来调控这两个基因的表达进而对植物的发育产生影响, *miR167*界定*ARF6*和*ARF8*在雄蕊和胚珠中的正确表达区域(Wu等2006), 确保萼片、花瓣、花药、雌蕊和胚珠的正常发育, 包括其外在形状、内部细胞大小、花粉粒萌发率以及柱头、胚轴的长短等等性状(图1-B) (Ru等2006)。当然, 除了*miR167*以外还有一些其他的miRNA是通过影响生长素应答对植物发育(包括花发育)产生广泛的影响。如*miR393*通过结合生长素受体家族基因*TIR1* (*TRA-NSPORT INHIBITOR RESPONSE 1*)和*AFB* (*AUXIN SIGNALING F-BOX*)来维持花的正常发育(Nag和Jack 2010), 但是其具体调节机制还有待考证。

7 *miR169*抑制金鱼草和矮牵牛花被中C功能基因的活性

被子植物花器官发育的ABC模型指出, A和C功能基因的活性相互拮抗, A功能基因抑制C功能

基因在第1、2轮花器官中表达, C功能基因抑制A功能基因在第3、4轮花器官中表达。拟南芥A功能基因对C功能基因的活性抑制是由*AP2*和一系列其他的调节因子如LUG (LEUNIG)、SEU (SUESS)、SAP (STERILE APETALA)和ANT (AINTEGUMENTA)来完成的(Goodrich等1997; Liu等1995; Franks等2002)。*CURLY LEAF* (*CLF*)也是*AG*基因的抑制者, 能够抑制*AG*在第1和2轮中异位表达(Goodrich等1997)。在金鱼草中, *AP2*的同源基因*LIP1* (*LIPLESS1*)和*LIP2* (*LIPLESS2*)在决定花器官特征属性方面具有一定的功能冗余, 但并不能抑制C功能基因在第1和2轮中异位表达(Keck等2003), 而是由*FIS* (*FISTULATA*)、*STY* (*STYLOSA*)和*CHO* (*CHORIPETALA*)介导了对C功能基因的表达抑制(Jack 2004)。金鱼草和矮牵牛中C功能基因的活性分别由*BL* (*BLIND*)和*FIS*编码的*miR169*所抑制。*miR169*的靶基因是NF-YA (又称为HAP2或CBF-B)转录因子家族成员, 这类转录因子可与*AG*直系同源基因(如*PLE*/*pMADS3*等)第2个内含子的CCAAT盒结合(Cartolano等2007)。在拟南芥中, *miR169*调控参与防御干旱胁迫的*NFYA5*基因, 但是不能调控抑制C功能基因活性的*NFYA*。由此推测, *miR169*主要在金鱼草和矮牵牛花被中通过抑制C功能基因活性维持花的正常发育。

8 *miR319a*调节拟南芥花器官的大小和形状

*miR319*基因家族包括3个成员: *miR319a*、*miR319b*和*miR319c*。过量表达*miR319a*基因可使植株叶片边缘呈现锯状化的*jaw-D*表型, 推定*miR319a*基因在叶片发育过程中起作用(Palatnik等2003)。但是, 启动子活性检测结果表明, *miR319a*、*miR319b*和*miR319c*基因主要在花发育晚期花序的特定部位表达(Nag等2009), 并证明*miR319a*在调控花器官的大小和形状方面也发挥关键作用。在花序中过量表达和沉默*miR319a*基因, TCP (TEOSINTE BRANCHED/CYCLOIDEA/PCF)类转录因子TCP2、TCP3、TCP4、TCP10和TCP24的mRNA水平相对于野生型花序中的mRNA水平分别表现为下降或者上升, 表明*miR319a*可能通过调控*TCP*基因而在花发育过程中起作用。这些*TCP*基因对细胞生长和促进细胞增殖起负调控作用(Crawford等2004; Efroni等2008; Luo等1999)。

*miR319a*功能缺失突变体的花瓣宽度变小、花瓣和雄蕊长度减小(Nag等2009)。时空表达模式分析表明, *miR319c*在靠近幼叶的区域表达, 主要在叶片发育过程中起作用; 而*miR319a*不在叶片中表达。尽管3个*miR319*基因均在花序中表达, 但是*miR319a*在发育早中期的花瓣和雄蕊中高丰度表达。*tcp4*等位基因的*miR319*结合位点突变能够互补*miR319a*功能缺失突变的表型(Nag等2009; Palatnik等2007)。*tcp4*突变体的花发育正常, 而*tcp4/miR319a*双突变后能对*miR319a*突变体的花发育缺陷产生强烈的抑制作用。因此, 在花发育中*TCP4*是*miR319a*的关键靶基因。

在拟南芥雄蕊发育过程中, AP2类转录因子DRNL起着至关重要的作用, 为了分离其他与DRNL一同在花器官发育过程中起作用的因子, Nag等(2009)通过增强子筛选和图位克隆的方法获得$miR319a^{129}$基因。$miR319a^{129}$基因由成熟的*miR319a*基因的第12个碱基位置G突变成A而形成的。在花瓣和雄蕊发育过程中, $miR319a^{129}$基因主要通过直接调节TCP转录因子和间接调节DRNL转录因子而影响花瓣和雄蕊的形状(图1-B), 并且与B功能MADS-box基因*AP3*存在着间接的联系(Nag等2009)。

9 *miR159*通过结合*MYB*类因子发挥其在花发育方面的宽泛作用

*miR319a*基因对于雄蕊发育的调节主要依赖于该基因对于MYB转录因子MYB33和MYB65的交叉调控, 而这种调节作用很弱, 远远低于与*miR319*家族序列极其相似的*miR159*家族基因对MYB33和MYB65的调节作用。*miR159*家族包括*miR159a*、*miR159b*和*miR159c*, 是植物王国里一类最古老的miRNA (Axtell 2008), 也是拟南芥中含量最丰富的miRNA (Kasschau等2007; Rajagopalan等2006; Nakano等2006)。*MYB33*、*MYB65*、*MYB81*、*MYB97*、*MYB101*、*MYB104*和*MYB120*是*miR159*的靶基因, 但是遗传学分析表明*miR159*主要通过调节*MYB33*、*MYB65*和*MYB101*/*DUO1* (*DUO POLLEN1*)的表达来发挥其在花发育方面(包括花药的正常发育)方面的作用(图1-B) (Millar和Gubler 2005)。

*miR159a*过表达植株的雄蕊发育不全、育性降低。*miR159a*或*miR159b*功能缺失突变体不能形成完整的花; *miR159a*/*miR159b*双突变体植株矮

化、顶端优势降低、育性降低、种子形状不规则。*miR159a*和*miR159b*不在花药中表达, 而靶基因*MYB33*和*MYB65*在花药中表达, 从而保证花药的正常发育(Allen等2007)。尽管*miR159c*对于花发育所起的调节作用是很微弱的, 但是功能互补实验证明, 足够水平的*miR159c*还是可以沉默*MYB33*和*MYB65*基因的(Allen等2010)。在转基因大麦中, 花药*GAMYB*-like基因的表达水平以及*miR159c*的调节作用是非常重要的(Murray等2003), 反映了*miR159c*可能作为谷类作物花药发育的重要调控因子。

作为分子开关, *miR159*可以完全沉默营养组织中的*MYB33*和*MYB65*。但是, 在种子中, 这种基因沉默效率有所减弱。在萌发的种子中, *miR159*和*MYB33*能够在糊粉层和胚中共转录(Alonso-Peral等2012), *miR159*表现出*MYB33*一致的时空表达模式。由此可见, miRNA介导的基因沉默效率不仅仅取决于miRNA的量和靶基因的转录水平, 可能还存在其他的调控机制。

10 展望

作为诱导植物开花和控制花器官发育的重要调控因子, miRNA为人们更加深入地研究植物开花的多样化及花器官发育提供了新的线索。已有的研究证实, *miR156*、*miR159*、*miR164*、*miR172*和*miR319*在拟南芥和玉米的花发育过程中发挥着关键作用(Wu等2009), 此外, 拟南芥中的部分miRNA具有花粉特异表达的特性(Grant-Downton等2009), 它们在植物生长发育过程中的功能及作用机制逐渐得以阐释, 但大多数miRNA的作用机理尚不明晰。

虽然已知miRNA发挥作用的机制包括降解靶mRNA、抑制靶mRNA的翻译以及对基因座位上的DNA进行甲基化修饰。但是, 还未通过分析靶基因的蛋白表达模式来确定靶基因翻译抑制的程度。而且对大多数miRNA家族的研究仅限于对其结构的简单分析(Rajagopalan等2006)。此外, 对调控*miRNA*基因表达的调控因子、*miRNA*基因的时空表达模式及内源激素、生物胁迫或非生物胁迫如何影响*miRNA*基因表达还知之甚少。

miRNA信号调控网络十分复杂, 许多miRNA可以调控相同的靶基因, 例如, *miR166*和*miR172*可以共同控制花器官特征属性MADS-box基因*AG*; *miR172*和*miR156*共同的靶基因为*SPL3/4/5*基因。目前植物miRNA的研究主要集中于模式植物, 如拟南芥、玉米、水稻等, 但是在山核桃(*Carya cathayensis*) (Wang等2012)、葡萄(*Vitis vinifera*) (Sun等2012)、猕猴桃(*Actinidia*) (Varkonyi-Gasic等2012)、夏堇(*Torenia fournieri*) (Shikata等2012)也有miRNA调控花发育的报道。miRNA参与被子植物花发育的分子机制还有待于进一步深入探讨, 如由基部被子植物到核心真双子叶植物进化过程中, miRNA的作用机制、功能演化是否存在协同进化的关系? 在不完全花(雌雄同株、雌雄异株)形成过程中, miRNA是否参与其中? 为深入了解miRNA在植物花发育中的功能与作用机制, 需要拓宽miRNA功能性研究的平台, 探明miRNA及其调控靶基因的功能趋异、活性模式建立的分子事件, 为探究花发育及花器官属性决定与miRNA的关联提供理论线索。

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