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·热点评·

“先驱”转录因子LEC1在早期胚胎重置春化状态的机制

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摘要 开花是植物由营养生长阶段向生殖生长阶段转变的重要过程, 长时间低温处理即春化对开花起到非常重要的促进作用。春化控制的拟南芥(*Arabidopsis thaliana*)开花中, 阻抑型转录因子FLC是重要的关节点, 春化记忆依赖于对该基因的控制。何跃辉研究组之前对拟南芥的研究揭示了转录因子VAL1或VAL2可以识别负调控开花的关键基因*FLC*成核区的顺式DNA元件, 协同PRC2复合体在春化过程中沉默*FLC*基因的表达, 并在随后的常温下继续维持*FLC*基因沉默直至受精结束, 使植物产生春化记忆。但在下一代中如何擦除这种记忆功能, 使*FLC*重新被激活, 以防止植物在过冬前或过冬时开花, 相关机制目前并不清楚。近期, 该研究组揭示了在植物胚胎发育早期一个种子特有的“先驱”转录因子参与擦除春化记忆, 重新激活*FLC*基因的分子机制, 并解析了胚胎中的基因激活传递到后胚胎发育(营养生长期)的表观遗传机理。该研究是开花领域的重要突破, 为作物开花调控的生产应用提供了新思路。

关键词 染色体重塑, 表观调控, 春化作用, 春化记忆

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中国科学院上海生命科学研究院植物逆境生物学研究中心何跃辉研究组近期揭示了有关植物早期胚胎染色质状态重编程的新机制, 同时阐述了胚胎中的基因激活传递到后胚胎发育时期的表观遗传机理(图1), 该研究是开花调控分子与遗传机制的重要突破(Tao et al., 2017)。开花是植物由营养生长向生殖生长转变的重要过程。此过程受到外部环境因子和植物内在发育状态的双重复杂精准调控。这种内外协调的作用模式使得植物能在合适的时刻开花, 从而最大程度地确保生殖发育的成功及繁衍。拟南芥(*Arabidopsis thaliana*)中, 开花诱导主要由4种途径调控, 即赤霉素途径、自主途径、光周期途径和春化途径(Fornara et al., 2010)。其中, 春化作用在禾本科植物和拟南芥中都得到了广泛研究。

冬性和二年生植物需要经历一段时间持续环境低温的诱导, 才能从营养生长转入生殖生长, 该过程即为春化作用。一年生植物接受春化后在当代会产生记忆, 减数分裂形成的配子受精后, 此记忆会被擦除, 进而产生新的一代, 即形成*FLC* (*FLOWERING LOCUS C*)沉默的解除状态。春化作用的分子与表观遗传控制机理在双子叶(拟南芥)和单子叶(小麦(*Triticum aestivum*))植物中是完全不同的模式。小麦春化模式代表了一类春化调控分子遗传机制。在小麦中, 春化促进开花途径包括*VRN1* (*VERNALIZATION1*)、*VRN2*、*VRN3*和*VRN-D4* (*VERNALIZATION D4*)等春化基因的调控(Yan et al., 2003, 2006; Dubcovsky et al., 2006; Kippes et al., 2015)。其中, *VRN1*编码1个类似FRUITFULL的MADS-box转录因子, 该转录因子在春化过程中起至关重要的促进作用。小麦春化调控基因*VER2* (*VERNALIZATION-RELATED 2*)具有部分取代春化的功能(Yong et al., 2003), 其编码的凝集素蛋白VER2在春化处理后发生磷酸化修饰, 并进入细胞核识别糖基化修饰的RNA结合蛋白TaGRP2 (Glycine-Rich RNA-binding Protein), TaGRP2进一步识别春化基因*TaVRN1* Pre-mRNA第1内含子的critical region中RIP3基序, 进而控制其转录与开花启动(Xing et al., 2009; Xiao et al., 2014)。TaGRP2结合的RIP3基序决定着小麦的春冬性, 在小麦育种中具有非常重要的作用(Kippes et al., 2015)。关键蛋白的磷酸化(如VER2)和糖基化(如GRP2)可能参与复杂的春化记忆过程。

拟南芥开花受到*FLC*基因的抑制, 而*FLC*对开花

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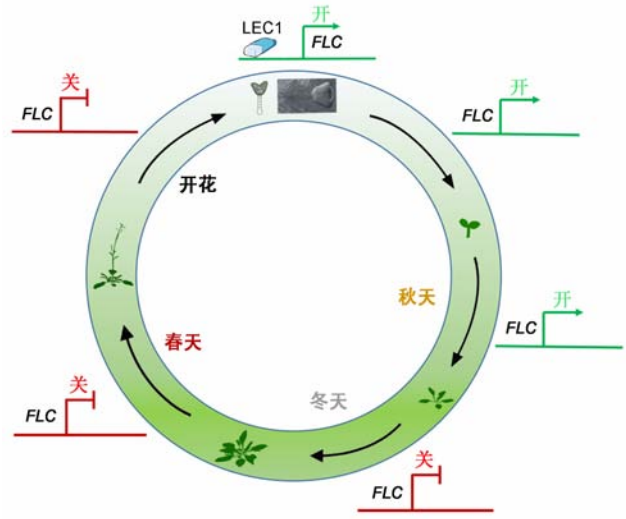

图1 “先驱”转录因子LEC1 NF-Y在拟南芥生命周期中控制*FLC*表达示意图

在胚胎发育早期，1个种子特有的“先驱”转录因子LEC1重新激活*FLC*的表达，并通过H3K36me3修饰促进染色体活性状态的建立，擦除H3K27me3修饰的沉默状态。*FLC*的激活状态通过细胞分裂传递，持续整个胚胎发育时期以及发育后期(幼苗期)，形成了苗期的“胚胎*FLC*表达记忆”。冬天低温使得*FLC*染色体状态因H3K27me3修饰转变成沉默状态，且在春季回暖后*FLC*基因沉默能够被维持，即形成春化记忆作用，该春化记忆作用直到胚胎发育早期才被LEC1擦除。

Figure 1 Schematic model for the seed-specific LEC1 NF-Y-mediated the expression of *FLC* throughout the life cycle in Arabidopsis

The “pioneer” transcription factor LEC1 NF-Y expressed in pro-embryo de novo activates *FLC* expression through the establishment of the active chromatin state marked with H3K36me3 and consequently resetting the silenced state marked with H3K27me3, which is maintained in seed development and passed on to seedling stages, leading to an embryonic memory of *FLC* activation in post-embryonic life. After a prolonged cold expose (winter), *FLC* was silenced by the increase of the repressive mark H3K27me3 at *FLC*. And this silenced state is maintained upon returning to warmth, named “vernalization memory”, which was deleted by LEC1 in proembryo.

的抑制作用随着低温处理时间的延长会被解除。在低温条件下，组蛋白修饰和非编码RNA共同调控*FLC*的基因沉默(Hepworth and Dean, 2015)。非编码*FLC*转录本*COLDAIR*可能通过募集PRC2 (Polycomb Repressive Complex 2)复合体，使*FLC*基因染色质组蛋白H3第27位的赖氨酸三甲基化(H3K27me3)修饰增加而发生基因沉默(Heo and Sung, 2011; Csorba et al., 2014)。2016年，何跃辉团队和John Innes Centre的Caroline Dean团队分别发现了1个记忆顺式元件和反式作用因子协同Polycomb复合体，它们对*FLC*周围的组蛋白进行H3K27me3表观修饰，导致*FLC*基因表达关闭，使植物在温暖的季节能够从营养生长阶段转入生殖生长阶段。在拟南芥营养生长时期，一类B3转录因子VAL1或VAL2可以识别负调控开花的关键基因*FLC*成核区的顺式DNA元件，并招募PRC2复合体催化H3K27me3，从而沉默*FLC*的表达，且在恢复常温条件后*FLC*基因的沉默能够被维持，即春化记忆作用。正是春化记忆作用的存在使得植物具备了在经历漫长冬天低温处理后，能够在来年春季开花的能力(Qüesta et al., 2016; Yuan et al., 2016)。然而在开花后及下一代种子中春化记忆如何被擦除，*FLC*基因如何被重新激活并不清楚。

近期，何跃辉研究组发现了1个种子独有的“先驱”转录因子(pioneer transcription factor) LEC1 (LEAFY COTYLEDON1)能够在受精后使得沉默状态的*FLC*基因被重新激活(Tao et al., 2017)。一年生越冬生态型拟南芥的FRI (FRIGIDA)，在低温来临之前通过促进*FLC*基因组蛋白发生活化状态修饰的酶类(如H3K36甲基转移酶EFS (SDG8))富集，使*FLC*基因上调到一个很高的水平(Kim et al., 2009; He, 2012; Crevillén et al., 2014)。低温状态下，PRC2复合体在*FLC*成核区富集，并使其周围组蛋白发生H3K27me3修饰，*FLC*基因处于沉默状态，且在恢复常温后通过有丝分裂依然维持这种沉默状态。之前有报道，H3K27去甲基化酶ELF6 (EARLY FLOWERING 6)参与受精后在下一代重置*FLC*沉默状态(Crevillén et al., 2014)，但通过对弱突变体*elf6-3*和敲除突变体*elf6-4*中*FLC*基因在春化前后及受精后的表达情况进行分析，发现春化记忆仍被擦除(Tao et al., 2017)，可见有其它因子主导*FLC*的重新激活。

他们通过*FLC::GUS*转基因苗GUS染色，发现*FLC*在胚胎发育早期(原胚)被重新激活。一个种子特有的“先驱”转录因子LEC1 (NF-Y类)及其同源基因在胚胎发育早期表达，且LEC1在胚胎发育过程中起主要的调控作用(Kwong et al., 2003)。为了探索NF-Y类转录因子在激活*FLC*方面的潜在功能，何跃辉研究

组将*FRI-Col*、*FLC::GUS*和*lec1*分别进行杂交，在其后代种子中，经RNA定量检测发现*FLC*的表达在*lec1*突变体胚胎发育早期以及胚胎整个发育时期受到抑制，且*LEC1*的同源基因*L1L* (*LEC1 LIKE*)能够部分加强*lec1*的表型。由此他们发现NF-Y类转录因子LEC1及其同源基因在胚胎发育早期能够被重新激活并促进*FLC*基因的表达。

他们用LEC1-FLAG转基因苗进行ChIP实验，发现LEC1转录因子在*FLC*启动子区富集，并识别*FLC*启动子区的CCAAT基序，该基序为FLC在胚胎发育早期被重新激活所必需。之后，他们通过DEX异源诱导表达LEC1，发现LEC1-NF-Y转录因子可直接激活*FLC*的表达，且这种激活状态能够从胚胎发育早期开始，一直持续到营养生长发育时期。该研究组又通过组蛋白表观修饰检测发现，LEC1能够使*FLC*基因染色质上促进表达的H3K36me3修饰及H3K4me3修饰增加，抑制转录的H3K27me3修饰被逐渐消除，进而擦除春化记忆作用，并最终重新激活*FLC*的表达。他们进一步实验发现，种子特异表达的转录因子LEC1通过富集H3K36甲基转移酶EFS和染色体重塑复合体亚基SWR1c，在胚胎形成时期维持*FLC*的活性染色体状态。*FLC*的激活表达持续整个胚胎发育时期，并且此激活状态在种子发芽出苗后，因组蛋白标记在细胞分裂中的传递继续维持，形成了苗期的“胚胎*FLC*激活表达记忆”，使得*FLC*不仅在种子发育时期表达，在营养生长时期也表达，进而防止植物在过冬前或过冬时发生由营养生长阶段向生殖生长阶段转变。

这一创新性研究不仅揭示了植物开花后胚胎发育早期逐渐擦除“低温记忆”，并重新设置了激活*FLC*基因表达的分子机制，而且解析了胚胎发育早期*FLC*基因激活传递到发育后期的表观遗传机理，是该领域的一项重大突破。该研究在开花调节以及表观遗传调控方面具有重要的理论指导意义，同时也为作物开花调控的生产应用提供了新的理论依据。

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Mechanism of The “Pioneer” Transcription Factor LEC1 in Resetting Vernalized State in Early Embryos

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Abstract Flowering is an important process for plants to switch from vegetative to reproductive phase. Vernalization is a process whereby plants acquire the ability to flower after exposure to a prolonged cold temperature. In Arabidopsis, inhibitor-type transcription factor *FLOWERING LOCUS C* (*FLC*) is a critical point in vernalization-mediated flowering pathway. Previous studies in *Arabidopsis thaliana* revealed that two homologous epigenome readers, VAL1 and VAL2, recognize a *cis* DNA element in the nucleation region for Polycomb group (PcG) silencing at the key floral repressor *FLC*, engaging Polycomb group proteins to induce epigenetic silencing of *FLC* by histone 3 lysine trimethylation (H3K27me3) during vernalization. This silencing is maintained in subsequent growth and development under normal temperature, namely vernalization memory. How to delete vernalization memory in the next generation to de novo activate *FLC* expression, preventing the offspring from flowering before or during winter, is not clear. Recently, Chinese scientist have found that a seed-specific transcription factor LEAFY COTYLEDON1 (LEC1) functions in deleting vernalization memory and reactivating the expression of *FLC* in the pro-embryo by resetting the chromatin states from the silenced state (marked by H3K27me3) to an active state (H3K36me3). This study provides important understanding of molecular and genetic mechanisms for flowering control by vernalization, and a novel strategy to genetically manipulate crop flowering times for the benefit of agricultural production, which is a great breakthrough of this field.

Key words chromatin reprogramming, epigenetic regulation, vernalization, vernalization memory

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