

木材形成过程中木质素生物合成的表观遗传调控

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摘要: 木质素是木材的主要成分, 也是限制木材转化效率的关键因素。其生物合成受转录因子(TF) – DNA 相互作用的转录调控。然而, 这些相互作用在木质素生物合成中的表观遗传机制仍然在很大程度上未知。解析其调控功能, 将为揭示木材形成的分子机制和创制材质材性优异的林木新品种提供线索。本研究以杨树为对象, 通过酵母单杂交、染色质免疫沉淀和凝胶迁移实验, 分析了关键的木材形成转录因子 *PtrbZIP44-A1*, 与木质素生物合成相关酶基因 *PtrCCoAOMT2* 和 *PtrCCR2* 启动子的相互作用。此外, 利用酵母双杂交、双分子荧光互补、生化分析、瞬时转化和 CRISPR 介导的杨树遗传转化, 分析了组蛋白去乙酰化酶 *PtrHDA15* 与 *PtrbZIP44-A1* 之间的蛋白相互作用, 以及 *PtrCCoAOMT2* 和 *PtrCCR2* 启动子区染色质上的组蛋白修饰水平。得出以下结论: (1) 转录因子 *PtrbZIP44-A1* 可以直接结合并抑制 *PtrCCoAOMT2* 和 *PtrCCR2* 的表达。(2) *PtrbZIP44-A1* 与组蛋白去乙酰化酶 *PtrHDA15*, 这一表观遗传抑制因子发生相互作用; *PtrHDA15* 被 *PtrbZIP44-A1* 招募后进行染色质组蛋白修饰, 从而抑制 *PtrCCoAOMT2* 和 *PtrCCR2* 的表达, 减少木质素的沉积。(3) 在过量表达 *PtrbZIP44-A1* 或 *PtrHDA15* 的转基因杨树株系中, *PtrCCoAOMT2* 和 *PtrCCR2* 启动子上的组蛋白乙酰化水平降低, 导致其表达和木质素含量下降。而在功能缺失的 *ptrbzip44-a1* 和 *ptrhda15* 突变体中, *PtrCCoAOMT2* 和 *PtrCCR2* 启动子上的组蛋白乙酰化水平升高, 其表达水平增加, 并且木质素含量也提高。本研究揭示了一种抑制木质素生物合成的表观遗传调控机制。这一发现可能有助于填补杨树木材形成过程中表观遗传调控与木质素生物合成之间的知识空白。

Epigenetic regulation of lignin biosynthesis in wood formation

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Abstract: Lignin, a major wood component, is the key limiting factor for wood conversion efficiency. Its biosynthesis is controlled by transcriptional regulatory networks involving transcription factor (TF)–DNA interactions. However, the epigenetic mechanisms underlying these interactions in lignin biosynthesis remain largely unknown. Understanding these functions in wood formation regulation can offer crucial insights into the molecular mechanisms of wood formation and facilitate the creation of new wood species with superior material properties. In this study, *Populus trichocarpa* served as the model organism. Using yeast one-hybrid, chromatin immunoprecipitation, and electrophoretic mobility shift assays, we identified that *PtrbZIP44-A1*, a key wood-forming TF, directly interacts with the promoters of *PtrCCoAOMT2* and *PtrCCR2*, genes involved in the monolignol biosynthetic pathway. We employed yeast two-hybrid, bimolecular fluorescence complementation, biochemical

analyses, transient and CRISPR-mediated transgenesis in *P. trichocarpa* to analyze how PtrHDA15 interacts with PtrbZIP44-A1 and alters chromatin histone modifications at the promoters of *PtrCCoAOMT2* and *PtrCCR2*. (1) PtrbZIP44-A1 directly binds and represses *PtrCCoAOMT2* and *PtrCCR2*. (2) PtrHDA15, a histone deacetylase, acts as an epigenetic inhibitor and is recruited by PtrbZIP44-A1 for chromatin histone modifications to repress *PtrCCoAOMT2* and *PtrCCR2*, leading to reduced lignin deposition. (3) In transgenic plants overexpressing *PtrbZIP44-A1* or *PtrHDA15*, histone acetylation at the promoters of *PtrCCoAOMT2* and *PtrCCR2* decreased, resulting in reduced gene expression and lignin content. Conversely, in loss-of-function *ptrbzip44-a1* and *ptrhda15* mutants, increased histone acetylation at *PtrCCoAOMT2* and *PtrCCR2* promoters enhanced target gene expression and lignin content. This study uncovered an epigenetic mechanism that suppresses lignin biosynthesis. This finding may help fill a knowledge gap between epigenetic regulation and lignin biosynthesis during wood formation in *Populus*.