



植物次生细胞壁生物合成的转录调控网络

张雨^{1, 2, 3}, 赵明洁^{1, 2, 3}, 张蔚^{1, 2, 3*}

¹华中农业大学园艺林学学院, 武汉 430070; ²园艺植物生物学教育部重点实验室, 武汉 430070

³农业农村部华中都市农业重点实验室, 武汉 430070

摘要 植物次生细胞壁包含纤维素、半纤维素和木质素, 赋予细胞壁机械强度及疏水性, 这种特性对植物直立生长、水分和营养物质运输以及抵御生物和非生物胁迫十分重要。该文总结了调控次生细胞壁生物合成的转录因子及其调控机制, 包括NAC转录因子调控次生壁合成的一级开关作用, AtMYB46/AtMYB83及其下游调控因子的二级开关作用, 以及其它转录因子对次生壁生物合成的调控作用, 并对未来研究内容和方法进行了展望, 以期为进一步系统理解次生细胞壁生物合成的转录调控网络提供参考。

关键词 NAC转录因子, MYB转录因子, 次生细胞壁, 生物合成, 调控网络

张雨, 赵明洁, 张蔚 (2020). 植物次生细胞壁生物合成的转录调控网络. 植物学报 55, 351–368.

细胞壁是位于植物细胞膜外的一层较厚、较坚韧并且略具弹性的结构, 为植物细胞所特有, 是区别于动物细胞的主要特征之一。植物不同组织的细胞具有不同类型的细胞壁, 根据其成分及其在生长过程中是否延伸可分为2种类型: 初生细胞壁(primary cell wall, PCW)和次生细胞壁(secondary cell wall, SCW) (以下简称次生壁)。PCW是指细胞分裂后期细胞板形成后, 由原生质体分泌物质在中层的表面最初阶段所沉积的壁, 弹性较大, 普遍存在于所有植物细胞中。SCW比PCW更厚, 沉积在PCW与细胞膜之间, 主要成分包括纤维素、半纤维素和木质素。SCW只沉积于特殊类型的细胞, 如管状分子(tracheary elements, TEs)和纤维细胞的内部(Cosgrove and Jarvis, 2012)。SCW在特化细胞中具有特殊的重要性, 如具有支撑作用的细胞和参与水分输导的细胞。研究表明, 以NAC (NAM、ATAF1/2和CUC2)和MYB为核心成员的转录因子对植物次生壁的形成发挥关键的调控作用。此外, 这2类转录因子对次生壁的调控不仅存在于双子叶植物如陆地棉(*Gossypium hirsutum*)中, 也存在于单子叶植物如水稻(*Oryza sativa*)和二穗短柄草(*Brachypodium distachyon*)中; 不仅存在于草

本植物如拟南芥(*Arabidopsis thaliana*)和紫花苜蓿(*Medicago sativa*)中, 也存在于木本植物如毛果杨(*Populus trichocarpa*)、白桦(*Betula platyphylla*)和桉树(*Eucalyptus robusta*)中, 表明NAC和MYB转录因子在调控次生壁生物合成方面具有功能保守性。本文综述了调控植物次生壁生物合成的一级开关和二级开关, 以及其它对次生壁生物合成起调控作用的转录因子的研究进展, 旨在进一步厘清次生壁合成过程中的转录因子在调控网络中的层级关系, 并深化对调控网络的整体认识。

1 调控植物次生壁生物合成的一级开关

NAC转录因子是植物一类特有的转录因子, 其家族成员众多。NAC一词源于最早发表的3个具有NAC结构域转录因子的首字母缩写, 分别是矮牵牛(*Petunia hybrida*)中的NAM (NO APICAL MERISTEM), 以及拟南芥中的ATAF1/2 (*Arabidopsis thaliana* ACTIVATION FACTOR 1/2)和CUC2 (CUP-SHAPED COTYLEDON 2) (Souer et al., 1996; Aida et al., 1997)。NAC转录因子的N端为高度保守的功能结构域, 与核定位、DNA结合以及蛋白互作二聚体的形成有关; 而

收稿日期: 2019-07-15; 接受日期: 2020-03-23

基金项目: 国家自然科学基金(No.31772342)

* 通讯作者。E-mail: zhangw@mail.hzau.edu.cn

C端为转录激活域(Hao et al., 2010), 其序列组成和长度具有高度变异性, 能够激活或抑制靶基因的转录活性(Ernst et al., 2004)。

自第1个NAC转录因子从矮牵牛中克隆后, 相继在模式植物(拟南芥、水稻和毛果杨等)、农作物(玉米(*Zea mays*)、小麦(*Triticum aestivum*)和大豆(*Glycine max*))以及园艺作物(葡萄(*Vitis vinifera*)、番茄(*Lycopersicon esculentum*)和草莓(*Fragaria × ananassa*)等)中发现多个NAC转录因子。研究表明, NAC转录因子在植物生长发育(Olsen et al., 2005)、胁迫应答(Christianson et al., 2010; Tran et al., 2010; Nakashima et al., 2012; Puranik et al., 2012; Shao et al., 2015)以及激素信号转导(Yang et al., 2011)等过程中均发挥重要调控作用。

1.1 拟南芥AtVNS家族

VNS (VND、NST/SND和SMB (SOMBRERO))基因家族在次生壁形成中发挥关键调控作用, 为次生壁合成调控网络的转录因子开关。自首次从百日草(*Zinnia elegans*)中发现与植物次生壁形成相关的NAC转录因子以来(Demura et al., 2002), 已获得一系列与次生壁合成相关的NAC转录因子, 将其依次命名为VND1–7 (VASCULAR-RELATED NAC DOMAIN 1–7) (Kubo et al., 2005)。其中, VND6和VND7是调控木质部导管形成的核心开关。在拟南芥中超量表达VND6引起后生木质部加厚, 而超量表达VND7则导致原生木质部加厚(Kubo et al., 2005)。VND1–5正向调控纤维细胞次生壁的沉积(Zhou et al., 2014)。此外, VND1–3在子叶木质部导管元件分化中也起关键作用。在拟南芥vnd1/vnd2/vnd3三突变体中, 拟南芥幼苗子叶的木质部导管元件分化受到强烈抑制(Tan et al., 2018)。综上, VND蛋白是木质部导管细胞分化的关键调控因子。

NST1 (NAC SECONDARY WALL THICKENING PROMOTING FACTOR 1)、NST2和SND1 (NST3/SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN 1)为次生壁合成的关键转录因子(Zhong et al., 2006; Mitsuda et al., 2007; Zhong and Ye, 2015)。NST1和NST2调控花药开裂所必需的花药内皮层细胞的次生壁增厚(Mitsuda et al., 2005)。同时, NST2也参与茎秆纤维细胞次生壁合成

的调控(Zhong and Ye, 2015)。NST2在维管束间纤维细胞和木质部纤维细胞中高度表达, 当NST1、NST2和NST3/SND1三者同时发生突变时, 纤维细胞次生壁完全缺失, 表明NST2、NST1以及NST3/SND1协同调控纤维次生壁的合成(Zhong and Ye, 2015b)。

NST3 (又称SND1或ANAC012) (*Arabidopsis* NAC DOMAIN CONTAINING PROTEIN 012)是1个可以双向调控次生壁形成的NAC转录因子。SND1在茎维管束间纤维细胞和木质纤维细胞中特异表达, 通过显性抑制SND1导致纤维细胞次生壁增厚显著下降(Zhong et al., 2006)。研究表明, NST3/SND1是NST1的同源基因, NST3/SND1和NST1在调控拟南芥果实中瓣膜边缘次生壁的形成和促进植物次生壁增厚方面功能冗余(Zhong et al., 2007a, 2008; Mitsuda et al., 2007; Mitsuda and Ohme-Takagi, 2008)。SND1基因过量表达能够激活与次生壁合成相关基因的表达, 从而引起木质部导管细胞次生壁的大量沉积(Zhong et al., 2006; Ko et al., 2007; Mitsuda et al., 2007)。综上, NST3/SND1可以正向调控植物纤维细胞和木质部导管细胞次生壁的合成。然而, SND1超量表达植株中纤维细胞次生壁的增厚却被强烈抑制。具体表现为维管束间纤维细胞的细胞壁非常薄; 而木质纤维细胞几乎没有次生壁的形成(Zhong et al., 2006; Ko et al., 2007), 表明SND1基因的适度表达对纤维细胞次生壁的正常沉积至关重要(Zhong et al., 2006)。而SND1基因的超量表达会引起木质部导管细胞次生壁的大量沉积, 推测这是纤维细胞次生壁受到抑制后的一种弥补机制(Ko et al., 2007)。此外, Tan等(2018)利用体外植物激素KDB诱导系统培养nst1/nst3突变体的子叶, 结果发现, 相比野生型, nst1/nst3双突变体中木质部导管分化程度更高, 表明NST1和NST3对木质部导管分化发挥负向调控作用。总之, SND1/NST3/ANAC012可以负向调控维管束间纤维和木质纤维细胞的次生壁增厚。由此推测, NSTs的异位表达因细胞类型的不同而引起次生壁增厚的模式不同, 同一基因对于不同组织部位所起到的作用有所差别。综上所述, NST1、NST2和NST3为调控植物次生壁合成中不可或缺的转录因子。

此外, NAC转录因子家族IIB分支蛋白SMB (SOMBRERO)、BRN1 (BEARSKIN1)和BRN2在促进细胞分化、根冠成熟以及产生功能性根冠所需的细

胞壁分离等方面功能冗余。对其进行超量表达时,也能诱导植物次生细胞壁的异位沉积(Willemsen et al., 2008; Bennett et al., 2012),表明其与VND和NST转录因子的功能相似。在毛果杨基因组中含有16个VNS基因,也称WNDs(WOOD ASSOCIATED NAC DOMAIN TRANSCRIPTION FACTORS)基因(Zhong et al., 2010a)。其中,8个VNS基因(VNS01–08)属于AtVND亚族,4个VNS基因(VNS09–12)属于AtNST亚族,4个VNS基因(VNS13–16)属于AtSMB亚族(Ohtani et al., 2011)。上调表达VNS10和VNS11引起纤维素、半纤维素和木质素的异位沉积,使木质部和韧皮部组织中纤维细胞次生壁增厚(Zhong et al., 2011; Zhao and Bartley, 2014)。杂交杨(*P. tremula* × *P. tremuloides*) *vns09/vns10/vns11/vns12*四突变体中木质部木纤维、木质部射线薄壁细胞和韧皮部纤维中的SCW出现缺失,只有靠近导管细胞的一些木质纤维出现了次生壁的沉积(Takata et al., 2019),说明杨树中VNS基因协同调控次生壁的形成。上述结果表明,VNS蛋白诱导次生细胞壁合成的作用是保守的,并且它们在进化上可能来源于同一祖先(Nakano et al., 2015)。

1.2 NAC转录因子的调控机制

1.2.1 转录及转录后水平的调控

研究发现,VNS转录因子通过与其下游基因启动子上特殊位点结合的方式来调控下游基因的表达,进而调控细胞次生壁合成(Zhong et al., 2010a, 2010b; Ohashi-Ito et al., 2010; McCarthy et al., 2011; Endo et al., 2015)。VND6/7和NST1–3等转录因子的靶基因启动子序列均含有SNBE (secondary wall NAC-binding element)基序,为19 bp的核心序列(T/A)NN(C/T)(T/C/G)TNNNNNNNA(A/C)GN(A/C/T)(A/T) (Zhong et al., 2010b)。所有AtVNDs基因都能直接结合到AtVND7启动子上的SNBE基序激活其表达(Zhou et al., 2014; Endo et al., 2015)。VND6也能够结合其下游基因启动子上的TERE (tracheary element-regulating *cis*-element)序列,调控木质部管状分子的分化(Ohashi-Ito et al., 2010)。TERE (CTTGAAAGCAA)序列对TE的特异分化具有重要作用(Pyo et al., 2007; Ohashi-Ito et al., 2010)。

其它物种中也有类似结合元件。棉花中1个NAC

转录因子GhFSN1 (fiber secondary cell wall-related NAC1)通过激活其下游与棉花纤维次生壁合成相关基因的表达,正向调控棉花纤维次生壁发育(Zhang et al., 2018b)。酵母单杂交实验显示,GhFSN1能够与其自身及其下游基因GhKNL1、GhMYBL1、GhGUT1、GhDUF231L和GhIRX12的启动子结合。凝胶迁移率实验表明,GhFSN1能直接结合到其下游基因启动子内的SNBE基序上。进一步通过定点突变的方法,鉴定出SNBE基序包含1个13 bp的核心序列,即(C/T)(C/G/T)TN(A/T)(G/T)(A/C/G)(A/G)(A/T/G)(A/T/G)AAG (Zhang et al., 2018b)。因此,VNS转录因子通过与其下游基因启动子上SNBE/TERE基序结合来调控次生壁的形成。

转录后调控对调节VNS转录因子的活性也发挥重要作用。毛果杨VNS基因*PtWND1B/PtVNS11/PtSND1-A2*含有选择性剪接变异体,且在不同组织中的表达丰度不同(Li et al., 2012b; Zhao and Bartley, 2014)。这类小变异体的蛋白产物缺乏C端结构域,但能够结合到PtVNS的全长蛋白上。研究发现,截短的*PtWND1B/PtVNS11/PtSND1-A2*通过PtVNS蛋白抑制其自身的转录激活活性(Li et al., 2012b),从而抑制毛果杨中纤维细胞的次生壁加厚(Zhao and Bartley, 2014)。这种选择性剪接完全依赖于*PtWND1B/PtVNS11/PtSND1-A2*的内含子序列,能够特异性调控毛果杨纤维细胞次生壁的形成。对VNS基因的深入研究将有助于更好地理解可变剪接对VNS活性的调控。

1.2.2 蛋白质相互作用与翻译后修饰

研究发现,NAC转录因子可与自身或其它蛋白形成同源或异源二聚体(Olsen et al., 2005; Weiner et al., 2012)。例如,VND和NST蛋白可以相互结合形成异源二聚体,也可以形成同源二聚体(Yamaguchi et al., 2008; Li et al., 2012)。NAC转录因子通过形成二聚体来调节其转录活性。VND7与其互作蛋白VNI2结合形成二聚体后,VND7的转录激活活性受到抑制,最终导致木质部导管细胞的分化受到抑制。VNI1及其同源基因ANAC103不仅能够与VND7相互作用,也能和VND1/2/3互作(Yamaguchi et al., 2015)。但与VNI2的负向调控作用不同,VNI1和ANAC103具有转录激活活性,通过调节多种NAC转录因子的转录活性促

进各种类型细胞的分化(Yamaguchi et al., 2015)。研究表明,当多个VND和NST基因同时表达时,应考虑到二聚体之间可能产生的效应(Nakano et al., 2015)。目前普遍的认识是,二聚体的形成能够极大地提高NAC转录因子对次生壁调控网络中下游基因的调控效率,从而更好地满足植物的需求。

棉花NAC转录因子GhFSN1不仅能够与其自身形成同源二聚体,也可与同家族的其它成员(GhFSN2)以及泛素结合酶E2形成异源二聚体,由此推测GhFSN1可能存在蛋白酶体介导的泛素化调控途径(Zhang et al., 2018b)。从mRNA和蛋白质水平对不同处理条件下GhFSN1的表达量进行检测,结果表明,在棉花纤维发育过程中,GhFSN1转录因子的活性存在由蛋白酶体介导的潜在调控机制,其降解可能通过蛋白酶体介导的泛素化途径实现(Zhang et al., 2018b)。

1.2.3 植物激素及环境条件的调控路径

植物激素尤其是生长素,对维管组织的分化具有重要作用(Fukuda, 2004)。LBD15/ASL11 (Lateral Organ Boundaries Domain 15/Asymmetric Leaves2-like-11)、LBD18/ASL20和LBD30/ASL19已被证明参与木质部管状分子的形成(Soyano et al., 2008; Ohashi-Ito et al., 2010; Zhong et al., 2010b; Yamaguchi et al., 2011)。LBD15/ASL11为AtVND7和AtSND1的直接靶基因(Zhong et al., 2010b), LBD30/ASL19和LBD18/ASL20是AtVND6和AtVND7的直接靶基因(Soyano et al., 2008; Yamaguchi et al., 2011),能被AtVND6和AtVND7诱导上调表达,从而诱导不同类型的细胞产生次生壁的异位沉积。此外,超量表达LBD18/ASL20也能够引起AtVND7的异位表达(Soyano et al., 2008)。因此,ASL基因与AtVND6和AtVND7形成一条反馈通路,协同调控管状分子的分化。此外,LBD18/ASL20也能够被生长素上调(Soyano et al., 2008)。由此暗示在VND和LBD/ASL之间可能存在由生长素介导的反馈调控(Nakano et al., 2015)。

此外,赤霉素对次生壁中的主要组成部分纤维素的合成发挥重要作用。纤维素的合成受到一系列CESAs基因调控(Taylor et al., 1999, 2000, 2003; Doblin et al., 2002; Williamson et al., 2002)。Huang

等(2015)发现了水稻中由GA-SLR1 (SLENDER RICE 1)介导的连接一级开关NAC转录因子和二级开关MYB转录因子的复合调控网络,即NAC29/31-MYB61-CESA调控通路。赤霉素能够激活CESAs基因的表达,进而促进纤维素的合成,GA和GA信号抑制子slr1介导的信号通路是纤维素合成所必需的。NAC29/31也是CESAs基因的调控元件,NAC29/31通过与MYB61启动子的SNBE基序结合促进MYB61的表达,进而激活CESAs基因的转录。而在GA和NAC-MYB转录因子之间,SLR1起到了桥梁作用。SLR1能够结合NAC29/31的DNA结合域,从而阻碍三者(NAC-MYB-CESA)的级联调控路径,通过抑制纤维素的合成抑制次生壁的形成(Huang et al., 2015)。

综上,在次生壁合成的转录调控网络中,一级开关转录因子NAC作为网络中枢,连接下游转录因子和植物激素等内部和外部因素,协同调控次生壁合成。

2 调控植物次生壁生物合成的二级开关

MYB转录因子广泛存在于高等植物中,其主要的结构特征为N端具有高度保守的DNA结合结构域(MYB结构域)。根据MYB蛋白含有的MYB结构域数量可将其分为4类:1R-MYB/MYB-related、R2R3-MYB、3R-MYB和4R-MYB(4个R1/R2的重复)。其中,R2R3-MYB转录因子的数目最多,其功能和调控机理的研究也更为深入。已有研究表明,MYB转录因子在植物次生壁生物合成途径中扮演着重要角色(Liu et al., 2015a)。

2.1 拟南芥AtMYB46和AtMYB83

拟南芥AtMYB46和AtMYB83是2个功能冗余的R2R3-MYB转录因子。AtMYB46和AtMYB83位于次生壁生物合成调控网络中的第2级,是SND1的直接靶基因,也是调控拟南芥次生壁形成的节点基因(Zhong et al., 2007a; Ko et al., 2009, 2012; McCarthy et al., 2009)。不仅SND1,SND1的同源基因NST1/2和VND6/7也能够直接调控AtMYB46和AtMYB83的表达(Zhong et al., 2007a, 2010c; McCarthy et al., 2009; Ohashi-Ito et al., 2010; Yamaguchi et al., 2011)。

AtMYB46和AtMYB83在花序茎的纤维细胞和导管细胞中特异表达。将其在拟南芥中超量表达,转基

因植株中纤维素、木质素和木聚糖的生物合成途径被激活, 导致非厚壁细胞中次生壁的异位沉积; 而 *AtMYB46* 或 *AtMYB83* 的显性抑制植株表现为纤维细胞和导管细胞的次生壁增厚显著减弱 (Zhong et al., 2007a; McCarthy et al., 2009)。此外, 在 *myb46/myb83* 双突变体中, 拟南芥导管细胞中的次生壁沉积受到严重影响, 导致突变株幼苗生长停滞 (McCarthy et al., 2009)。

一级开关基因 *VNS* 在维管束植物中十分保守 (Nakano et al., 2015)。与此类似, 调控网络的第2级开关 *MYB46/MYB83* 在维管植物中也非常保守。例如, *PtrMYB3* 和 *PtrMYB20* 是拟南芥 *AtMYB46/AtMYB83* 的同源基因, 参与毛果杨次生壁的合成与调控。当其在拟南芥中超量表达时, 能够同时激活纤维素、木聚糖和木质素的生物合成途径, 也能够激活与次生壁合成相关基因启动子的表达 (McCarthy et al., 2010)。此外, 水稻 *OsMYB46* 和玉米 *ZmMYB46* 是拟南芥 *AtMYB46/AtMYB83* 的直系同源基因。在拟南芥中超量表达 *OsMYB46* 或 *ZmMYB46* 能够激活整个次生壁的合成途径 (Zhong et al., 2011)。*OsMYB46* 和 *ZmMYB46* 作为 *OsSWNs* 与 *ZmSWNs* 下游的直接靶基因, 其启动子上也含有 *SNBE* 位点 (Zhong et al., 2011)。

2.2 拟南芥 *AtMYB46* 和 *AtMYB83* 调控的下游基因

AtMYB46 与 *AtMYB83* 作为二级调控开关, 具有承上启下的关键作用, 其上游既受到 *NAC* 转录因子一级开关的调控, 也能调控一系列位于其下游与次生壁合成相关基因的表达。由于 *AtMYB46* 和 *AtMYB83* 功能冗余, 二者调控的下游转录因子基因也有所重叠 (McCarthy et al., 2009; Ko et al., 2009)。

2.2.1 拟南芥 *AtMYB46* 和 *AtMYB83* 调控的转录因子基因

前期已鉴定出多个位于 *AtMYB46* 和 *AtMYB83* 下游的转录因子基因, 包括 *AtMYB4*、*AtMYB7*、*AtMYB32*、*AtMYB42*、*AtMYB43*、*AtMYB52*、*AtMYB54*、*AtMYB85*、*AtMYB58*、*AtMYB63* 和 *AtMYB103* 等一系列 *MYB* 基因, 以及其它类型的转录因子, 如 *TZF* (Tandem CCCH Zinc Finger) 锌指蛋白基因和 *KNA-T7* (*KNOTTED Arabidopsis THALIANA 7*) 基因 (Z-

hong et al., 2007b, 2008; Ko et al., 2009, 2014; Nakano et al., 2010)。其中, *AtMYB4*、*AtMYB7* 和 *AtMYB32* 在二级结构上具有相似的抑制元件, 被认为在次生壁生物合成中起负调控作用 (Jin et al., 2000; Preston et al., 2004; Ko et al., 2009; Wang and Dixon, 2012)。*AtMYB4* 特异性地抑制木质素单体合成相关基因 *C4H* 的表达 (Jin et al., 2000), 而 *AtMYB7* 和 *AtMYB32* 能够负调控木质素合成相关基因的表达 (Preston et al., 2004)。*AtMYB4* 在毛果杨中的同源基因 *PdMYB4*, 在次生壁合成过程中也发挥负调控作用, 表明其功能保守 (Tang et al., 2015)。此外, 超量表达 *PdMYB4* 还能够抑制与纤维素和木聚糖合成相关基因的表达 (Tang et al., 2015), 这在一定程度上反映出不同物种中基因功能的进化与分化。此外, 拟南芥 *AtMYB58*、*AtMYB63* 和 *AtMYB85* 及其在其它物种中的直系同源基因较特异地调控木质素的生物合成 (Bomal et al., 2008; Zhong et al., 2008; Cassan-Wang et al., 2013), 三者调控次生壁形成过程中的木质素合成方面可能存在功能冗余 (Zhou et al., 2009)。

AtMYB42、*AtMYB43*、*AtMYB52* 和 *AtMYB54* 均在木质部组织中优势表达 (Zhong et al., 2008), 然而至今人们对这些基因在次生壁形成过程中的功能还存在争议。通过使用嵌合抑制子沉默技术 (chimeric repressor gene silencing technology, CRES-T) 显著抑制 *AtMYB52* 或 *AtMYB54*, 花序茎维管束间纤维细胞和木质纤维细胞的次生壁增厚受到强烈抑制, 说明 *AtMYB52* 和 *AtMYB54* 参与植物次生壁增厚; 然而, *AtMYB52* 和 *AtMYB54* 的超量表达却对次生壁合成无显著影响 (Zhong et al., 2008)。这表明个别基因的高表达不足以引起次生壁的异位沉积, 但其正常表达对于次生壁的形成具有重要作用 (Zhong et al., 2008)。另有关于 *AtMYB52* 的研究却认为, *AtMYB52* 对次生壁的合成起负调控作用, 原因是拟南芥突变体 *myb52* 中出现了木质素的异位沉积; 而且与次生壁合成相关基因的表达均大幅提升 (Cassan-Wang et al., 2013)。Cassan-Wang 等 (2013) 给出了比较合理的解释: *AtMYB52* 编码转录抑制因子, 因此当其与 *EAR* 基序形成嵌合蛋白时, *AtMYB52* 转变为高效的负调控因子, 表现出更强烈的转录抑制活性, 从而抑制纤维细胞次生壁的增厚。此外, 在 *AtMYB46/AtMYB83* 的调控下,

*AtMYB43*的表达水平上调,但*AtMYB43*基因具体的生物学功能还有待进一步探究(Nakano et al., 2010)。

*AtMYB103*主要在维管束间纤维细胞和木质部组织中表达,超量表达*AtMYB103*可显著增加转基因株系中木质部纤维细胞和维管束间纤维细胞次生壁的厚度。而且*AtMYB103*可以在体外激活纤维素合酶*CESA8*基因的启动子,因此其最初被认为是特异性调控纤维素生物合成的调控因子(Zhong et al., 2008)。研究证实*AtMYB103*是*AtMYB46/83*的靶基因(Nakano et al., 2010; Yamaguchi and Demura, 2010; Yamaguchi et al., 2011),同时也是受*SND1*、*NST1/2*以及*VND6/7*直接调控的靶基因(Zhong et al., 2008)。Öhman等(2013)发现*myb103*突变体中1个编码细胞色素P450亚酶的基因*F5H* (*FERULATE-5-HYDROXYLASE*)的表达量显著下降,导致紫丁香基木质素(syringyl lignin, S-木质素)含量大幅降低。这说明*AtMYB103*是*F5H*表达以及合成S-型木质素所必需的,该转录因子不仅对次生壁的生长结构有影响,也能调控木质素单体组分的合成过程。

此外,*AtMYB46*和*AtMYB83*还能调控*KNOX*家族的*KNAT7*基因和具有C3H锌指结构域的*AtC3H14*基因。*KNAT7*既是*AtMYB46*和*AtMYB83*的靶基因(Zhong et al., 2007b, 2008; McCarthy et al., 2009; Ko et al., 2014),也是受*SND1*、*NST1*、*VND6*和*VND7*直接调控的靶基因(Zhong et al., 2008)。最早研究发现,*KNAT7*作为转录抑制因子发挥作用(Brown et al., 2005; Li et al., 2011, 2012),且其抑制活性可通过与*OFP4* (*OVATE FAMILY PROTEIN 4*)和*OFP1*蛋白的互作而增强(Li et al., 2011)。超量表达*KNAT7*引起维管束间纤维细胞壁厚度下降(Li et al., 2012)。*knat7*功能缺失突变体木质部不规则,且表现出严重的木质部塌陷(Brown et al., 2005; Li et al., 2012)。然而,其纤维细胞的次生壁厚度却有所增加,并且伴有木质素含量的增加以及纤维素、木质素和木聚糖生物合成基因表达量的上升(Li et al., 2012)。银腺杂种杨(*P. alba* × *P. glandulosa*)的*KNAT2/6b*通过调控一级开关*NAC*转录因子抑制木质部导管的细胞分化及次生壁沉积,从而抑制次生壁合成(Zhao et al., 2020)。此外,*KNAT7*还能正向调控木聚糖的生物合成(He et al., 2018)。*KNAT7*能够激活木聚糖生物合成基因的启动子,包括*IRX9* (*IRREGULAR XY-*

LEM 9)、*IRX10*、*IRX14L* (*IRREGULAR XYLEM 14-LIKE*)和*FRA8* (*FRAGILE FIBER 8*)。综上,*KNAT7*既能作为转录抑制子也能作为转录激活因子调控次生壁的合成,这取决于不同组织和细胞中的转录因子组分(He et al., 2018)。

*KNAT7*能与多种转录因子发生互作,形成复合体参与次生壁的合成。有研究证明,*KNAT7*能与*AtMYB75*在体外发生相互作用(Bhargava et al., 2010),也能在体内互作形成功能复合体,调控拟南芥花序茎的维管组织和种皮,从而调控次生壁的形成(Bhargava et al., 2013)。研究表明,*BLH6* (*BELL1-LIKE HOMEODOMAIN*)蛋白能够与*KNAT7*特异结合,负向调控次生壁的形成(Liu et al., 2015b)。*BLH6*是转录抑制子,*BLH6-KNAT7*复合体能够增强*KNAT7*和*BLH6*的抑制活性。超量表达*KNAT7*和*BLH6*引起维管束纤维次生壁厚度下降(Liu et al., 2015b)。进一步研究表明,*OFP1*和*OFP4*能够增强*BLH6*的抑制活性。因此,*OFP*可能作为*BLH6-KNAT7*复合物的组成部分形成多蛋白转录调控复合体,特异性地调控某个细胞类型或发育阶段的次生壁合成(Liu and Douglas, 2015)。由于*KNAT7*存在多个互作蛋白,研究者认为*KNAT7*可以根据不同的细胞类型,通过与不同的蛋白发生相互作用,进而调控次生壁的合成(Li et al., 2012; Liu and Douglas, 2015)。此外,*KNAT7*还受到*AtMYB61*的调控。*AtMYB61*调控木质部分化,通过某个特定的基序AC元件结合到*KNAT7*的启动子上,共同调控拟南芥子叶维管系统、木质部和种皮形成(Romano et al., 2012)。最近在毛果杨中发现的1个*MYB6*转录因子也能够与*KNAT7*相互作用,通过负调控拟南芥和毛白杨(*P. tomentosa*)中木质素生物合成的代谢通路抑制次生壁的合成(Wang et al., 2019)。上述研究表明,*KNAT7*能够通过调控纤维细胞和木质部导管细胞次生壁增厚调控次生壁的形成。*KNAT7*可能通过靶向不同的基因调控不同细胞类型次生细胞壁沉积的不同方面。由于*KNAT7*存在多个互作蛋白,*KNAT7*可能通过与不同的互作蛋白形成功能复合体,在不同类型的细胞中发挥不一样的功能,最终共同形成反馈调控环,协同调控次生壁的形成。

植物特异性串联CCCH锌指蛋白基因处于MYB转录因子的下游,参与次生壁合成。拟南芥*AtC3H14*是*AtSND1*以及*AtMYB46*的直接靶基因(Ko et al.,

2009), 能够激活与纤维素、半纤维素和木质素合成相关基因的表达(Ko et al., 2009; Kim et al., 2014b)。AtC3H14既能直接结合到纤维素与木质素合成相关基因的启动子上, 也能结合到聚半乳糖醛酸酶ADPG1的RNA上。因此, AtC3H14可能参与次生壁生物合成基因的转录和转录后调控(Kim et al., 2014b)。Chai等(2014)在白杨(*P. deltoides*)中也鉴定出2个C3H锌指蛋白基因(*PdC3H17/18*), 能够激活与纤维素、木聚糖和木质素合成相关基因的表达。其上游转录因子PdMYB3/21通过与*PdC3H17/18*的启动子结合, 调控其表达水平。水稻中1个非典型的C3H锌指蛋白IIP4能够与次生壁合成网络中的一级调控因子及二级调控因子发生相互作用, 进而抑制次生壁的合成(Zhang et al., 2018a)。由此表明, C3H锌指蛋白在次生壁合成中起桥梁作用, 深入探究其作用机制将有助于进一步完善次生壁生物合成的调控网络。

2.2.2 拟南芥AtMYB46和AtMYB83的调控机制

AtMYB46和AtMYB83不仅能调控转录因子基因, 也能调控一系列与次生壁合成相关的基因。且二者的靶基因启动子上都具有7个核苷酸(ACC[A/T]A[A/C][T/C])的特异结合元件, 即SMRE (secondary wall MYB-responsive element)基序。作为调控植物次生壁生物合成的二级开关, AtMYB46和AtMYB83均通过与下游基因的SMRE基序相结合激活下游基因的表达, 实现对次生壁生物合成的转录调控(Zhong and Ye, 2012)。例如, AtMYB46的下游转录因子基因, 包括*AtMYB43*、*AtMYB58*、*AtMYB63*和*KNAT7*均含有AtMYB46结合的SMRE基序(Kim et al., 2012; Zhong and Ye, 2012)。此外, *AtMYB46*和*AtMYB83*的毛果杨同源基因*PtrMYB2/3/20/21*、桉树*EgMYB2*以及松树(*Pinus taeda*) *PtMYB4*均能结合到其下游靶基因的SMRE基序, 进而激活下游基因的表达(Zhong et al., 2013)。由此表明, 在草本植物拟南芥和木本植物中, MYB46及其同源基因均通过结合SMRE基序来激活其下游基因的表达, 暗示MYB46及其同源基因在草本和木本植物中功能保守。

Kim等(2012)通过分析*AtC3H14*基因的启动子区域, 鉴定出1个AtMYB46特异识别的顺式作用元件M46RE (MYB46-responsive *cis*-regulatory element), 该元件为含有8个核苷酸([A/G][G/T][A/T]GGT[A/

G])的核心基序, 是AtMYB46实现转录调控的必要和充分条件。例如, 3种纤维素合酶CESA4、CESA7和CESA8的启动子上均含有M46RE基序, 若该基序发生突变, AtMYB46便无法有效地与这3个基因的启动子结合, 说明AtMYB46与其启动子的结合依赖于完整的M46RE基序(Kim et al., 2013a, 2013b)。另一个纤维素类合酶A9 (CELLULOSE SYNTHASE-LIKE A9, CSLA9)为参与拟南芥花序轴内初生细胞壁和次生细胞壁甘露聚糖的主要合酶(Liepmann et al., 2005; Goubet et al., 2009)。AtMYB46通过与M46RE基序相互作用, 结合到CSLA9基因的启动子上, 当超量表达*AtMYB46*时, 甘露聚糖的含量显著增加(Kim et al., 2014c)。此外, MYB46也能直接激活木聚糖和木质素生物合成基因的表达, 超量表达MYB46能增加木聚糖和木质素的含量(Kim et al., 2014a)。上述研究表明, *AtMYB46/83*通过与其下游基因启动子上特定的SMRE/M46RE序列结合, 调控次生壁的形成。

3 其它转录因子对次生壁生物合成的调控作用

除了前文所述的一级开关NAC转录因子、二级开关AtMYB46/AtMYB83及其下游调控因子外, 还有VNS蛋白调控的下游转录因子AtSND2和AtSND3等, 以独立于AtMYB46/AtMYB83的方式调控次生壁生物合成的MYB转录因子(包括AtMYB20、AtMYB26、AtMYB69、AtMYB75和AtMYB99等), WRKY和bHLH (basic helix loop-helix)类转录因子以及HD-ZIP III转录因子和miR165/166。

3.1 正向调控因子AtSND2和AtSND3

AtSND2和AtSND3为SND1的下游转录因子(Zhong et al., 2006, 2007a)。AtSND3除了是SND1的直接靶基因外, 也是SND1的同源基因NST1、NST2、VND6和VND7的直接靶基因(Zhong et al., 2008)。AtSND2参与几乎所有与次生壁形成相关的调控进程。例如, 纤维素、木聚糖和甘露聚糖的生物合成, 木质素聚合和细胞壁修饰(Hussey et al., 2011)。在桉树中过量表达*AtSND2*也能增加桉树纤维细胞次生壁的厚度, 表明*AtSND2*的功能在草本和木本植物中比较保守(Hussey et al., 2011)。但是, 将*AtSND2*的毛果杨同

源基因*PopNAC154*在毛果杨中超量表达,转基因植株的木质部次生壁厚度并未发生明显变化(Grant et al., 2010)。最近,在水稻中分离了1个*AtSND2*的同源基因*OsSND2*,该基因能正向调控水稻的次生壁形成。*OsSND2*还能与*OsMYB61*等多个调控次生壁生物合成的MYB基因启动子直接结合。简而言之,*OsSND2*是一个调控次生壁生物合成的开关因子(Ye et al., 2018)。上述研究表明,*AtSND2*及其同源基因在不同物种中作用的重要程度有所不同,这可能取决于不同物种中其它调控因子的作用效果。

3.2 负向调控因子*AtXND1*和*VNI2*

与植物次生壁合成相关的NAC转录因子SWNs (secondary wall-related NAC transcription factors) (Zhong et al., 2010a; Zhong and Ye, 2014)中,既有正向调控因子(如VNS蛋白),也有负向调控因子,如*XND1* (XYLEM NAC DOMAIN 1)和*VNI2* (VND-INTERACTING 2)。*XND1*也称为*ANAC104*,最初从拟南芥中鉴定出来(Ooka et al., 2003),由于其在木质部中特异表达,又将其命名为*XND1* (Zhao et al., 2005)。*XND1*起初被认为与拟南芥叶片衰老有关(Guo et al., 2004),但之后的研究发现*XND1*作为负调控因子,通过特异调控木质部导管的分化进而调控植物细胞次生壁的形成(Zhao et al., 2008, 2017; Tang et al., 2018)。研究者在其它物种中鉴定出*AtXND1*的同源基因,均以负向调控的方式参与细胞次生壁的合成,说明其功能在不同类型的物种中保守性较高(Grant et al., 2010; Li et al., 2014)。

*XND1*受*VND7*直接调控(Zhong et al., 2010b);反之,在导管分子分化期间,*XND1*也能抑制*VND7*的表达(Zhao et al., 2017)。研究发现,*XND1*拥有高度保守的C端,其内部存在4个能与细胞周期和分化调控因子RBR (RETINOBLASTOMA-RELATED)发生相互作用的基序,分别为CKII-acidic、LXCXE、E2F^{TD}-LIKE和LXCXE-mimic。其中,LXCXE或LXCXE-mimic基序的完整性对于*XND1*和RBR的互作十分关键,直接决定了*XND1*调控木质部管状分子分化作用的强弱。当LXCXE或LXCXE-mimic基序出现碱基突变,或者LXCXE基序缺失时,*XND1*对木质部管状分子的抑制作用则会下降甚至消失,导致相应的超量表达表型也减弱或消失。*XND1*的C端所含基序能与RBR发生

相互作用,从而特异地抑制木质部细胞的分化(Zhao et al., 2017)。

*VNI2*在根和花茎的木质部以及韧皮部细胞中都有表达。*VNI2*能与VND家族蛋白*VND7*、*VND1-5*以及其它NAC蛋白发生相互作用,但*VNI2*与*VND7*结合的亲和性高于其它NAC蛋白。组成型超量表达*VNI2*的植株,其幼苗期根中木质部导管的正常发育受到抑制。此外,在*VND7*启动子的控制下,C-端截短的*VNI2*能抑制根部和地上部分木质部导管的正常发育。*VNI2*与*VND7*结合后,抑制了*VND7*的转录激活活性,从而抑制*VND7*下游靶基因的表达,最终抑制木质部的合成(Yamaguchi et al., 2010c)。上述结果表明,*VNI2*作为1个转录抑制子调控木质部细胞特异化,是VND的负向调控因子(Yamaguchi et al., 2010c)。

3.3 调控次生壁形成的其它MYB转录因子

AtMYB20、*AtMYB26*、*AtMYB69*、*AtMYB75*和*AtMYB99*转录因子不受二级转录开关*AtMYB46*/*AtMYB83*的调控。其中,*AtMYB20*和*AtMYB69*在木质部细胞中优势表达(Zhong et al., 2006, 2007a, 2007b, 2008)。显性抑制分析表明,*AtMYB69*参与调控次生壁的形成(Zhong et al., 2008),但是具体的调控路径还不十分清楚。*AtMYB75*最初被鉴定为花青素合成的正向调控因子,因此被命名为PAP1 (PRODUCTION OF ANTHOCYANIN PIGMENT 1) (Borevitz et al., 2000)。之后发现*AtMYB75*还能负调控木质部纤维和维管束间纤维的次生壁形成,作为抑制因子调控木质素的合成(Bhargava et al., 2010)。Nakano等(2010)发现在木质部导管细胞的体外分化过程中,*AtMYB99*基因的表达上调,并且早期就能在木质部导管细胞中检测到*AtMYB99*的表达,这暗示其可能参与导管次生壁的形成。*AtMYB26*是1个调控花药内壁次生壁形成的转录因子。拟南芥*myb26*突变体中花药内壁的次生壁不能正常形成,导致花药开裂失败,最终引起雄性不育(Dawson et al., 1999; Steiner-Lange et al., 2003)。超量表达*AtMYB26*能诱导次生壁的异位沉积(Yang et al., 2007)。这2个表型分别与*nst1/nst2*双突变体及超量表达*AtNST1/AtNST2*植株的表型类似(Mitsuda et al., 2005)。需要指出的是,*AtNST1*的过表达能诱导*AtMYB26*的表达(Mitsuda et al., 2005),且*AtMYB26*的过表达也能引起*AtNST1*和

*AtNST2*表达上调(Yang et al., 2007)。上述研究表明,就花药内壁而言,NAC与MYB之间形成了一种正向的反馈调控回路,而不是转录调控的级联反应。

3.4 参与次生壁合成的WRKY和bHLH类转录因子

研究表明,WRKY和bHLH类转录因子也参与对木质素生物合成的调控。在拟南芥中,WRKY12以负向调控的方式参与茎髓组织薄壁细胞的次生壁加厚(Wang et al., 2010)。WRKY12在拟南芥的髓组织中特异表达,WRKY12的功能缺失会引起次生壁异常加厚,同时伴随着木质素、木聚糖和纤维素的异位沉积,说明WRKY12发挥抑制茎髓组织薄壁细胞次生壁加厚的作用(Wang et al., 2010)。此外,在苜蓿中发现了WRKY12的同源基因*MtSTP*。苜蓿*mtstp-1*突变体髓细胞的木质化程度显著高于野生型,随后发现*MtSTP*编码1个WRKY转录因子(Wang et al., 2010)。WRKY12基因突变导致NST2及其它与次生壁合成相关转录因子的表达量上升。凝胶迁移实验表明,WRKY12与NST2的启动子结合从而抑制其表达。由此表明,WRKY12能够负向调控次生壁的合成(Wang et al., 2010)。此外,蛋白质与DNA互作分析表明,在真核生物中十分保守的转录因子E2Fc负向调控植物体内的核内复制,可能是次生壁合成的1个关键转录因子(Taylor-Teeple et al., 2015)。在不同情况下,E2Fc能对*AtVND6*和*AtVND7*起到激活或抑制作用,而且E2Fc能够结合到除*AtVND*外其它在木质部中特异表达的转录因子基因的启动子上(Taylor-Teeple et al., 2015)。

bHLH转录因子能分别与NAC和MYB转录因子发生相互作用,调控次生壁合成。Yan等(2013)利用高粱(*Sorghum bicolor*) *bmr* (*brown midrib*)突变体与野生型构建了1个差减文库,从中分离到1个bHLH类转录因子SbbHLH1。进一步将其在拟南芥中进行超量表达,SbbHLH1与MYB转录因子竞争性地结合到与木质素合成相关基因的启动子上,进而抑制木质素的合成。或者,SbbHLH1与MYB转录因子形成复合体,抑制其活性从而抑制木质素合成相关基因的表达,进而抑制木质素的合成。当木质素含量过低时,SbbHLH1又作为信号激活MYB转录因子的表达,说明其对木质素的合成起负调控作用(Yan et al., 2013)。拟南芥中另外2个bHLH转录因子MYC2/4与一

级调控转录因子NST1调控次生壁的形成(Zhang et al., 2018d)。在蓝光信号条件下,蓝光受体CRY1 (CRYPTOCHROME1)能够激活其下游基因MYC2/4的表达,使其结合到NST1基因的启动子上,从而激活一系列与次生壁合成相关转录因子的表达,促进次生壁细胞的增厚(Zhang et al., 2018d)。综上,bHLH转录因子与NAC-MYB转录因子形成了层级路径调控次生壁的合成。

3.5 参与次生壁调控的HD-ZIP III转录因子和miR-165/166

拟南芥中HD-ZIP III TFs (Class III homeodomain leucine zipper transcription factors)参与维管束分化和次生壁合成(Baima et al., 2001; Ohashi-Ito and Fukuda, 2003; Ohashi-Ito et al., 2005)。HD-ZIP III TFs包括5个成员,分别是REV/IFL1 (REVOLUTA/INTERFASCICULAR FIBERLESS 1)、PHB (PHABULOSA)、PHV (PHAVOLUTA)、CORONA (CAN/AtHB15)和AtHB8 (Du and Wang, 2015)。其中,REV/IFL1、PHB和PHV在维管束的分化和形成中存在功能冗余。CAN/AtHB15负向调控次生壁的形成,在*athb15tu*突变体中,2个NAC关键转录因子SND1和NST2表达上调(Du et al., 2015)。而AtHB8作为正向调控因子与生长素信号互作调控维管组织的发育,超量表达*AtHB8*可以促进木质部的分化(Baima et al., 2001)。此外,从HD-ZIP III TFs同源基因在百日草和水稻中所起的作用可以看出,在不同物种中其对维管束组织分化和形成的功能保守(Ohashi-Ito and Fukuda, 2003; Ohashi-Ito et al., 2005; Itoh et al., 2008)。

激活标记突变体(activation tagged mutants)的鉴定和分析明确了miR156/166在维管发育中的作用(Du and Wang, 2015)。miR156/166通过与HD-ZIP III TFs中的START (steroidogenic acute regulatory protein-related lipid transfer)结构域上的特定序列结合,进而调控其表达水平(Mallory et al., 2004)。MiR165b、miR166a和miR166g的激活降低了PHB、PHV和AtHB15的转录本水平。因此,当START结构域发生无义突变或是点突变时,miR156/166无法正常与其结合,HD-ZIP III TFs的转录本水平上升,进而影响维管束组织的发育进程(Kim et al., 2005; Williams et al., 2005; Du and Wang, 2015)。

4 总结与展望

近20年来,得益于遗传学和分子生物学的迅猛发展,植物次生壁生物合成的转录调控研究取得了空前巨大的进展。尤其在模式植物拟南芥中,结合突变体筛选和全基因组信息,已获得多个植物细胞壁合成相关基因,明确了NAC和MYB类转录因子在维管束组织的木质部导管、纤维细胞和花药皮层次生细胞壁加厚等过程中的核心作用,以及其它转录因子在此过程中的调控作用,并解析了这些调控因子之间的层级关系(Zhong and Ye, 2014; Nakano et al., 2015; Yang and Wang, 2016),由此植物次生细胞壁生物合成的调控网络逐渐清晰和明朗。本文综述了以拟南芥为代表的植物中细胞壁合成转录调控的研究进展,并基于此,绘制了次生壁合成的调控网络(图1A, B)。NAC转录因子作为调控次生壁合成的一级转录开关,不同的成员所起作用不同。VNDs主要调控导管元件的分化与形成,而NSTs则主要调控纤维细胞次生壁的形成。然而,这两类转录因子是否有功能上的重叠还需进一步研究。虽然在导管分子分化过程中,VNDs基因活性处于动态变化状态,而NSTs则无明显变化。然而,在分化为维管束导管的细胞中却检测出了NSTs启动子的活性(Mitsuda et al., 2005, 2007),表明NSTs可能在维管束导管次生壁的形成中具有一定作用。二级开关转录因子MYB46/83在纤维细胞和导管细胞中均发挥作用,且两者所调控的下游转录因子基因和次生壁合成相关合酶基因主要通过调控木质素和纤维素的合成调控次生壁的沉积。其它转录因子通过与一级和二级开关转录因子相互作用,形成一个错综复杂的反馈调控网络,共同调控次生壁的形成。其中,植物激素(如生长素和赤霉素)以及外界环境(如蓝光)对于次生壁的合成也起到了一定的作用,作为响应因子促进次生壁的合成。值得思考的是,同一转录因子在不同的细胞类型中可能发挥不同功能,甚至功能截然相反。由此说明次生壁合成调控网络非常复杂。

研究表明,次生壁加厚现象除了在维管束中导管和纤维细胞中存在,在树叶、种皮、花药以及果实中石细胞的皮层细胞里也有发生(Mitsuda and Ohme-Takagi, 2008)。此外,除了拟南芥,在其它物种(如水稻、棉花)甚至是非维管束植物(如小立碗藓(*Physcomitrella patens*))中也存在类似的转录调控途径(Xu et al., 2014)。在木本植物中也发现了一系列与拟南芥

调控网络中一级开关、二级开关以及其它调控因子的同源基因,其中,一级和二级转录因子功能的保守性相对较高(Zhang et al., 2018c)。由此可见,由NAC-MYB转录因子介导的次生壁合成调控网络在大多数物种中均比较保守。当然,除了转录因子功能的相似性外,不同物种不同组织的不同结构也会存在一定的差异。例如,禾本科植物中次生壁的结构和形成模式与双子叶植物拟南芥有所不同,这也暗示两者的次生壁合成调控网络存在差异(Handakumbura and Hazen, 2012; Rao and Dixon, 2018)。

由于次生细胞壁含有较多的纤维素、半纤维素及木质素,因而是植物生物量的主要来源之一。例如,水稻、玉米和小麦等农作物的秸秆就属于农业生态系统中十分宝贵的生物质能资源。然而,农作物生产首先需要满足人类的食品需求。相较之下,由于木本植物能产生大量的木质纤维素,因此木材生物量作为一种可再生的、成本效益高的生物能源和工业资源,预计将成为下一代生物燃料的原材料之一。但是,来源于木质纤维素的生物乙醇要比来源于粮食作物的昂贵许多(Mosier et al., 2005)。为了降低生物燃料转换的成本,利用转基因技术改善和提高木材的质量和数量显得尤为重要。已有研究提出并验证了人工重建次生细胞壁的可能性,这将为生产生物乙醇和其它化学品的新原料提供理论依据(Sakamoto and Mitsuda, 2014)。Sakamoto等(2016)利用拟南芥NST3/SND1基因的启动子驱动水稻中NST3/SND1的同源基因,发现其能增加杂交杨的生物量且不影响其生长发育。进一步通过组织化学法染色表明其在杂交杨次生木本组织中具有依赖性表达模式(Takata et al., 2017)。这表明AtNST3/SND1基因的启动子将成为表达特定效应基因以修饰木材次生细胞壁组分和生物量的有效工具。多年生草本柳枝稷(*Panicum virgatum*)也被认为是生物燃料的主要可再生和可持续原料作物之一。PvSWNs和PvMYB46A为拟南芥中SWNs和MYB46/83的同源基因,作为转录开关因子调控次生壁合成(Zhong et al., 2015)。另一项研究中,通过调控WRKY基因在玉米、柳枝稷和苜蓿中的表达实现了作物生物量质量和数量的显著提高(Gallego-Giraldo et al., 2016)。因此,明确次生壁合成途径中的关键调控因子,解析次生细胞壁合成途径,可为植物生物量的遗传改良及生产应用提供理论依据。

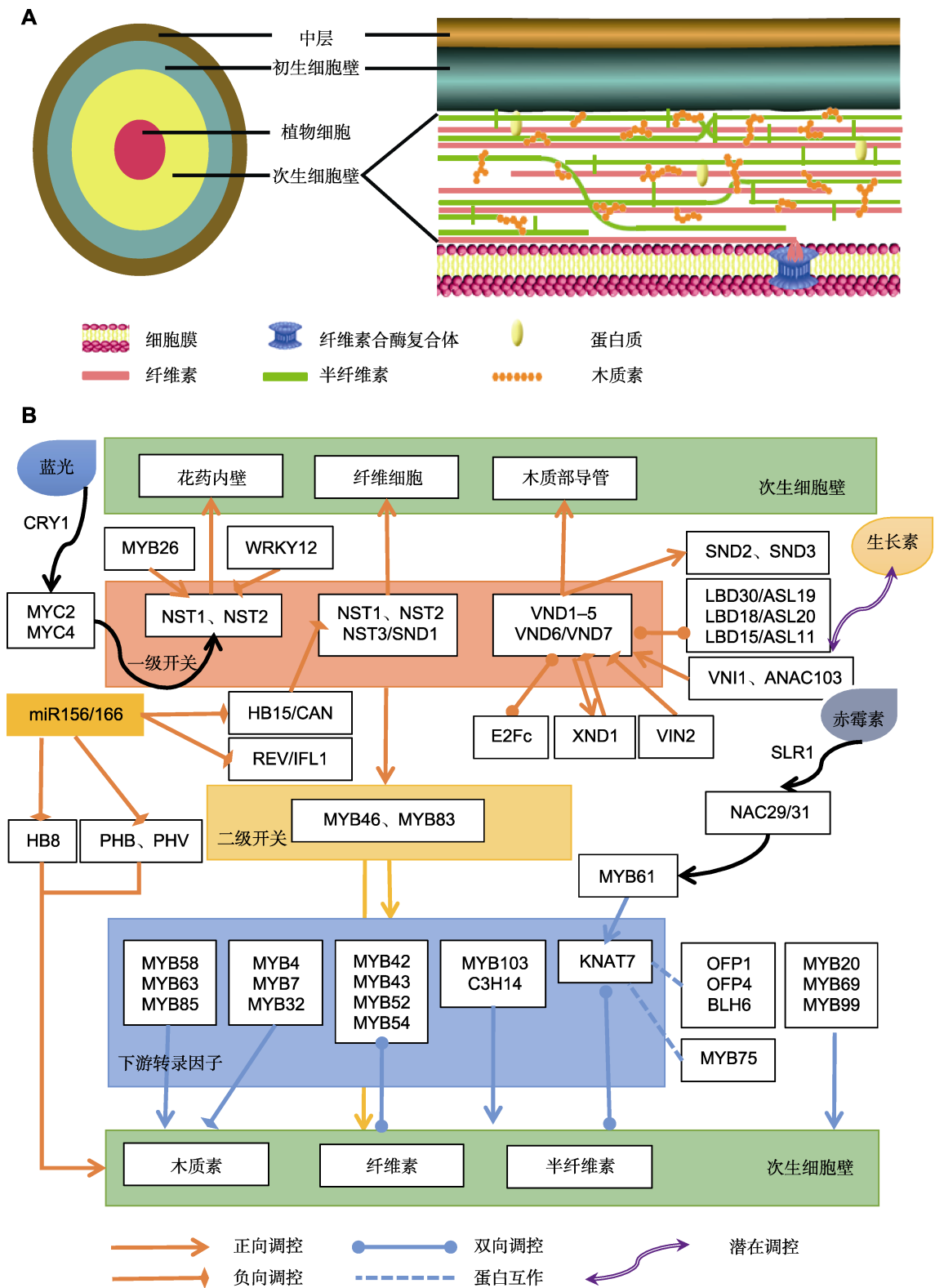


图1 拟南芥次生细胞壁结构及其生物合成转录调控网络
(A) 次生细胞壁结构示意图; (B) 次生细胞壁合成转录调控网络图

Figure 1 Structure of secondary cell wall and its biosynthetic transcriptional regulatory network in *Arabidopsis thaliana*
(A) Structural sketch of the secondary cell wall; (B) Transcriptional regulatory network of the secondary cell wall biosynthesis

此外,阐明次生壁生物合成的调控网络,对于经济树种如桉树和洋槐(*Robinia pseudoacacia*),经济作物如棉花、苧麻(*Boehmeria nivea*)和亚麻(*Linum usitatissimum*),以及观赏植物如月季(*Rosa chinensis*)、玫瑰(*R. rugosa*)和野蔷薇(*R. multiflora*)的品质性状改良具有十分重要的意义。以棉花为例,棉纤维是由胚珠外珠被表皮细胞在受精前后经分化突起、伸长和细胞壁增厚而形成。棉纤维次生壁的最大特点是组成简单,主要由纤维素构成,没有木质素沉积。而次生壁合成时期决定了棉纤维的强度。月季花色丰富,花型多变,但茎秆多皮刺,使得其在栽种管理和切花采摘、包装过程中存在诸多不便,并带来安全隐患。而采用机械法去除皮刺又会对花枝造成伤害,从而缩短瓶插寿命。有研究表明,月季皮刺的主要成分为木质素、纤维素、半纤维素及木栓质(李慧等, 2012)。通过观察木质素沉积部位,发现木质素的转移方向为刺顶部向刺基部沉积,表明皮刺的硬化与木质素的积累有关(Asano et al., 2008)。因此,将有望通过生物技术手段调控皮刺中木质素的合成,从而调控皮刺的生长与硬化过程。

不同物种中的次生壁合成调控网络存在保守的途径,也可能还存在特异性的调控途径。随着研究的不断深入和系统化,通过蛋白质相互作用分析、共表达分析,并综合运用基因组、转录组以及蛋白质组等分析方法,将更好地揭示不同物种中各层级调控因子在次生壁生物合成过程中的功能。

参考文献

- 李慧, 刘凤荣, 郝琳, 高彬, 严珊, 王玲, 马男, 赵梁军, 杨春起 (2012). 月季皮刺的组织结构与化学组成. 园艺学报 39, 1321–1329.
- Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M (1997). Genes involved in organ separation in *Arabidopsis*: an analysis of the cup-shaped cotyledon mutant. *Plant Cell* 9, 841–857.
- Asano G, Kubo R, Tanimoto S (2008). Growth, structure and lignin localization in rose prickles. *Bull Fac Agric Saga Univ* (93), 117–125.
- Baima S, Possenti M, Matteucci A, Wisman E, Altamura MM, Ruberti I, Morelli G (2001). The *Arabidopsis* ATHB-8 HD-zip protein acts as a differentiation-promoting transcription factor of the vascular meristems. *Plant Physiol* 126, 643–655.
- Bennett T, van den Toorn A, Sanchez-Perez GF, Campilho A, Willemsen V, Snel B, Scheres B (2012). SOMBRERO, BEARSKIN1, and BEARSKIN2 regulate root cap maturation in *Arabidopsis*. *Plant Cell* 22, 640–654.
- Bhargava A, Ahad A, Wang SC, Mansfield SD, Haughn GW, Douglas CJ, Ellis BE (2013). The interacting MYB75 and KNAT7 transcription factors modulate secondary cell wall deposition both in stems and seed coat in *Arabidopsis*. *Planta* 237, 1199–1211.
- Bhargava A, Mansfield SD, Hall HC, Douglas CJ, Ellis BE (2010). MYB75 functions in regulation of secondary cell wall formation in the *Arabidopsis* inflorescence stem. *Plant Physiol* 154, 1428–1438.
- Bomal C, Bedon F, Caron S, Mansfield SD, Levasseur C, Cooke JEK, Blais S, Tremblay L, Morency M, Pavy N, Grima-Pettenati J, Séguin A, MacKay J (2008). Involvement of *Pinus taeda* MYB1 and MYB8 in phenylpropanoid metabolism and secondary cell wall biogenesis: a comparative *in planta* analysis. *J Exp Bot* 59, 3925–3939.
- Borevitz JO, Xia YJ, Blount J, Dixon RA, Lamb C (2000). Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. *Plant Cell* 12, 2383–2393.
- Brown DM, Zeef LAH, Ellis J, Goodacre R, Turner SR (2005). Identification of novel genes in *Arabidopsis* involved in secondary cell wall formation using expression profiling and reverse genetics. *Plant Cell* 17, 2281–2295.
- Cassan-Wang H, Goué N, Saidi MN, Legay S, Sivadon P, Goffner D, Grima-Pettenati J (2013). Identification of novel transcription factors regulating secondary cell wall formation in *Arabidopsis*. *Front Plant Sci* 4, 189.
- Chai GH, Qi G, Cao YP, Wang ZG, Yu L, Tang XF, Yu YC, Wang D, Kong YZ, Zhou GK (2014). Poplar PdC3H17 and PdC3H18 are direct targets of PdMYB3 and PdMYB21, and positively regulate secondary wall formation in *Arabidopsis* and poplar. *New Phytol* 203, 520–534.
- Christianson JA, Dennis ES, Llewellyn DJ, Wilson IW (2010). ATAF NAC transcription factors: regulators of plant stress signaling. *Plant Signal Behav* 5, 428–432.
- Cosgrove DJ, Jarvis MC (2012). Comparative structure and biomechanics of plant primary and secondary cell walls. *Front Plant Sci* 3, 204.
- Dawson J, Sözen E, Vizir I, Waeyenberge SV, Wilson ZA, Mulligan BJ (1999). Characterization and genetic mapping of a mutation (*ms35*) which prevents anther dehiscence in *Arabidopsis thaliana* by affecting secondary wall thickening in the endothecium. *New Phytol* 144, 213–222.
- Demura T, Tashiro G, Horiguchi G, Kishimoto N, Kubo

- M, Matsuoka N, Minami A, Nagata-Hiwatashi M, Nakamura K, Okamura Y, Sassa N, Suzuki S, Yazaki J, Kikuchi S, Fukuda H (2002). Visualization by comprehensive microarray analysis of gene expression programs during transdifferentiation of mesophyll cells into xylem cells. *Proc Natl Acad Sci USA* **99**, 15794–15799.
- Doblin MS, Kurek I, Jacob-Wilk D, Delmer DP (2002). Cellulose biosynthesis in plants: from genes to rosettes. *Plant Cell Physiol* **43**, 1407–1420.
- Du Q, Avci U, Li SB, Gallego-Giraldo L, Pattathil S, Qi LY, Hahn MG, Wang HZ (2015). Activation of *miR165b* represses *AtHB15* expression and induces pith secondary wall development in *Arabidopsis*. *Plant J* **83**, 388–400.
- Du Q, Wang HZ (2015). The role of HD-ZIP III transcription factors and *miR165/166* in vascular development and secondary cell wall formation. *Plant Signal Behav* **10**, e10-78955.
- Endo H, Yamaguchi M, Tamura T, Nakano Y, Nishikubo N, Yoneda A, Kato K, Kubo M, Kajita S, Katayama Y, Ohtani M, Demura T (2015). Multiple classes of transcription factors regulate the expression of VASCULAR-RELATED NAC-DOMAIN 7, a master switch of xylem vessel differentiation. *Plant Cell Physiol* **56**, 242–254.
- Ernst HA, Olsen AN, Skriver K, Larsen S, Lo Leggio L (2004). Structure of the conserved domain of ANAC, a member of the NAC family of transcription factors. *EMBO Rep* **5**, 297–303.
- Fukuda H (2004). Signals that control plant vascular cell differentiation. *Nat Rev Mol Cell Biol* **5**, 379–391.
- Gallego-Giraldo L, Shadle G, Shen H, Barros-Rios J, Corrales SF, Wang HZ, Dixon RA (2016). Combining enhanced biomass density with reduced lignin level for improved forage quality. *Plant Biotechnol J* **14**, 895–904.
- Goubet F, Barton CJ, Mortimer JC, Yu XL, Zhang ZN, Miles GP, Richens J, Liepman AH, Seffen K, Dupree P (2009). Cell wall glucomannan in *Arabidopsis* is synthesised by CSLA glycosyltransferases, and influences the progression of embryogenesis. *Plant J* **60**, 527–538.
- Grant EH, Fujino T, Beers EP, Brunner AM (2010). Characterization of NAC domain transcription factors implicated in control of vascular cell differentiation in *Arabidopsis* and *Populus*. *Planta* **232**, 337–352.
- Guo Y, Cai Z, Gan S (2004). Transcriptome of *Arabidopsis* leaf senescence. *Plant Cell Environ* **27**, 521–549.
- Handakumbura PP, Hazen SP (2012). Transcriptional regulation of grass secondary cell wall biosynthesis: playing catch-up with *Arabidopsis thaliana*. *Front Plant Sci* **3**, 74.
- Hao YJ, Song QX, Chen HW, Zou HF, Wei W, Kang XS, Ma B, Zhang WK, Zhang JS, Chen SY (2010). Plant NAC-type transcription factor proteins contain a NARD domain for repression of transcriptional activation. *Planta* **232**, 1033–1043.
- He JB, Zhao XH, Du PZ, Zeng W, Beahan CT, Wang YQ, Li HL, Bacic A, Wu AM (2018). KNAT7 positively regulates xylan biosynthesis by directly activating *IRX9* expression in *Arabidopsis*. *J Integr Plant Biol* **60**, 514–528.
- Huang DB, Wang SG, Zhang BC, Shangguan KK, Shi YY, Zhang DM, Liu XL, Wu K, Xu ZP, Fu XD, Zhou YH (2015). A gibberellin-mediated DELLA-NAC signaling cascade regulates cellulose synthesis in rice. *Plant Cell* **27**, 1681–1696.
- Hussey SG, Mizrahi E, Spokevicius AV, Bossinger G, Berger DK, Myburg AA (2011). *SND2*, a NAC transcription factor gene, regulates genes involved in secondary cell wall development in *Arabidopsis* fibres and increases fibre cell area in *Eucalyptus*. *BMC Plant Biol* **11**, 173.
- Itoh JI, Hibara KI, Sato Y, Nagato Y (2008). Developmental role and auxin responsiveness of class III homeodomain leucine zipper gene family members in rice. *Plant Physiol* **147**, 1960–1975.
- Jin HL, Cominelli E, Bailey P, Parr A, Mehrkens F, Jones J, Tonelli C, Weisshaar B, Martin C (2000). Transcriptional repression by AtMYB4 controls production of UV-protecting sunscreens in *Arabidopsis*. *EMBO J* **19**, 6150–6161.
- Kim J, Jung JH, Reyes JL, Kim YS, Kim SY, Chung KS, Kim JA, Lee M, Lee Y, Kim VN, Chua NH, Park CM (2005). MicroRNA-directed cleavage of *ATHB15* mRNA regulates vascular development in *Arabidopsis* inflorescence stems. *Plant J* **42**, 84–94.
- Kim WC, Kim JY, Ko JH, Kang H, Han KH (2014a). Identification of direct targets of transcription factor MYB46 provides insights into the transcriptional regulation of secondary wall biosynthesis. *Plant Mol Biol* **85**, 589–599.
- Kim WC, Kim JY, Ko JH, Kang H, Kim J, Han KH (2014b). AtC3H14, a plant-specific tandem CCCH zinc-finger protein, binds to its target mRNAs in a sequence-specific manner and affects cell elongation in *Arabidopsis thaliana*. *Plant J* **80**, 772–784.
- Kim WC, Kim JY, Ko JH, Kim J, Han KH (2013a). Transcription factor MYB46 is an obligate component of the transcriptional regulatory complex for functional expression of secondary wall-associated cellulose synthases in *Arabidopsis thaliana*. *J Plant Physiol* **170**, 1374–1378.
- Kim WC, Ko JH, Han KH (2012). Identification of a cis-acting regulatory motif recognized by MYB46, a master

- transcriptional regulator of secondary wall biosynthesis. *Plant Mol Biol* **78**, 489–501.
- Kim WC, Ko JH, Kim JY, Kim J, Bae HJ, Han KH** (2013b). MYB46 directly regulates the gene expression of secondary wall-associated cellulose synthases in *Arabidopsis*. *Plant J* **73**, 26–36.
- Kim WC, Reca IB, Kim Y, Park S, Thomashow MF, Keegstra K, Han KH** (2014c). Transcription factors that directly regulate the expression of CSLA9 encoding mannan synthase in *Arabidopsis thaliana*. *Plant Mol Biol* **84**, 577–587.
- Ko JH, Jeon HW, Kim WC, Kim JY, Han KH** (2014). The MYB46/MYB83-mediated transcriptional regulatory programme is a gatekeeper of secondary wall biosynthesis. *Ann Bot* **114**, 1099–1107.
- Ko JH, Kim WC, Han KH** (2009). Ectopic expression of MYB46 identifies transcriptional regulatory genes involved in secondary wall biosynthesis in *Arabidopsis*. *Plant J* **60**, 649–665.
- Ko JH, Kim WC, Kim JY, Ahn SJ, Han KH** (2012). MYB46-mediated transcriptional regulation of secondary wall biosynthesis. *Mol Plant* **5**, 961–963.
- Ko JH, Yang SH, Park AH, Lerouxel O, Han KH** (2007). ANAC012, a member of the plant-specific NAC transcription factor family, negatively regulates xylary fiber development in *Arabidopsis thaliana*. *Plant J* **50**, 1035–1048.
- Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, Ito J, Mimura T, Fukuda H, Demura T** (2005). Transcription switches for protoxylem and metaxylem vessel formation. *Genes Dev* **19**, 1855–1860.
- Li EY, Bhargava A, Qiang WY, Friedmann MC, Forneris N, Savidge RA, Johnson LA, Mansfield SD, Ellis BE, Douglas CJ** (2012). The Class II KNOX gene *KNAT7* negatively regulates secondary wall formation in *Arabidopsis* and is functionally conserved in *Populus*. *New Phytol* **194**, 102–115.
- Li EY, Wang SC, Liu YY, Chen JG, Douglas CJ** (2011). OVATE FAMILY PROTEIN 4 (OFP4) interaction with KNA-T7 regulates secondary cell wall formation in *Arabidopsis thaliana*. *Plant J* **67**, 328–341.
- Li W, Huang GQ, Zhou W, Xia XC, Li DD, Li XB** (2014). A cotton (*Gossypium hirsutum*) gene encoding a NAC transcription factor is involved in negative regulation of plant xylem development. *Plant Physiol Biochem* **83**, 134–141.
- Liepmann AH, Wilkerson CG, Keegstra K** (2005). Expression of *cellulose synthase-like (Csl)* genes in insect cells reveals that *CslA* family members encode mannan synthases. *Proc Natl Acad Sci USA* **102**, 2221–2226.
- Liu JY, Osbourn A, Ma PD** (2015a). MYB transcription factors as regulators of phenylpropanoid metabolism in plants. *Mol Plant* **8**, 689–708.
- Liu YY, Douglas CJ** (2015). A role for OVATE FAMILY PROTEIN 1 (OFP1) and OFP4 in a BLH6-KNAT7 multi-protein complex regulating secondary cell wall formation in *Arabidopsis thaliana*. *Plant Signal Behav* **10**, e1033126.
- Liu YY, You SJ, Taylor-Teeple M, Li WL, Schuetz M, Brady SM, Douglas CJ** (2015b). BEL1-LIKE HOMEODOMAIN6 and KNOTTED ARABIDOPSIS THALIANA7 interact and regulate secondary cell wall formation via repression of *REVOLUTA*. *Plant Cell* **26**, 4843–4861.
- Mallory AC, Reinhart BJ, Jones-Rhoades MW, Tang GL, Zamore PD, Barton MK, Bartel DP** (2004). MicroRNA control of *PHABULOSA* in leaf development: importance of pairing to the microRNA 5' region. *EMBO J* **23**, 3356–3364.
- McCarthy RL, Zhong RQ, Fowler S, Lyskowski D, Piyasena H, Carleton K, Spicer C, Ye ZH** (2010). The poplar MYB transcription factors, PtrMYB3 and PtrMYB20, are involved in the regulation of secondary wall biosynthesis. *Plant Cell Physiol* **51**, 1084–1090.
- McCarthy RL, Zhong RQ, Ye ZH** (2009). MYB83 is a direct target of SND1 and acts redundantly with MYB46 in the regulation of secondary cell wall biosynthesis in *Arabidopsis*. *Plant Cell Physiol* **50**, 1950–1964.
- McCarthy RL, Zhong RQ, Ye ZH** (2011). Secondary wall NAC binding element (SNBE), a key *cis*-acting element required for target gene activation by secondary wall NAC master switches. *Plant Signal Behav* **6**, 1282–1285.
- Mitsuda N, Iwase A, Yamamoto H, Yoshida M, Seki M, Shinozaki K, Ohme-Takagi M** (2007). NAC transcription factors, NST1 and NST3, are key regulators of the formation of secondary walls in woody tissues of *Arabidopsis*. *Plant Cell* **19**, 270–280.
- Mitsuda N, Ohme-Takagi M** (2008). NAC transcription factors NST1 and NST3 regulate pod shattering in a partially redundant manner by promoting secondary wall formation after the establishment of tissue identity. *Plant J* **56**, 768–778.
- Mitsuda N, Seki M, Shinozaki K, Ohme-Takagi M** (2005). The NAC transcription factors NST1 and NST2 of *Arabidopsis* regulate secondary wall thickenings and are required for anther dehiscence. *Plant Cell* **17**, 2993–3006.
- Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapfel M, Ladisch M** (2005). Features of promising technologies for pretreatment of lignocellulosic biomass.

- Bioresour Technol* **96**, 673–686.
- Nakano Y, Nishikubo N, Goué N, Ohtani M, Yamaguchi M, Katayama Y, Demura T** (2010). MYB transcription factors orchestrating the developmental program of xylem vessels in *Arabidopsis* roots. *Plant Biotechnol* **27**, 267–272.
- Nakano Y, Yamaguchi M, Endo H, Rejab NA, Ohtani M** (2015). NAC-MYB-based transcriptional regulation of secondary cell wall biosynthesis in land plants. *Front Plant Sci* **6**, 288.
- Nakashima K, Takasaki H, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K** (2012). NAC transcription factors in plant abiotic stress responses. *Biochim Biophys Acta (BBA)-Gene Regul Mech* **1819**, 97–103.
- Ohashi-Ito K, Fukuda H** (2003). HD-ZIP III homeobox genes that include a novel member, *ZeHB-13 (Zinnia)/ATHB-15 (Arabidopsis)*, are involved in procambium and xylem cell differentiation. *Plant Cell Physiol* **44**, 1350–1358.
- Ohashi-Ito K, Kubo M, Demura T, Fukuda H** (2005). Class III homeodomain leucine-zipper proteins regulate xylem cell differentiation. *Plant Cell Physiol* **46**, 1646–1656.
- Ohashi-Ito K, Oda Y, Fukuda H** (2010). *Arabidopsis* VASCULAR-RELATED NAC-DOMAIN6 directly regulates the genes that govern programmed cell death and secondary wall formation during xylem differentiation. *Plant Cell* **22**, 3461–3473.
- Öhman D, Demedts B, Kumar M, Gerber L, Gorzsás A, Goeminne G, Hedenström M, Ellis B, Boerjan W, Sundberg B** (2013). MYB103 is required for *FERULATE-5-HYDROXYLASE* expression and syringyl lignin biosynthesis in *Arabidopsis* stems. *Plant J* **73**, 63–76.
- Ohtani M, Nishikubo N, Xu B, Yamaguchi M, Mitsuda N, Goué N, Shi FS, Ohme-Takagi M, Demura T** (2011). A NAC domain protein family contributing to the regulation of wood formation in poplar. *Plant J* **67**, 499–512.
- Olsen AN, Ernst HA, Leggio LL, Skriver K** (2005). NAC transcription factors: structurally distinct, functionally diverse. *Trends Plant Sci* **10**, 79–87.
- Ooka H, Satoh K, Doi K, Nagata T, Otomo Y, Murakami K, Matsubara K, Osato N, Kawai J, Carninci P, Hayashizaki Y, Suzuki K, Kojima K, Takahara Y, Yamamoto K, Kikuchi S** (2003). Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. *DNA Res* **10**, 239–247.
- Preston J, Wheeler J, Heazlewood J, Li SF, Parish RW** (2004). AtMYB32 is required for normal pollen development in *Arabidopsis thaliana*. *Plant J* **40**, 979–995.
- Puranik S, Sahu PP, Srivastava PS, Prasad M** (2012). NAC proteins: regulation and role in stress tolerance. *Trends Plant Sci* **17**, 369–381.
- Pyo H, Demura T, Fukuda H** (2007). TERE; a novel *cis*-element responsible for a coordinated expression of genes related to programmed cell death and secondary wall formation during differentiation of tracheary elements. *Plant J* **51**, 955–965.
- Rao X, Dixon RA** (2018). Current models for transcriptional regulation of secondary cell wall biosynthesis in grasses. *Front Plant Sci* **9**, 399.
- Romano JM, Dubos C, Prouse MB, Wilkins O, Hong H, Poole M, Kang KY, Li EY, Douglas CJ, Western TL, Mansfield SD, Campbell MM** (2012). AtMYB61, an R2R3-MYB transcription factor, functions as a pleiotropic regulator via a small gene network. *New Phytol* **195**, 774–786.
- Sakamoto S, Mitsuda N** (2014). Reconstitution of a secondary cell wall in a secondary cell wall-deficient *Arabidopsis* mutant. *Plant Cell Physiol* **56**, 299–310.
- Sakamoto S, Takata N, Oshima Y, Yoshida K, Taniguchi T, Mitsuda N** (2016). Wood reinforcement of poplar by rice NAC transcription factor. *Sci Rep* **6**, 19925.
- Shao HB, Wang HY, Tang XL** (2015). NAC transcription factors in plant multiple abiotic stress responses: progress and prospects. *Front Plant Sci* **6**, 902.
- Souer E, Van Houwelingen A, Kloos D, Mol J, Koes R** (1996). The no apical meristem gene of petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. *Cell* **85**, 159–170.
- Soyano T, Thitamadee S, Machida Y, Chua NH** (2008). *ASYMMETRIC LEAVES2-LIKE19/LATERAL ORGAN BOUNDARIES DOMAIN30* and *ASL20/LBD18* regulate tracheary element differentiation in *Arabidopsis*. *Plant Cell* **20**, 3359–3373.
- Steiner-Lange S, Unte US, Eckstein L, Yang CY, Wilson ZA, Schmelzer E, Dekker K, Saedler H** (2003). Disruption of *Arabidopsis thaliana* MYB26 results in male sterility due to non-dehiscent anthers. *Plant J* **34**, 519–528.
- Takata N, Awano T, Nakata MT, Sano Y, Sakamoto S, Mitsuda N, Taniguchi T** (2019). *Populus* NST/SND orthologs are key regulators of secondary cell wall formation in wood fibers, phloem fibers and xylem ray parenchyma cells. *Tree Physiol* **39**, 514–525.
- Takata N, Sakamoto S, Mitsuda N, Taniguchi T** (2017). The *Arabidopsis* NST3/SND1 promoter is active in se-

- condary woody tissue in poplar. *J Wood Sci* **63**, 396–400.
- Tan TT, Endo H, Sano R, Kurata T, Yamaguchi M, Ohtani M, Demura T** (2018). Transcription factors VND1–VND3 contribute to cotyledon xylem vessel formation. *Plant Physiol* **176**, 773–789.
- Tang N, Shahzad Z, Lonjon F, Loudet O, Vailleau F, Maurel C** (2018). Natural variation at *XND1* impacts root hydraulics and trade-off for stress responses in *Arabidopsis*. *Nat Commun* **9**, 3884.
- Tang XF, Zhuang YM, Qi G, Wang D, Liu HH, Wang KR, Chai GH, Zhou GK** (2015). Poplar PdMYB221 is involved in the direct and indirect regulation of secondary wall biosynthesis during wood formation. *Sci Rep* **5**, 12240.
- Taylor NG, Howells RM, Huttly AK, Vickers K, Turner SR** (2003). Interactions among three distinct CesA proteins essential for cellulose synthesis. *Proc Natl Acad Sci USA* **100**, 1450–1455.
- Taylor NG, Laurie S, Turner SR** (2000). Multiple cellulose synthase catalytic subunits are required for cellulose synthesis in *Arabidopsis*. *Plant Cell* **12**, 2529–2539.
- Taylor NG, Scheible WR, Cutler S, Somerville CR, Turner SR** (1999). The *irregular xylem3* locus of *Arabidopsis* encodes a cellulose synthase required for secondary cell wall synthesis. *Plant Cell* **11**, 769–779.
- Taylor-Teeple M, Lin L, de Lucas M, Turco G, Toal TW, Gaudinier A, Young NF, Trabucco GM, Veling MT, Lamothe R, Handakumbura PP, Xiong G, Wang C, Corwin J, Tsoukalas A, Zhang L, Ware D, Pauly M, Kliebenstein DJ, Dehesh K, Tagkopoulos I, Breton G, Pruneda-Paz JL, Ahnert SE, Kay SA, Hazen SP, Brady SM** (2015). An *Arabidopsis* gene regulatory network for secondary cell wall synthesis. *Nature* **517**, 571–575.
- Tran LS, Nishiyama R, Yamaguchi-Shinozaki K, Shinozaki K** (2010). Potential utilization of NAC transcription factors to enhance abiotic stress tolerance in plants by biotechnological approach. *GM Crops* **1**, 32–39.
- Wang HZ, Avci U, Nakashima J, Hahn MG, Chen F, Dixon RA** (2010). Mutation of WRKY transcription factors initiates pith secondary wall formation and increases stem biomass in dicotyledonous plants. *Proc Natl Acad Sci USA* **107**, 22338–22343.
- Wang HZ, Dixon RA** (2012). On-off switches for secondary cell wall biosynthesis. *Mol Plant* **5**, 297–303.
- Wang LJ, Lu WX, Ran LY, Dou LW, Yao S, Hu J, Fan D, Li CF, Luo KM** (2019). R2R3-MYB transcription factor MYB6 promotes anthocyanin and proanthocyanidin biosynthesis but inhibits secondary cell wall formation in *Populus tomentosa*. *Plant J* **99**, 733–751.
- Welner DH, Lindemose S, Grossmann JG, Møllegaard NE, Olsen AN, Helgstrand C, Skriver K, Leggio LL** (2012). DNA binding by the plant-specific NAC transcription factors in crystal and solution: a firm link to WRKY and GCM transcription factors. *Biochem J* **444**, 395–404.
- Willemsen V, Bauch M, Bennett T, Campilho A, Wolkenfelt H, Xu J, Haseloff J, Scheres B** (2008). The NAC domain transcription factors FEZ and SOMBRERO control the orientation of cell division plane in *Arabidopsis* root stem cells. *Dev Cell* **15**, 913–922.
- Williams L, Grigg SP, Xie MT, Christensen S, Fletcher JC** (2005). Regulation of *Arabidopsis* shoot apical meristem and lateral organ formation by microRNA miR166g and its AtHD-ZIP target genes. *Development* **132**, 3657–3668.
- Williamson RE, Burn JE, Hocart CH** (2002). Towards the mechanism of cellulose synthesis. *Trends Plant Sci* **7**, 461–467.
- Xu B, Ohtani M, Yamaguchi M, Toyooka K, Wakazaki M, Sato M, Kubo M, Nakano Y, Sano R, Hiwatashi Y, Murata T, Kurata T, Yoneda A, Kato K, Hasebe M, Demura T** (2014). Contribution of NAC transcription factors to plant adaptation to land. *Science* **343**, 1505–1508.
- Yamaguchi M, Demura T** (2010). Transcriptional regulation of secondary wall formation controlled by NAC domain proteins. *Plant Biotechnol* **27**, 237–242.
- Yamaguchi M, Kubo M, Fukuda H, Demura T** (2008). VASCULAR-RELATED NAC-DOMAIN 7 is involved in the differentiation of all types of xylem vessels in *Arabidopsis* roots and shoots. *Plant J* **55**, 652–664.
- Yamaguchi M, Mitsuda N, Ohtani M, Ohme-Takagi M, Kato K, Demura T** (2011). VASCULAR-RELATED NAC-DOMAIN 7 directly regulates the expression of a broad range of genes for xylem vessel formation. *Plant J* **66**, 579–590.
- Yamaguchi M, Nagahage ISP, Ohtani M, Ishikawa T, Uchimiya H, Kawai-Yamada M, Demura T** (2015). *Arabidopsis* NAC domain proteins VND-INTERACTING1 and ANAC103 interact with multiple NAC domain proteins. *Plant Biotechnol* **32**, 119–123.
- Yamaguchi M, Ohtani M, Mitsuda N, Kubo M, Ohme-Takagi M, Fukuda H, Demura T** (2010c). VND-INTERACTING2, a NAC domain transcription factor, negatively regulates xylem vessel formation in *Arabidopsis*. *Plant Cell* **22**, 1249–1263.
- Yan L, Xu CH, Kang YL, Gu TW, Wang DX, Zhao SY, Xia GM** (2013). The heterologous expression in *Arabidopsis*

- thaliana* of sorghum transcription factor SbbHLH1 down-regulates lignin synthesis. *J Exp Bot* **64**, 3021–3032.
- Yang CY, Xu ZY, Song J, Conner K, Barrena GV, Wilson ZA** (2007). *Arabidopsis* MYB26/MALESTERILE35 regulates secondary thickening in the endothecium and is essential for anther dehiscence. *Plant Cell* **19**, 534–548.
- Yang JH, Wang HZ** (2016). Molecular mechanisms for vascular development and secondary cell wall formation. *Front Plant Sci* **7**, 356.
- Yang SD, Seo PJ, Yoon HK, Park CM** (2011). The *Arabidopsis* NAC transcription factor VNI2 integrates abscisic acid signals into leaf senescence via the *COR/RD* genes. *Plant Cell* **23**, 2155–2168.
- Ye YF, Wu K, Chen JF, Liu Q, Wu YJ, Liu BM, Fu XD** (2018). OsSND2, a NAC family transcription factor, is involved in secondary cell wall biosynthesis through regulating MYBs expression in rice. *Rice* **11**, 36.
- Zhang DM, Xu ZP, Cao SX, Chen KL, Li SC, Liu XL, Gao CX, Zhang BC, Zhou YH** (2018a). An uncanonical CCCH-tandem zinc-finger protein represses secondary wall synthesis and controls mechanical strength in rice. *Mol Plant* **11**, 163–174.
- Zhang J, Huang GQ, Zou D, Yan JQ, Li Y, Hu S, Li XB** (2018b). The cotton (*Gossypium hirsutum*) NAC transcription factor (FSN1) as a positive regulator participates in controlling secondary cell wall biosynthesis and modification of fibers. *New Phytol* **217**, 625–640.
- Zhang J, Xie M, Tuskan GA, Muchero W, Chen JG** (2018c). Recent advances in the transcriptional regulation of secondary cell wall biosynthesis in the woody plants. *Front Plant Sci* **9**, 1535.
- Zhang Q, Xie Z, Zhang R, Xu P, Liu HT, Yang HQ, Doblin MS, Bacic A, Li LG** (2018d). Blue light regulates secondary cell wall thickening via MYC2/MYC4 activation of the *NST1*-directed transcriptional network in *Arabidopsis*. *Plant Cell* **30**, 2512–2528.
- Zhao CS, Avci U, Grant EH, Haigler CH, Beers EP** (2008). XND1, a member of the NAC domain family in *Arabidopsis thaliana*, negatively regulates lignocellulose synthesis and programmed cell death in xylem. *Plant J* **53**, 425–436.
- Zhao CS, Craig JC, Petzold HE, Dickerman AW, Beers EP** (2005). The xylem and phloem transcriptomes from secondary tissues of the *Arabidopsis* root-hypocotyl. *Plant Physiol* **138**, 803–818.
- Zhao CS, Lasses T, Bako L, Kong DY, Zhao BY, Chanda B, Bombarely A, Cruz-Ramírez A, Scheres B, Brunner AM, Beers EP** (2017). XYLEM NAC DOMAIN 1, an angiosperm NAC transcription factor, inhibits xylem differentiation through conserved motifs that interact with RETINOBLASTOMA-RELATED. *New Phytol* **216**, 76–89.
- Zhao KM, Bartley LE** (2014). Comparative genomic analysis of the R2R3 MYB secondary cell wall regulators of *Arabidopsis*, poplar, rice, maize, and switchgrass. *BMC Plant Biol* **14**, 135.
- Zhao YQ, Song XQ, Zhou HJ, Wei KL, Jiang C, Wang JN, Cao Y, Tang F, Zhao ST, Lu MZ** (2020). *KNAT2/6b*, a class I KNOX gene, impedes xylem differentiation by regulating NAC domain transcription factors in poplar. *New Phytol* **225**, 1531–1544.
- Zhong RQ, Demura T, Ye ZH** (2006). SND1, a NAC domain transcription factor, is a key regulator of secondary wall synthesis in fibers of *Arabidopsis*. *Plant Cell* **18**, 3158–3170.
- Zhong RQ, Lee C, McCarthy RL, Reeves CK, Jones EG, Ye ZH** (2011). Transcriptional activation of secondary wall biosynthesis by rice and maize NAC and MYB transcription factors. *Plant Cell Physiol* **52**, 1856–1871.
- Zhong RQ, Lee C, Ye ZH** (2010a). Functional characterization of poplar wood-associated NAC domain transcription factors. *Plant Physiol* **152**, 1044–1055.
- Zhong RQ, Lee C, Ye ZH** (2010b). Global analysis of direct targets of secondary wall NAC master switches in *Arabidopsis*. *Mol Plant* **3**, 1087–1103.
- Zhong RQ, Lee C, Ye ZH** (2010c). Evolutionary conservation of the transcriptional network regulating secondary cell wall biosynthesis. *Trends Plant Sci* **15**, 625–632.
- Zhong RQ, Lee C, Zhou JL, McCarthy RL, Ye ZH** (2008). A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in *Arabidopsis*. *Plant Cell* **20**, 2763–2782.
- Zhong RQ, McCarthy RL, Haghighat M, Ye ZH** (2013). The poplar MYB master switches bind to the SMRE site and activate the secondary wall biosynthetic program during wood formation. *PLoS One* **8**, e69219.
- Zhong RQ, Richardson EA, Ye ZH** (2007a). Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of *Arabidopsis*. *Planta* **225**, 1603–1611.
- Zhong RQ, Richardson EA, Ye ZH** (2007b). The MYB46 transcription factor is a direct target of SND1 and regulates secondary wall biosynthesis in *Arabidopsis*. *Plant Cell* **19**, 2776–2792.
- Zhong RQ, Ye ZH** (2012). MYB46 and MYB83 bind to the SMRE sites and directly activate a suite of transcription

- factors and secondary wall biosynthetic genes. *Plant Cell Physiol* **53**, 368–380.
- Zhong RQ, Ye ZH** (2014). Secondary cell walls: biosynthesis, patterned deposition and transcriptional regulation. *Plant Cell Physiol* **56**, 195–214.
- Zhong RQ, Ye ZH** (2015). The *Arabidopsis* NAC transcription factor NST2 functions together with SND1 and NST1 to regulate secondary wall biosynthesis in fibers of inflorescence stems. *Plant Signal Behav* **10**, e989746.
- Zhong RQ, Yuan YX, Spiekerman JJ, Guley JT, Egbosiba JC, Ye ZH** (2015). Functional characterization of NAC and MYB transcription factors involved in regulation of biomass production in Switchgrass (*Panicum virgatum*). *PLoS One* **10**, e0134611.
- Zhou JL, Lee C, Zhong RQ, Ye ZH** (2009). MYB58 and MYB63 are transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in *Arabidopsis*. *Plant Cell* **21**, 248–266.
- Zhou JL, Zhong RQ, Ye ZH** (2014). *Arabidopsis* NAC domain proteins, VND1 to VND5, are transcriptional regulators of secondary wall biosynthesis in vessels. *PLoS One* **9**, e105726.

Transcriptional Regulatory Network of Secondary Cell Wall Biosynthesis in Plants

Yu Zhang^{1, 2, 3}, Mingjie Zhao^{1, 2, 3}, Wei Zhang^{1, 2, 3*}

¹College of Horticulture & Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, China

²Key Laboratory of Horticultural Plant Biology, Ministry of Education, Wuhan 430070, China

³Key Laboratory of Urban Agriculture in Central China, Ministry of Agriculture and Rural Affairs, Wuhan 430070, China

Abstract Plant secondary cell walls (SCWs) contain cellulose, hemicellulose and lignin, which endow the cell walls with mechanical strength and hydrophobicity. This characteristic is very important for plant upright growth, water and nutrient transport, and resistance to biotic and abiotic stresses. In this review, we summarize the transcription factors regulating SCW biosynthesis and their regulatory mechanisms, including NAC transcription factors functioning as first-layer master switch, the AtMYB46/AtMYB83 and their downstream regulators serving as secondary-layer master switch, as well as the other transcription factors involved in the regulation of biosynthesis of the SCW. The future research contents and methods are also prospected in order to provide reference for further research on the transcriptional regulatory network of SCW biosynthesis.

Key words NAC transcription factor, MYB transcription factor, secondary cell wall, biosynthesis, regulation network

Zhang Y, Zhao MJ, Zhang W (2020). Transcriptional regulatory network of secondary cell wall biosynthesis in plants. *Chin Bull Bot* **55**, 351–368.

* Author for correspondence. E-mail: zhangw@mail.hzau.edu.cn

(责任编辑: 朱亚娜)