细胞骨架参与调控细胞壁形成的研究进展

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摘要 植物细胞壁是地球上最丰富的可再生资源,也是植物细胞区别于动物细胞的特殊结构之一,它与细胞质膜及细胞骨架共同构成了植物细胞表面的细胞壁-质膜-细胞骨架连续体。细胞壁为植物细胞提供外部支撑结构,细胞骨架则在细胞内构成内部网络支架结构。近年来,有关植物细胞骨架调控细胞壁形成的研究有了很大进展,本文从细胞骨架参与细胞壁物质膜泡运输、细胞骨架调控纤维素微纤丝沉积、细胞骨架调控次生细胞壁加厚以及细胞骨架参与细胞壁形成信号的调控等方面进行了阐述和总结,并对今后的研究方向进行了展望。

关键词 细胞壁, 细胞骨架, 次生壁加厚, 细胞壁信号

植物细胞壁是由纤维素微纤丝与半纤维素交联成网状,并由果胶多糖与少量结构蛋白填充于网状结构的空隙而形成的复杂结构^[1]。它参与调控植物细胞的大小和形状,提供结构支撑,并在生物与非生物胁迫中起到防御作用^[2~4]。作为一种以多糖为基础的复杂的胞外基质,细胞壁是生产生物燃料和化学品的巨大生物物质资源,其形成及调控机制的研究对细胞壁的高效利用与新能源开发具有重要意义^[5]。

植物细胞壁可以分为初生细胞壁与次生细胞壁。初生细胞壁主要由纤维素、半纤维素和果胶3种多糖组成,此外,还包括蛋白质和一些化合物^[6]。次生细胞壁是在初生壁与细胞质膜之间加厚的细胞壁,次生壁大多数不含果胶,但是含有木质素。一般初生细胞壁的合成与细胞的伸长和分裂等密切相关,决定组织器官的大小,而次生细胞壁的沉积于植物细胞特异分化

后发生,有助于提高植物机械强度及生物量^[7]。

植物细胞壁是原生质体向外运输物质并在胞外沉积的产物。细胞壁的形成过程不仅包括细胞壁物质大分子的合成,还包括这些组分的前体向沉积所在部位的转运,以及最终在细胞壁形成位点的沉积与结合^[8]。植物细胞中,细胞壁、质膜与细胞骨架构成了细胞表面细胞壁-质膜-细胞骨架连续体,细胞壁为植物细胞提供外部支撑结构,包括微管与微丝在内的细胞骨架则在细胞内构成高度动态的内部网络支架结构,并能将合成细胞壁的相关组分拴缚到质膜,对细胞壁物质运输与沉积进行时空调控而参与细胞壁的形成^[9]。此外,细胞壁不断受到细胞外激素、机械刺激以及细胞生长限制因素等的影响,细胞骨架及相关蛋白可以响应这些细胞壁信号,并影响细胞壁的形成^[10~12]。本文介绍了细胞骨架参与调控细胞壁形成的最新研究进

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展, 着重就细胞骨架在细胞壁物质膜泡运输与纤维素微纤维定向沉积中的功能、细胞骨架调控次生细胞壁加厚过程以及细胞骨架参与细胞壁信号调控等方面进行了综述。

1 细胞骨架参与细胞壁物质膜泡的运输

纤维素是植物细胞壁的主要成分, 在植物生长发育中起重要作用^[6,13]。纤维素由 β -1,4-葡聚糖链组成, 通过纤维素合酶复合体(cellulose synthase complexes, CSCs)在细胞质膜上合成。纤维素合酶(cellulose synthase, CESA)蛋白在内质网合成。冰冻蚀刻技术及活细胞定量成像技术均表明, CESA在高尔基体组装成CSC, 有实验表明CSC也可能在内质网进行组装^[14-17], 随后被转运到质膜。半纤维素和果胶多糖在高尔基体中合成, 细胞壁的结构蛋白同其他分泌途径的蛋白一样, 在内质网合成, 并于内质网与高尔基体进行加工^[18]。半纤维素、果胶多糖以及细胞壁结构蛋白均通过囊泡介导的运输途径分泌到细胞外基质和细胞间隙^[19], 这些细胞壁的不同组分在胞外沉积而形成复杂的细胞壁网络结构。

细胞骨架在CSC靶向质膜过程中发挥重要的作用。植物细胞中, 肌球蛋白介导的高尔基体囊泡运输过程依赖于微丝骨架^[20], 微丝骨架参与CSC的运输。CSC在质膜上的分布与高尔基体在细胞周质的位置有关, 破坏微丝骨架会引起富含CSC的高尔基体囊泡的聚集, 使得CSC在质膜分布不均^[21,22]。研究发现, 胞质含CSC的囊泡沿亚周质微丝进行转运, 使用肌动蛋白聚合抑制剂LatB(latrunculin B)破坏微丝, 或者在肌动蛋白的突变体 $act2act7$ 中, 均可发现CSC到质膜的运输效率降低, 总纤维素显著减少^[22]。近期的研究发现, 在肌动蛋白的马达蛋白——肌球蛋白myosin XI三突变体中, 含有纤维素合酶的囊泡在细胞周质积累, CSC转运效率降低, 质膜CSC丰度减少, 同时纤维素合成也显著减少。说明肌动蛋白及其马达蛋白myosin XI通过调节含纤维素合酶囊泡的胞吐作用, 介导了CSC向质膜的转运^[23]。微管骨架及其马达蛋白被认为能够通过锚定作用减缓细胞器或囊泡的运输速率^[24], 从而有利于细胞壁物质的精确分泌。通过荧光蛋白标记技术对CSC的胞内转运进行研究, 发现细胞周质区域一些CSC囊泡可以沿单根微管运动, 其运动速率与微管的

解聚速率一致。解聚微管或使用紫杉醇稳定微管, 均能影响囊泡的形成与运动速率^[16,25]。

组成CSC的CESA在内质网合成, 在内质网或高尔基体被组装成CSC^[14-16], CESA或CSC被转运到高尔基体, 定位于高尔基体的蛋白STL(stello)在CSC的组装和转运过程中发挥重要的功能。CSC还包括有一些辅助蛋白, 例如KOR(korrigan)和纤维素合酶伴侣蛋白(companion of cellulose synthase, CC)。目前已发现, 纤维素合酶伴侣蛋白1(companion of cellulose synthase 1, CC1)和纤维素合酶伴侣蛋白2(companion of cellulose synthase 2, CC2)沿着微管与CSC结合。研究表明, CC1和CC2均能在体外直接结合微管, 促进微管的形成^[26]。CSC被转运到周质微管^[17], 这一过程被认为借助于CESA互作蛋白1(cellulose synthase interacting 1, CSI1)。CSI1在体外与微管结合, 荧光标记的CSI1蛋白与CESA3和CESA6在细胞质膜共定位, 并具有与CESA3和CESA6相似的动力学^[27-29]。CSI1连接CSC与周质微管, 为纤维素微纤维与微管排列一致提供了基础^[30-32]。csi1突变体中, CSC与微管排列的一致性被破坏, 说明CSI1对于CSC与微管的一致性排列是必需的^[27]。外泌复合体(exocyst complex)和PATROL1也参与了CSC囊泡转运过程(图1)。外泌复合体通过与微管以及CSI1的相互作用, 增强了囊泡与质膜的拴缚与融合, PATROL1包含一个Munc13结构域, 这个结构域可能对CSCs向细胞膜的插入具有重要意义^[30]。

细胞壁形成过程中, 包括果胶和半纤维素在内的非纤维素类多糖由高尔基体膜定位的糖基转移酶合成后分泌到质外体^[19,33,34]。反面高尔基体网络(trans Golgi network, TGN)囊泡的转运异常将会破坏细胞壁的完整性。定位于TGN的 H^+ -ATP酶 $\alpha 1$ 亚基(vacuolar H^+ -ATPase subunit $\alpha 1$, VHA- $\alpha 1$)的破坏, 导致囊泡的分泌转运以及胞吞过程异常, 引起细胞伸长缺陷和纤维素缺乏^[35,36]。多种类型的细胞中, 微丝骨架在细胞壁物质的分泌过程中发挥重要的作用。当以LatB破坏微丝骨架后, 果胶沉积异常^[37]。非纤维素多糖结合到纤维素微纤维时, 会降低纤维素的结晶度, 这一过程依赖于微管对于新生细胞壁物质的组织^[38]。微管结合蛋白MOR1(microtubule organization 1)的温度敏感突变体mor1在限制温度下, 周质微管密度降低, 非微管结合的CSC数量增加, 非纤维素多糖对纤维素生物合成和结晶位点的靶向性降低, 增加了纤维素结晶度^[38]。这

图 1 细胞骨架参与细胞壁形成示意图。细胞壁形成过程中, 包括果胶和半纤维素在内的非纤维素类多糖及细胞壁结构蛋白等细胞壁合成所需组分以分泌泡形式沿细胞骨架转运, 并以胞吐作用运输到胞外。纤维素合酶CESA在内质网合成, 并在内质网或高尔基体组装成CSC, 含CSC的高尔基体囊泡沿着肌动蛋白丝运输, 肌球蛋白myosin XI参与了这一过程。高尔基体定位的STL蛋白在CSC转运过程中发挥重要作用。外泌复合体和PATROL1也参与了CSC囊泡转运过程。除了CESA外, CSC复合体还含有KOR和CC等辅助蛋白。含CSC的囊泡会转运到周质微管, 在微管上停靠, 并在exocyst complex和相关蛋白指导下插入质膜, CESA互作蛋白CSI1参与了这一过程, CSI1与微管结合, 引导CSC沿着微管移动。质膜CSC可以通过网格蛋白介导的胞吞作用回收再利用或者降解

Figure 1 Schematic diagram of the role of the cytoskeleton in cell wall formation. During cell wall formation, non-cellulose polysaccharides including pectin and hemicellulose, as well as structural proteins are packaged in vesicles and move along the cytoskeleton, then were released to the extracellular space via exocytosis. CESA is synthesized in the endoplasmic reticulum (ER) and assembles in Golgi, and possibly in the ER. CSC containing vesicles traffic along actin filaments and myosin XI is involved in this process. The activity of Golgi-localized STL is necessary to maintain CSC trafficking. Exocyst complex and PATROL1 also participate in CSC vesicle transport. In addition to the CESA subunits, the CSC contains accessory proteins including KOR and CC. CSC are typically delivered to sites of cortical microtubules and pause at cortical microtubules. The insertion of CSC into plasma membranes appears to be mediated by the Exocyst complex and related proteins. CSI1 interacts with both CSC and microtubules and directs the co-alignment between CSCs and microtubule. CSC is directed by microtubules and once the CSC is delivered to the plasma membrane, cellulose synthesis commences. The CSC could be internalized, possibly via clathrin-mediated endocytosis

一结果支持了周质微管也指导半纤维素和果胶胞吐位置的推论. 周质微管可能将所有细胞壁合成所需材料定位于相同部位, 从而促进纤维素结晶时将所有多糖细胞壁成分结合到新生的细胞壁基质中^[11]. 近来的研究发现, 沿周质微管运动的马达蛋白AtKINESIN-4A/FRA1(fragile fiber 1), 其突变体细胞壁中纤维素总量没有变化, 但是果胶等非纤维素多糖发生了改变, 并且细胞壁在细胞扩展时缺乏弹性. 微管及其马达蛋白Kinesin-4参与含非纤维素多糖囊泡的转运, 影响了植物细胞壁的合成与特性^[39].

2 细胞骨架调控纤维素微纤丝的定向沉积

细胞壁形成过程中, 纤维素微纤丝通过质膜定位的CSC合成. 周质微管限制了CSC对质膜的插入位置, 当以黄草消(oryzalin)处理解聚微管后, CSC的线性分布受到破坏, 呈现均匀分布模式, 而以紫杉醇稳定微管后, 增强了CSC的线性排布^[16]. 一旦CSCs被转运到质膜上, 便可通过磷酸化等被激活, 并开始合成纤维素^[21,40,41]. 纤维素在细胞壁内的沉积具有一定的方向性和排列方式, 在伸长的细胞中, 纤维素微纤丝的排列垂直于细胞的伸长方向, 这种排列与细胞周质微管的排列方式一致. 借助于荧光蛋白YFP标记的CESA6和活细胞成像技术, Paredez等人^[15]在拟南芥下胚轴中观察到CSC沿着周质微管在质膜呈线性运动, 并且证实纤维素微纤丝的排列受周质微管排列的引导.

微管骨架在决定CSC在质膜的运动速度与方向中发挥重要作用, CSC沿着质膜下方周质微管的轨迹运动, 纤维素合酶在质膜运动的速度和方向与纤维素微纤丝形成的速度与方向密切相关. 荧光蛋白标记CESA的追踪实验证明, CSC沿着荧光标记的微管骨架呈现双向运动, 利用抑制剂使微管紊乱, 或者由于一些因素使微管重排, 均能导致CSC的轨迹与纤维素微纤丝的变化^[15,42,43]. CSI1除了在CSC转运过程中发挥作用外, 也参与周质微管对CSC运动的引导过程^[27,32]. 新形成的纤维素微纤丝插入细胞壁, 进一步驱动了纤维素合酶沿微管的运动, 该过程由周质微管通过CSI1/POM2引导. *csi1*突变体造成了CSC从周质微管上脱离, 细胞生长异常^[44]. 此外, 纤维素合酶与微管解耦连蛋白(cellulose synthase-microtubule uncouplings, CMUs)沿着周质微管分布, *cmu*突变体中, 周质微管横向移位,

细胞扭曲, 微管对CSC的引导受到破坏. 因此, CMU可以稳定周质微管, 使其能够承受纤维素合成过程中CSC运动产生的力, 从而指导纤维素的合成和植物的各向异性生长^[45].

CSC停止合成纤维素后将从质膜内化, 其内化可能通过网格蛋白介导的胞吞作用完成^[41,46,47]. 在纤维素合成抑制剂处理下, CSC会内化进入细胞内特定区室, 即微管相关的纤维素合酶区室(microtubule-associated cellulose synthase compartments, MACS), MACS的运动依赖于周质微管的动态变化^[16], 当微管解聚时, MACS形成减少, 表明CSCs内化过程中, 微管骨架可能发挥了促进的作用, 这一过程可能通过与特定微管结合蛋白结合, 或者与募集MACS的包被蛋白相关. 因此, 微管骨架还可能参与了CSC从质膜内化的过程^[16].

3 细胞骨架调控次生细胞壁加厚

植物细胞分裂后形成初生细胞壁, 在一些特殊组织中, 细胞停止分裂后在初生细胞壁与质膜之间形成次生细胞壁. 次生细胞壁是植物特异分化后产生的细胞结构, 在植物加固机械支撑力、管状分子运输水分、光合作用产物的储存等方面发挥重要的功能^[48].

成熟木质部由多种类型的细胞组成, 其中研究最多的是管状分子(tracheary elements, TEs), 管状分子是木质部中高度特化的细胞, 包括导管和管胞, 在植物体中由下至上输送水分和无机盐^[49]. 管状分子细胞表现出非常典型的次生细胞壁加厚的特征, 其细胞壁加厚形式有环形、螺旋状、网状或点状等^[50,51]. 这些次生壁的加厚与微管骨架密切相关, 微管骨架通过确定次生壁沉积位置参与调控次生壁的加厚^[52].

高度动态的微管骨架在次生细胞壁加厚之前, 于次生壁加厚区域下方排列成束^[53], 作为次生壁沉积的支架, 确定了次生壁的位置^[54]. 成束排列的微管使CSCs被限定于次生壁加厚的区域^[55]. 大量研究表明, 周质微管在管状分子的发育过程中, 不仅标记了纤维素在次生壁中的沉积位置, 而且还标记了半纤维素等其他细胞壁成分的沉积位置^[56]. 微管解聚剂甲基氨草磷(amiprophos methyl, APM)破坏微管后, 干扰了纤维素和半纤维素的沉积, 而半纤维素的沉积模式与纤维

素完全一致, 表明微管也决定着除纤维素外的其他细胞壁成分的沉积^[55]。CSC和半纤维素都通过高尔基囊泡运输, 而高尔基囊泡需靶向细胞膜的特定区域。因此, 管状分子中的周质微管标记了许多组分的囊泡转运位点^[55]。但在次生壁沉积完成之前, 微管的排布现象已消失或者微管已经丢失, 说明微管很可能只在次生壁沉积的早期阶段是必需的^[57]。

Oda等人^[58]发现, 外泌复合体亚基EXO70A1(exocyst subunits EXO70A1)通过卷曲螺旋蛋白1(vesicle tethering 1, VETH1)和卷曲螺旋蛋白2(vesicle tethering 2, VETH2), 与保守的低聚高尔基复合体蛋白COG2(conserved oligomeric Golgi)相互作用, 富集到培养拟南芥木质部细胞的周质微管上。EXO70A1对分泌囊泡靶向质膜特定区域起重要作用(图2)。当*exo70A1*突变后, 导致细胞壁形态异常, 大量囊泡积累在发育的木质部细胞中^[59]。

无论是初生细胞壁还是次生细胞壁中, CSCs都与位于质膜下的周质微管密切相关^[15,55,60]。在次生细胞壁的合成中, oryzalin破坏微管导致次生细胞壁区域的带状CSCs消失^[55,61,62]。初生细胞壁中, 这种微管指导CSCs排列的机制依赖于纤维素合成酶相互作用蛋白CSII/POM2和CSI3^[27,29,32,63,64]。目前已经证明, 类似的机制在次生细胞壁中也同样存在。在拟南芥的次生细胞壁中, CSII/POM2在微管下富集, RNAi(RNA interference, RNAi)沉默CSII/POM2的细胞中观察到异常的次生细胞壁模式^[65]。在次生细胞壁形成的早期, CSII/POM2的缺失导致CSC与微管解耦联, 进一步证明了这些蛋白在次生细胞壁形成中发挥作用^[66]。

微管结合蛋白参与管状分子的发育过程^[65], 与管状分子不同形式的次生壁加厚相关。微管结合蛋白MAP65通过在微管之间形成交叉桥而实现微管的交叉连接^[67]。从百日草中分离到的MAP65在百日草维管束的木质部细胞中表达, 并且在百日草管状分子分化过程中上调, 表明其在百日草管状分子分化时与微管成束相关。当在拟南芥悬浮细胞中过表达MAP65时, 引起周质微管成束, 出现与管状分子分化时一致的现象^[68]。微管结合蛋白CORD1(cortical microtubule disordering 1, CORD1)被发现在拟南芥木质部导管中调节周质微管的组织, 其与周质微管结合, 对于后生木质部导管次生壁的点状加厚是必需的。CORD1的异位表达能够使周质微管从质膜区域脱离, 周质微管的平行

排列受到影响, 无序性增强而使纹孔面积增大^[69]。拟南芥微管结合蛋白AIR9(auxin-induced in root cultures, AIR)调节管状分子的类型, 过表达AIR9导致孔纹管状分子增加, 而RNAi沉默AIR9导致螺纹管状分子增加^[65]。棉花中微管结合蛋白GhCLASPs(cytoplasmic linker-associated proteins, CLASPs)主要在次生壁加厚阶段表达, 通过与微管相互作用而影响纤维素合成与沉积, 从而影响纤维的发育与强度^[70]。MAP70-5编码一种植物特异的微管蛋白结合蛋白, 被认为能够调控次生壁加厚模式, MAP70-5在拟南芥管状细胞分化时期特异性表达, 其过表达能够促进螺纹管状分子的形成^[71]。微管结合蛋白MIDD1(microtubule depletion domain 1)及驱动蛋白13A(kinesin-13A)能够与小G蛋白ROP11(rho-like GTPase from plant 11)形成复合体, 定位于无次生壁加厚的区域。活化的ROP11通过其效应子MIDD1和Kinesin-13A诱导微管解聚, 阻止其所在部位的次生壁沉积, 从而形成了不同的次生壁加厚模式^[72~74]。

目前有关微丝在管状分子次生壁加厚中的功能少见报道。早期有研究发现, 微丝在百日草的管状分子形成过程中也表现出类似于微管的排布模式。此外, 在百日草的管状分子分化过程中, 运用细胞松弛素B(cytochalasin B)破坏微丝, 阻碍了微管从纵向到横向的重定位, 次生壁模式异常, 说明微丝在微管重定位中发挥重要调控作用^[75,76]。对拟南芥根和树木的研究表明, 微丝在管状分子分化过程中主要是纵向排列, 说明微丝主要在细胞壁物质囊泡的运输中发挥作用^[77,78]。具有较薄次生壁和异常微丝排布的突变体中, 微管的模式正常, 暗示微丝在次生细胞壁加厚过程中可能独立于微管行使功能^[78,79]。

4 细胞骨架参与细胞壁形成信号的调控

细胞骨架、细胞壁及其之间的细胞质膜构成了植物细胞表面的细胞壁-质膜-细胞骨架连续体。在动物细胞中, 整联蛋白作为细胞外基质与细胞骨架之间的跨膜连接物, 能够将胞外信号传递至细胞骨架, 在多种生物学过程中发挥重要作用^[80]。植物细胞中, 也发现存在与动物细胞整联蛋白相似的类整联蛋白(integrin-like protein)^[81], 研究表明, 植物中类整联蛋白与动物中整联蛋白的作用机制十分相似^[81,82]。植物细胞中

图 2 植物细胞骨架参与次生细胞壁加厚. 植物次生细胞壁加厚过程中, 周质微管决定了次生壁沉积的位置. 周质微管骨架在次生壁加厚区域下方组装排列成紧密的微管束, 确定了次生壁的位置. 低聚高尔基复合体蛋白COG2与卷曲螺旋蛋白VETH1/2形成复合物, 外泌复合体与VETH-COG复合物相互作用, 富集到周质微管, 将包含CSCs的囊泡拴系到次生壁沉积区域, CSCs沿微管合成纤维素并在该区域沉积

Figure 2 Plant cytoskeletons involve in secondary cell wall thickening. In the process of plant secondary cell wall thickening, cortical microtubules define the sites of secondary cell wall deposition. Cortical microtubules become bundled and line the plasma membrane at the region of secondary cell wall formation. COG2 protein forms a complex with VETH1/2 associated with cortical microtubules. Exocyst complex interacts with the VETH-COG complex and promotes tethering of the CSC vesicles at the sites of secondary cell wall thickening. CSCs synthesize cellulose microfibrils along cortical microtubules

的一种类整联蛋白AT14A, 其序列和结构与动物细胞整联蛋白相似, 拟南芥悬浮细胞中AT14A的过表达或突变缺失, 均导致细胞微丝骨架与微管骨架的异常, 细胞壁厚度也发生改变. AT14A可能是细胞壁-质膜-细胞骨架连续体的成员之一, 并以类似动物细胞整联蛋白的方式调节细胞壁和细胞骨架的关系, 在细胞壁-质膜-细胞骨架连续体之间传递跨膜信号^[83]. 此外, 拟南芥微丝结合蛋白AtFH1(formin homology 1, FH1)能够同时结合细胞壁以及微丝骨架, 感受胞外信号, 调节周质微丝的组装而引发细胞反应^[84].

细胞壁形成过程中, 纤维素微纤维的沉积模式决定了细胞的生长方向, 周质微管在纤维素定向沉积过程中发挥关键作用^[15,37]. 周质微管的排列方式受到激素等多种外界信号的调控, 生长素诱导拟南芥根和下胚轴细胞的周质微管从垂直于细胞伸长方向的横向排布向平行于细胞伸长方向的纵向排布转变, 赤霉素则诱导微管从纵向排列向横向排列转变^[69,85]. 研究表明, 激素信号能够通过微管结合蛋白调节周质微管的排列, 从而影响CSCs的轨迹, 进一步导致纤维素微纤维的排列发生变化而影响细胞的生长^[64]. 例如, 乙烯信

号途径激活下游转录因子EIN3/EIN1(ethylene insensitive 3/EIN3-like 1), 活化的转录因子结合到微管结合蛋白WDL5 (wave-dampened 2-like 5)的启动子区域, 上调WDL5的表达. WDL5是一个微管成束蛋白, 通过调控微管成束和稳定微管, 使周质微管从横向排列向纵向排列转变, 与此相一致, 乙烯能够抑制拟南芥黄化下胚轴生长^[86]. 油菜素内脂信号促进黄化下胚轴快速生长, 这一过程中, MDP40(microtubule destabilizing protein 40)作为该信号途径下游转录因子BZR1(brassinazole resistant 1)的靶基因而上调表达, 继而通过维持微管横向排列的动力学特征促进下胚轴细胞的伸长^[87].

细胞骨架在机械应力响应中也具有重要作用. 在人工施加机械压力时, 周质微管沿着压力的方向排列^[12,88]. 在各向异性生长的器官中, 组织内的细胞与周围细胞紧密结合而产生各向异性张力, 因此, 在自然条件下, 微管的组织排布也会受到机械张力的影响. 研究发现, 机械张力能够激活微管切割蛋白katanin p60亚基KTN1, 通过切割微管而改变微管动力学, 为周质微管自组装成平行束状提供更多的微管蛋白亚基, 从而促进周质微管的有序化排布^[89], 进一步通过引导纤维素微纤丝的沉积而促进细胞壁的合成, 而合成的细胞壁又会产生机械张力, 反之影响微管的排布.

5 展望

植物细胞壁是地球上最丰富的可再生生物能源物质, 解析细胞壁形成机理, 有利于为其开发与利用提供理论基础. 细胞壁结构复杂, 多糖与蛋白质成分以错综复杂的网状结构连接在一起. 细胞壁成分的合成、转运和组装都是动态并可调节的, 其过程决定了细胞的生长和形态. 细胞骨架是细胞内高度动态的蛋白纤维网络结构, 参与物质运输、信号传导等许多重要的生命活动过程. 以往的研究提高了人们对细胞骨架参与调控植物细胞壁形成的认识, 但是仍然有很多问题亟待探究. 首先, 参与初生壁与次生壁形成的CSCs由不同的纤维素合酶组装形成, 鉴定和发现更多的与不同CSCs和微管及微丝骨架相互作用的蛋白, 将有利于回答有关不同CSCs向靶膜的转运以及微管如何介导次生壁沉积的问题; 其次, 借助于新型的超高分辨率显微技术, 在原位活体细胞中可视化初生壁合成向次生壁合成的转化过程, 追踪这一过程中细胞骨架以及细胞壁组分的动态, 有利于更深入地了解植物细胞骨架如何参与调控细胞壁的形成; 最后, 细胞壁形成过程中的细胞骨架动态检测并结合建模分析, 有助于深入精细解析细胞壁结构. 总之, 深入解析细胞骨架对细胞壁的合成、次生壁加厚及细胞壁合成网络信号的调控, 对细胞壁开发与利用具有重要意义.

参考文献

- 1 Bosch M, Mayer C D, Cookson A, et al. Identification of genes involved in cell wall biogenesis in grasses by differential gene expression profiling of elongating and non-elongating maize internodes. *J Exp Bot*, 2011, 62: 3545–3561
- 2 Somerville C, Bauer S, Brininstool G, et al. Toward a systems approach to understanding plant cell walls. *Science*, 2004, 306: 2206–2211
- 3 Malinovsky F G, Fangel J U, Willats W G T. The role of the cell wall in plant immunity. *Front Plant Sci*, 2014, 5: 178
- 4 Le Gall H, Philippe F, Domon J M, et al. Cell wall metabolism in response to abiotic stress. *Plants*, 2015, 4: 112–166
- 5 Pauly M, Keegstra K. Plant cell wall polymers as precursors for biofuels. *Curr Opin Plant Biol*, 2010, 13: 304–311
- 6 McFarlane H E, Döring A, Persson S. The cell biology of cellulose synthesis. *Annu Rev Plant Biol*, 2014, 65: 69–94
- 7 Schuetz M, Smith R, Ellis B. Xylem tissue specification, patterning, and differentiation mechanisms. *J Exp Bot*, 2012, 64: 11–31
- 8 Robinson D G. Plant cell wall synthesis. *Adv Bot Res*, 1977, 5: 89–151
- 9 Liu Z, Persson S, Zhang Y. The connection of cytoskeletal network with plasma membrane and the cell wall. *J Integr Plant Biol*, 2015, 57: 330–340
- 10 Zhang R, Li L G. Research progress of the plant cell wall signaling (in Chinese). *J Plant Physiol*, 2018, 54: 1254–1262 [张蕊, 李来庚. 植物细胞壁信号研究进展. *植物生理学报*, 2018, 54: 1254–1262]
- 11 Bashline L, Lei L, Li S, et al. Cell wall, cytoskeleton, and cell expansion in higher plants. *Mol Plant*, 2014, 7: 586–600
- 12 Sampathkumar A, Krupinski P, Wightman R, et al. Subcellular and supracellular mechanical stress prescribes cytoskeleton behavior in

- Arabidopsis* cotyledon pavement cells. *eLife*, 2014, 3: e01967
- 13 Wallace I S, Somerville C R. A blueprint for cellulose biosynthesis, deposition, and regulation in plants. In: Fukuda H, ed. *Plant Cell Wall Patterning and Cell Shape*. Hoboken, NJ: John Wiley & Sons, Inc, 2014. 65–97
 - 14 Haigler C H, Brown Jr. R M. Transport of rosettes from the Golgi apparatus to the plasma membrane in isolated mesophyll cells of *Zinnia elegans* during differentiation to tracheary elements in suspension culture. *Protoplasma*, 1986, 134: 111–120
 - 15 Paredez A R, Somerville C R, Ehrhardt D W. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science*, 2006, 312: 1491–1495
 - 16 Crowell E F, Bischoff V, Desprez T, et al. Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in *Arabidopsis*. *Plant Cell*, 2009, 21: 1141–1154
 - 17 Rudolph U. Occurrence of rosettes in the ER membrane of young *Funaria hygrometrica* protonemata. *Naturwissenschaften*, 1987, 74: 439
 - 18 Richter S, Voss U, Jürgens G. Post-Golgi traffic in plants. *Traffic*, 2009, 10: 819–828
 - 19 Worden N, Park E, Drakakaki G. Trans-Golgi network—An intersection of trafficking cell wall components. *J Integr Plant Biol*, 2012, 54: 875–886
 - 20 Tominaga M, Ito K. The molecular mechanism and physiological role of cytoplasmic streaming. *Curr Opin Plant Biol*, 2015, 27: 104–110
 - 21 Gutierrez R, Lindeboom J J, Paredez A R, et al. *Arabidopsis* cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. *Nat Cell Biol*, 2009, 11: 797–806
 - 22 Sampathkumar A, Gutierrez R, McFarlane H E, et al. Patterning and lifetime of plasma membrane-localized cellulose synthase is dependent on actin organization in *Arabidopsis* interphase cells. *Plant Physiol*, 2013, 162: 675–688
 - 23 Zhang W, Cai C, Staiger C J. Myosins XI are involved in exocytosis of cellulose synthase complexes. *Plant Physiol*, 2019, 179: 1537–1555
 - 24 Cai G, Cresti M. Are kinesins required for organelle trafficking in plant cells? *Front Plant Sci*, 2012, 3: 170
 - 25 Wightman R, Turner S. Trafficking of the plant cellulose synthase complex. *Plant Physiol*, 2010, 153: 427–432
 - 26 Endler A, Kesten C, Schneider R, et al. A mechanism for sustained cellulose synthesis during salt stress. *Cell*, 2015, 162: 1353–1364
 - 27 Li S, Lei L, Somerville C R, et al. Cellulose synthase interactive protein 1 (CS11) links microtubules and cellulose synthase complexes. *Proc Natl Acad Sci USA*, 2011, 109: 185–190
 - 28 Mei Y, Gao H B, Yuan M, et al. The *Arabidopsis* ARCP protein, CS11, which is required for microtubule stability, is necessary for root and anther development. *Plant Cell*, 2012, 24: 1066–1080
 - 29 Bringmann M, Li E, Sampathkumar A, et al. POM-POM2/cellulose synthase interacting1 is essential for the functional association of cellulose synthase and microtubules in *Arabidopsis*. *Plant Cell*, 2012, 24: 163–177
 - 30 Zhu X, Li S, Pan S, et al. CS11, PATROL1, and exocyst complex cooperate in delivery of cellulose synthase complexes to the plasma membrane. *Proc Natl Acad Sci USA*, 2018, 115: E3578–E3587
 - 31 Bringmann M, Landrein B, Schudoma C, et al. Cracking the elusive alignment hypothesis: the microtubule-cellulose synthase nexus unraveled. *Trends Plant Sci*, 2012, 17: 666–674
 - 32 Gu Y, Kaplinsky N, Bringmann M, et al. Identification of a cellulose synthase-associated protein required for cellulose biosynthesis. *Proc Natl Acad Sci USA*, 2010, 107: 12866–12871
 - 33 Cosgrove D J. Growth of the plant cell wall. *Nat Rev Mol Cell Biol*, 2005, 6: 850–861
 - 34 Driouich A, Follet-Gueye M L, Bernard S, et al. Golgi-mediated synthesis and secretion of matrix polysaccharides of the primary cell wall of higher plants. *Front Plant Sci*, 2012, 3: 79
 - 35 Dettmer J, Hong-Hermesdorf A, Stierhof Y D, et al. Vacuolar H⁺-ATPase activity is required for endocytic and secretory trafficking in *Arabidopsis*. *Plant Cell*, 2006, 18: 715–730
 - 36 Brûx A, Liu T Y, Krebs M, et al. Reduced V-ATPase activity in the trans-Golgi network causes oxylipin-dependent hypocotyl growth inhibition in *Arabidopsis*. *Plant Cell*, 2008, 20: 1088–1100
 - 37 Rounds C M, Hepler P K, Winship L J. The apical actin fringe contributes to localized cell wall deposition and polarized growth in the lily pollen tube. *Plant Physiol*, 2014, 166: 139–151
 - 38 Fujita M, Lechner B, Barton D A, et al. The missing link: Do cortical microtubules define plasma membrane nanodomains that modulate cellulose biosynthesis? *Protoplasma*, 2012, 249: 59–67
 - 39 Kong Z, Ioki M, Braybrook S, et al. Kinesin-4 functions in vesicular transport on cortical microtubules and regulates cell wall mechanics during

- cell elongation in plants. *Mol Plant*, 2015, 8: 1011–1023
- 40 Chen S, Ehrhardt D W, Somerville C R. Mutations of cellulose synthase (CESA1) phosphorylation sites modulate anisotropic cell expansion and bidirectional mobility of cellulose synthase. *Proc Natl Acad Sci USA*, 2010, 107: 17188–17193
- 41 Sánchez-Rodríguez C, Ketelaar K D, Schneider R, et al. Brassinosteroid insensitive2 negatively regulates cellulose synthesis in *Arabidopsis* by phosphorylating cellulose synthase 1. *Proc Natl Acad Sci USA*, 2017, 114: 3533–3538
- 42 Anderson C T, Carroll A, Akhmetova L, et al. Real-time imaging of cellulose reorientation during cell wall expansion in *Arabidopsis* roots. *Plant Physiol*, 2010, 152: 787–796
- 43 Chan J, Crowell E, Eder M, et al. The rotation of cellulose synthase trajectories is microtubule dependent and influences the texture of epidermal cell walls in *Arabidopsis* hypocotyls. *J Cell Sci*, 2010, 123: 3490–3495
- 44 Lei L, Singh A, Bashline L, et al. Cellulose synthase interactive1 is required for fast recycling of cellulose synthase complexes to the plasma membrane in *Arabidopsis*. *Plant Cell*, 2015, 27: tpc.15.00442
- 45 Liu Z, Schneider R, Kesten C, et al. Cellulose-microtubule uncoupling proteins prevent lateral displacement of microtubules during cellulose synthesis in *Arabidopsis*. *Dev Cell*, 2016, 38: 305–315
- 46 Gadeyne A, Sánchez-Rodríguez C, Vanneste S, et al. The TPLATE adaptor complex drives clathrin-mediated endocytosis in plants. *Cell*, 2014, 156: 691–704
- 47 Sánchez-Rodríguez C, Shi Y, Kesten C, et al. The cellulose synthases are cargo of the TPLATE adaptor complex. *Mol Plant*, 2018, 11: 346–349
- 48 Huang C, Li L G. Research progress on regulation of plant secondary cell wall thickening. *Plant Physiol J*, 2016, 52: 8–18 [黄成, 李来庚. 植物次生细胞壁加厚调控研究进展. *植物生理学报*, 2016, 52: 8–18]
- 49 Turner S, Gallois P, Brown D. Tracheary element differentiation. *Annu Rev Plant Biol*, 2007, 58: 407–433
- 50 Růžicka K, Ursache R, Hejátko J, et al. Xylem development—From the cradle to the grave. *New Phytol*, 2015, 207: 519–535
- 51 Zhang B C, Zhou Y H. Plant cell wall formation and regulation (in Chinese). *Sci Sin Vitae*, 2015, 45: 544–556 [张保才, 周奕华. 植物细胞壁形成机制的新进展. *中国科学: 生命科学*, 2015, 45: 544–556]
- 52 Vukašinović N, Oda Y, Pejchar P, et al. Microtubule-dependent targeting of the exocyst complex is necessary for xylem development in *Arabidopsis*. *New Phytol*, 2017, 213: 1052–1067
- 53 Oda Y, Hasezawa S. Cytoskeletal organization during xylem cell differentiation. *J Plant Res*, 2006, 119: 167–177
- 54 Baskin T I. On the alignment of cellulose microfibrils by cortical microtubules: A review and a model. *Protoplasma*, 2001, 215: 150–171
- 55 Watanabe Y, Meents M J, McDonnell L M, et al. Visualization of cellulose synthases in *Arabidopsis* secondary cell walls. *Science*, 2015, 350: 198–203
- 56 Hogetsu T. Mechanism for formation of the secondary wall thickening in tracheary elements: microtubules and microfibrils of tracheary elements of *Pisum sativum* L. and *Commelina communis* L. and the effects of amiprofosmethyl. *Planta*, 1991, 185: 190–200
- 57 Roberts A W, Frost A O, Roberts E M, et al. Roles of microtubules and cellulose microfibril assembly in the localization of secondary-cell-wall deposition in developing tracheary elements. *Protoplasma*, 2004, 224: 217–229
- 58 Oda Y, Iida Y, Nagashima Y, et al. Novel coiled-coil proteins regulate exocyst association with cortical microtubules in xylem cells via the conserved oligomeric Golgi-complex 2 protein. *Plant Cell Physiol*, 2015, 56: 277–286
- 59 Li S, Chen M, Yu D, et al. EXO70A1-mediated vesicle trafficking is critical for tracheary element development in *Arabidopsis*. *Plant Cell*, 2013, 25: 1774–1786
- 60 Fujita M, Himmelspach R, Hocart C H, et al. Cortical microtubules optimize cell-wall crystallinity to drive unidirectional growth in *Arabidopsis*. *Plant J*, 2011, 66: 915–928
- 61 Gardiner J C, Taylor N G, Turner S R. Control of cellulose synthase complex localization in developing xylem. *Plant Cell*, 2003, 15: 1740–1748
- 62 Wightman R, Turner S R. The roles of the cytoskeleton during cellulose deposition at the secondary cell wall. *Plant J*, 2008, 54: 794–805
- 63 Lei L, Li S, Gu Y. Cellulose synthase interactive protein 1 (CS11) mediates the intimate relationship between cellulose microfibrils and cortical microtubules. *Plant Signal Behav*, 2012, 7: 714–718
- 64 Lei L, Li S, Du J, et al. Cellulose synthase INTERACTIVE3 regulates cellulose biosynthesis in both a microtubule-dependent and microtubule-independent manner in *Arabidopsis*. *Plant Cell*, 2013, 25: 4912–4923
- 65 Derbyshire P, Ménard D, Green P, et al. Proteomic analysis of microtubule interacting proteins over the course of xylem tracheary element formation in *Arabidopsis*. *Plant Cell*, 2015, 27: 2709–2726

- 66 Schneider R, Tang L, Lampugnani E R, et al. Two complementary mechanisms underpin cell wall patterning during xylem vessel development. [Plant Cell](#), 2017, 29: 2433–2449
- 67 Chan J, Jensen C G, Jensen L C W, et al. The 65-kDa carrot microtubule-associated protein forms regularly arranged filamentous cross-bridges between microtubules. [Proc Natl Acad Sci USA](#), 1999, 96: 14931–14936
- 68 Mao G, Buschmann H, Doonan J H, et al. The role of MAP65-1 in microtubule bundling during *Zinnia* tracheary element formation. [J Cell Sci](#), 2006, 119: 753–758
- 69 Sasaki T, Fukuda H, Oda Y. CORTICAL MICROTUBULE DISORDERING1 is required for secondary cell wall patterning in xylem vessels. [Plant Cell](#), 2017, 29: 3123–3139
- 70 Zhu S H, Xue F, Li Y J, et al. Identification and functional characterization of a microtubule-associated protein, GhCLASP2, from upland cotton (*Gossypium hirsutum* L.). [Front Plant Sci](#), 2018, 9: 882
- 71 Pesquet E, Korolev A V, Calder G, et al. The microtubule-associated protein AtMAP70-5 regulates secondary wall patterning in *Arabidopsis* wood cells. [Curr Biol](#), 2010, 20: 744–749
- 72 Oda Y, Fukuda H. Initiation of cell wall pattern by a Rho- and microtubule-driven symmetry breaking. [Science](#), 2012, 337: 1333–1336
- 73 Oda Y, Fukuda H. Rho of plant GTPase signaling regulates the behavior of *Arabidopsis* kinesin-13A to establish secondary cell wall patterns. [Plant Cell](#), 2013, 25: 4439–4450
- 74 Sugiyama Y, Wakazaki M, Toyooka K, et al. A novel plasma membrane-anchored protein regulates xylem cell-wall deposition through microtubule-dependent lateral inhibition of Rho GTPase domains. [Curr Biol](#), 2017, 27: 2522–2528.e4
- 75 Kobayashi H, Fukuda H, Shibaoka H. Reorganization of actin filaments associated with the differentiation of tracheary elements in *Zinnia* mesophyll cells. [Protoplasma](#), 1987, 138: 69–71
- 76 Kobayashi H, Fukuda H, Shibaoka H. Interrelation between the spatial disposition of actin filaments and microtubules during the differentiation of tracheary elements in cultured *Zinnia* cells. [Protoplasma](#), 1988, 143: 29–37
- 77 Chaffey N, Barlow P, Barnett J. A cytoskeletal basis for wood formation in angiosperm trees: The involvement of microfilaments. [Planta](#), 2000, 210: 890–896
- 78 Hu Y, Zhong R, Morrison III W H, et al. The *Arabidopsis* *RHD3* gene is required for cell wall biosynthesis and actin organization. [Planta](#), 2003, 217: 912–921
- 79 Zhong R, Burk D H, Nairn C J, et al. Mutation of SAC1, an *Arabidopsis* SAC domain phosphoinositide phosphatase, causes alterations in cell morphogenesis, cell wall synthesis, and actin organization. [Plant Cell](#), 2005, 17: 1449–1466
- 80 Palazzo A F, Eng C H, Schlaepfer D D, et al. Localized stabilization of microtubules by integrin- and FAK-facilitated Rho signaling. [Science](#), 2004, 303: 836–839
- 81 Knepper C, Savory E A, Day B. *Arabidopsis* NDR1 is an integrin-like protein with a role in fluid loss and plasma membrane-cell wall adhesion. [Plant Physiol](#), 2011, 156: 286–300
- 82 Sakurai M, Pak J Y, Muramatsu Y, et al. Integrin-like protein at the invaginated plasma membrane of epidermal cells in mature leaves of the marine angiosperm *Zostera marina* L. [Planta](#), 2004, 220: 271–277
- 83 Lü B, Wang J, Zhang Y, et al. AT14A mediates the cell wall-plasma membrane-cytoskeleton continuum in *Arabidopsis thaliana* cells. [J Exp Bot](#), 2012, 63: 4061–4069
- 84 Martinière A, Gayral P, Hawes C, et al. Building bridges: formin1 of *Arabidopsis* forms a connection between the cell wall and the actin cytoskeleton. [Plant J](#), 2011, 66: 354–365
- 85 Vineyard L, Elliott A, Dhingra S, et al. Progressive transverse microtubule array organization in hormone-induced *Arabidopsis* hypocotyl cells. [Plant Cell](#), 2013, 25: 662–676
- 86 Ma Q, Sun J, Mao T. Microtubule bundling plays a role in ethylene-mediated cortical microtubule reorientation in etiolated *Arabidopsis* hypocotyls. [J Cell Sci](#), 2016, 129: 2043–2051
- 87 Wang X, Zhang J, Yuan M, et al. *Arabidopsis* microtubule destabilizing protein40 is involved in brassinosteroid regulation of hypocotyl elongation. [Plant Cell](#), 2012, 24: 4012–4025
- 88 Hamant O, Inoue D, Bouchez D, et al. Are microtubules tension sensors? [Nat Commun](#), 2019, 10: 2360
- 89 Uyttewaal M, Burian A, Alim K, et al. Mechanical stress acts via Katanin to amplify differences in growth rate between adjacent cells in *Arabidopsis*. [Cell](#), 2012, 149: 439–451

Role of cytoskeleton in plant cell wall formation

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Cell wall, which is one important and characteristic structure of plant cells, represents the most abundant renewable resource on the earth. It exists as part of a continuum with the plasma membrane and cytoskeleton. The cell wall provides external support and the cytoskeletons including the microtubules and the microfilaments constitute an internal framework. In recent years, great progress has been made in the understanding of the roles of cytoskeleton involved in regulating plant cell wall formation. In this review, we summarized the role of cytoskeleton in trafficking of vesicles containing cell wall precursors and in the oriented deposition of cellulose microfibrils, the regulation of secondary cell wall thickening by the cytoskeleton, and the involvement of cytoskeleton in cell wall signaling. In the end, we also give some prospects for the future studies.

cell wall, cytoskeleton, secondary cell wall thickening, cell wall signaling

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