Cell Chemical Biology

Preview

TITLE

A "Double Click" for Illuminating Plant Cell Walls

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SUMMARY

In this issue of *Cell Chemical Biology*, **Lion et al. (2017)** report a multiplexed labeling method to visualize plant cell wall lignification *in vivo*. This approach uses two different lignin precursor analogs tagged with azide and alkyne reporters that can be independently incorporated into cell walls, then differentially derivatized *in vivo* via two bioorthogonal click reactions, i.e., strain-promoted and copper-assisted azide-alkyne cycloadditions.

MAIN TEXT

Lignification, i.e., deposition of the phenolic lignin polymer in plant secondary cell walls, is an innovative biological mechanism that early terrestrial plants acquired for land colonization. The molecular process involved in the synthesis of lignin polymer is essential and highly conserved in all vascular plants. Lignified cell walls are most prominently found in vascular tissues, such as xylem tracheids and structural fibers, where lignin plays essential roles in water conductivity, mechanical support, and pathogen defense. Up to one-third of dry plant can be composed of lignin, and thus this polymer is believed to be the second most abundant organic molecule on earth, after cellulose. Lignin biosynthesis has been a major research focus, not only because

of its fundamental importance in understanding plant life and evolution, but also because of its economic importance associated with agro-industrial utilizations of plant biomass (**Rinaldi et al., 2016**).

Cell wall lignification is a dynamic and complex process that successively involves the synthesis of lignin monomers (i.e., monolignols) inside the cells, translocation of monolignols to apoplastic cell wall domains, and finally polymerization of monolignols to form lignin polymers. The lignin polymerization of three canonical monolignols, coniferyl, sinapyl, and *p*-coumaryl alcohols, proceeds via combinatorial radical coupling reactions initiated by wall-bound laccases and/or peroxidases, giving rise to guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) lignin polymer units, respectively. Despite extensive biochemical and genetic studies, many of the molecular events that occur during lignification remain elusive. For example, our knowledge regarding monolignol transportation is quite limited. Several different models of monolignol export out of the cell have been proposed; passive diffusion or active transport assisted by specific membrane proteins, such as ATP-binding cassette transporters and proton-dependent transporters, may be involved in this process. There also is a long-standing debate on whether monolignols are transported as free forms or as their glucosides. Moreover, little is known about how plants execute the spatiotemporally controlled lignin deposition, i.e., transportation and polymerization of different monolignol types in specific cell wall domains at specific times during development. Coordinated regulations of the monolignol metabolic supply and the polymerization machinery localization may dictate this process (**Barros et al., 2015**).

Recently, chemical biology-based techniques have provided plant biologists with unique opportunities to scrutinize the elusive lignification processes more closely. Dye-tagged monolignol mimics have been developed for probing the interaction of monolignols with lignin polymerization enzymes *in vitro* as well as for imaging cell wall lignification *in vivo*. With the latter approach, fluorescence imaging using the probes has been successful in providing spatiotemporal information about lignin formation in several live plant and cell systems (**Tobimatsu et al., 2013**). Nevertheless, this method still suffers from the intrinsic drawbacks

associated with having the dye attached directly to monolignol. In particular, the bulky fluorescent dye likely limits the ability of the probe to accurately report the *in planta* movement of natural monolignol. Inspired by numerous successful applications in other biomolecular imaging studies (**Sletten and Bertozzi, 2011**), a methodology using bioorthogonal chemical reporters has been implemented in lignin imaging studies to overcome the limitation imposed by large probes (**Tobimatsu et al., 2014; Bukowski et al., 2014**). In this approach, biocompatible monolignol mimics that have been modified with chemical reporter groups, such as azides and alkynes, are metabolically introduced into lignifying cell walls, where they are subsequently derivatized via bioorthogonal click chemistry for fluorescence labeling. Compared with the previous method using fluorescence-tagged monolignols, tagging these monomers with simpler and more inert chemical reporters enhances the probe's biocompatibility and ability to precisely report lignification processes *in planta*.

In this issue of *Cell Chemical Biology*, **Lion et al. (2017)** report a combination of two versatile click reactions, i.e., strain-promoted azide-alkyne cycloaddition (SPAAC) and copper-assisted azide-alkyne cycloaddition (CuAAC) reactions, for the concurrent detection of two different metabolically incorporated monolignol mimics in one experiment. In their strategy named BLISS (Bioorthogonal Labeling Imaging Sequential Strategy), two different monolignol mimics tagged with azide and alkyne reporters are independently incorporated into plant cell walls, where they are then sequentially derivatized via SPAAC and CuAAC with fluorophores bearing activated cyclooctyne and azide groups, respectively. As a proof of concept, a series of labeling experiments using an azide-tagged H-type and an alkyne-tagged G-type monolignol mimics were conducted in flax (*Linum usitatissimum* L.) plant systems. For the success of BLISS, SPAAC labeling for the azide reporter must precede CuAAC labeling for the alkyne reporter; the first copper-free SPAAC allows a selective derivatization of the azide reporter without activating the proximate alkyne reporter, which must be left intact for the second CuAAC labeling with a Cu(I) catalyst. **Lion et al. (2017)** firmly established that H- and G-type monolignol mimics can be individually incorporated into the flax cell wall lignins

and simultaneously, but differentially, co-visualized by two-color fluorescence detection at subcellular levels and in a live-cell environment.

By taking advantage of BLISS, **Lion et al. (2017)** examined the incorporation of H- and G-type monolignol mimics in flax lignifying tissues. As in most angiosperms, the lignin in the flax xylem is a G-rich G/S-type lignin with only minor levels of H-type units (usually less than a few percent). In spite of such uneven lignin composition, BLISS experiments determined that exogenously supplied H- and G-type monolignol mimics are similarly incorporated into the same xylem cell wall compartments at comparable levels. This result suggests that the flax xylem cells indeed possess polymerization machinery capable of producing both H- and G-type lignin polymers. Therefore, the deposition of the two different lignin polymers is not necessarily regulated by the monomer specificity of the polymerization machinery, but is more likely controlled by the metabolic supply of monomers, e.g., by the controlled expressions of genes responsible for monolignol synthesis and/or transportation. **Lion et al. (2017)** also demonstrated that flax bast fibers, which uniquely produce thick secondary cell walls devoid of lignins, failed to incorporate the monolignol mimics. Thus, the “hypoliginification” in the flax bast fibers might be regulated by transcriptional interferences in the construction of the lignin polymerization machinery, rather than only by the metabolic supply of monolignols. In line with this, upregulations of genes associated with the peroxidase/H_2O_2 monolignol oxidation system were observed in the stems of flax *lbf1* mutants, which show ectopic lignification in their bast fibers (**Chantreau et al., 2014**).

Overall, while it is still one of the rare attempts to apply chemical biology techniques in plant research, BLISS could be a powerful addition to the chemical toolkit of plant biologists especially for investigating the dynamics of plant cell wall formation. In principle, this approach is applicable to various two-color fluorescence imaging studies by using a pair of modified precursors for biomolecules that make up plant cell walls, including all the three lignin monomers and possibly some sugar precursors for wall polysaccharides, which have been also demonstrated to be accessible by click methodology (**Andeson et al., 2012**; **Zhu et al., 2016**). Such imaging approaches, in combination with other biochemical, cell biological, and

transgenic plant approaches, should facilitate studies of the molecular mechanisms involved in plant cell wall formation and ultimately enable more efficient and precise manipulation of cell wall structure and architecture for better utilizations of plant biomass.

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