Lignification and Oxidative Enzymes: Localization, Localization, Localization!¹

Lignification of cell walls is a pivotal process in plant life. Lignin is a biopolymer that provides additional stability to secondary cell walls (SCWs), enables vascular cells to transport water, and protects the plant from pathogenic attack (Tobimatsu and Schuetz, 2019). It is produced from monolignols that are synthesized in the cytosol and subsequently secreted to the apoplast, where they are cross-linked via radical-radical coupling. Oxidative enzymes, laccases (LAC) and peroxidases (PRX), as well as reactive oxygen species (ROS) such as H₂O₂, are required for this polymerization step. The polymerized lignin is incorporated into the SCW, where it cross-links the different cell wall polysaccharides. However, only certain cell types have lignified SCWs, and even in these specialized cells there can be local differences in lignification between the walls. Lignification therefore appears to be a tightly directed and precisely localized event. From recent work it has emerged that this precise targeting is in part achieved by specific localizations of the oxidative enzymes. In this regard, the lab of A. Lacey Samuels has previously described the localization of LAC4 and PRX64 to either the entire SCW (LAC4) or just to the middle lamella and cell corners (PRX64; Schuetz et al., 2014; Yi Chou et al., 2018). Building on this work, they now set out to investigate the localization between the walls.

Having demonstrated these specific localizations for the different enzymes, the authors move on to test for actual oxidizing activity in these regions. Incubating several more LAC and PRX enzymes involved in lignification to their specific subdomain of the SCW, bringing us closer to visualizing lignin polymerization on a subcellular level (Hoffmann et al., 2020).

In this issue of Plant Physiology, Hoffmann et al. (2020) use expression data for different LAC and PRX genes, data on coexpression of these genes with other SCW synthesis components, and known phenotypes of mutants to narrow down the long list of Arabidopsis (Arabidopsis thaliana) LAC and PRX family members to eight prime candidates, which might be the central enzymes facilitating the lignification of xylem cells and interfascicular fibers in Arabidopsis stems. This thorough analysis forms the basis of the work presented here, and the authors have included a helpful table summarizing LAC and PRX gene identifiers, expression patterns, and coexpression data as well as stem mutant phenotypes.

In their bioinformatic analysis, Hoffmann et al. (2020) additionally identified LAC10, PRX42, PRX52, PRX71, and PRX72 as the most promising candidates to study lignification in stems. Tagging these proteins with the mCherry fluorophore, they then visualized the localization of these eight enzymes in cells of the Arabidopsis stem, subdomains of the SCW, as well as comparing several developmental stages. They found that LAC17 and PRX72 localize to the SCW of xylem vessels and fiber cells, while PRX64 and PRX71 localize to the cell corners and middle lamella of fiber cells. LAC10, PRX42, and PRX52 localize to nonlignified tissues and may therefore be active in other pathways. Interestingly, LAC4 is distributed homogenously along the SCW of protoxylem vessel elements at the earliest developmental stages and then additionally localizes specifically to the cell corners and middle lamella of fiber cells, before it also shows a homogenous distribution in the SCW of these cells for the remaining developmental stages (Fig. 1). LAC4 may therefore play a role in lignification of the SCW in both vessel elements and fiber cells during different stages of development.

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stem sections with the PRX-oxidation substrate TMB (3,3′,5,5′-tetramethylbenzidine) and exogenous H$_2$O$_2$ results in oxidized TMB in all cell walls, independent of lignification, indicating that PRXs are generally present in all walls. When the stem sections were incubated with TMB alone, however, oxidation was limited to the lignified SCW, overlapping with the localization of the different LAC and PRX enzymes. This observation demonstrates that it is not just the localization of the enzymes to these specific regions, but also targeted release of endogenous H$_2$O$_2$ into the apoplast in these domains, that contributes to the specific lignification pattern.

Hoffmann et al. (2020) have identified LAC and PRX enzymes (as well as combinations thereof) involved in lignification of SCW at different developmental stages and in specific subdomains of the SCW, which will help to refine our model of lignification. They further demonstrate that it is not just the spatial distribution of the different enzyme sets and the targeted secretion of monolignols to these regions, but also localized ROS accumulation, that is required to achieve highly localized lignification. This is in agreement with recent findings that lignification of the Casparian strip is also dependent on targeted ROS accumulation and oxidative enzyme localization (Fujita et al., 2020).

The findings presented here also demonstrate that the many LAC and PRX family members are not merely redundant to each other but appear to fulfill specific functions in specific domains and at specific time points. It will be interesting to learn more about how the enzymes are directed toward their specific domains and how they are sequestered there. In this regard, LAC4, which changes its intramural localization from the corners of fiber cells to a homogenous distribution, will be an especially interesting candidate. Overall, the results in this study represent another step toward understanding lignin polymerization at the subcellular level.

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