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Cellular interactions during tracheary elements formation and function

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The survival of higher plant species on land depends on the development and function of an efficient vascular system distributing water and minerals absorbed by roots to all aerial organs. This conduction and distribution of plant sap relies on specialized cells named tracheary elements (TEs). In contrast to many other cell types in plants, TEs are functionalized by cell death that hollows the cell protoplast to make way for the sap. To maintain a stable conducting function during plant development, recovery from vascular damages as well as to adapt to environmental changes, TEs are completely dependent on direct cellular interactions with neighboring xylem parenchyma cells (XPs).

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Introduction

The colonization of land by plants coincided with the acquisition of a vascular system dedicated to water and mineral conduction named xylem (from Greek *xylos* for wood). In this tissue, the 'plumbing pipe cells' are the tracheary elements (TEs) — named by the first plant anatomists based on their overall morphological resemblance with insect trachea and their transverse orientated rib reinforcements [1]. In contrast to many other plant cell types, TEs are functionalized by programmed cell death (PCD) resulting in hollow cell cadavers with only a remaining cell wall delimiting the sap conducting cylinder [2]. The driving force responsible for plant sap upward transport in TEs is a negative pressure created by a gradient of decreasing water potential (Ψh) between the soil and the atmosphere interconnected by strands of TEs: like sucking water through a straw [3]. Furthermore, along the vascular path the lateral transport of the sap in tissues is mediated by side Ψh gradients created mainly by the osmotic pressure of TE neighboring xylem parenchyma cells (XPs). Xylem is an open vascular system in which the conducted sap water is released in the atmosphere by evapotranspiration at a rate depending on abscisic acid (ABA) sensitive stomatal conductance [4]. Xylem is remarkable compared to other biological vascular systems: sap is transported through unadjustable dead conduits in an open system at a rate dependent on environmental factors (humidity and temperature); it is therefore highly susceptible to cavitation and embolism [5]. Moreover the fact that death enables the functional state of TEs challenges TE cell autonomous capacity to adapt to any environmental changes. The entire TE function in plants therefore relies on complete assistance by other living cell types which will (i) modulate TE structural composition/features for optimal sap conduction, (ii) adapt TE sap content and flow rate relative to changing environmental conditions and (iii) control TE recovery or decommission in response to environment and biotic interactions. Other than with precursor cells and living TEs in earlier stages of differentiation, TE cellular interactions occur mainly with neighboring XPs next to dead TEs and/or linked to TEs by the sap path.

TE engineering design

While other plant cell differentiations require metabolic activity to fulfill their specialized function, TE formation leads to functional cell corpses with specific morphological features optimized for sap conduction including (i) a hollowed content made by PCD to enable the sap to fill the emptied cell lumen, (ii) reinforced lateral cell walls with thick secondary cell wall depositions to maintain the cell lumen open during sap conduction and (iii) modified and thinned primary cell walls in-between reinforcements to enable lateral sap distribution [6,7]. The original TE blueprint, developed by ancestral protracheophytes like *Aglaophyton* and *Horneophyton*, used the cell wall remains of cells having committed PCD to form a conducting cylinder [8]. Improvements during evolution enabled TEs to sustain the negative pressure associated with sap conduction. As a result TE side walls are reinforced by 2–3 μm thick secondary cell wall reinforcements composed of polysaccharidic polymers cellulose and hemicelluloses as well as polyphenolic polymer lignin. Disturbances of any of TE secondary cell wall polymer deposition leads to collapsed TEs unable to withstand the negative pressure associated to the sap flow [9–11]. These TE secondary cell walls are organized in specific ordered patterns describing annular, spiral, scalariform, reticulate or pitted motifs leaving space for

lateral sap movements through unthickened areas in-between secondary cell wall depositions. Each TE pattern — ordered cell wall organization alternating secondary and modified primary cell walls — is controlled by a specific microtubule network [12,13]. The unthickened primary cell walls are modified by changing pectin esterification degree and are thinned to increase lateral porosity [14,15]: this is an ancestral feature already found in primitive TEs of fossil tracheophytes such as *Sennicaulis*, *Gosslingia* and *Psilophyton* [16]. To form a vascular system, TEs assemble into a continuous network. In certain species, TE modified primary cell walls lignify to reinforce the mechanical cohesion between adjoining TEs in the vascular network [17]. The interconnection between TEs to form a continuous vascular network is enabled through (i) the lateral residual primary cell wall bordered by the secondary cell walls of two adjoining TEs named bordered pit membrane [18], and through (ii) TE perforated ends in species where TEs assemble longitudinally to form vessels named perforation plates [19].

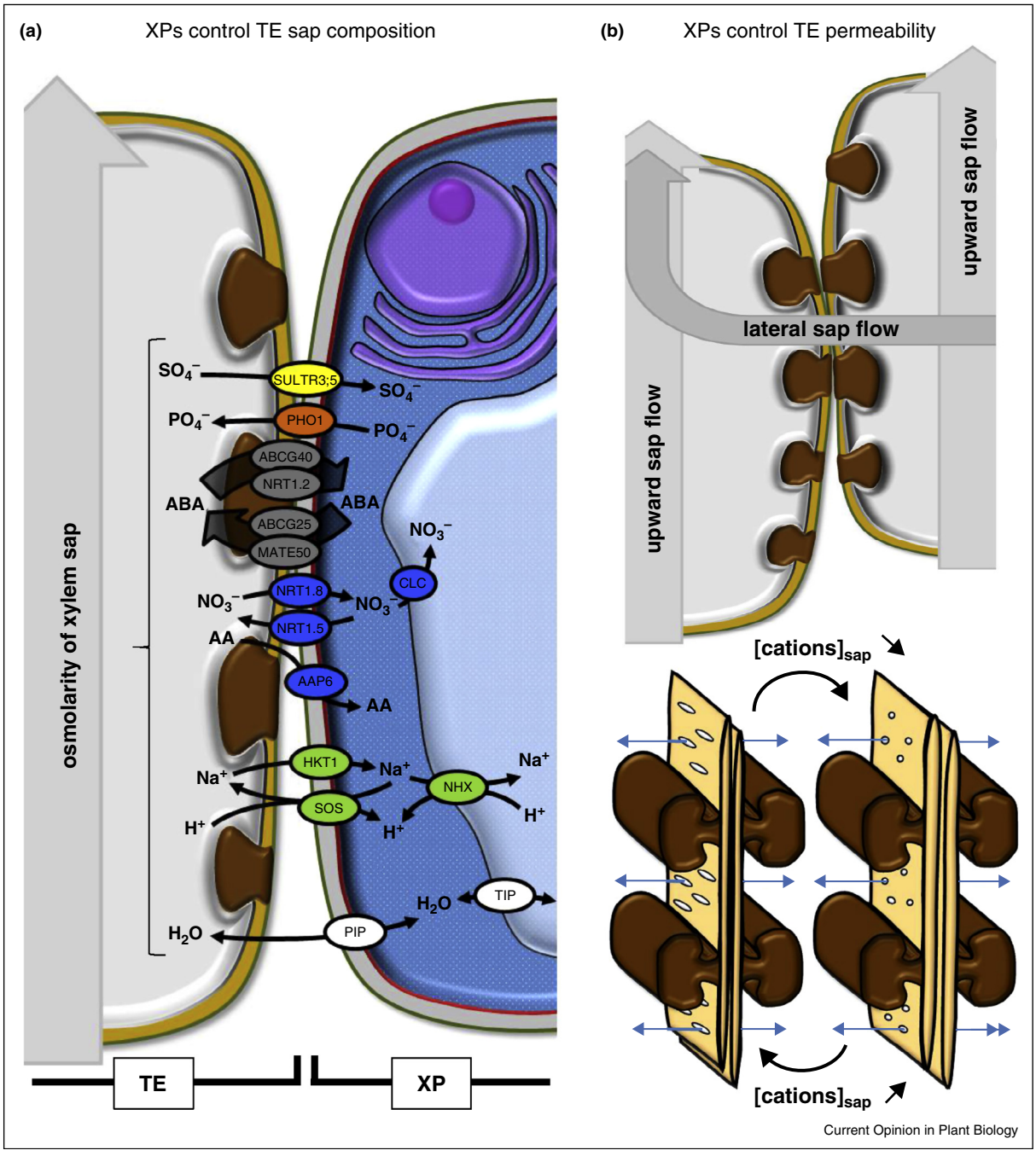
Cellular interactions controlling TE conductivity

The rate of sap conduction through plant TEs depends on the gradient of Ψh between soil and atmosphere and can be partly modulated by stomatal conductance. Drought and salt stresses alter the Ψh gradient, submitting TEs to greater compression constraints and increasing the sap flow rate if unchanged. As TEs are dead cells, their adaptation to any external constraint will exclusively depend on TE-neighboring XPs which can adapt the sap Ψh by loading or unloading compounds into the sap to regulate the flow rate (Figure 1a). The ionic strength, pH and metabolite content loaded into the sap are controlled by various influx and efflux transporters at the plasma and vacuole membrane of XPs [20–30]. XPs can thus compensate salt stress by specifically unloading the sap salt excess using influx transporters such as HKT1 in *Arabidopsis* [20]. In stressed conditions, XPs will actively regulate the TE sap flow rate by (i) remotely modifying stomatal conductance and by (ii) locally altering TE lateral permeability: similarly to closing-down the tap and limiting leaks (Figure 1). XP remote regulation of stomatal conductance is mediated by the release of ABA into the TE sap stream which triggers stomatal closure [4]. Whether XPs directly synthesize ABA or regulate ABA concentration in the sap is still debated, as the localization of ABA biosynthetic genes has been reported to be in both XPs and in phloem companion cells [31,32] (Figure 1a). XP, however, actively regulates ABA sap content using plasma membrane localized influx transporters NRT1.2/AIT1 and ABC-G40 as well as efflux transporters ABC-G25 and DTX/Multidrug and Toxic Compound Extrusion (MATE)-50 in *Arabidopsis* [33–36]. Mutants disrupted in DTX/MATE-50 exhibit more tolerance to drought with a reduced stomatal conductance [36] whereas mutants disrupted in ABC-G40 are more sensitive to drought, with an increased stomatal conductance [34]. Additionally, XP modification of the sap conduction rate is also mediated by an active local 'ionic effect' of the sap content on the hydraulic properties of TE bordered pits (Figure 1b). The mechanisms hypothesized to explain the local modification of TE pit permeability induced by XPs are (i) a sap cation-induced modification of the thickness and porosity of the pectic hydrogel composing TE pits [37,38] and/or (ii) a change in electroviscosity of the sap flow due to the sap ionic and pH content which alters the pectin charge of the TE pits [39•]. It is so far unknown if TE pit pectin composition can be altered after TEs are formed; nevertheless, a mutation in xylem expressed Polygalacturonase (PG) AT1G19170 in *Arabidopsis* exhibits an increased TE hydraulic resistance to extreme environmental changes [18]. XPs therefore locally regulate the sap pH and cation contents, which modifies the charge and water imbibition of the pectin in TE pits, to fine-tune TE lateral permeability and conductance.

Cellular interactions controlling TE structure

As the death of TEs enables their conducting function, it was previously assumed that once formed no further structural changes of the vascular conduit cells composition could occur. This restriction greatly limits the long-term adaptability of the vascular conduit cell to the increasing structural constraints associated to plant growth and development. The rigidity and compression resistance of TEs are due to lignin deposition which responds to environmental changes, increasing during drought [40]. It has been observed that reduction in TE lignin content directly affects TE hydraulic properties in poplar [41]. The lignification of TE secondary cell walls has recently been shown to occur *post-mortem* in *Arabidopsis* and *Zinnia elegans*: extending TE structural changes far beyond TE lifespan [12,42••,43••]. TE *post-mortem* lignification operates in differentiating cell cultures of *Zinnia* by a direct cellular interaction with neighboring XPs which provide both lignin monomers (monolignols or dilignols) and reactive oxygen species (ROS) to dead lignifying TEs (Figure 2a) [43••,44,45]. The mechanisms enabling XPs to export monolignols to TEs could partly be due to other active transporters: for instance ABC-G29 in *Arabidopsis* can transport non-methoxylated monolignol — *p*-coumaryl alcohol — which forms *p*-hydroxyphenyl (H) residues in the lignin polymer [46]. ROS production is directly dependent on plasma membrane localized NADPH oxidase (NO_x) which actively exports superoxide $O_2^{\bullet -}$ to TE apoplast [43••]. This $O_2^{\bullet -}$ can then be dismutated by apoplastic superoxide dismutase (SOD) into oxygen (O_2) and hydrogen peroxide (H_2O_2) [47]. Phenoloxidases (O_2-dependent laccases and H_2O_2-dependent peroxidases) use XP-provided substrates to pursue the lignification of the secondary cell walls of dead TEs [48]. The mechanisms controlling the interaction of dead TEs with XPs to

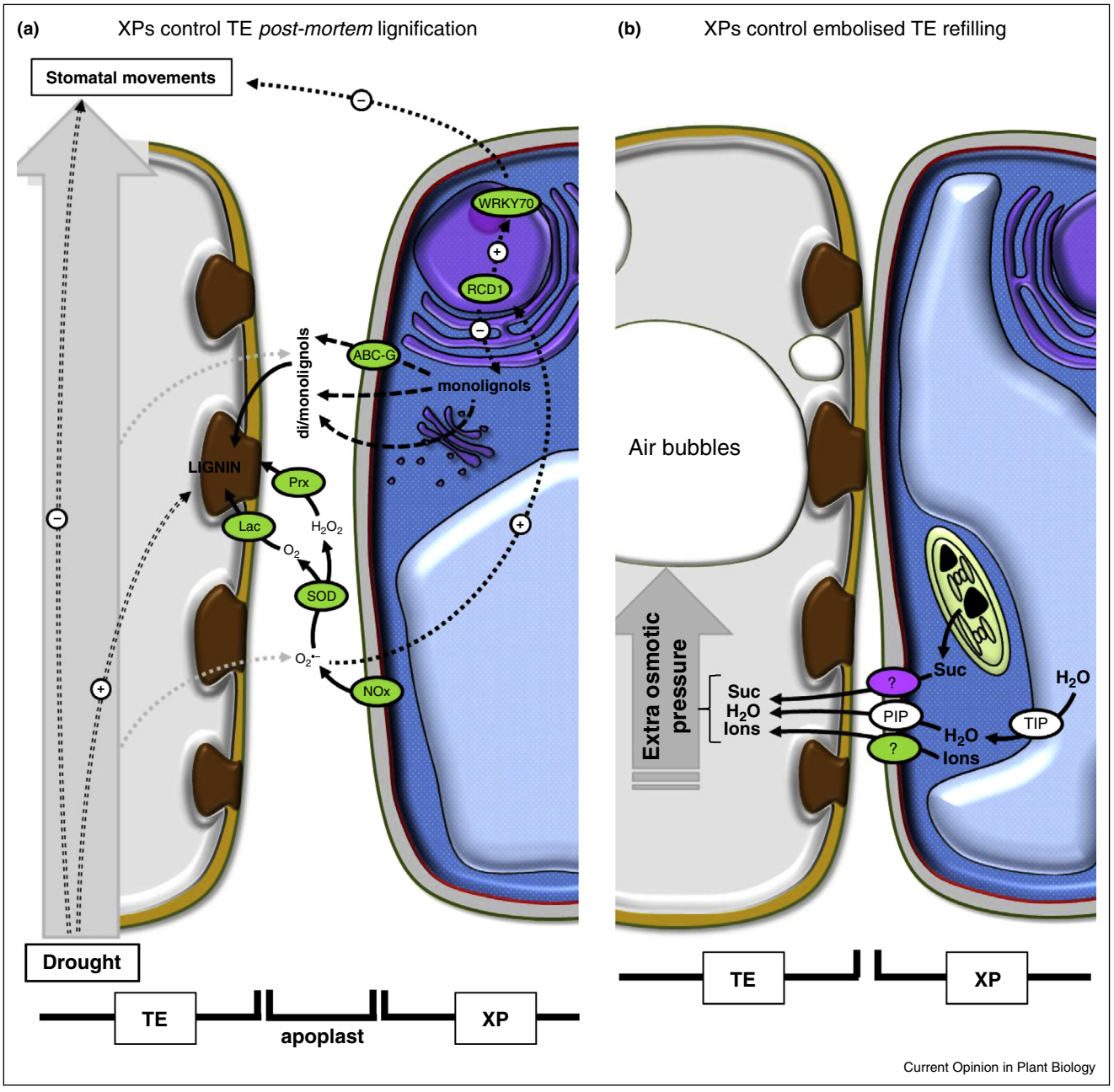
Figure 1



Cellular interactions between XPs and TEs to fine-tune TE sap composition and TE hydraulic conductance properties. The mineral content of TE sap is directly controlled by plasma membrane localized transporter proteins which load or unload specific compounds (e.g. ions and hormones) into the TE sap **(a)**. Long-distance regulation of TE sap conductance is dependent on XP-derived abscisic acid (ABA) released in the sap which controls stomatal opening. Lateral sap flow between TEs through bordered pits is dependent on sap cation(s) concentration which modulates the degree of pectin swelling and porosity of TE pits **(b)**.

enable lignification appear to be dependent on genes responding to the proper progression of TE PCD. When TE PCD is pharmacologically altered in *Zinnia* TE cell cultures, *post-mortem* lignification of TEs is prevented and XP gene expressions are altered [48]. Radical-induced Cell Death1 (RCD1) is one of the XP expressed genes responding to TE cell death; mutations disrupting RCD1 in *Arabidopsis* increase the xylem lignin quantity and

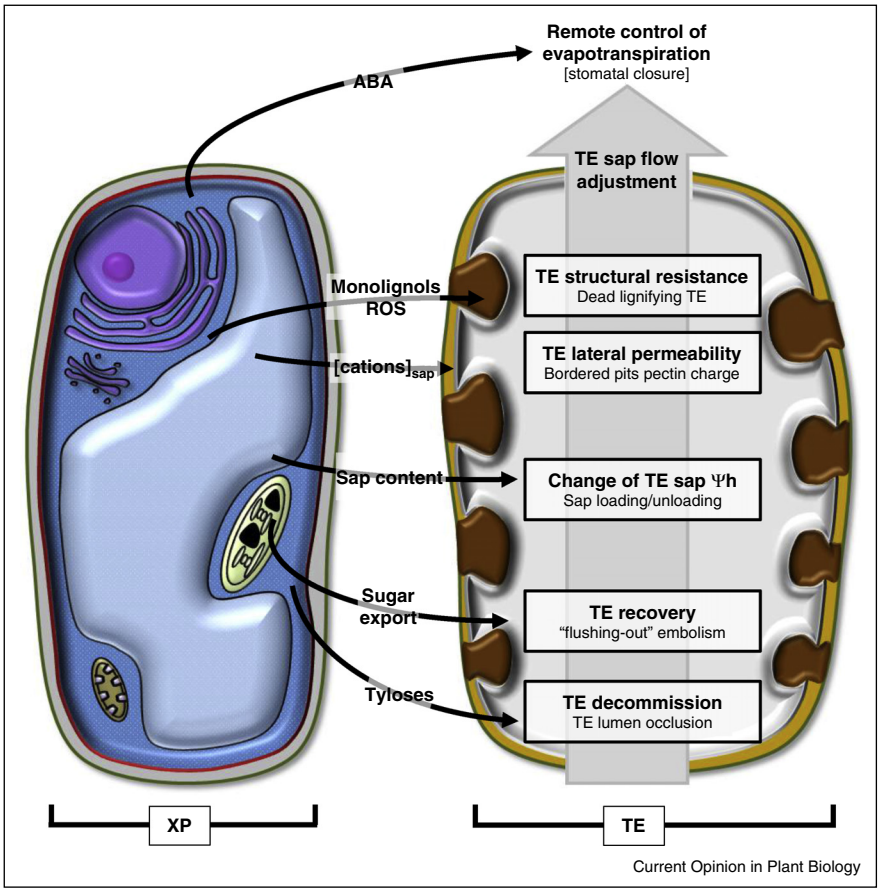
Figure 2



Cellular interactions between XPs and TEs during long-term modification of TEs as well as during recovery from embolism. XP cooperation during TE *post-mortem* lignification is mediated by XP-derived production and export of lignin monomers (monolignols and dilignols) through passive, vesicular and/or ABC transporters to the apoplast as well as extracellular export of superoxide through NADPH oxidase (NO_x): these substrates are then used by peroxidase(s) (Prx) and laccase(s) (Lac) to specifically lignify TE secondary cell wall thickenings **(a)**. In cases of extreme conditions causing cavitation of the sap, air bubbles causing embolism completely occlude TE lumen **(b)**. XPs capable of perceiving disturbance in TE sap flow (probably by local increase of sugar molecules) will locally increase sap osmotic pressure with sugars and ions to 'flush out' the occluding air bubble and refill the TE lumen.

H residue incorporation [43••]. Interestingly, mutations in RCD1 confer over-sensitivity to $O_2^{\bullet-}$ and induce spontaneous cell death lesions [49], placing RCD1 in the pathway controlling XP viability status in response to changes in the local TE sap environment [50]. Transcriptomic analyses have demonstrated that the transcriptional network downstream of RCD1 transits through the transcription factor WRKY70 [50] which, when disrupted,

Figure 3



Summary of the different cellular interactions between XPs and TEs to ensure proper physiological function of the hydro-mineral vascular system through (i) structural reinforcement of vascular conducts, (ii) changes of lateral permeability and (iii) sap Ψh as well as (iv) recovery in case of embolism and even (v) occlusion for TE decommission.

exhibits a lower tolerance to drought stress and a modified stomatal conductance [51] (Figure 2a). XP mediated *post-mortem* lignification of TEs represents then another crucial cell interaction mechanism enabling long-term irreversible modifications of TE hydraulic properties during plant development.

Cellular interactions controlling TE function

The major differences in fluid dynamics between animal and plant vascular systems are due to (i) the fixed diameter of plant conduits and (ii) the irregular sap flow rate in plants due to external environmental conditions (temperature and humidity). In any conducting system, fluid flow rate must remain laminar otherwise causing cavitation leading to embolized conduits unable to fulfill vascular conduction. As xylem is an open vascular system, brutal environmental changes suddenly altering the sap flow rate increase the sensitivity of plants to embolism [5]. The recovery mechanism of embolized TEs relies exclusively on neighboring XPs which control the TE refilling process. The analysis of sap composition in embolized TEs of poplar revealed that XPs actively export sucrose, ions and H^+ into embolized TE lumen to locally decrease the sap Ψh causing massive water influx to 'flush out' air bubbles [52•] (Figure 2b). The release of sucrose associated with embolism recovery could derive from the XP plastidial starch reserves which have been shown to reduce concomitantly with TE refilling [53]. The local water influx for the refilling of TEs uses Plasma Intrinsic Proteins (PIPs) water channels enabling the water movement from the surrounding XPs [54,55] — appearing as droplets on embolized TE lateral walls [56]. The expression reduction or disruption of PIP genes in *Arabidopsis* and poplar increase vulnerability to TE embolism [54,55]. Furthermore, the XP-induced Ψh decrease during the refilling of embolized TEs will also modify the pectin hydrogel of TE pits causing an additional influx of water [57]. In the case of unrecoverable TE damage or vascular pathogen infection, XPs will decommission TEs by occluding TE lumen with tyloses outgrowing from neighboring XPs which have passed through TE pits [58,59]. Pectin-rich gels and gums accumulate around intruding tyloses to completely seal off TE lumen, completely abolishing the TE conducting function

[59]. The formation of XP-derived tyloses responds to vascular wilt pathogens invading the vascular network [60] but also responds to TE structural defect as shown in poplars genetically reduced in lignin which exhibit increased numbers of tylosed TEs [58]. XPs therefore control the fate of damaged TEs by either restoring or occluding their conducting function.

Conclusions

Although TEs are corpses, they are far from being an inert tube inadaptable to any changes. Their formation and function rely essentially on assistance of the surrounding XPs which modify both TE long-term structural characteristics and fine-tune TE conductivity in response to environmental changes (Figure 3). Knowledge about mechanisms enabling XPs to perceive the state and function of their neighboring TEs open new research perspectives to uncover the coordination between the multiple cell types of plant vascular tissue. As the plant xylem governs both plant nutrition and biomass production, tailoring the cellular interactions between XPs and TEs represents means for the optimization of agronomical plant nutrition and productivity in response to changing environmental conditions.

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- •• of outstanding interest

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