Update on Reactive Oxygen Species

Role of Reactive Oxygen Species during Cell Expansion in Leaves¹[OPEN]

Romy Schmidt, Alicja B. Kunkowska, and Jos H.M. Schippers*
Institute of Biology I, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

The conditions under which plants grow greatly fluctuate and require that plants continuously monitor their environment and adjust their developmental program accordingly. Recent advances have indicated a clear and distinct role for reactive oxygen species (ROS) in both environmental stress sensing and guiding plant development. Leaf growth is a flexible process in which the final shape and size of the organ is tailored to the environment. Both during development under controlled conditions as well as during abiotic stress, cell expansion in leaves is part controlled through the regulation of apoplastic ROS homeostasis. The effect of different ROS types on cell wall properties is well documented, but how plants control apoplastic ROS homeostasis is poorly understood. Furthermore, ROS appear to influence other cellular processes that guide cell expansion, including water uptake and cytoskeleton dynamics. Here, an overview of our current understanding on the role of ROS in leaf cell expansion is given and avenues for future research directions are highlighted.

ROS have long been documented as damaging molecules that especially accumulate during stress in plants. Abiotic stresses, such as drought and salinity, are characterized by an initial growth reduction of leaves and the induction of programmed cell death under prolonged stress conditions (Loggini et al., 1999; Hernández et al., 2001). The notion that alterations in the growth rate of leaves correlate with ROS homeostasis have paved the way for a more fundamental role of ROS in the regulation of plant development. Although during abiotic stress ROS levels rise, this does not necessarily apply to the growth zone of the leaf. For instance, studies on leaf expansion in maize (Zea mays) under saline conditions revealed that not an increase in ROS but a decrease caused retarded leaf growth (Rodríguez et al., 2004). Moreover, exposure of maize to salinity or drought causes an increase in the antioxidant capacity of the leaf and thereby restricts cell expansion (Bernstein et al., 2010; Kravchik and Bernstein, 2013; Avramova et al., 2015). Then again, an increase in ROS levels also can result in a restriction of cell growth under abiotic stress conditions (MacAdam and Grabber, 2002; Simonovicova et al., 2004), indicating that ROS have a dual role in the regulation of cell expansion. Still, ROS homeostasis does not act alone, as abiotic stresses like salinity, drought, or osmotic stress all interfere with the water balance and cause a reduction in cell turgor, which affects the mechanical power of the cell to expand (Schopfer, 2006).

The initial observations under abiotic stress have attracted a broad research interest in the relationship between cell growth and ROS. Nowadays, it is clear that ROS homeostasis does not only control growth under stress conditions but also during plant development. ROS, like hydrogen peroxide (H₂O₂), hydroxyl radicals (OH), and superoxide radicals (O₂⁻), have been implicated in developmental processes and emerged as key signaling molecules in plants (Schmidt and Schippers, 2015). For instance, during root hair and pollen elongation, ROS play fundamental roles in the spatial regulation of polar cell growth (Takeda et al., 2008; Kaya et al., 2014). Still, the role of ROS in the regulation of leaf development remains unclear. The leaf emerges at the flank of the shoot apical meristem and at first grows through active cell division (Beemster et al., 2005; Polyn et al., 2015; Schippers et al., 2016). The final size of the leaf is determined by the subsequent

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* Address correspondence to schippers@bio1.rwth-aachen.de.
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ADVANCES

- The differential regulation of cell wall extensibility by ROS is a fundamental and conserved feature to control plant cell size and shape.
- Apoplastic H₂O₂ is taken up by plant cells, resulting in potential downstream signaling events.
- Recent work shows that transcriptional regulation of ROS homeostasis is part of developmental programs that control organ size.
expansion of leaf cells, which accounts for up to 95% of final leaf area. Enlargement of leaf cells is driven by two major regulators: turgor pressure and cell wall dynamics (Gonzalez et al., 2012). In addition, cell expansion requires the synthesis and distribution of new biomaterials to sustain growth.

Developmental, biochemical, and abiotic stress studies have revealed the importance of ROS homeostasis in the apoplast for the regulation of cell expansion (Fry, 1998; Rodríguez et al., 2002; Lu et al., 2014; Avramova et al., 2015). Here, we highlight known and potential roles for ROS in the main processes that control cell enlargement during leaf growth.

**ROS IN THE APOPLAST: THE OXIDANT’S PLAYGROUND**

Cell expansion is the result of a delicate balance between wall relaxation and wall stiffening (Wolf et al., 2012). The primary cell wall consists out of cellulose, hemicellulose, pectin, and structural proteins (Geisler et al., 2008). In primary cell walls, the cellulose matrix is cross-linked by hemicellulose and pectin molecules, in such a fashion that it provides mechanical strength but still allows for cell expansion. Loosening of the cell wall is in part regulated by two classes of proteins (expansins and xyloglucan endotransglucosylases/hydrolases) that act on the interaction between xyloglucans, the major hemicellulose polymer, and the cellulose network (Park and Cosgrove, 2012). Studies on leaf expansion in maize under saline conditions revealed a decreased O$_2^-$-derived OH production in the apoplast and a consequent reduction in leaf growth (Rodriguez et al., 2004). Interestingly, it was found that OH promotes cell elongation by loosening the cell wall through oxidative cleavage of polymers like xyloglucan and pectin (Fry, 1998; Müller et al., 2009). In the presence of transition metals, the Haber-Weiss reaction can convert H$_2$O$_2$ and O$_2^-$ anions to OH, a reaction that can be performed both nonenzymatically as well as enzymatically (Chen and Schopfer, 1999). Although, H$_2$O$_2$ is a prerequisite for the formation of OH and thereby for oxidative cell wall loosening, a buildup of H$_2$O$_2$ causes cross-linking of the cell wall and restricts elongation growth (Schopfer, 1996). Indeed, during salt, drought, or osmotic stress, an increase in the level of apoplastic H$_2$O$_2$ has been noticed and was linked to the inhibition of leaf growth (Lin and Kao, 2001; Kravchik and Bernstein, 2013; Avramova et al., 2015). These observations argue that the apoplastic balance between OH and H$_2$O$_2$ regulates cell expansion during leaf growth, but how is ROS homeostasis regulated in the apoplast?

This fundamental question is complex to answer, as large apoplastic and membrane protein families contribute to ROS production (Fig. 1). Among the enzymes that contribute to apoplastic ROS homeostasis are peroxidases (POXs), amine oxidases, oxalate oxidases, and NADPH oxidases (Kärkönen and Kuchitsu, 2015). NADPH oxidases are plasma membrane-located enzymes that, in a Ca$^{2+}$-dependent manner, produce O$_2^-$ in the apoplast (Fig. 1). The O$_2^-$ dismutates into H$_2$O$_2$, which can cause cross-linking of the cell wall or is converted into the cell wall loosening OH (Richards et al., 2015). During biotic stress, NADPH oxidases produce an oxidative burst to eliminate invading pathogens and send stress signals (Suzuki et al., 2011); during abiotic stress, they appear to mainly act as stress signal amplifiers to promote adaptive responses. If NADPH oxidase-derived O$_2^-$ is required for cell wall stiffening by H$_2$O$_2$, then loss-of-function mutants would be expected to develop more elongated cells. In

**Figure 1.** Cell wall remodeling and apoplastic ROS homeostasis. ROS have a dual role in regulating cell expansion. NADPH oxidase-derived ROS can both promote as well as restrict cell wall extensibility. H$_2$O$_2$ derived from the dismutation of O$_2^-$ or produced through the action of cell wall-located POXs, polyanine oxidases (POA), or copper-containing amine oxidase (COA) results in dehydration and cross-linking of cell wall polymers. On the other hand, H$_2$O$_2$ and O$_2^-$ can act as substrates for the Haber-Weiss reaction or pH-dependent POXs, leading to the production of highly reactive OH. The OH results in cleavage of cell wall polymers and thereby promotes cell wall loosening. The pH-dependent formation of OH by POXs can operate in parallel to the pH-dependent activation of EXPANSINs (EXPs) to stimulate cell growth. ROS channeling from NADPH oxidases to POXs is mediated by CASP-LIKE proteins [CASP(L)s], allowing for control over the balance between H$_2$O$_2$ and OH production. In addition, apoplastic OH provokes activation of the Ca$^{2+}$ transporter ANN1. The influx of Ca$^{2+}$ might act as a feed-forward signal, since it can activate NADPH oxidases.
contrast, mutations in NADPH oxidases have been shown to impair the elongation of root hairs and pollen tubes (Foreman et al., 2003; Takeda et al., 2008; Kaya et al., 2014) and the growth of leaves (Chaouch et al., 2012). Thus, NADPH oxidases are positive regulators of cell growth. So, how can plants control the transition of NADPH oxidase-derived O$_2^-$ into OH to promote cell expansion? A recent report suggests that NADPH oxidase-derived ROS is channeled to apoplastic peroxidases during lignification in roots, by a protein scaffold that brings both proteins in close proximity (Fig. 1; Lee et al., 2013). NADPH oxidase/peroxidase scaffolds have evolved in animals as single proteins, the DUOX class of NADPH oxidases. Still, DUOX proteins are absent in plants, indicating a necessity for a flexible protein scaffold. The identified scaffold proteins belong to the CASPARIAN STRIP MEMBRANE DOMAIN PROTEIN (CASP) family, which, in Arabidopsis, has 20 members (Roppolo et al., 2014). Therefore, it would be interesting to determine which CASP-LIKE proteins might regulate ROS channeling from NADPH oxidases during leaf growth.

The secreted class III POXs in Arabidopsis (Arabidopsis thaliana) consists of 73 members with largely unexplored biochemical properties (Passardi et al., 2004). Early reports on cell growth revealed an inverse correlation between cell growth and POX activity (Fry, 1986; Goldberg et al., 1987). Also, during abiotic stress, POX activity increases and can cause lignification and rigidification of the cell wall, limiting leaf growth (Heggie et al., 2005; Lee et al., 1987). Also, during abiotic stress, POX activity is correlated with cell growth revealed an inverse correlation between cell growth and POX activity (Fry, 1986; Goldberg et al., 1987). Thus, the role of POXs in modulating cell expansion is an area of active research.

The accumulation of H$_2$O$_2$ due to the accumulation of H$_2$O$_2$ (Lu et al., 2007). In Arabidopsis, several POX genes have been linked to the regulation of cell expansion during leaf development. Plants overexpressing POX57 or POX71 show a reduced leaf size due to the accumulation of H$_2$O$_2$ (Lu et al., 2014; Raggi et al., 2015). Interestingly, both POXs only affect cell expansion, but not proliferation, indicating that POXs might be specifically employed during the regulation of cell growth. However, not all apoplastic POXs generate H$_2$O$_2$; some do the opposite while others convert H$_2$O$_2$ and O$_2^-$ into OH (Chen and Schopfer, 1999; Liszky et al., 2003). The enzymatic generation of OH in the apoplast by POXs requires a low pH, which can be achieved through the action of auxin (Schopfer et al., 2002). Still, it is not known which of the 73 class III POX proteins contribute to OH formation.

The accumulation of OH in the apoplast activates the atypical Ca$^{2+}$-permeable ANNEXIN1 (ANN1) channel (Laobavisit et al., 2012). Studies on ANN1 in Arabidopsis indicate that loss-of-function mutants have reduced vegetative growth (Konopka-Postupolska et al., 2009). The influx of Ca$^{2+}$ might act as an amplification mechanism for the production of OH, as it activates NADPH oxidases (Renew et al., 2005). Intriguingly, ANN1 is a multifunctional protein that also exhibits peroxidase activity (Gorecka et al., 2005). As stated above, a low pH can promote OH production by several apoplastic POXs, suggesting that ANN1 might also contribute to the production of apoplastic ROS. This assumption would be worth to test as it would place the action of ANN1 into a different perspective, allowing for a feed-forward model in which NADPH oxidase-dependent OH production is amplified by the activation of ANN1.

Next to NADPH oxidases and POXs, other enzymes also contribute to ROS homeostasis in the apoplast. Both amine and oxalate oxidases have a major impact on apoplastic H$_2$O$_2$ production (Kärkönen and Kuchitsu, 2015). Two types of amine oxidases, copper-containing oxidases and polyamine oxidases, catalyze the oxidative deamination of di- and polyamines, resulting in the formation of H$_2$O$_2$ (Ghuge et al., 2015). To what extent these enzymes contribute to growth regulation during development still needs to be further explored.

Among the large number of proteins that control ROS homeostasis at the cell wall, several have now been demonstrated to have a role in regulating cell expansion during leaf growth. Still, additional research efforts are needed to understand how a cell manages to produce ROS in a specific and timely manner to control cell growth. The recent discovery of potential protein scaffolds that modulate ROS channeling at the apoplast represent attractive new avenues for future research efforts.

### WATER UPTAKE AND TRANSPORT OF H$_2$O$_2$

Leaf growth relies on active water uptake through controlled transport across membranes (Steudle and Frensch, 1996). Water uptake by cells from the apoplast is facilitated by aquaporins (AQPs). Interestingly, AQPs contribute to the permeability of lipid membranes for a variety of neutral molecules, which are, next to water itself, carbon dioxide and H$_2$O$_2$ (Bienert and Chaumont, 2014). Thus, movement of apoplastic H$_2$O$_2$ relies on an active transport system, which might serve several functions (Fig. 2). For several AQPs, roles in leaf growth have been demonstrated (Chaumont et al., 1998; Lee et al., 2012). Overexpression of PIP1;2 from Arabidopsis in tobacco (Nicotiana tabacum) speeds up whole-plant growth and increases leaf dry matter accumulation under non-stress conditions. Thus, water transport via AQPs represents a rate-limiting step for growth (Aharon et al., 2003).

The transport of H$_2$O$_2$ across membranes via plant AQPs was first demonstrated using yeast (Bienert et al., 2007; Dynowski et al., 2008). Expression of Arabidopsis PIP2;1, PIP2;4, TIP1;1, or TIP1;2 increased intracellular accumulation of H$_2$O$_2$ (Bienert et al., 2007). The comparable dipole moment and molecular diameter of H$_2$O$_2$ and H$_2$O allow these molecules to undergo hydrogen bonds with residues at the inner surface of the AQP pore (Bienert and Chaumont, 2014). Consistently, mutation of a cytosolic His residue within AtPIP2;1 impedes both water transport and H$_2$O$_2$ uptake (Dynowski et al., 2008).

Considering the negative role of H$_2$O$_2$ on cell wall extensibility, it was assumed that H$_2$O$_2$ might also interfere with AQP activity. Indeed, H$_2$O$_2$ treatment of the alga Chara corallina significantly reduced the water permeability of the plasma membrane (Henzler et al., 2004). Interestingly, AQPs contain redox-sensitive Cys residues that form disulphide bonds between PIP monomers, which could be potential targets of H$_2$O$_2$ to deactivate the channel (Bienert et al., 2014). Thus, NADPH oxidase/peroxidase scaffolds have evolved in animals as single proteins, the DUOX class of NADPH oxidases. Still, DUOX proteins are absent in plants, indicating a necessity for a flexible protein scaffold. The identified scaffold proteins belong to the CASPARIAN STRIP MEMBRANE DOMAIN PROTEIN (CASP) family, which, in Arabidopsis, has 20 members (Roppolo et al., 2014). Therefore, it would be interesting to determine which CASP-LIKE proteins might regulate ROS channeling from NADPH oxidases during leaf growth.

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et al., 2012). However, mutation of these conserved Cys residues into redox-insensitive residues did not affect the channeling activity of maize PIPs under oxidizing or reducing conditions in oocytes (Bienert et al., 2012). Still, to determine whether this is also the case in plant cells, one should complement specific 

PIPs move into or out of membrane microdomains, allowing a dynamic distribution between the plasma membrane and intracellular compartments to control water permeability of the cell (Boursiac et al., 2008; Wudick et al., 2015). Abiotic stress conditions, which result in increased $\text{H}_2\text{O}_2$ levels, promote PIP endocytosis. Moreover, intracellular accumulation of PIPs in Arabidopsis upon salt stress was prevented in the presence of catalase (Boursiac et al., 2008). Furthermore, a phosphoproteomics study points to oxidative stress-induced posttranslational modifications of PIPs that initiate endocytosis (Prak et al., 2008). Differential phosphorylation of two Ser residues within the C terminus of AtPIP2;1 upon $\text{H}_2\text{O}_2$ treatment correlated with redistribution of AQPs from the plasma membrane to intracellular vesicles (Prak et al., 2008). A lower PIP abundance at the plasma membrane ultimately results in diminished water transport through the membrane (Wudick et al., 2015). Thus, $\text{H}_2\text{O}_2$ not only restricts cell expansion by cross-linking the cell wall, but potentially also reduces water transport across the membrane, by promoting the endocytosis of PIPs (Fig. 2).

**Figure 2.** $\text{H}_2\text{O}_2$ modulates water uptake by aquaporins. Turgor pressure-driven cell expansion is controlled by AQPs, which facilitate the transport of $\text{H}_2\text{O}$ across the plasma membrane. Uptake of extracellular $\text{H}_2\text{O}_2$, derived from either NADPH oxidases or apoplastic POXs, across the membrane is regulated by AQPs. In the apoplast, a buildup of $\text{H}_2\text{O}_2$ limits cell growth by cross-linking the cell wall. In addition, the influx of $\text{H}_2\text{O}_2$ might also limit the water uptake as it appears to activate the endocytosis of AQPs, regulating their abundance at the plasma membrane. Thus, apoplastic $\text{H}_2\text{O}_2$ could cause both cell wall stiffening and the inhibition of water uptake.

**ROS AND THE REGULATION OF THE CYTOSKELETON AND VESICLE TRANSPORT DURING EXPANSION**

Cell size and shape is controlled by the extension of the cell wall (Smith and Oppenheimer, 2005). The building blocks for cell growth are delivered by Golgi-derived vesicles that are transported to the cell surface through the action of the cytoskeleton. The cytoskeleton allows for controlling the growth direction of cells by guiding the delivery of Golgi-derived vesicles and the cellulose synthase machinery (Geisler et al., 2008). Uniform cell enlargement in all directions is called isotropic expansion (Fig. 3A), while cell growth along a preferred axis, or in a preferred direction, is known as anisotropic expansion (Crowell et al., 2010). During leaf growth, mesophyll cells mainly show isotropic expansion while epidermal cells show mainly anisotropic expansion (Fu et al., 2002). Still, in single cells, both isotropic and anisotropic growth can occur at the same time (Crowell et al., 2010).

The importance of microtubules (MTs) and actin filaments in the regulation of cell expansion has been widely demonstrated. For leaf development, ACTIN2 and ACTIN7 also are required for optimal growth (Gilliland et al., 2002) together with numerous tubulin genes (Ishida et al., 2007; Komis et al., 2011). In contrast to its name, the cytoskeleton consists of a highly dynamic and mobile network of protein polymers. MTs and actin filaments exhibit a treadmilling movement caused by a fast rate of net polymerization at the plus
end and a slower rate of depolymerization at the minus end (Blanchoin et al., 2010). Interestingly, microtubules and actin filaments are known to be very sensitive to ROS, and emerging evidence suggests that redox cues impact on cytoskeleton dynamics (Livanos et al., 2012). Chemical interference with ROS homeostasis results in the reorientation and replacement of MTs in plants. For instance, NADPH oxidase activity has been shown to modulate MT and actin polymerization upon abiotic stress or treatment with cytoskeletal toxins (Yao et al., 2011; Liu et al., 2012). Interestingly, tolerance of plants toward cold stress is associated with the stability of microtubules (Wang and Nick, 2001). Plants that maintain microtubule stability show cold-resistant leaf expansion (Ahad et al., 2003). Oxidative stress as part of abiotic stress can result in oxidative modifications of tubulin and actin proteins (Fig. 3B; Wang et al., 2012) and reorientation of the cytoskeleton.

Next to a direct effect of H$_2$O$_2$ on cytoskeleton polymers, it also modulates the activity of microtubule/actin-associated proteins (MAPs; Fig. 3B). MAPs are direct targets of MAPK cascades (Komis et al., 2011), of which some have a role in ROS signaling (Schmidt and Schippers, 2015). Arabidopsis Nucleus- and Phragmoplast-Localized Kinase1-Related Protein Kinase1 (ANP1), ANP2, and ANP3 encode MAP3Ks that are activated by H$_2$O$_2$, and initiate a signaling cascade involving MKK6 and the MAPKs MAPK3, MAPK4, and MAPK6 (Kovtun et al., 2000; Beck et al., 2010). ANPs are required during cytokinesis, but also regulate pavement cell expansion, since anp2anp3 mutants show aberrant epidermal cell morphology (Beck et al., 2010). Notably, downstream the MAPK cascade act two conserved key regulators of cortical MT array organization, the microtubule-associated proteins MAP65-1 and MAP65-2, which both have been implicated in ROS responses (Lucas et al., 2011; Zhu et al., 2013; Livanos et al., 2012). Potentially, H$_2$O$_2$ sensed by the ANP cascade modulates cytoskeleton dynamics during cell proliferation and expansion (Fig. 3B). Additional research efforts are needed to understand the importance of the ANP cascade in ROS signaling during cell growth.

The Golgi is a highly diverse organelle with roles in many different physiological and molecular processes within the plant, including cell growth (Fu et al., 2002). Still, a potential role of the Golgi in oxidative stress responses in plants was presented only recently (Lee et al., 2015). The trans-Golgi network regulates cell expansion by directing vesicle trafficking and targeting (Gendre et al., 2011). Secretion of pectin and hemicellulose, the two major cell wall polysaccharides,
is controlled by a trans-Golgi network-localized complex formed by YPT/RAB GTPase Interacting Protein 4a (YIP4a), YIP4b, and ECHIDNA (ECH; Gendre et al., 2013). In addition, the vacuolar H⁺-ATPase subunit a₁ (VHAa1) colocalizes with the ECH/YIP4 complex. As chemical inhibition of VHAa1 activity mimics ech/yip4 mutants, the proteins appear to act in the same pathway (Gendre et al., 2011). Since VHAa1 activity is redox regulated (Tavakoli et al., 2001), it might represent an integration site for ROS within the trafficking network. Treatment of Arabidopsis with H₂O₂ results in Cys oxidation of VHA subunits (Waszczak et al., 2014), which inactivates their H⁺-pumping activity (Tavakoli et al., 2001). Thus, oxidative stress might act as a brake on vesicle trafficking, which could hamper cell expansion. The mobile secretory vesicle compartments (SVCs) move toward the cell surface to interact with the exocyst complex (Hála et al., 2008) and deliver cell wall material during growth. Rho of plant (ROP) GTPases, together with their effector proteins, have been shown to interact with exocyst subunits to mediate directed cell growth (Lavy et al., 2007; Uhrig and Hülskamp, 2014). Next to a role for ROP proteins in vesicle trafficking, they have also been implemented in the regulation of actin and MTs during leaf pavement cell morphogenesis (Fu et al., 2002; Oda and Fukuda, 2012). On the one hand, activated ROP2/4 promotes the formation of F-actin networks required for the lateral outgrowth of a lobe (Fu et al., 2005); on the other hand, activation of ROP6 promotes the MT-severing activity of KATANIN1 (KTN1), which results in the reassembly and alignment of MTs to restrict cell growth in the neck regions of the developing pavement cell (Fig. 3, B and C). Furthermore, NADPH oxidases are activated by a direct interaction with ROP proteins, indicating an intimate relation between the regulation of ROS homeostasis and the diverse functions of ROP during cell elongation (Wong et al., 2007). How H₂O₂ and NADPH oxidase activity regulate cytoskeleton dynamics and vesicle trafficking is poorly understood and requires extensive research efforts in the future.

TRANSCRIPTIONAL REGULATION OF ROS HOMEOSTASIS DURING LEAF EXPANSION

Under nonstress conditions, the final size of plant leaves is predictable due to the strict genetic control of cell proliferation and expansion. Although many transcription factors regulating cell proliferation have been identified (Polyn et al., 2015), those controlling leaf expansion growth are largely lacking (Powell and Lenhard, 2012).

Thus far, only two transcription factors specifically regulating leaf cell expansion were reported: KUODA1 (KUA1) and ZHD5 (Lu et al., 2014; Hong et al., 2011). ZHD5 belongs to the group of zinc finger homeodomain (ZHD) transcription factors and its activity is regulated by a mini zinc finger protein (MIF1). Scanning electron microscopy of epidermal cells of ZHD5 overexpression lines revealed bigger cell sizes compared to the wild type (Hong et al., 2011). Overexpression of the non-DNA binding MIF1 causes a great decrease in leaf area due to the inhibition of ZHD5 (Hong et al., 2011; Hu et al., 2008). Still, the factors causing cell enlargement and the direct targets of ZHD5 are not known (Hong et al., 2011).

KUA1 is a MYB-like transcription factor that is upregulated during expansion growth of the leaf (Lu et al., 2014). Overexpression of KUA1 results in an increase in leaf cell size compared to the wild type, while kua1 mutants show a decrease in cell size. Detailed analysis revealed that KUA1 negatively regulates POX activity by direct repression of seven POX genes during leaf growth (Lu et al., 2014). As mentioned above, class

Figure 4. Transcriptional regulation of ROS homeostasis during leaf expansion. Cell expansion is restricted by cell wall stiffening due to the action of H₂O₂. During cell expansion in Arabidopsis, the levels of apoplastic H₂O₂ are maintained low through the action of the transcription factor KUA1. KUA1 represses the expression of seven POX genes (POX7, POX8, POX10, POX30, POX35, POX44, and POX57), which produce H₂O₂ in the apoplast. The repression of the POX genes is crucial for cell wall relaxation and expansion.
III POXs can promote cell wall loosening or cross-linking depending on the apoplastic conditions. KUA1-regulated POXs increase apoplastic H$_2$O$_2$ levels, which restricts expansion (Fig. 4). Therefore, KUA1 positively regulates leaf growth by lowering the levels of apoplastic H$_2$O$_2$ and promoting cell wall relaxation (Lu et al., 2014). Still, as H$_2$O$_2$ acts as a precursor for the production of OH, it is likely that the regulation of POX activity within the cell wall is more complex.

Interestingly, in roots, a transcription factor of the bHLH family similarly acts on POX gene expression. UPBEAT (UPB1) is mainly expressed in the root elongation zone and its overexpression causes a decrease in root cell size, but increased cell proliferation, resulting in longer roots compared to the wild type (Tsukagoshi et al., 2010). Among the direct target genes of UPB1, 21 encode POXs, including POX57, which is also targeted by KUA1 in leaves (Tsukagoshi et al., 2010; Lu et al., 2014). While KUA1-regulated POXs promote cell wall stiffening in leaves, the POXs controlled by UPB1 in roots mainly cause cell wall relaxation by either scavenging H$_2$O$_2$ or promoting the formation of OH. In both cases, loss of the transcriptional regulators results in increased H$_2$O$_2$ levels and decreased cell size, indicating that the biochemical action of H$_2$O$_2$ in roots and leaves on cell enlargement is conserved. Notably, UPB1 was shown to act independently from plant hormones, while overexpression of KUA1 is accompanied by an increase in auxin (Kwon et al., 2013). As KUA1 and UPB1 show no homology, it indicates that plants evolved diverse transcriptional regulators to control POXs.

So far, to our knowledge, KUA1 is the first described transcription factor with a clearly defined role in modulating leaf cell expansion through the regulation of ROS homeostasis. In accordance with the successful identification of transcription factors controlling cell proliferation (Polyn et al., 2015), it is expected that future research will reveal additional transcriptional regulators of leaf cell expansion.

CONCLUSIONS AND PERSPECTIVES

Since the initial discovery that ROS play pivotal roles in the regulation of root hair and pollen tube elongation, a picture of the complex interplay between ROS and growth is starting to emerge. That said, here, we have specifically highlighted our current understanding of the regulation of cell expansion by ROS homeostasis during leaf growth. Although the exact molecular details are incomplete, it is clear that ROS are implemented in all processes that control leaf expansion. The modulation of cell wall extensibility and water uptake by ROS are convincing examples of how fine-tuning of ROS signaling modulates growth. Still, our current understanding only hints to a role of ROS in cytoskeleton dynamics and vesicle trafficking (see Outstanding Questions). Nonetheless, the field of ROS-regulated development foresees a fruitful future with many novel molecular mechanisms and transcriptional regulators to be identified that will provide fundamental understanding of the regulation of multicellular development.

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OUTSTANDING QUESTIONS

- Although several apoplastic and plasma membrane-bound proteins with a role in ROS homeostasis have been linked to growth processes, a systematic analysis of all these proteins is required to fully understand the impact of apoplastic ROS homeostasis on plant development.
- Does ROS channeling occur in the apoplast? Recent work suggests that NADPH oxidase-derived superoxide is conveyed to apoplastic peroxidases through scaffold proteins.
- Exploring the interaction between cytoskeleton dynamics and ROS may reveal fine-tuning of molecular mechanisms during cell growth.
- While transcriptional networks regulating plant growth have been in part well defined, it is largely unknown how these modulate ROS homeostasis to guide cell growth.
Role of ROS in Cell Growth


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