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Review

ROS-mediated redox signaling during cell differentiation in plants ☆

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ABSTRACT

Background: Reactive oxygen species (ROS) have emerged in recent years as important regulators of cell division and differentiation.
Scope of review: The cellular redox state has a major impact on cell fate and multicellular organism development. However, the exact molecular mechanisms through which ROS manifest their regulation over cellular development are only starting to be understood in plants. ROS levels are constantly monitored and any change in the redox pool is rapidly sensed and responded upon. Different types of ROS cause specific oxidative modifications, providing the basic characteristics of a signaling molecule. Here we provide an overview of ROS sensors and signaling cascades that regulate transcriptional responses in plants to guide cellular differentiation and organ development.
Major conclusions: Although several redox sensors and cascades have been identified, they represent only a first glimpse on the impact that redox signaling has on plant development and growth.
General significance: We provide an initial evaluation of ROS signaling cascades involved in cell differentiation in plants and identify potential avenues for future studies. This article is part of a Special Issue entitled Redox regulation of differentiation and de-differentiation.

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1. Introduction

The role of reactive oxygen species (ROS) as signaling molecules is conserved between all aerobic organisms [1–3]. Both anaerobic metabolism and aerobic metabolism involve oxidation–reduction (redox) reactions, however, the switch to oxygen-based metabolism greatly promoted these reactions in aerobes and resulted in the production of ROS from electron-transfer reactions with oxygen [2]. As maintaining redox homeostasis is essential, cells evolved sophisticated scavenging mechanisms to detoxify ROS [3]. This in turn might have been the key to employ certain ROS as signaling molecules to monitor internal and external conditions to guide and fine-tune cellular responses. As many proteins are redox sensitive [4–6], they might form the basic components for ROS sensing and signaling networks throughout the cell. Still, the signaling pathways that harness the reactivity of ROS are still rather poorly explored in plants.

At first glance, the reactive nature of ROS renders them indiscriminate and unsuitable as signaling molecules [7]. The prerequisite for a messenger is a specific interaction (non-covalent binding) with an effector (receptor) molecule. Plant cells produce different types of ROS with type-specific properties and origin sites within the cell. In the light, both the chloroplast and the peroxisome are the main sites of ROS production in plants [8]. Photoreduction of O_2 in the chloroplasts results in the formation of singlet oxygen ($^1O_2$) at photosystem II [9] and superoxide ($O_2^{\bullet -}$) at photosystems I and II [10,11]. High levels of hydrogen peroxide (H_2O_2) are produced in peroxisomes by the glycolate oxidase during photorespiration [12] and through β-oxidation of long-chain fatty acids [13]. The protein complexes that form the mitochondrial electron transport chain (mtETC) are prone to generate $O_2^{\bullet -}$ as a byproduct during energy metabolism [14]. In the endoplasmic reticulum (ER), H_2O_2 is produced during oxidative protein folding [15]. Next to that, plant cells produce apoplastic ROS in the form of $O_2^{\bullet -}$ by plasma membrane-located NADPH oxidases [16] and H_2O_2 through the action of class III peroxidases [17,18]. Of note, $O_2^{\bullet -}$ readily dismutates to H_2O_2, either enzymatically or non-enzymatically [19,20]. Furthermore, in the presence of reduced transition metal ions, e.g. Ti^{3+}, Fe^{2+} or Cu^{+}, H_2O_2 will give rise to highly reactive hydroxyl radical (OH•) via the Fenton and Haber–Weiss reactions [21]. Although cells also produce reactive nitrogen species, we focus in this review only on the sensing and roles of ROS.

Gene expression studies on plants treated with various types of ROS clearly show that each provokes unique and overlapping transcriptional responses [22–25], indicating that each ROS type is perceived specifically. These distinct responses can be attributed to the inherited chemical nature of each ROS type, including reactivity, lifetime and target specificity. In Fig. 1, an overview of the distinct properties of ROS is presented. Singlet oxygen, produced through the light reactions in the chloroplast or via the Haber–Weiss reaction [26] has a lifetime of 3.0–3.5 μs and can travel up to 150 nm [27]. Similarly, the unstable

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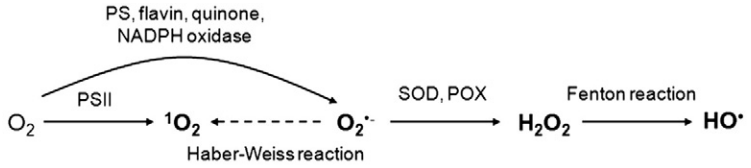

Properties				
Lifetime (μs)	3.0 - 3.5	2.0 - 4.0	> 1000	0.001
Target range (nm)	150	50 - 500	> 1000	0.001
Targets				
DNA	+	-	-	++
Lipids	PUFA	-/+	-/+	++
Carbohydrates	-	-	-	++
Protein	+	Fe-S	Cys	++

Fig. 1. Common ROS and their biochemical properties. The scheme above the table indicates the (enzyme-dependent) formation of the different ROS types from molecular oxygen. These are the superoxide anion ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO•). Abbreviations: PS, photosystem; SOD, superoxide dismutase; POX, peroxidase.

$O_2^{\bullet-}$ has a short lifetime of 2.0–4.0 μs [28] and, depending on the cellular environment, can travel 50–500 nm [29,30]. In addition, for H_2O_2 it has been shown that it can travel across the membrane through the action of aquaporins [31], underlining its role as a suitable molecule for cell-to-cell signaling [32]. The in vivo lifetime of OH• is around 1 ns, which limits diffusion to very short distances of less than 1.0 nm [33]. The stability and distance that the different ROS can travel are important aspects for a signaling molecule. Still, the key feature of a signaling molecule is the ability to specifically interact with a target. Interestingly, inside a cell, ROS are maintained at a low level, which facilitates specific reactions at the atomic level with cellular targets. Among ROS, HO• appears to be unsuitable as a signaling molecule, since it readily reacts with all types of cellular components, including nucleic acids, lipids, proteins and carbohydrates. Nonetheless, HO• has biological significance as it appears to cooperate in loosening of the cell wall and the transport of Ca^{2+} and K^+ at the plasma membrane [33,34]. Interestingly, the other ROS appear to have a preferred cellular target with which they react (Fig. 1) besides general scavenging compounds. Although $^1O_2$ is quite reactive, its preferred targets are polyunsaturated fatty acids (PUFA) [35]. Especially linoleic acid (18:2) and linolenic acid (18:3), constituting the major fatty acids in plant membrane galactolipids (thylakoid membrane) and phospholipids (all other membranes) [36], are particularly susceptible to $^1O_2$. Lipid peroxidation in leaves by $^1O_2$ results in the formation of different types of hydroxyoctadecatrieonic (HOTE) lipid hydroperoxides, including 10-HOTE and 15-HOTE [37]. In contrast, $O_2^{\bullet-}$ and H_2O_2 modify proteins, whereby the first reacts with iron–sulfur clusters ([Fe–S] clusters) and the latter with Cys residues [7]. This target preference is exploited by bacteria that evolved specific one-component signaling systems to discriminate and regulate distinct responses towards these two types of ROS [1]. The specific action of ROS on single amino acids (the atomic level) and co-factors implies that a huge number of proteins can act as ROS-specific receptors. In contrast to classical hormone signaling pathways, which have distinct receptors and primary response factors, redox signaling is extremely complex. Even so, as different ROS provoke specific responses, the signaling network must be highly coordinated and specialized.

Here we focus on ROS signaling mechanisms that result in transcriptional control over plant cellular differentiation and development. We emphasize the mechanistic features and pathways underlying redox sensing. Furthermore, as the understanding of redox signaling in differentiation in plants is still at its initial stage, we highlight current findings, potential mechanisms and pathways.

2. Molecular ROS sensing and signaling mechanisms

The hallmark for ROS sensing and signaling is the paradigm of $O_2^{\bullet-}$ and H_2O_2 sensing and signaling in bacteria. Two redox-sensitive transcription factors (TFs) were found to specifically respond to each of the two different ROS. The first, SoxR, is an Fe–S cluster-containing redox-sensitive TF that acts as a homodimer, and upon oxidation of the Fe–S cluster becomes transcriptionally active [38]. The second redox-sensitive TF, OxyR, is activated through the formation of a disulfide bond upon oxidation with H_2O_2, and is deactivated through enzymatic reduction by glutaredoxin [39]. Both $O_2^{\bullet-}$ and H_2O_2 sensing occur through reversible protein modification.

In contrast to prokaryotes, the eukaryotic cell is highly organized with specialized compartments, including the nucleus, peroxisome, mitochondria, and in plants the chloroplast. Whereas in bacteria ROS signaling appears to rely mainly on one-component systems, for example a redox-sensitive transcriptional regulator (Fig. 2), in eukaryotes redox signaling pathways tend to be more complex. In both cases, a redox sensor delivers information about ROS levels into a regulatory network resulting in a cellular response. In principle, two types of redox sensors exist, static and kinetic [40]. The kinetic sensor is an integral part of a thiol-disulfide/ROS redox cascade with high substrate affinity, while a static sensor is synchronized with the redox state of another redox component and itself does not participate in the electron flow [40].

One-component redox signaling systems are based on redox-sensitive TFs. On the other hand, non-redox-sensitive TFs involved in redox signaling function as output for upstream redox-sensitive signaling pathways. These pathways contain as an initiator a redox-sensitive component. One of the best studied upstream signaling cascades that can directly modulate TF activity is the mitogen activated protein kinase (MAPK) cascade (Fig. 2) [1]. The MAPK cascade is a well-defined integrator of ROS signals in plants, yeast and animals but is not found in prokaryotes [41–43]. Although MAPK cascades are generally accepted as ROS signaling pathways, it has remained unclear if a MAPKKK can function as a redox sensor, or if additional upstream factors activate the MAPKKK in a redox-dependent manner. In conjunction, protein phosphatases (PPs), which can next to TFs also target MAPK proteins [44,45], appear to be deactivated upon a ROS burst. Recently, it was found that the proteolytic-controlled release of the membrane-bound TFs ANAC053 and ANAC089 occurs in a redox-dependent manner [46,47]. Another mechanism covering redox signaling is initiated through lipid peroxidation by $^1O_2$ causing the formation of HOTE. Lipid

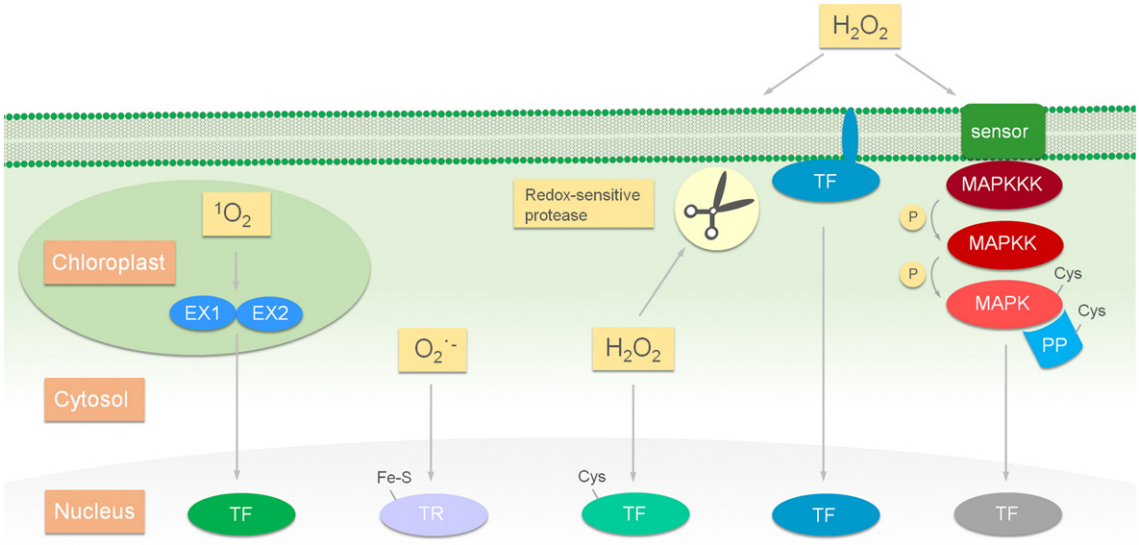

Fig. 2. Schematic representation of ROS signaling pathways. The figure depicts one-component ROS signaling systems and multi-component systems. In all cases, effector proteins convert the redox signal into a transcriptional response. From left to right: chloroplasts represent the main site of $^1O_2$ production in the cell. Transcriptional responses induced by $^1O_2$ depend on the activity of EXECUTOR1 (EX1) and EX2. Two types of one-component ROS cascades exist, those for $O_2\bullet^-$ contain an Fe–S cluster (transcriptional regulator, TR) and those for H_2O_2 have a redox-sensitive Cys residue. In other cases, redox sensor proteins transmit a ROS signal through a signaling cascade containing multiple components. Depicted here are two examples, the first involves a redox-sensitive protease that releases a membrane-tethered transcription factor (TF) through proteolytic cleavage. In some cases, oxidation promotes cleavage by the protease and in other cases the protease is only active under reducing conditions. Another example of a ROS-related signaling pathway involves the MAPK cascade. Typically, MAPKKK is located at the plasma membrane and interacts with a ROS sensor protein. Upon a ROS signal, the MAPKKK is activated and phosphorylates a MAPKK, which in turn phosphorylates a MAPK. Here the MAPK is kept in an inactive state through the action of a redox-sensitive protein phosphatase (PP). The activated MAPK phosphorylates a TF and thereby induces a transcriptional response.

hydroperoxides appear to be linked to the activity of two redundant chloroplastic proteins [48], EXECUTER 1 (EX1) and EX2, which subsequently transmit a signal to the nucleus resulting in a transcriptional response (Fig. 2).

2.1. Redox sensing by Fe–S cluster-containing proteins

Although in prokaryotes a clear role for Fe–S cluster-containing redox-sensitive TFs in sensing and signaling of $O_2\bullet^-$ has been established, an equivalent system in eukaryotes is still elusive. To date, around 100 Fe–S cluster-containing proteins have been identified in plants, which mainly play a role in energy metabolism, phytohormone and coenzyme synthesis, DNA repair and translation [49]. As the occurrence of an Fe–S cluster cannot be predicted from the primary amino acid sequence, it is expected that many more Fe–S proteins are to be discovered.

Arguably, the relative short lifetime of $O_2\bullet^-$ and the high level of compartmentalization in the eukaryotic cell might restrict its diffusion and therefore limit its signaling role to being merely a precursor of H_2O_2. Still, the occurrence of CuZn-SOD in the nuclei of spinach leaves indicates that $O_2\bullet^-$ is present within this compartment [50]. Next to that, Fe–S proteins are found in the plant nucleus, suggesting that $O_2\bullet^-$ might directly affect nuclear processes including transcription. Among the Fe–S proteins localized in the nucleus is a family of DNA glycosylases that act at the epigenetic level to regulate transcription through demethylation of DNA [51]. As changes in DNA methylation are commonly observed during biotic and/or abiotic stresses [52], it would be interesting to determine to which extend the stress-induced DNA methylation pattern is a consequence of a change in the redox status of the cell.

2.2. Reversible oxidative modification of proteins

The most common reversible oxidative modification is the formation of a disulfide bond through the oxidation of Cys residues by H_2O_2 [4]. Reversible ROS-induced thiol modifications were initially found as detoxification mechanisms for peroxides by 2-Cys-peroxiredoxins [53]. In general, the reaction of ROS with a Cys residue causes the formation of sulfenic acid (Cys-SOH), an unstable oxidation intermediate [4]. The Cys-SOH residue readily reacts with nearby Cys residues, resulting in the formation of inter- or intramolecular disulfide bonds [54]. Furthermore, Cys-SOH residues can react with GSH causing protein glutathionylation. Both the formation of a disulfide bond and glutathionylation are reversible protein modifications which can alter protein conformation and/or protein properties. In contrast to phosphorylation, glutathionylation causes a more profound structural change as a bulky tripeptide and two negative charges are added to the protein [54]. Similarly, oxidative modification of Met, resulting in the formation of methionine sulfoxide (Met-SO), has a profound effect on protein conformation and activity [55]. The reversion of oxidized Cys and Met residues relies on the action of two types of conserved thiol oxidoreductases, the thioredoxins (TRXs) and glutaredoxins (GRXs) [56], although it has to be noted that, for the reversion of oxidized Met, a specific Met-SO reductase cooperates with TRX and GRX [55]. TRX and GRX reduce target proteins by initiating a thiol-disulfide exchange reaction, causing their own oxidation. Reduction of either GRX or TRX goes at the expense of NADPH or ferredoxin [1,56]. Recently, a sulfenomemining study in *Arabidopsis* was performed using a novel probe to trap sulfenylated proteins in vivo, revealing that at least 100 proteins are specifically oxidized upon H_2O_2 treatment [57]. This initial study already indicates that many proteins are redox sensitive, and that in one way or the other they might all be candidate redox sensors. However, one should differentiate here between general redox sensing and redox signaling. Although both rely on reversible protein modifications, redox sensing is a means to adjust protein activity throughout the cell while redox signaling involves a discrete specific cue that activates or deactivates a pathway in a redox-dependent manner. Below we use the term redox sensor specifically for proteins that are activated or deactivated by a redox signal to provoke a transcriptional response. For this we will first focus on characterized plant

redox-sensitive TFs, which represent the one-component system. Next to that, we specifically discuss several other potential redox signaling cascades that form multi-component systems.

2.2.1 . One-component system, redox-sensitive transcription factors

Transcriptional control is central to the adaptation of a plant towards a change in the cellular redox state. TFs represent the molecular hardware through which a gene regulatory network can be modulated [58]. Efforts to characterize the role of TFs in different developmental or physiological processes have resulted in an impressive amount of identified key regulators. Still, the molecular mechanisms that control the activity of a specific TF are less well explored. Here we give an overview of TFs that are sensitive to post-translational modifications induced by the redox status. These redox-sensitive TFs can act as redox sensors and thereby directly transmit a ROS signal into the transcriptional regulatory network of plants.

The potential regulatory function of the redox status on the activity of TFs in plants was, among others, initially demonstrated for two sunflower homeodomain TFs [59]. One TF belongs to the HD-zip II family while the other is a member of the HD-zip IV family, which contain conserved Cys residues in their dimerization domains. Under reducing conditions, the proteins are present as monomers and bind DNA; however, upon oxidation, dimers are formed and the TFs can no longer bind DNA. Interestingly, DNA binding of both redox-sensitive TFs is promoted in the presence of TRX, linking their action to the cellular redox network. In analogy, redox regulation for *Arabidopsis* HD-zip III class homeodomain proteins was demonstrated to rely on two conserved Cys residues [60]. The authors showed redox regulation for PHAVOLUTA (PHV) (Table 1), a redox-sensitive TF involved in embryo patterning, organ polarity, vascular development, and meristem function [61]. Of note, HD-zip III redox-sensitive TFs, including REVOLUTA (REV) and PHABULOSA, contain at their C-terminus a PAS-like domain [62]. PAS-like domains can function among others as ROS sensors through redox-sensitive Cys residues, and the one found in REV was shown to act as a negative regulator of its activity [63]. Next to that, redox sensitivity of a subset of plant R2R3 MYB TFs depends on the presence of a pair of conserved Cys residues in the R2 domain [64]. Under oxidizing conditions, the Cys residues form an intramolecular disulfide bond inhibiting DNA binding. The effect of this modification for *Arabidopsis* R2R3 MYB TFs has not been demonstrated so far. On the other hand, MYB2, an *Arabidopsis* TF containing a single Cys residue in its R2 domain, is sensitive to redox modification by nitric oxide [65]. Nitric oxide results in S-nitrosylation of the Cys residue inhibiting DNA binding of MYB2. The TEOSINTE BRANCHED1-CYCLOIDEA-PROLIFERATING CELL FACTOR1 (TCP) TFs constitute a family of plant developmental regulators that can be divided into two classes [66]. Most members of the class I TCP TFs have a conserved Cys residue in their DNA binding and dimerization domain [67]. Under oxidative conditions, TCP15 and TCP20 form homodimers and their DNA binding activity is decreased. Mutation of the conserved Cys into Ser results in DNA binding under oxidizing conditions, indicating that this Cys residue mediates the redox sensitivity of TCP15 and TCP20. Another group of redox-sensitive TFs is found among the bZIP family of transcriptional regulators. The G-group of bZIP TFs consists of five members in *Arabidopsis* of which bZIP16, bZIP68 and GBF1 show redox-dependent DNA binding activity [68]. Plants overexpressing the wild-type form of bZIP16 show an increase in hypocotyl length. In contrast, constitutive expression of a bZIP16 protein lacking the Cys residue has no growth-promoting effect, indicating that the redox sensitivity of bZIP16 is important *in planta*. The AP2 TF RAP2.4a was initially identified as a transcriptional regulator of 2-Cys peroxiredoxin in *Arabidopsis* [69]. Interestingly, DNA binding of RAP2.4a depends on the formation of a homodimer. Under reducing conditions, RAP2.4a exists as a monomer, which is unable to bind DNA. Upon a redox shift to more oxidized conditions, the TF dimerizes and binds DNA; however, upon oxidative stress, it oligomerizes and DNA binding is lost again. Another redox-based sensing mechanism involves the translocation of a protein from the cytosol to nucleus. Under reducing conditions, the TF HEAT SHOCK FACTOR 8 (HSF8) resides in the cytosol [40]. Upon H_2O_2 treatment, HSF8 translocates from the cytosol to the nucleus, which depends on the presence of specific Cys residues.

The above examples clearly illustrate that the simplest solution for redox signal transduction, the one-component system, is commonly used by many different types of plant TFs.

Table 1
Redox-sensing transcription factors in *Arabidopsis*.

AGI	Name	Function	Reference
AT1G30490	PHV	Meristem development and functioning; organ polarity and vascular development.	[60]
AT1G36060	RAP2.4a	Redox regulation and photosynthesis	[69]
AT1G32150	bZIP68	Unknown	[68]
AT1G67970	HSFA8	Redox regulation and oxidative stress	[40]
AT1G69690	TCP15	Cell proliferation, endoreduplication	[67]
AT2G35530	bZIP16	Light and hormone signaling	[68]
AT2G47190	MYB2	Abiotic stress (drought, hypoxia, salt stress)	[65]
AT3G27010	TCP20	Cell cycle	[67]
AT4G36730	GBF1	Light signaling	[68]

2.2.2 . Multi-component systems, MAPKs, phosphatases and proteases

Although multi-component signaling systems are elegant solutions for signal transduction, they probably represent more specialized pathways that transduce ROS signals highly specifically. One enigmatic multi-component ROS signal relay found in all eukaryotes is the MAPK cascade. However, it appears that MAPK cascades act as mediators between sensor proteins and effector proteins [1], as so far direct activation of MAPKKKs by ROS has not been demonstrated in plants. The best studied ROS-related MAPK cascade in plants is the MEKK1-MKK1/2-MPK4 module. It was shown that H_2O_2-induced activation of MPK4 depends on MEKK1, and that *mpk4* and *mekk1* mutants accumulate H_2O_2 [70]. Interestingly, MEKK1 is located at the plasma membrane, where it interacts with MKK1 and MKK2 [71]. However, the interaction between MKK1/2 and MPK4 was observed both at the plasma membrane and the nucleus. Recent reports indicate that the CALCIUM/CALMODULIN-REGULATED RECEPTOR-LIKE KINASE (CRKL1) is required for the activation of MEKK1 during cold stress [72] and that it can directly phosphorylate MEKK1 [73]. As an H_2O_2 burst is commonly observed upon abiotic stress application, the activation of MEKK1 by CRLK1 might be redox mediated. Of note, MPK4, which acts downstream of MEKK1, contains a redox-sensitive Cys, which is readily oxidized upon treatment of plants with H_2O_2 [57]. Since H_2O_2 treatment of *mekk1* mutant plants does not result in the activation of MPK4 [70], it is unlikely that the detected Cys oxidation of MPK4 results in its activation. Especially when one considers that extracellular H_2O_2 can enter the cell through aquaporins [31], the oxidation of MPK4 should still occur in *mekk1*. Next to MPK4, also MPK2 and MPK7 get sulfenylated upon H_2O_2 treatment [57]. Both MPK2 and MPK7 are activated by exposure of plants to H_2O_2 [74,75] and they act downstream of the H_2O_2-activated MKK3. If the observed redox modification of MPK2 and MPK7 has an effect on their activity remains to be analyzed. Regarding the oxidative modification of MPKs, it is interesting to note that the activity of OsMAPK5 in rice is inhibited in the presence of reduced OsTRX23 [76]. However, upon oxidation, the activity of OsMAPK5 is restored, which is in support of its role during ROS-dependent salt stress signaling in rice [77]. Many different classes of proteins are known to act as substrates for MAPKs. To provoke a transcriptional response, the targets of the MAPK cascade are effector TFs (Fig. 2), which convert the initial redox signal into a transcriptional response.

The derepression of MAPK signaling pathways also depends on the inactivation of MAPK phosphatases [45] (Fig. 2). Several reports in the literature indicate that oxidative stress can inhibit PP activity. For example, the PROTEIN TYROSINE PHOSPHATASE 1 (PTP1) acts as a negative

regulator of MPK3 and MPK6. Under oxidative conditions, PTP1 can no longer dephosphorylate MPK3 and MPK6 [45], which is in line with the recent observation that PTP1 is undergoing thiol oxidation by H_2O_2 *in planta* [57]. In addition, MPK4 activity is regulated by the PP2C phosphatase AP2C1 and the DUAL-SPECIFICITY PROTEIN PHOSPHATASE 1 [78,79]. Interestingly, also type-2 PPs are sulfenylated by H_2O_2 treatment [57], suggesting that also MPK4 activity is in part relieved through the inactivation of PPs by ROS. The potential positive regulation of MAPKs together with the negative regulation of PPs by the redox status provides a double-edged sword to rapidly relief the repression of this type of ROS signaling cascades.

Other ROS signaling cascades rely on the redox-dependent release of membrane-bound TFs through proteolytic cleavage (Fig. 2). Among the NAC TF family, 13 members were found to contain a putative transmembrane domain in their C-terminal regions [80]. One of these, ANAC053, was shown to control ROS homeostasis during drought stress in plants by regulating the expression of NADPH oxidase encoding genes [81]. Both the proteolytic release of ANAC053 as well as its expression is stimulated by H_2O_2 treatment, constituting a positive feedback loop, which eventually results in ROS-mediated programmed cell death [46,82]. In contrast, ANAC089, another membrane-tethered TF, is only released during reducing conditions [47]. Interestingly, ANAC089 is a negative regulator of the expression of *STROMAL ASCORBATE PEROXIDASE* (*sAPX*). Upon oxidative stress, the release of ANAC089 is inhibited and *sAPX* expression increases. The oxidative environment of the ER is required for correct protein folding by stimulating disulfide bond formation [15]. A group of bZIP proteins containing a membrane anchor are localized to the ER-membrane and released during the so-called unfolded protein response [83,84]. Interestingly, the proteolytic release of bZIP28 is promoted by the treatment with the reducing agent dithiothreitol (DTT) [84]. Moreover, the redox-dependent proteolytic release of TFs involved in the unfolded protein response is highly conserved between eukaryotes [15,83]. Still, in most cases, the protease that mediates proteolytic cleavage of the signaling TFs is unknown, as well as the redox-dependent mechanisms controlling their activity.

3. Redox regulation of cell proliferation

In eukaryotes, cells divide when they reach a certain size or when they are stimulated by internal or extracellular triggers such as hormones [85]. As sessile organisms, plants continuously monitor environmental conditions and adjust growth accordingly. In most cases, a stress initially inhibits growth and, therefore, the regulation of the cell cycle involves both proliferative signals as well as stress signals. As in other eukaryotes, the components controlling the cell cycle in plants are well defined [86]. However, the influence of redox signals on these components is hardly explored in plants [87]. In yeast, cell proliferation is closely linked to metabolism such that cell division occurs during the oxidative phase of respiratory metabolism, while DNA synthesis and mitosis occur during the reductive phase of metabolism [88]. In principle, cell cycle transitions are based on the sequential expression of cyclins and activation of cyclin-dependent kinases (CDKs), while checkpoints that monitor fidelity act on cyclins and CDKs to control transition from one phase to the next [89]. From studies on human cells it is clear that each phase of the cell cycle has a subset of redox-sensory proteins that allows the cell to respond to changes in the redox status [85]. This monitoring system permits the cell to withdraw from the cycle at any time in response to endogenous dysregulation of signaling or exogenous insults [89]. Although the molecular mechanisms involving redox regulation during the cell cycle in plants are scarcely explored, several genetic and biochemical studies support the notion that the redox cycle within the cell cycle is conserved. Cell division in plants primarily occurs in apical meristems and organ primordia. Below, we will separately discuss the effect of the redox status during cell proliferation in roots, shoots and leaf primordia.

3.1. Root apical meristem

The root apical meristem consists of proximal meristem (PM) cells from which all root tissues originate, and a quiescent center (QC), which is essential to maintain root meristem organization [90]. QC cells differ from rapidly dividing PM cells by possessing relatively long cell proliferation times of up to 200 h due to a cell cycle trapped at the G_1/S transition. Remarkably, their cell cycle activity depends on the intracellular redox status [91,92]. As demonstrated by redox-sensitive fluorescent dyes, QC cells are predominantly oxidized as compared to PM cells, e.g. possessing >30-fold more H_2O_2 [92]. The level of oxidized ascorbic acid (dehydroascorbic acid; DHA) is more than 1.000-fold increased in QC cells as compared with PM cells; also the ratio of ascorbic acid (AA)/DHA is lower within this cell type. In addition to DHA, glutathione is predominantly present in its oxidized form glutathione disulfide (GSSG) in QC cells [92]. One reason for this observation is that NADPH, mandatory for the regeneration of GSH from GSSG via glutathione reductase [93], is below detectable levels present in QC cells. Therefore, the decreased level of GSH might contribute to the cessation of the QC cell cycle activity at the G_1 stage [92]. Interestingly, these observations are in line with fluctuations in the redox status during the cell cycle progression in yeast and animals [85], where the G_1 stage is also known as the oxidative phase of the cell cycle. Moreover, proliferating *Arabidopsis* cell cultures show an accumulation of AA in the nucleus during the initial stage of the cell cycle, which is followed by a progressive increase in the level of GSH [94]. Recruitment of cytosolic GSH to the nucleus is mandatory for ongoing cell proliferation, as depletion of GSH traps the cell cycle in the G_1 phase [95,96], in a similar manner as seen for the QC cells. The regulation of meristem activity by redox intermediates is supported by the knock-out mutant *root meristemless1* (*rml1*) lacking the first enzyme involved in GSH biosynthesis. The depleted GSH levels in *rml1* come along with the inability to establish an active root meristem and application of GSH rescues the growth phenotype [95].

Interestingly, the redox status of QC and PM cells correlates with endogenous levels of the phytohormone auxin [92]. Auxin affects root growth in a dose-dependent manner; at very low concentration it stimulates root elongation, while increasing concentrations block root growth [97]. Its concentration in the oxidized QC is rather high as compared with PM cells. Perturbation of the polar auxin transport by root cap decapitation or treatment of intact roots with N-1-napthylphthalamic acid (NPA) changes the redox status of the QC to a more reduced state, thereby inducing cell division [92]. On the other hand, PM cells get more oxidized and are subsequently slowed down in their proliferation, a phenomenon which is attributed to a shift of the auxin maximum from the columella/QC to the PM zone. The exact mechanism by which auxin accomplishes changes in both redox status and cell cycle activity of the QC is elusive. However, AA and/or GSH represent good candidates to link auxin with QC performance. Notably, in cultured maize root tissues, auxin stimulates the de novo biosynthesis and enzymatic activity of ascorbate oxidase (AAO), which oxidizes AA to DHA and may thereby contribute to the oxidative status of QC cells [91]. As in turn AAO is capable of degrading auxin, a sophisticated mechanism for the regulation of auxin levels and redox homeostasis within the QC is established [98]. In addition, ROS appear to affect the amount and direction of auxin transport through post-translational modification of auxin influx and efflux carriers [99]. Triple mutant plants lacking the TRX reductase genes *NTRA* and *NTRB* and the weak GSH biosynthesis gene mutant allele *cad2* exhibit impaired auxin transport and reduced lateral root formation [100]. Interference with the redox homeostasis in the root tip by treatment with buthionine sulphoximine (BSO; GSH biosynthesis inhibitor) causes the disappearance of the auxin efflux carriers PIN1, PIN2 and PIN7 from the plasma membrane in a dose-dependent manner without affecting their gene expression [101]. Addition of the reducing compound DTT to BSO-treated roots restored the accumulation of the efflux carriers at

the plasma membrane, indicating that PIN positioning is redox controlled. In line with this finding is the notion that H_2O_2 inhibits exocytosis of PIN1 [102]. At the transcriptional level, the specification of the root stem cell niche is regulated by two pathways. One auxin-dependent network that is regulated by PLETHORA (PLT) TFs [103] and the WUSCHEL-RELATED HOMEOBOX 5 (WOX5) TF [104], and an auxin-independent SHORTROOT (SHR)- and SCARECROW (SCR)-regulated pathway [105]. So far, a direct involvement of redox regulation for these TFs has not been shown, although it is possible that the QC-specific WOX5 operates only under oxidizing conditions.

3.2. Shoot apical meristem and leaf primordia

The shoot apical meristem (SAM) gives rise to all aboveground organs of the plant. Whereas maintenance of the stem cell pool mainly depends on the action of cytokinin, the formation and positioning of organs is largely controlled by auxin [106]. The shoot meristem is organized in a central zone (CZ), which contains slowly dividing stem cells (SC) [90], which is surrounded by a peripheral zone (PZ), where cells are more rapidly dividing. Furthermore, the stem cell zone can be divided into three layers (L1, L2, and L3), and a meristem organizing center (OC) (Fig. 3A). Loss of the homeodomain TF SHOOTMERISTEMLESS (STM) results in the depletion of the pool of stem cells, which can be rescued by cytokinin [106]. The stem cell pool in the different meristem layers of the SAM is positively regulated by the homeodomain TF WUSCHEL (WUS), which is mainly active in the OC. On the other hand, the signaling peptide CLAVATA 3, present in the CZ, suppresses stem cell activity. In part, SAM maintenance is redox-regulated, as indicated by the *ntra/ntrb/cad2* triple mutant which exhibits pin-shaped inflorescences [100]. Still, relatively little is known about the distribution of the redox pools within the SAM. Recently, a high resolution gene expression map of the SAM has been established by using cell type-specific transcriptome profiling [107]. Interestingly, when looking at the average expression level of *GSH1*, then it is the highest in the OC (WUS) as compared to the surrounding L1, L2 layers and the adjacent SC (Fig. 3B). Moreover, other anti-oxidant genes like *CATALASE3* (*CAT3*), *TRX5* and *GLUTATHIONE REDUCTASE 1* (*ATGR1*) are all much higher expressed in the OC as compared to any of the other domains of the SAM. In general, higher expression of these genes indicates an increased generation of ROS [25,108]. Not all ROS-related genes show this distribution, as *ASCORBATE PEROXIDASE 1* (*APX1*) is relatively stably expressed throughout the SAM (Fig. 3B). The CZ shows a slower rate of cell division as compared to the surrounding PZ, but cell proliferation in the OC is inhibited by the action of WUS. Recently, it was found that the regulation of stem cell proliferation in the CZ and maintenance of the OC is regulated by the interplay of WUS with the bHLH TF HECTATE 1 [109]. All in all, the low proliferation rates of the OC seem to overlap with an increased redox potential, although further research in this direction is needed to uncover the exact role of redox status on SAM maintenance.

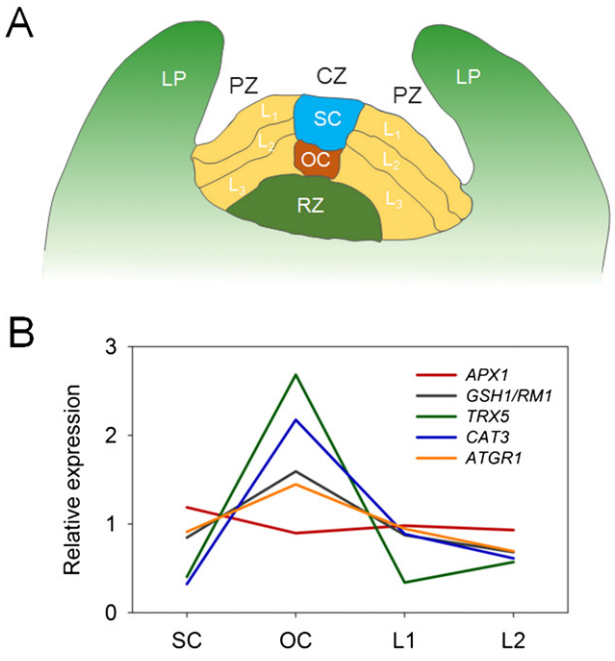

Fig. 3. Organization of the shoot apical meristem and ROS distribution. A) The maintenance of the pool of stem cells (SC) in the central zone (CZ) of the meristem relies on signals arriving from the organizing center (OC). The CZ is surrounded by a peripheral zone (PZ) of three layers of more rapidly dividing cells (L_1, L_2 and L_3) from which leaf primordia (LP) derive, and an underlying rib zone (RZ) which gives rise to stem tissue. B) Distribution of ROS transcript levels throughout different cell populations within the shoot apical meristem. Relative average expression level of five ROS transcripts is shown as based on data published by Yadev et al., 2014. The labels on the X-axis correspond to the different cell populations.

Redox-sensitive HD-Zip III TFs play a dual role, as on the one hand they act as regulators of meristem functioning and on the other hand they promote adaxial cell fate in organ primordia [110]. The action of HD-zip III TFs is antagonized by KANADI TFs, which promote abaxial cell fate in organ primordia and negatively regulate meristem formation [110,111]. The triple homozygous *ntra/ntrb/rml1* mutant fails to form active organ primordia [112]. The initiation of leaf primordia mainly depends on the action of two interacting TFs, the MYB TF ASSYMETRIC LEAVES 1 (AS1) and the LOB domain TF AS2, which repress STM function [113]. *AS2* is directly activated by BLADE-ON-PETIOLE1 (BOP1) and BOP2, two NPR1-like proteins [114,115]. NPR1 is one of the best studied redox-sensitive transcriptional regulators, which exists in its oxidized state as an oligomeric complex in the cytosol, but upon pathogen attack, it is reduced by TRXs and the subsequent free monomers translocate to the nucleus [116]. The two redox-sensitive Cys residues of NPR1 are conserved in BOP1 and BOP2, indicating that a redox signal might mediate the establishment of the leaf primordia by inhibiting meristem identity. Subsequent primordia outgrowth is the consequence of extensive cell proliferation. In several studies, TCP TFs from *Arabidopsis* have been reported to regulate cell proliferation in various tissues, including young internodes, leaves and flowers, which is achieved by the direct or indirect regulation of cell cycle genes [117–120]. Among them, the redox-sensitive TCP15 and TCP20, two class I TCPs, were shown to directly control the expression of *CYCA2;3*, *RETINOBLASTOMA-RELATED* (*RBR*) genes and *CYCB1;1*, respectively (Fig. 4A) [117,120]. Most class I TCPs have a Cys residue within their DNA-binding and dimerization domain which, when oxidized, e.g. by H_2O_2 or GSSG treatment, abolishes binding to DNA [67]. Since oxidizing conditions cause cell cycle cessation [95], it is plausible that this is a consequence of the inhibition of TCP15 and TCP20 which regulate cell cycle target genes. In line with this observation is that cell proliferation in leaf primordia stops at the onset of chloroplast maturation [121]. As photosynthetically active chloroplasts represent together with peroxisomes the major source of ROS production in a plant cell exposed to light, they might limit proliferation through the production of redox signals. Although many other components regulate cell proliferation in leaves, it is often unexplored whether their activity is under redox control.

4. Redox switch during the transition from proliferation to elongation

After several rounds of proliferation, cells exit the cell cycle in the transition zone after which they elongate/expand and acquire a specific differentiation. The transition zone in roots is visually easily recognized, but also during the formation of leaves a kind of transition zone exists. Interestingly, the transition from cell proliferation to elongation is in part redox-regulated through the modulation of class III heme peroxidases (POXs) in roots of *Arabidopsis* [122]. POXs can either produce or scavenge H_2O_2, depending on the chemical environment [123]. The

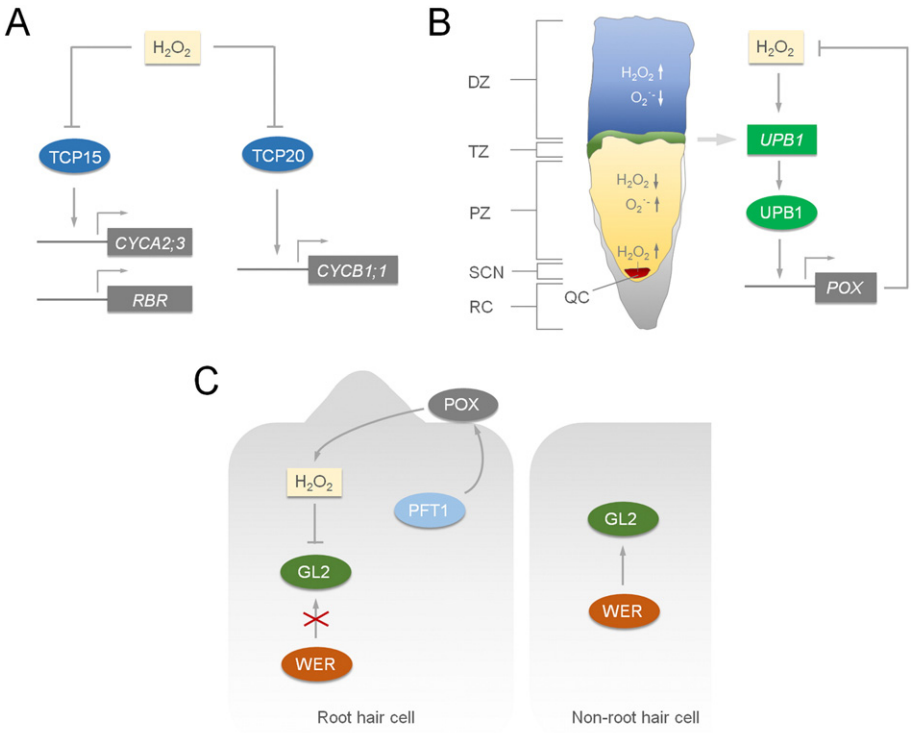

Fig. 4. Redox regulation during cell proliferation and differentiation. A) Cell proliferation during leaf growth is positively regulated by redox-sensitive TCP TFs. Both TCP15 and TCP20 positively regulate the cell cycle by inducing the expression of the A-type cyclin *CYCA2*;3 and B-type cyclin *CYCB1*;*1*, respectively. Furthermore, *RETINOBLASTOMA-RELATED* (*RBR*) acts downstream of TCP15. As the DNA-binding activity of both TCP TFs is inhibited by H_2O_2, they act as a potential redox checkpoint within the cell cycle. B) Model of redox-regulated root growth. The quiescent centre (QC) is required to keep the surrounding stem cell niche (SCN) in an undifferentiated state. QC cells display very low cell cycle activity, which is characterized by a highly oxidized cellular environment. Stem cell daughters divide in the proliferation zone (PZ), which is characterized by a relative high level of $O_2^{\bullet-}$. Once these cells reach the transition zone (TZ), they encounter an increased level of H_2O_2 and stop dividing and begin to elongate and differentiate in the differentiation zone (DZ). The bHLH TF UPB1 regulates meristem size by balancing the $O_2^{\bullet-}/H_2O_2$ ratio through modulating the expression of *PEROXIDASE* (*POX*) genes. C) Root hair initiation is negatively regulated by GLABRA2 (GL2). In epidermal cells that are destined to become hair cells, the induction of *GL2* expression by WEREWOLF (WER) is inhibited. At the same time, the mediator complex subunit PFT1 activates the expression of genes encoding H_2O_2— producing POXs. The increased H_2O_2 level mediates root hair differentiation, potentially through inactivating remaining GL2 protein through oxidation of redox-sensitive Cys residues.

expression of a subset of class III *POX* genes that contribute to H_2O_2 scavenging is repressed by the bHLH TF UPBEAT1 (UPB1) (Fig. 4B) [122]. In the absence of a functional UPB1 protein, the root meristem is enlarged and the onset of cell elongation and differentiation is delayed. Overexpression of *UPB1* results in an earlier transition from proliferation to differentiation, causing a decreased meristem size. The *upb1-1* knockout mutant has higher $O_2^{\bullet-}$ but lower H_2O_2 levels in its root as compared to the wild type, while overexpression of *UPB1* results in an opposite effect (i.e., decrease in $O_2^{\bullet-}$ and an increase in H_2O_2). Therefore, the ratio between both ROS seems to be important for exiting the proliferative phase and initiating cell differentiation. Notably, *UPB1* expression is induced by H_2O_2, forming a feedback loop within the signaling cascade (Fig. 4B). Furthermore, the observed redox switch at the transition zone appears to function independently from cytokinin or auxin as *upb1-1* mutant roots still show normal growth responses to these hormones [122].

In contrast to root growth, leaf growth is determinate and the final size of the leaf is the result of both proliferation and cellular expansion [18]. The transition from cell proliferation to expansion involves many factors and proceeds in a basipetal manner starting from the tip [124]. Initially, it was thought that transition proceeds gradually, but new insights demonstrated that the position of the cell cycle arrest front during leaf growth is rather stable and rapidly disappears once a certain number of cells has been reached [121,125]. One of the factors that regulate transition is the maturation of chloroplasts [121]. Norflurazon (NF) treatment of seedlings represses the expression of nuclear-encoded chloroplast genes and causes photo-oxidative stress, which impedes chloroplasts development [126]. Especially the inhibition of carotenoid synthesis readily enhances the photosensitizing activity of the chlorophyll and causes a concomitant increase in $^1O_2$ production [126,127]. NF treatment delays the onset of leaf cell expansion, indicating that a retrograde signal from the chloroplast can control timing of the transition in leaves [121]. Interestingly, the *ex1*/*ex2* mutant suppresses in part the effect of NF application [126], indicating that the retrograde signal is in part $^1O_2$-mediated. Furthermore, the redox-sensitive TF TCP20 directly represses four bHLH TF genes required for chloroplast differentiation during the transition phase, which are also repressed by NF treatment [128]. Despite a lack of down-regulation of *TCP20*, the expression of the four bHLH genes is strongly induced at the onset of cell expansion and sink-source transition [128]. Potentially, a change in the redox status could post-transcriptionally inactivate TCP20 [67] to allow transition to the next phase. Taken together, it appears that in leaves, ROS mediate the transition from the proliferation phase to the expansion phase, although this needs to be explored in more depth in future studies.

5. Redox signaling and regulation during cell expansion

Expansion of differentiated tissues is mainly driven by two processes, vacuolar enlargement to facilitate the uptake of water and solutes [129], and the extension of the cell wall [130]. Apoplastic ROS have a direct effect on the stiffness of the cell wall, thereby either promoting or restricting cellular extension. The production of H_2O_2 in the cell wall promotes cross-linking of cell wall components and restricts growth [18,123]. On the other hand, cell wall POXs can also generate $OH^{\bullet}$ using $O_2^{\bullet-}$ and H_2O_2 as substrates, which cleaves cell wall polysaccharides and acts as a wall-loosening agent that promotes expansion growth [131]. A study in rice revealed the presence of an apoplastic

TRX, which is needed for maintaining extracellular redox homeostasis and plant growth [132]. Still, how the redox status in the apoplast is controlled and monitored is far from being understood. While during expansion growth, the enlargement of the vacuole is mainly achieved through water uptake, a new role for the vacuole has emerged in which it sequestrates GSSG to avoid an excessively oxidizing cytosolic redox potential [133]. These observations already indicate the potential influence of redox changes on cellular expansion.

5.1. Root and root hair cell elongation

A main factor for root growth is the increase in cell volume. This requires among others loosening of the cell wall matrix [134]. The involvement of ROS-producing or -scavenging class III POXs in root cell elongation was shown for POX33 and POX34, two homologous cell wall-located POXs in *Arabidopsis* [135]. Reducing the expression of *POX33* or *POX34* significantly decreased root cell length, while overexpression of *POX34* resulted in an increased cell length as compared with the wild type [135]. Therefore, both POXs are involved in growth-promoting redox changes in roots. Interestingly, roots of the *mpk6* mutant are less sensitive to root growth inhibition by exogenous H_2O_2 [136]. This is in part due to increased activity of POX34 in the *mpk6* background, linking ROS signaling with redox-modulated growth. POX33 and POX34 are positive regulators of root cell expansion, similarly, three other *POX* genes (*POX39*, *POX40* and *POX57*), which are directly repressed by UPB1 [122], have a positive effect on root length. The length of the first mature cortical root cell is decreased in *UPB1* overexpression plants, but increased in *upb1-1*. Since both H_2O_2 and salicylhydroxamic acid (SHAM) treatment reduce cell length, the POXs acting downstream of UPB1 are required for H_2O_2 scavenging.

Root hairs are initiated by a hair-forming epidermal cell and elongate through tip growth [137]. Hairs of the *Arabidopsis* mutant *root hair defective 2* (*rhd2*) set off correctly but they do not extend [138]. Map-based cloning revealed that the *RHD2* gene encodes a NADPH oxidase with similarity to respiratory burst oxidases (RBOHs). The NADPH oxidase-produced ROS burst promotes Ca^{2+} influx that leads to a depolarization of the plasma membrane [138]. In addition, RBOH proteins contain EF-hand domains in their cytoplasmic tail, which bind Ca^{2+} and promote further ROS production [139]. In addition, loss of *RHD2* results in smaller cells in the root elongation zone, indicating that NADPH oxidases might be more generally involved in cell elongation in plants. Indeed, tip growth as observed in root hairs is highly similar to that found in pollen tubes, which also depends on ROS generation by NADPH oxidases [140]. Tip growth involves rapid modulation of the cell wall, which affects the Ca^{2+} influx that is promoted by NADPH oxidases [141]. Interestingly, the growth defects observed in the *rhd2* mutant is caused by a disturbed microtubule organization [142,143]. Similarly, diphenyleneiodonium (DPI) treatment, a chemical inhibitor of NADPH oxidases, causes macrotubule formation. On the other hand, NADPH oxidase-mediated ROS accumulation can result in depolymerization of microtubules [144]. Thus, depending on the redox balance, the organization of the tubulin cytoskeleton is altered. Intriguingly, in maize, the MICROTUBULE-ASSOCIATED PROTEIN 65 (MAP65) was found to be required for brassinosteroid-mediated ROS production through the activation of NADPH oxidase expression [145]. Moreover, a ROS-responsive MAPK was shown to be required for the H_2O_2 signal propagation by MAP65. This implies that a ROS signal generated by NADPH oxidases is involved in the organization of the cytoskeleton during growth. Recently, it was found that the effect of RHD2 on microtubule organization is mediated by a ROS-responsive p38-like MAPK [146]. Inhibition of the p38-like MAPK activity mitigated the effect of both the *rhd2* mutant and H_2O_2 application on microtubule organization, indicating that the p38-like MAPK functions as a redox sensor. The interplay between ROS homeostasis and cytoskeleton structure might be involved in fine-tuning cell growth in plants.

5.2. Leaf cell expansion

The regulation and timing of plant growth is controlled by the circadian clock. In dicots, leaf expansion rates are the highest in the morning and depend on the function of CIRCADIAN-CLOCK ASSOCIATED 1 (CCA1) [147]. Recently, a clock-regulated MYB-like TF, KUODA1 (KUA1), was found to function as a positive regulator of leaf cell expansion by modulating apoplastic ROS homeostasis [18]. Similar to UPB1, KUA1 represses the expression of a subset of class III *POX* genes during the growth of the leaf. The *kua1* mutant has smaller leaves than the wild type, a result of a specific decrease in cell size. Moreover, *kua1* shows elevated levels of H_2O_2 and increased activity of class III POXs. Treatment with SHAM, a chemical that among others inhibits POX activity, recovers the mutant phenotype. Thus, in contrast to POXs regulated by UBP1, those acting downstream of KUA1 are involved in H_2O_2 production and cell wall stiffening in leaves. As KUA1 function depends on CCA1 [18], it indicates that the circadian clock regulates cell expansion by modulating ROS homeostasis. Indeed, CCA1 has been recently established as a redox gatekeeper that controls the transcriptional response towards ROS [108]. The transcriptional response of genes upon ROS treatment depends on the time of the day. Exposure of plants to ROS in the morning results generally in an up-regulation of ROS-related genes; however, this transcriptional response does not occur when plants are treated in the afternoon or during the night. In contrast, plants that constitutively express *CCA1* show an induction of ROS-related genes upon treatment independent from time, indicating that the clock gates redox responses [108]. Although other components play a crucial role in plant cell expansion, for instance expansins and xyloglucan endotransglucosylase-hydrolases, there has been no report that shows that they act in a redox-dependent manner.

6. Redox signaling during (de)differentiation in plants

Plant cells are considered to be totipotent, which, when in culture, can dedifferentiate into an undifferentiated cell mass of which new plants can be regenerated [148]. *In planta*, differentiation of cells into specific tissues depends in part on positional cues and ROS distribution [149]. Cell differentiation in plants has been mainly studied during the development of root hairs and trichomes. Here we focus on a few cases that reveal the role of ROS distribution and sensing during (de)differentiation.

6.1. Root hair and lateral root differentiation

As indicated above, NADPH oxidase activity is required for root hair elongation [138]. Although the *rhd2* mutant appears to be unaffected in the establishment of the root hair-initiating cell, there are clear indications that ROS are actively involved in this differentiation process. Root hair cell differentiation is inhibited by the homeodomain TF GLABRA2 (GL2), which is positively regulated by the MYB TF WEREWOLF (WER) [150]. In epidermal cells that are destined to form a root hair, the expression of *GL2* is switched-off by blocking the positive effect of WER (Fig. 4C). Interestingly, GL2 appears to be a member of the redox-sensitive homeodomain TFs, whose DNA-binding ability is inhibited upon oxidative stress [59]. This feature is especially interesting as it was recently shown that increased levels of H_2O_2 are required for the differentiation of root hair cells [151]. Loss of the transcriptional co-regulator PHYTOCHROME AND FLOWERING TIME1 (PFT1) impairs root hair formation as compared to wild type. Expression analysis revealed that PFT1 mainly regulates ROS-related genes, including those encoding POXs and NADPH oxidases. The decreased expression of *POX* genes correlated with lower levels of H_2O_2, while increased expression of NADPH oxidases overlapped with higher levels of $O_2^{\bullet -}$ [151]. Subsequently, the authors showed that the root hairless phenotype of *pft1* can be rescued by exogenous application of H_2O_2 and DPI, indicating that the balance between H_2O_2 and $O_2^{\bullet -}$ regulates root hair

differentiation. Here we propose that the ROS-mediated effect on root hair differentiation might be in part through the regulation of GL2 activity (Fig. 4C).

In many plant species, root architecture is modified through the de novo formation of lateral roots, which initiate from founder cells in the main root [152]. An S-PHASE KINASE-ASSOCIATED PROTEIN 2 (SKP2B):GFP marker line was exploited to specifically isolate RNA from cells involved in the initiation of lateral roots [153,154]. Subsequent expression analysis revealed lateral root primordia-specifically enriched genes, including a set of ROS-related genes and *UPB1*. In line, ROS accumulate during later root differentiation, indicating a potential involvement for redox regulation in this process. Indeed, *pox7* and *pox57* mutant plants displayed a reduced number of lateral roots [154]. Thus, the differentiation of lateral roots depends on ROS signaling and homeostasis, which is at least in part controlled by the action of UPB1. Still, more insights are needed to determine the exact mechanisms of redox regulation during lateral root differentiation.

6.2. Cell dedifferentiation and tissue regeneration

Dedifferentiation of plant tissues in vitro involves the ability of previously differentiated cells (those being quiescent and which have enlarged vacuoles) to start to divide again [148]. One way of inducing dedifferentiation is by placing tissue explants on callus-inducing medium, resulting in cells that show features similar to meristematic cells and have lost their differentiated cell morphology [155]. Because calli can be induced to form shoots and roots, all plant cells have been traditionally thought of as being totipotent [148]. Recently, it has become apparent that calli derive from only a single cell type (xylem pole pericycle-like cells), regardless of the origin of the tissues (leaf, root, or petals) [156,157]. Moreover, the processes that are induced during callus formation are highly similar to those observed *in planta* during lateral root initiation and formation [156]. Thus, it appears that callus formation does not really involve dedifferentiation of plant cells, but rather involves the differentiation of mitotic competent cells.

In contrast to callus formation, different types of somatic plant cells can be induced to form embryos, so-called somatic embryogenesis [158]. Embryo development from differentiated somatic cells in plants is the best proof for their totipotent capacity. Remarkably, the developmental switch initiating somatic embryogenesis is triggered by transient strong stress and/or high non-physiological concentrations of growth regulators. Abiotic stress is an important factor for the induction of somatic embryogenesis in *Arabidopsis*, including osmotic, heavy metal ions and dehydration stresses [159]. The fact that H_2O_2 treatment can induce somatic embryogenesis indicates a role for ROS regulation during this process [160]. This is actually not surprising, when one considers the ubiquitous role of H_2O_2 during abiotic stress signaling and development. Recent evidence for the potential promoting effect of oxidative stress on the initiation of somatic embryogenesis comes from a study with an ascorbic acid biosynthesis mutant [161]. The *vitamin C deficient 2-5* (*vtc2-5*) mutant shows an improved formation of somatic embryos as compared to the wild type, indicating that an increased redox ratio of the ascorbate pool stimulates dedifferentiation of plant cells. Of note, the expression of *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE4* (*SERK4*) and *SERK5* is up-regulated in *vtc* mutants [162]. As *SERK* expression is a hallmark for embryogenic cells in culture [163], it appears that the higher expression of these genes in *vtc* mutants might promote cellular reprogramming. The involvement of redox signaling during somatic embryogenesis is still largely unexplored, but additional efforts on this phenomenon can reveal how ROS mediates plant developmental plasticity.

7. Conclusion

Based on current literature it is clear that ROS-mediated redox signaling plays an essential role during cell differentiation in plants. The diverse types of ROS and their cellular compartmentalization provide some specificity for the complex redox signaling network that is present throughout the cell. The complexity of this signaling network is especially typified by the huge amount of (potential) redox sensors present within the cell. Interestingly, oxidative modification of proteins can result in very different outcomes. For example, oxidation can result in protein inactivation, oligomerization, translocation or cleavage. Owing to the fact that only a fraction of the ROS signaling cascades during cell differentiation has been resolved, there will be exciting times lying ahead of us.

In conclusion, to obtain a better understanding of redox sensing and signaling during cell differentiation, we should first identify the ROS entry points in known regulatory networks (i.e., hormone signaling pathways).

Abbreviations

DPI	diphenyleneiodonium
DTT	dithiothreitol
ER	endoplasmic reticulum
GRX	glutaredoxin
GSH	glutathione
GSSG	oxidized glutathione
HOTE	hydroxyoctadecatrieonic
H_2O_2	hydrogen peroxide
MAPK	mitogen activated protein kinase
Met-SO	methionine sulfoxide
NF	norflurazon
PP	protein phosphatase
POX	heme peroxidase
PUFA	polyunsaturated fatty acids
QC	quiescent center
RBOH	respiratory burst oxidase
ROS	reactive oxygen species
SHAM	salicylhydroxamic acid
$^1O_2$	singlet oxygen
Cys-SOH	sulfenic acid
$O_2^{\bullet-}$	superoxide
TF	transcription factor
TRX	thioredoxin

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