Title: Secondary sulfur metabolism in cellular signalling and oxidative stress responses

Authors: Kai Xun Chan¹²,*, Su Yin Phua¹², and Frank van Breusegem¹².

Affiliations:
¹Ghent University, Department of Plant Biotechnology and Bioinformatics, Technologiepark 71, 9052 Ghent, Belgium
²VIB Center for Plant Systems Biology, Technologiepark 71, 9052 Ghent, Belgium
*Corresponding author

Contact: KXC (kaixun.chan@psb.ugent.be); SYP (suyin.phua@psb.ugent.be); FVB (frank.vanbreusegem@psb.ugent.be)
+32 (0)9 331 39 24

Running Title: Secondary S metabolites in stress cellular signalling

Highlight
Secondary sulfur metabolism produces several metabolites which regulate various aspects of cellular signalling and homeostasis in response to environmental perturbations.

Abstract
The sulfur metabolism pathway in plants produces a variety of compounds that are central to the acclimation response to oxidative stresses such as drought and high light. Primary sulfur assimilation provides the amino acid cysteine, which is utilized in protein synthesis and as a precursor for the cellular redox buffer glutathione. In contrast, the secondary sulfur metabolism pathway produces sulfated compounds such as glucosinolates and sulfated peptides, as well as a corresponding by-product 3’-phosphoadenosine 5’-phosphate (PAP). Emerging evidence over the past decade has shown that secondary sulfur metabolism also has a crucial engagement during oxidative stress. This occurs across various cellular, tissue and organismal levels including chloroplast-to-nucleus retrograde signalling events mediated by PAP, modulation of
hormonal signalling by sulfated compounds and PAP, control of physiological responses such as stomatal closure, and potential regulation of plant growth. In this review, we examine the contribution of the different components of plant secondary metabolism to oxidative stress homeostasis, and how this pathway is metabolically regulated. We further outline the key outstanding questions in the field that are necessary to understand how and why this ‘specialized’ metabolic pathway plays significant roles in plant oxidative stress tolerance.

Key words
Sulfur, metabolism, 3’-phosphoadenosine 5’-phosphate, retrograde signalling, sulfotransferase, APK, SAL1, drought, oxidative stress

Abbreviations

ABA   abscisic acid
APK   Adenosine Phosphosulfate Kinase
APS   adenosine phosphosulfate
GSH   glutathione
JA    jasmonic acid
OH-JA hydroxyl-jasmonic acid
PAP   3’-phosphoadenosine 5’-phosphate
PAPS  3’-phosphoadenosine 5’-phosphosulfate
PAPST 3’-phosphoadenosine 5’-phosphosulfate Transporter
ROS   reactive oxygen species
SA    salicylic acid
SOT   Sulfotransferase
TPST  Tyrosyl Protein Sulfotransferase
XRN   exoribonuclease
Introduction

Plant responses to environmental conditions that invoke reactive oxygen species (ROS) over-accumulation leading to photo-oxidative stress in leaves, such as drought and high light, and combinations thereof, involve coordinated acclimation processes across biochemical, cellular and physiological levels. Processes that are activated include the synthesis and downstream signalling of hormones (Galvez-Valdivieso et al., 2009), alterations in metabolism of nutrients such as sulfur (Malcheska et al., 2017; Rizhsky et al., 2004), chloroplast-to-nucleus retrograde signalling (Chan et al., 2016b), secondary messenger signalling involving reactive oxygen species (ROS) and Ca\(^{2+}\) (Steinhorst and Kudla, 2013). Each of these processes impinge on physiological responses such as stomatal closure (Murata et al., 2015) and regulation of plant growth (Bechtold and Field, 2018). Intriguingly, multiple facets of the plant response to oxidative stress are regulated by components of sulfur metabolism (Estavillo et al., 2011; Speiser et al., 2018; Zechmann, 2014).

Plants assimilate sulfur in the form of sulfate anions, which are first taken up into root cells and then transported between and within plant cells via four groups of sulfate transporters (SULTRs) with differing subcellular localizations and substrate affinities (Takahashi et al., 2011). The sulfate is first activated in the cytosol and chloroplasts to adenosine phosphosulfate (APS) through the action of ATP sulfurylases (ATPSs) (Figure 1). APS contains a high-energy P-S bond which enables it to act downstream as an S-donor in the contrasting branches of a bifurcated metabolic pathway. In the primary branch, the sulfate moiety of APS is successively reduced and integrated into the carbon skeleton of O-acetyleserine for the synthesis of cysteine. Cysteine serves as a substrate for biosyntheses of methionine, as well as the cellular redox buffer glutathione (GSH) (Takahashi et al., 2011), which has well-established important roles in redox control during growth and development and oxidative stress homeostasis (Mhamdi and Van Breusegem, 2018). Alternatively, APS can enter the secondary sulfur metabolism pathway to be phosphorylated by APS kinase (APK) enzymes in the cytosol and chloroplasts to produce 3’-phosphoadenosine 5’-phosphosulfate (PAPS) (Mugford et al., 2009). The chloroplastic PAPS can be transported to the cytosol by at least two chloroplast envelope-localized PAPS Transporters (PAPSTs) (Ashykhmina et al., 2018; Gigolashvili et al., 2012), while cytosol-to-
Golgi PAPS is hypothesized to occur though the transporter is yet to be identified. The activated sulfur in PAPS is then transferred to various acceptor molecules including hormones, xenobiotics and growth-regulating peptides by cytosolic and Golgi-resident sulfotransferases (SOTs) (Hirschmann et al. (2014)). As a by-product, 3'-phosphoadenosine 5'-phosphate (PAP) is formed, which is dephosphorylated by the SAL1 phosphatase to adenosine monophosphate (AMP) in chloroplasts and mitochondria (Estavillo et al., 2011). (Figure 1).

Interestingly, plastidial APS is utilized by both primary and secondary sulfur metabolism (Figure 1), which necessitates coordination between these pathways at multiple levels, although the mechanism(s) are not fully understood (Kopriva et al., 2012). For example, loss of APK activity at the entry point into secondary sulfur metabolism re-directs sulfur flux into cysteine and GSH synthesis (Mugford et al., 2011). Conversely, loss of SAL1 activity leads to decreased levels of both primary and secondary sulfur metabolites, as well as that of sulfate (Lee et al., 2012). Therefore, the control of sulfur partitioning between primary and secondary sulfur metabolism still remains to be elucidated, particularly during oxidative stress conditions such as drought stress when multiple sulfur metabolites participate in the cellular response (Chan et al., 2013). Indeed, evidence over the past decade increasingly point towards crucial roles for secondary sulfur metabolites in oxidative stress signalling and responses.

**APS Kinases: Facilitators of sulfur flux into secondary sulfur metabolites**

The APK enzymes constitute a branching point of sulfur flux into secondary sulfur metabolism, as they direct sulfur away from primary sulfur assimilation through conversion of APS into PAPS. Three of the four APK isoforms in Arabidopsis (APK1, APK2, APK4) are localized to chloroplasts. APK1 and APK2 are the major isoforms with partially overlapping tissue expression patterns and functional redundancy (Mugford et al., 2009). This is evidenced by lack of visible growth phenotypes in any of the single apk genetic lesions and a dwarfed phenotype of apk1apk2 which possesses significantly lower levels of PAPS-requiring metabolites such as glucosinolates, and correspondingly accumulate the precursor desulfoglucosinolates (Mugford et al., 2009). Plastidial APK4 and cytosolic APK3 play relatively minor roles in the provision of PAPS (Mugford et al., 2010), since analysis of higher-order apk mutant combinations show that apk3apk4 is aphenotypic while apk1apk2apk3 and apk1apk2apk4 phenotypes are additive to apk1apk2. The decreased growth in plants with low APK activity is hypothesized to be caused at least in part by loss of sulfated peptides with growth-promoting roles (Mugford et al., 2009), but this has not been confirmed. Importantly,
these findings indicate that APK dependent PAPS production is rate-limiting for the production of downstream secondary sulfur metabolites catalyzed by the SOTs. Indeed, APK2 alone is not sufficient for PAPS provision since apk1apk3apk4 is embryo-lethal (Mugford et al., 2010) The contribution of APKs to oxidative stress tolerance has not been tested. Intriguingly, APK1 is inactivated by oxidation in vitro (Ravilious et al., 2012) (discussed in more detail below), and apk1apk2 accumulates higher levels of the redox buffer GSH. These findings may indicate that APK inactivation could be favoured under oxidative stress conditions. However, it can also be expected that severe limitation of PAPS supply can be deleterious since many of the SOT products can play important functions in response to oxidative stress as discussed below.

**Regulation of hormones, growth and stress homeostasis by sulfur-containing secondary metabolites and sulfotransferases**

In mammalian systems, the physiological functions of SOTs are relatively well-characterized, with known substrates including carbohydrates, proteoglycans, proteins, xenobiotics, and various steroid hormones (Gamage et al., 2006; Mueller et al., 2015). Diseases caused by loss-of-function of specific SOTs in humans are also described (Mueller et al., 2015). In contrast, plant SOT research still presents many unknowns. For example, there are 71 predicted SOTs in *Brassica napus*, but they remain poorly characterized with a few notable exceptions (Hirschmann and Papenbrock, 2015). Of the 22 SOTs encoded by the *Arabidopsis* genome, only ten have known in vitro substrates or in vivo physiological roles (Figure 2) (Hirschmann et al., 2014). Their substrates include desulfoglucosinolates, hormones or hormone derivatives (brassinosteroids, salicyclic acid, hydroxy-jasmonic acid), flavonoids, xenobiotics, and peptides (Hirschmann et al., 2014).

**Glucosinolates – defense compounds with additional roles in oxidative stress**

In *Brassicaceae*, the bulk of sulfur flux via the SOTs under steady-state conditions is utilized for the synthesis of glucosinolates, which are anti-herbivory defence compounds (reviewed by Kliebenstein et al in this issue). The final step of glucosinolate biosynthesis involves the transfer of the sulfate moiety from PAPS to desulfoglucosinolates by SOT16, SOT17 and SOT18 (Piotrowski et al., 2004). Interestingly, both foliar and root glucosinolate accumulation have been observed in several *Brassica* species subjected to abiotic stresses such as drought and salt stress (Del Carmen Martínez-Ballesta et al., 2013), and deletion of two MYB transcription factors which abolish accumulation of aliphatic glucosinolates was correlated with increased salt stress sensitivity (Martínez-Ballesta et al., 2015). The downstream breakdown products of
glucosinolates might be involved in the oxidative stress responses. The myrosinase enzyme TGG1 which degrades glucosinolates into active isothiocyanates is highly abundant in guard cells, its mutation led to loss of ABA sensitivity (Zhao et al., 2008), and exogenous application of isothiocyanates also closed stomata (Khokon et al., 2011). Exogenous isothiocyanate also increased NADPH oxidase activity and nitric oxide levels in guard cells (Khokon et al., 2011), and stimulates large-scale transcriptomic changes associated with oxidative stress responses (Kissen et al., 2016). It can be hypothesized that the effects of isothiocyanate is due to either the activation of specific signalling proteins, or the stimulation of cellular responses to the increased ROS and NO levels, or both. In animal systems the proposed modes of action for ingested isothiocyanates include direct binding to reactive thiol groups of proteins (Nakamura et al., 2018). Whether such mechanisms also hold true in plants will need further investigation.

**Growth regulating peptides**

Whereas the sulfation of desulfoglucosinolates by SOT16, 17 and 18 produce glucosinolates which are themselves inert until further activation by myrosinases, sulfation processes by other SOTs have been linked to direct modulation of bioactivity of their substrates. This is best examplified by the Tyrosyl Protein Sulfotransferase (TPST) (Komori et al., 2009). TPST-mediated sulfation of peptides phytosulfokine (PSK), plant peptide containing sulfated tyrosine 1 (PSY1) and root growth factor (RGF) is critical for the growth-promoting functions of these peptides (Komori et al., 2009; Matsuzaki et al., 2010; Zhou et al., 2010). Indeed, loss-of-function of TPST results in severe developmental defects, such as strongly decreased root growth and loss of stem cell identity in roots. The crucial role of TPST is exemplified by its strong evolutionary conservation pre-dating the emergence of land plants, in contrast to cytosolic SOTs such as SOT15 (Zhao et al., 2019). It has been hypothesized that TPST’s evolutionary conservation is due not just to its growth promoting effects, but also due to its involvement in stomatal regulation (Zhao et al., 2019) and in balancing the plant’s growth and stress responses (Figure 2). Its sulfated products, PSK and PSY1, function in both the induction of defense responses to pathogens and as growth factors; these two peptides also function antagonistically (reviewed in Sauter (2015)). This hypothesis is corroborated by the enhanced root growth sensitivity of tpst to copper deficiency compared to wild type (Wu et al., 2015). However, it is currently unknown whether TPST also coordinates growth and acclimation to other oxidative stresses such as drought or high light.

**Hormones and hormone derivatives**
SOTs are also capable of targeting at least four other classes of compounds involved in the coordination of growth and stress responses (Figure 2): brassinosteroids (SOT10, SOT12); salicylic acid (SA; sulfated by SOT12), flavonoids (SOT5, SOT8, SOT12, SOT13), and hydroxy-jasmonate (OH-JA; sulfated by SOT15). However, these reported substrates for SOTs have been mostly identified from in vitro assays and corresponding in vivo validation using genetic mutants have been largely lacking.

Brassinosteroids are regulators of growth and development (Wei and Li, 2016), but can also enhance oxidative stress tolerance through multiple mechanisms when applied exogenously (Sharma et al., 2017). SOT12 can sulfate multiple brassinosteroids in vitro but shows a preference for the brassinosteroid precursor 24-epicatasterone (Marsolais et al., 2007). Surprisingly, it is not known whether sot12 loss of function mutants display defects in brassinosteroid signalling. SOT10 prefers the biologically active end products of brassinosteroid biosynthesis such as 24-epibrassinolides and the naturally occurring (22R, 23R)-28-homobrassinosteroids. Interestingly, sulfation of 24-epibrassinolides can lead to suppression of its bioactivity (Rouleau et al., 1999). However, brassinosteroid-related phenotypes were not observed in sot10 loss-of-function or SOT10-overexpressing plants (Sandhu and Neff, 2013). These findings parallel that for the Brassica napus Sulfotransferase 3 (BnST3) and BnST4 enzymes, which are capable of targeting multiple brassinosteroids (Rouleau et al., 1999) but did not lead to BR-related phenotypes when over-expressed in Arabidopsis (Marsolais et al., 2004). Conjugation of polar moieties (e.g. through sulfation) to the relatively non-polar brassinosteroids has also been proposed to improve intracellular movement of brassinosteroids from their site of synthesis at the endoplasmic reticulum to their site of perception at the plasma membrane (Symons et al., 2008). Thus, it is suggested that brassinosteroid sulfotransferases regulate brassinosteroid activity, mobility and/or perception, although the exact mechanism(s) remain unknown.

Salicylic acid (SA) is a regulator of pathogen responses and cell death (Seyfferth and Tsuda, 2014). Baek et al. (2010) reported that SOT12 is able to transfer sulfate to SA. The authors proposed that sulfation of SA by SOT12 may be a key regulatory point for SA induction in response to pathogens, since sot12 sensitivity to the pathogen Pseudomonas syringae is accompanied by an inability to accumulate SA. The sot12 mutant is also more sensitive to some abiotic stresses such as salt stress. In rice, the Resistance to Rice Stripe Virus on Chromosome 11 (STV11) gene encodes a SOT that sulfates SA (Wang et al., 2014). STV11 mediates SA
accumulation *in vivo* and *Japonica* rice varieties lacking functional STV11 are sensitive to Rice Stripe Virus (Wang *et al.*, 2014).

Another known substrate for SOTs in *Arabidopsis* is hydroxyl-jasmonic acid (OH-JA), which functions synergistically and antagonistically to SA in regulating pathogen responses and cell death (Caarls *et al.*, 2015; Tamaoki *et al.*, 2013). Signalling by the biologically active JA can be controlled stepwise through enzymatic conjugation to amino acids, methylation, and hydroxylation to give rise to 12-hydroxy-JA (reviewed in Wasternack and Strnad (2016)). Interestingly, 12-hydroxy-JA and its related compound, 11-hydroxy-JA, can be further modified *via* sulfation by SOT15 to give rise to 12- and 11-hydroxy-JA sulfate respectively (Gidda *et al.*, 2003). Both 12-hydroxy-JA and 12-hydroxy-JA sulfate appear to be inactivated forms of JA, and they also down-regulate the expression of genes encoding for enzymes in JA biosynthesis (Miersch *et al.*, 2008). This suggests that 12-hydroxy-JA sulfate, and by extension SOT15, may constitute part of an “off” switch in JA signalling (Miersch *et al.*, 2008).

*Other stress-associated metabolites*

Flavonoids have diverse roles including UV protection, growth regulation and plant-microbe interactions (Falcone Ferreyra *et al.*, 2012). At least four SOTs are capable of sulfating a variety of flavonoids but with subtly different substrate preferences and enzymatic rates (Hashiguchi *et al.*, 2013; Hashiguchi *et al.*, 2014). Both SOT13 and SOT5 showed strong preference for the flavonol galangin, although the latter also has significant affinity for kaempferol. In contrast, the most preferred substrate for SOT12 is the flavonone naringenin, although it also processes galangin and kaempferol at similar rates to SOT13 and SOT5 (Hashiguchi *et al.*, 2013). Significantly, SOT12 is also active against quercetin, with a specific activity that is approximately 50% of that against its preferred substrate naringenin (Hashiguchi *et al.*, 2013). This raises the question whether SOT12 might also participate in auxin homeostasis *in vivo*, since quercetin-3-sulfate is proposed to regulate auxin transport in *Flaveria bidentis* (Ananvoranich *et al.*, 1994). However, auxin-related phenotypes have not been reported yet for *sot12*.

SOT12 is also capable to detoxify xenobiotic compounds produced by soil bacteria, such as the protein translation inhibitor cycloheximide (Chen *et al.*, 2015). While xenobiotic compounds are not regulators of plant growth and stress responses *per se*, these metabolites are also
produced endogenously in plants during high light stress and hence require detoxification (D’Alessandro et al., 2018).

**Biochemical basis for diversity of SOT substrates and functions**

The high number and variety of SOT substrates can be explained by the structural features of the SOT active site. Analysis of the SOT18 crystal structure reveals that plant SOTs have highly conserved catalytic residues and PAPS binding region which are also shared with orthologues from many different species (Hirschmann et al., 2017). In contrast, the binding site of the sulfate acceptor substrate along with three flexible loops gating the entrance to the active site are poorly conserved, thus providing the means for substrate specificity and specialization between SOTs (Hirschmann et al., 2017). Indeed, a small number of amino acid changes can lead to different activity levels and substrate preference of the same SOT enzyme from different Arabidopsis ecotypes (Klein and Papenbrock, 2009; Luczak et al., 2013); although these polymorphisms have not been mapped to the recent structural data. These findings could provide the basis for understanding the function and regulation of multi-substrate SOTs such as SOT12 during oxidative stresses. For example, the reported $K_M$ of SOT12 against 24-epicathasterone (6.9 µM) is several times lower compared to salicylic acid (440 µM), and the SOT12 specific activity against different flavonoids varies across two orders of magnitude (Baek et al., 2010; Hashiguchi et al., 2013; Marsolais et al., 2007). It will be interesting to explore the structural basis for these substrate preferences with respect to its protein structure.

**PAP-mediated chloroplast-to-nucleus retrograde signalling and physiological responses to abiotic stress**

While the different SOTs might process substrates with diverse chemical structures and roles in stress and growth homeostasis, one commonality shared between these enzymes is that they all consume PAPS, thereby producing PAP as a by-product (Figure 2). Early work on PAP-accumulating mutants showed that constitutively high PAP impedes auxotrophic growth in yeast and *E. coli* (Glaser et al., 1993; Masselot and De Robichon-Szulmajster, 1975; Neuwald et al., 1992). PAP most likely blocks sulfate assimilation through the inhibition of PAPS reductase in these organisms, which have a different sulfate assimilation pathway to plants and lack sulfotransferases (Murguia et al., 1995, 1996). In mammals, which lack a cysteine biosynthesis pathway but do possess sulfotransferases (Gamage et al., 2006; Mueller et al., 2015; Stipanuk, 2004), PAP accumulation inhibits sulfotransferase reactions and also interferes with protein translation through a sulfotransferase-independent mechanism (Frederick et al., 2008;
Hudson et al., 2013). PAP-accumulating mice are non-viable (Frederick et al., 2008; Hudson et al., 2013). In contrast, in plants PAP is not simply a toxic metabolite: although sal1 mutants in Arabidopsis show a dwarfed phenotype, PAP accumulation is not lethal and the mutants are also drought-tolerant (Estavillo et al., 2011; Phua et al., 2018a; Phua et al., 2018b; Wilson et al., 2009). Indeed, PAP acts as a chloroplast-to-nucleus retrograde signal during oxidative stresses such as drought and high light. Under these conditions, intracellular PAP accumulation and its perception in the nucleus activates stress-responsive gene expression, leading to acclimation responses and drought tolerance (Estavillo et al., 2011; Pornsiriwong et al., 2017).

Degradation, intracellular transport, and nuclear function of PAP

Unstressed plants maintain very low (almost undetectable) levels of PAP through the action of the PAP catabolic phosphatase SAL1, which hydrolyses PAP to AMP in chloroplasts and mitochondria (Estavillo et al., 2011; Quintero et al., 1996). During oxidative stress, however, PAP accumulates up to 30-fold higher under drought and by 50% higher under excess light (Estavillo et al., 2011). This accumulation is achieved at least in part via an oxidative post-translational inactivation of SAL1 (Chan et al., 2016a). Biochemical and structural analysis of the SAL1 protein show that formation of inter- and intra-molecular disulfide bonds in the SAL1 protein under oxidizing conditions decreases its capacity to degrade PAP. Modification of the redox-sensitive cysteines on SAL1 through glutathionylation similarly decreases its activity against PAP (Chan et al., 2016a). Redox regulation of SAL1 was also observed in vivo. Therefore, at least in chloroplasts, SAL1 functions as an oxidative stress and redox sensor by regulating PAP levels (Chan et al., 2016a; Estavillo et al., 2011) (Figure 3).

PAP is relocalised between organelles and the cytosol via the PAPS/PAP transporter 1 (PAPST1) and PAPST2, two antiporters which are able to exchange any two of PAPS, PAP, ATP and ADP (Ashykhmina et al., 2018; Gigolashvili et al., 2012). PAPST1 is localized to chloroplasts whereas PAPST2 is dual-targeted to both chloroplasts and mitochondria (Figure 3). Non-aqueous organelle fractionation experiments showed that PAP is present in the cytosol and the plastids, with lesions in PAPST2 causing altered intracellular distribution of PAP (Ashykhmina et al., 2018). Complementation experiments targeting SAL1 separately to either the chloroplast, cytosol or the nucleus in a sal1 loss-of-function mutant demonstrated that the different transgenic lines had almost equal reversion to the wild type phenotype (Estavillo et al., 2011). These results indicate that while PAPS and PAP are present predominantly in plastids and the cytosol, PAP can also move into the nucleus (Figure 3). The movement of PAP
between the nucleus and the cytosol is assumed to occur via passive diffusion through the nuclear membrane pores (Estavillo et al., 2011).

In the nucleus, PAP primarily targets 5’-3’ exoribonucleases XRN2 and XRN3, which have roles in the degradation of uncapped transcripts, removal of RNA Polymerase II (Pol II) from DNA to terminate transcription, and maintenance of gene silencing (Crisp et al., 2018; Gy et al., 2007). Inhibition of XRNs by PAP (Dichtl et al., 1997), leads to the activation of 25% of the high light stress transcriptome, including genes encoding antioxidant enzymes such as Ascorbate Peroxidase 2 (APX2) (Estavillo et al., 2011). Recent work by Crisp et al. (2018) and Krzyszton et al. (2018) provided valuable insights into the paradox of how specificity in the regulation of nuclear gene expression by PAP is achieved, given that the XRNs are not transcription factors which bind to specific DNA motifs. PAP-mediated inhibition of XRNs leads to inefficient removal of Pol II upon completion of transcription a particular gene, thereby facilitating polymerase read-through to the downstream gene and increasing transcription of this downstream gene. Indeed, the majority of the highest up-regulated genes in sal1 mutants are located downstream of an endogenously highly expressed gene. The effect of read-through on transcriptional up-regulation is also correlated with the distance between the upstream and downstream genes (Crisp et al., 2018). Of the genes up-regulated in sal1, 23% are directly up-regulated by read-through. Therefore, it is possible that genes up-regulated by Pol II read-through then lead to further up- and down-regulation of other genes via feedback effects or downstream signalling. Significantly, transcriptional read-through events were also observed in wild type plants during drought stress, indicating that Pol II regulation by PAP-XRN can be one of the mechanisms modulating gene expression for oxidative stress homeostasis (Crisp et al., 2018).

The characteristics of the PAP-responsive transcriptome are correlated with the increased oxidative stress tolerance phenotype in both sal1 and xrn2 xrn3 mutants. Constitutive up-regulation of stress homeostasis genes in these mutants lead to decreased hydrogen peroxide accumulation in response to high light stress, decreased ion leakage in response to osmotic stress, and accumulation of various osmoprotectants (Estavillo et al., 2011; Wilson et al., 2009). These biochemical alterations are in turn correlated with physiological outcomes such as increased drought tolerance, enhanced resistance to cadmium stress, and suppression of cell death (Bruggeman et al., 2016; Estavillo et al., 2011; Xi et al., 2016). Collectively, these results indicate that chloroplastic PAP-mediated signalling, via redox-inactivation of SAL1 and
intracellular PAP movement to the nucleus, can activate multiple molecular and physiological acclimation responses to oxidative stress.

**Beyond retrograde signalling: connections between PAP and other pathways**

The intersection(s) between PAP-mediated signalling and other stress signalling pathways are only just beginning to be defined. Pornsiriwong *et al.* (2017) showed that PAP complements, and participates in, the abscisic acid (ABA) signalling pathway in guard cells, which regulate water loss through stomata. Constitutive accumulation of PAP in *sal1* mutants rescued ABA sensitivity, stomatal closure and drought tolerance in ABA-insensitive mutants such as *open stomata 1 (ost1)*, which lacks a key activator kinase downstream of ABA perception. Exogenous PAP is also able to close stomata in both wild type and *ost1*. The *ost1 xrn2 xrn3* triple mutant also had restored ABA sensitivity and stomatal closure, indicating that PAP is restoring ABA sensitivity via inhibition of the XRNs. Activation of PAP-XRN signalling in guard cells up-regulates the expression of multiple genes involved in ABA signalling, including transcription factors and calcium signalling proteins such as *Calcium Dependent Protein Kinases 32 (CPK32)* and *CPK34*. Importantly, both CPK32 and CPK34 can phosphorylate, and activate, the SLAC1 anion channel whose activity is necessary for stomatal closure (Pornsiriwong *et al.*, 2017; Vahisalu *et al.*, 2008). Given that SLAC1 is also a phosphorylation target for the major ABA signalling kinase OST1 and other CPKs (Geiger *et al.*, 2010; Geiger *et al.*, 2009), these findings provide one mechanism by which PAP-mediated retrograde signalling complements hormonal signalling by converging upon common protein targets to regulate physiological responses to drought stress (Pornsiriwong *et al.*, 2017). Significantly, exogenous PAP application induces stomatal closure in representative species of all land plant clades, and PAP influences guard cell ion fluxes and ROS production in the same manner across multiple plant species (Zhao *et al.*, 2019). These findings, together with the targeting of SAL1 to plastids predating the emergence of stomata, indicate that the PAP-mediated retrograde signalling network had the capacity to be integrated with multiple cellular signalling networks throughout plant evolution (Zhao *et al.*, 2019).

The connections between PAP-mediated chloroplast communication and other signalling pathways still require extensive elucidation. Prolonged PAP accumulation exerts additional impacts on hormonal homeostasis and signalling. This was recently reviewed in Phua *et al.* (2018b) and thus will not be discussed in detail here. Nevertheless, it is intriguing to note that PAP accumulation alters JA metabolism and SA levels, and PAP also decreases glucosinolate
levels (Ishiga et al., 2017; Lee et al., 2012; Rodríguez et al., 2010). These alterations are linked to decreased plant immunity (Bruggeman et al., 2016; Ishiga et al., 2017), but have not been explored in the context of oxidative stress tolerance. PAP accumulation also increases tolerance to cadmium stress (Xi et al., 2016). The tolerance might be associated to altered levels, or signalling, of stress in the endoplasmic reticulum (ER), since sall shows lower induction of ER Unfolded Protein Response (UPR) marker genes in response to cadmium (Xi et al., 2016). However, direct links between chloroplastic PAP and ER signalling have not been demonstrated.

Co-operativity between secondary sulfur metabolites and intracellular signalling pathways

The substrates and/or products of the different sulfotransferases discussed above have partially overlapping functions in different cellular and physiological responses to oxidative stress. (Figure 2). In particular, it is noteworthy that PAP is involved in multiple physiological responses and would be produced regardless of which of the sulfotransferase(s) are activated, for example in the coordination of stress responses and growth (Figure 2). TPST and its sulfated peptide products are known regulators of growth especially in roots, while the putative SOT10/SOT12 substrate, brassinosteroids, are well-established regulators of root and shoot development. Similarly, sulfation of SA and 12-OH-JA by SOT12 and SOT15 respectively might affect the balance between SA and JA signalling in the regulation of stress responses and cell death. PAP accumulation concurrently up-regulates stress homeostasis genes and suppresses growth (Estavillo et al., 2011; Phua et al., 2018b; Rossel et al., 2006; Wilson et al., 2009). One possible mechanism by which PAP regulates growth and metabolism over short periods of drought stress is by extending the circadian period (Litthauer et al., 2018). Both sall and xrn mutants have extended circadian period and application of osmotic stress also prolongs the circadian clock (Litthauer et al., 2018). Primary metabolism and growth are strongly associated with circadian regulation; thus PAP together with other signal(s) might contribute to the regulation of these processes during oxidative stress (Jones, 2018). For instance, the effect of PAP on circadian rhythm is most pronounced under blue light (Litthauer et al., 2018). Enhanced activity of the blue light receptor proteins, cryptochromes, inhibits rosette growth (Lin et al., 1996); and cryptochromes have been implicated in responses to various oxidative stresses (D’Amico-Damião and Carvalho, 2018). PAP accumulation can suppress the abundance and/or downstream signalling of growth-promoting hormones such as gibberelic acid (GA) and auxin while up-regulating signalling by stress-responsive hormones such as
ABA (Phua et al., 2018b). Furthermore, the effects of PAP on growth can vary depending on its intracellular concentration. While strong PAP accumulation impedes growth, a small increase in cytosolic PAP pools can actually promote growth although the mechanism is currently unknown (Ashykhmina et al., 2018).

The involvement of secondary sulfur metabolites in guard cell regulation also highlights an interesting potential divergence in the roles of primary and secondary sulfur metabolism during drought. Various reports indicate that primary sulfur metabolism largely influences ABA synthesis; whereas secondary sulfur metabolism components are more prominent in ABA signalling. During drought, sulfate is actively loaded into xylem sap in a manner associated with ABA synthesis (Ernst et al., 2010). Indeed, sulfate up-regulates expression of ABA biosynthesis genes, and blocking sulfate transport into guard cells attenuates stomatal closure in leaves (Ernst et al., 2010; Malcheska et al., 2017). Sulfate and cysteine availability are important for ABA synthesis, with decreased sulfate and cysteine reducing ABA levels and impacting on stress tolerance (Cao et al., 2014). Direct feeding of cysteine to guard cells stimulates expression of the rate-limiting ABA biosynthesis gene NCED3, induces ABA accumulation, and closes stomata via the canonical ABA signalling pathway (Batool et al., 2018; Rajab et al., 2019). Accumulation of glutathione also leads to enhanced ABA levels, up-regulation of ABA synthesis and signalling genes at the translational level, and improved drought tolerance (Chen et al., 2011; Cheng et al., 2015). In contrast, PAP accumulation has variable impacts on ABA accumulation (Pornsiriwong et al., 2017; Rossel et al., 2006) but clearly activates multiple components of downstream ABA signalling via a parallel pathway that is independent of the canonical ABA signalling proteins (Pornsiriwong et al., 2017). The putative SOT substrates such as brassinosteroids and SA are also known to influence ABA-mediated guard cell responses (Ha et al., 2016; Prodhan et al., 2018).

Collectively, understanding the precise roles of PAP and other sulfotransferase substrate(s) and product(s) in stress and growth regulation networks may help to uncover how drought-stressed plants manage the dual problem of increased ROS production and diminished supply of water and carbon (Bechtold and Field, 2018).

**Regulatory mechanisms in secondary sulfur metabolism**

Secondary sulfur metabolism is tightly regulated at various levels (Figure 3). This regulation is important for at least two reasons. Metabolically, sulfur consumption through cysteine and
glutathione synthesis in primary assimilation needs to be coordinated with that of the aforementioned secondary sulfur metabolites (Chan et al., 2013; Mugford et al., 2011). In the context of cellular signalling and oxidative stress tolerance, metabolites such as PAP can play critical roles but also have strong impacts on various aspects of plant physiology (Phua et al., 2018b); thus their synthesis and degradation require tight control.

**Regulation of APKs catalyzing PAPS synthesis**

The biosynthesis of PAPS by the APK enzymes is subject to transcriptional and posttranslational control (Figure 3). The levels of *APK1* and *APK2* transcripts are regulated by MYB transcription factors which also regulate the glucosinolate biosynthesis genes (Yatusevich et al., 2010). In contrast, *APK3* seems to be only weakly activated, while *APK4* is not regulated, by these MYBs (Yatusevich et al., 2010). Low sulfur availability down-regulates *APK* expression alongside those of the glucosinolate genes, presumably to conserve sulfur for primary assimilation (Yatusevich et al., 2010). This down-regulation is mediated by the central transcription factor Sulfur Limitation 1 (SLIM1); as well as nuclear-localized transcriptional regulator proteins Sulfur Deficiency Induced 1 (SD1) and SDI2 which directly bind to, and inactivate, MYB28 (Aarabi et al., 2016; Maruyama-Nakashita et al., 2006). The APK enzymes are redox-sensitive *in vitro*; with cysteine disulfide-mediated dimerization strongly decreasing PAPS biosynthetic activity (Ravilious et al., 2012). This redox regulation appears to have evolved during the transition from cyanobacteria to land plants (Herrmann et al., 2015); and involves disulfide bonding between a cysteine present on a land plant-specific N-terminal domain with a cysteine located on the catalytic core. Disulfide bond formation inverts the binding affinities at the ATP/ADP and APS/PAPS sites (Ravilious and Jez, 2012; Ravilious et al., 2013). This altered order of adenosine binding decreases APK activity because binding of APS prior to ATP to the APK active site traps the enzyme in a dead-end complex (Ravilious and Jez, 2012; Ravilious et al., 2013). Due to the technical challenge to measure APK activity in leaf extract, the redox control of APK enzymes still awaits experimental validation *in vivo* (Mugford et al., 2009). During oxidative stress, it can be expected that APKs retain a degree of activity since the metabolites downstream of PAPS, such as glucosinolates and PAP, accumulate during drought (Estavillo et al., 2011; Mewis et al., 2012).

**Regulation of SOTs**

The transcriptional and posttranslational control of SOTs are still under-explored in the context of oxidative stress responses. Most SOTs have very low expression levels at unstressed...
conditions (Hirschmann et al., 2014). However, the expression of some SOTs can change significantly in response to oxidative stress and hormonal treatments (Baek et al., 2010; De Clercq et al., 2013; Ng et al., 2013).

The best-characterized SOTs at the level of transcriptional regulation are the glucosinolate-producing SOTs. Expression of SOTs 16, 17 and 18 is regulated by a group of six R2R3 MYB transcription factors including MYB28 and MYB29 (Burow et al., 2015; Frerigmann and Gigolashvili, 2014; Gigolashvili et al., 2009; Sønderby et al., 2010) (Figure 3). Similar to APK1 and APK2, the transcriptional repression of SOT17 and SOT18 under sulfur-deficient conditions is mediated by SDI1 and SDI2 through physical interaction with MYB28 (Aarabi et al., 2016). However, these SOTs do not seem to be targeted by the SLIM1 transcription factor since their repression by sulfur deficiency is unchanged in slim1 (Maruyama-Nakashita et al., 2006). Expression of SOT16 is also regulated through feedback regulation mediated by the glucosinolate biosynthesis gene 2-oxo acid-dependent dioxygenase 2 (AOP2) independent of MYB28 and MYB29 (Burow et al., 2015).

During drought stress, aliphatic glucosinolates were increased while indolic glucosinolates decreased in Arabidopsis phloem sap. The significance of these glucosinolate changes with regards to PAP levels is unclear, and it is currently not known whether and how the central transcriptional regulators SDI1/2 and SLIM1 might be involved in the drought stress response. Whether this process also involves differential regulation of SOTs 16, 17 and 18 will need to be investigated since SOT16 is specific for tryptophan-derived indolic desulfoglucosinolates whereas SOT17 and SOT18 process methionine-derived aliphatic desulfoglucosinolates (Klein and Papenbrock, 2009; Piotrowski et al., 2004). A recent survey of the sulfenylated plastid proteome shows that many amino acid metabolism enzymes, including those involved in tryptophan biosynthesis, contain oxidized cysteines after hydrogen peroxide treatment (De Smet et al., 2018). This raises the question whether GL synthesis and diversity could also be regulated upstream at the level of amino acid availability during oxidative stresses.

The expression of SOT12 is up-regulated in response to multiple abiotic stresses and hormonal treatments (Baek et al., 2010). Interestingly, SOT12 expression is also highly responsive to mitochondrial oxidative stress, for example in response to antimycin A treatment which blocks mitochondrial respiration (De Clercq et al., 2013; Ng et al., 2013). The SOT12 promoter contains a mitochondrial dysfunction motif (MDM) which is targeted by a group of ANAC
transcription factors responsive to mitochondrial oxidative stress (Figure 3). Mutation of the MDM in SOT12 abolishes its up-regulation under antimycin A treatment (De Clercq et al., 2013). Interestingly, the ANAC proteins regulating SOT12 expression are in turn regulated through protein-protein interactions by Radical-Induced Cell Death 1 (RCD1), a redox-sensitive WWE domain-containing protein which is suppressed by PAP accumulation (Shapiguzov et al., 2019). One recent hypothesis is that the SOT12 up-regulation could be linked to PAP signalling for maintaining mitochondrial homeostasis and suppression of cell death (Shapiguzov et al., 2019; Van Aken and Pogson, 2017). There is limited evidence for regulation of SOTs at the protein level through post-translational modifications, although enzymatic activity of SOTs is feedback-inhibited by high PAP levels (Hirschmann et al., 2017).

Regulation of SAL1 and PAP degradation

In contrast to SOTs, SAL1 transcript is readily detectable in multiple tissue types and developmental stages in unstressed conditions (Hruz et al., 2008). There is strong up-regulation of SAL1 during seed imbibition (Hruz et al., 2008) which also corresponds with the relatively higher SAL1 protein abundance in leaf tissue compared to dry seed (Pornsiriwong et al., 2017). Inducible or strong constitutive promoter-driven artificial micro RNA and RNA interference silencing lines of SAL1 show up to 90% reduction in SAL1 transcript abundance, but do not have a corresponding increase in PAP levels (Phua et al., 2018a). Furthermore, while PAP accumulation extends the circadian period, the expression of SAL1 is diurnal rather than circadian (Litthauer et al., 2018). Therefore, there is limited evidence for transcriptional regulation of SAL1 being a mechanism for regulation of PAP levels during stress. It is likely that the regulation of PAP is primarily driven by SAL1 enzymatic activity, which is consistent with SAL1’s relatively high affinity for PAP ($K_M < 10 \, \mu M$) and its inactivation via redox regulation (Chan et al., 2016a).

Under oxidative stress conditions, the regulation of SAL1 activity is largely mediated at the post-translational level through redox regulation rather than changes in protein abundance. The redox regulation of SAL1 is already discussed above in the context of PAP retrograde signalling, but two additional points are worth discussing. First, protein modelling through molecular dynamics simulations suggest that formation of the disulfide bonds in oxidized SAL1 decreases flexibility of Loop 1, a flexible protein loop that overhangs the active site (Chan et al., 2016a). Rigidification of Loop 1 is proposed to inhibit accessibility of the active site for substrate entry or product release (Chan et al., 2016a), and could represent an additional
regulatory target for modulation of SAL1 activity. Secondly, the redox midpoint potential (\(E_m\)) of SAL1 is approximately -308 mV and -284 mV at pH 7.5 for monomeric and dimeric SAL1 respectively. These values overlap with those of oxidation-inhibited APKs (-286 mV at pH 7.5) and oxidation-activated primary sulfur metabolism enzymes (-330 mV at pH 8.0 for APR and -318 mV at pH 7.0 for the glutathione biosynthesis enzyme GSH1) (Chan et al., 2016a; Hicks et al., 2007; Ravilious et al., 2012). This suggests that under oxidative stress, the redox status in the chloroplast may simultaneously regulate the activities of all three enzymes towards a coordinated sulfur consumption for protein and GSH synthesis, PAPS synthesis, and PAP accumulation.

**Transporters as enigmatic gatekeepers of organellar PAPS/PAP flux**

The coordination of PAP intracellular localization and movement via transporters is an emerging extra level of regulation for the secondary sulfur metabolism (Figure 1 and Figure 3). The localization of the PAPST1 and PAPST2 is consistent with the chloroplastic localization of APK1 and APK2 which are the major sources of PAPS for sulfation reactions (and most likely, PAP production); and with the chloroplastic-mitochondrial dual localization of SAL1 for PAP degradation. A simplistic model would have envisaged both transporters facilitating the export of PAPS from chloroplasts to the cytosol and import of cytosolic PAP into plastids for degradation. *In vitro* characterization of PAPST1 and PAPST2 show that both transporters are capable of transporting any two of ADP, ATP, PAP and PAPS in antiport mode. However, while recombinant PAPST1 shows a clear trend of substrate preference (ATP > PAPS > PAP >> ADP) when the counter-substrate is ATP; PAPST2 appears to have almost equal preference for PAP and ATP, with ADP and PAPS being only slightly less preferred. PAPST1 also shows an approximately nine-fold higher affinity for PAPS compared to PAPST2 (Ashykhmina et al., 2018; Gigolashvili et al., 2012).

Importantly, genetic data indicate that PAPST1 and PAPST2 play different roles *in vivo* (Ashykhmina et al., 2018). The papst1 mutant shows a significant decrease in glucosinolates, strong accumulation of desulfo-glucosinolates, up-regulation of glucosinolate biosynthetic genes, and accumulation of the primary sulfur metabolites cysteine and GSH. These features indicate that PAPST1 is important for the shuffling of PAPS produced in the chloroplast into the cytosol for utilization by SOTs for example in glucosinolate biosynthesis, and that its absence leads to re-direction of sulfur flux into primary sulfur metabolism (Gigolashvili et al., 2012). In contrast, papst2 only shows a small reduction in glucosinolates and correspondingly
low accumulation of desulfo-glucosinolates, with no up-regulation of glucosinolate biosynthetic genes. The accumulation of GSH and cysteine is also less marked in papst2. Therefore, PAPST2 is less important in provision of PAPS to the cytosol. Organelle fractionation experiments show higher cytosolic PAP accumulation in papst2, but not in papst1, compared to wild type plants (Ashykhmina et al., 2018). Importantly, crossing papst2, but not papst1, to the sal1 mutant allele fryl exacerbates cytosolic PAP accumulation and the fryl phenotype; and the frylpapst2 phenotype cannot be rescued by complementation with mitochondrial-targeted SAL1. Collectively, these results indicate that PAPST2 has a greater role in the transport of cytosolic PAP into organelles for degradation by SAL1, although PAPST1 is still able to transport PAP into the chloroplast. Indeed, targeting PAPST1 to the mitochondria also fails to rescue the papst2 phenotype, indicating independent roles for these two transporters (Ashykhmina et al., 2018). Therefore, it is possible that under oxidative stress conditions PAPST1 and PAPST2 contribute to the control of PAPS availability for sulfation and PAP localization for signalling respectively.

There are several unanswered questions with respect to PAPS/PAP transport (Figure 3). First, given the multiple possible substrates for PAPST1 and PAPST2, the kinetics and direction of transport for PAPS and PAP mediated by these two transporters during oxidative stress is difficult to predict (Ashykhmina et al., 2018). Second, PAPS is clearly needed in the Golgi for TPST-mediated sulfation reactions (Komori et al., 2009), but a PAPS/PAP transporter has not been identified for the Golgi. Third, that both PAPST2 and SAL1 co-localize to the mitochondria would suggest that either PAP has a metabolic role in this organelle, or that SAL1-PAP are involved in mitochondrial retrograde signalling (Van Aken and Pogson, 2017; Van Aken and Whelan, 2012). Fourth, low cytosolic PAP accumulation in papst2 enhances growth (Ashykhmina et al., 2018) (possibly through stimulation of SOTs) whereas high cytosolic PAP accumulation in sal1 mutants suppresses growth (Phua et al., 2018b). Whether this growth suppression in sal1 is due to the primed stress responses (Estavillo et al., 2011; Wilson et al., 2009), suppression of circadian rhythm (Litthauer et al., 2018), sulfur deficiency (Lee et al., 2012), alteration of hormonal signalling (Phua et al., 2018b), or all of the above, will need to be addressed. Since papst2 does not share the abovementioned sal1 phenotypes such as low sulfate and GSH content but both mutants do have lower glucosinolates (Ashykhmina et al., 2018; Lee et al., 2012), it is possible that different levels of cytosolic PAP activate different signalling pathways. The contribution of the PAPST proteins in regulating these different pathways during oxidative stress conditions will need to be critically addressed.
The road forward: how do we uncover new insights into secondary sulfur metabolism?

Some key questions in secondary sulfur metabolism (also indicated in Figure 3) that are still awaiting resolution include: 1) What are the functions and in vivo substrates of the SOTs? Would SOT functions dramatically differ in non-Brassicaceae species which lack glucosinolates? 2) What is the identity of the elusive Golgi PAPST(s)? 3) How is secondary sulfur metabolism coordinated across PAPS synthesis, PAP production and degradation, and intracellular shuttling of metabolites in response to different oxidative stresses? 4) Are there additional regulatory mechanisms beyond transcriptional control and redox modulation of protein activity? 5) What is the wider intersection network with other oxidative stress signalling pathways for plant acclimation to stress?

The plant sulfur metabolism pathways are extensively studied using a combination of biochemistry, protein structural biology and mutant characterization. These approaches provide a high degree of detail on individual protein(s) but are less helpful in identifying new pathway components. Most likely, the latter will be facilitated by utilization of “big data” and next-generation `omics technologies (reviewed in Kopriva et al. (2015)). For example, PAPST1 was identified through co-expression analysis of publically available transcriptome data for putative transporters strongly co-expressed with glucosinolate metabolism (Gigolashvili et al., 2012).

The increasing availability of genome sequences, for example through the 1000 Plants (1KP) project (Matasci et al., 2014), will allow investigation of how SOTs have diversified and identify “core” SOTs with key roles throughout evolution. A recent analysis shows that only TPST and SOTs 19-21 are evolutionarily conserved from lycophytes (moss) to higher land plants, whereas most of the cytosolic SOTs arose much later in evolution (Zhao et al., 2019); suggesting that SOTs 19-21 play (unknown) conserved roles in plant function. Mining of unbiased proteomics data could also reveal new questions. Our quick survey of SOTs 16-18 using the PTMViewer database (https://dev.bits.vib.be/ptm-viewer/index.php) (Willems et al., 2018) shows that these enzymes can be acetylated and/or contain reversibly oxidized cysteines: how do these posttranslational modifications affect protein function and overall regulation of the metabolic pathway? A complement of “new” and “old” strategies can help to reveal new insights into a pathway that is of secondary importance by classical definitions of metabolism, but that is clearly playing primary roles in plant acclimation to oxidative stresses and beyond.
Acknowledgements

KXC is funded by a Postdoctoral Fellowship from the Research Foundation – Flanders (FWO); grant number 12N4818N. FVB is funded by Fonds Wetenschappelijk Onderzoek (G0D7914N); Universiteit Gent (Bijzonder Onderzoeks Fonds, 01J11311); and Fonds De La Recherche Scientifique – FNRS. This work was supported by the Fonds Wetenschappelijk Onderzoek – Vlaanderen (FWO) and the Fonds de la Recherche Scientifique – FNRS under EOS Project No. 30829584.
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in supplying plastidic phosphoadenosine 5’-phosphosulfate to the cytosol. The Plant cell 24, 4187-4204.


Figure 1. The secondary sulfur metabolism pathway in *Arabidopsis*.

Inorganic sulfate anions taken up from the soil are transported into the cell via SULTR transporter proteins. ATP-consuming sulfate activation by ATPS isoforms in chloroplasts and the cytosol provides APS which feeds into either primary sulfur assimilation via APR for cysteine and glutathione synthesis, or into secondary sulfur metabolism through APK-mediated phosphorylation to PAPS. SOT-catalyzed reactions in the cytosol and Golgi transfer the S moiety from PAPS to acceptor compounds, thereby producing PAP as a by-product. PAP is degraded by SAL1 enzymes which are localized to both chloroplasts and mitochondria. The shuttling of PAPS and PAP between plastids and the cytosol is mediated by PAPST1 and PAPST2. Accumulated PAP can also traverse to the nucleus to alter RNA metabolism and gene expression. Transporters are shown as dark blue boxes, enzymes as light blue boxes, and metabolites as light yellow ovals. Black arrows indicate enzymatic reactions while blue arrows indicate metabolite movement. The un-identified Golgi PAPS/PAP transporter(s) and transport mechanism are indicated by a blue box with dashed outline and dashed blue lines respectively. Abbreviations: SULTR, sulfate transporter; ATPS, ATP sulfurylase; APS, adenosine phosphosulfate; APK, APS Kinase; APR, APS Reductase; PAPS, 3’-phosphoadenosine 5’-phosphosulfate; SOT, sulfotransferase; TPST, tyrosyl protein sulfotransferase; PAP, 3’-phosphoadenosine 5’-phosphate; PAPST, PAPS/PAP transporter; AMP, adenosine monophosphate; XRN, exoribonuclease; Cys, cysteine; GSH, glutathione.
Figure 2. Inter-cooperativity between secondary sulfur metabolism components and hormonal signalling for cellular homeostasis during oxidative stress.

The different secondary sulfur metabolism metabolites are able to intersect with different facets of oxidative stress signalling pathways or physiological responses mediated by hormonal signalling such as ABA, SA and JA. In several cases the putative substrates or products of SOTs converge on the same process. Note that a commonality shared between the diverse SOT reactions is that they all produce PAP as a by-product. PAP accumulation also impacts on the three aspects of oxidative homeostasis outlined above (see text). Metabolite and protein colour schemes are the same as for Figure 1, except that phytohormones are shown as purple ovals.

Abbreviations: ABA, abscisic acid; GLs, glucosinolates; SA, salicylic acid; JA. Jasmonic acid; OH-JA, hydroxy jasmonic acid; BR, brassinosteroids; Flv, flavonoids, PSK, Phytosulfokine; RGF, Root Growth Factor
Figure 3. Regulation of secondary sulfur metabolism components and SAL1-PAP retrograde signalling during oxidative stress.

During conditions of oxidative stress such as drought and high light, a shift in the chloroplast redox poise decreases SAL1 activity against PAP, and is expected to have similar effects on APK activity. The accumulated PAP is able to travel intracellularly and inhibit XRNs in the nucleus, causing RNA Pol II read-through and activation of gene expression. PAP is also able to feedback-inhibit SOT activity. Red diamonds indicate the outstanding questions relating to transcriptional regulation of SOTs, the identity and role of SOT substrates and products, redox regulation of APKs, and transport mechanisms of PAPS and PAP during oxidative stress.