Roles of ROS and redox in regulating cell-to-cell communication: Spotlight on viral modulation of redox for local spread

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Abstract

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Abstract:

Reactive oxygen species are important signaling molecules that influence many aspects of plant biology. One way in which ROS influence plant growth and development is by modifying intercellular trafficking through plasmodesmata (PD). Viruses have evolved to use plasmodesmata for their local cell-to-cell spread between plant cells, so it is therefore not surprising that they have found ways to modulate ROS and redox signaling to optimize plasmodesmata function for their benefit. This review examines how intracellular signaling via ROS and redox pathways regulate intercellular trafficking via PD during development and stress. The relationship between viruses and ROS-redox systems, and the strategies viruses employ to control PD function by interfering with ROS-redox in plants is also discussed.

Introduction

Reactive oxygen species (ROS) are oxygen-containing molecules that are highly reactive due to the presence of unpaired electrons. In plant cells, ROS are produced as natural byproducts of various metabolic processes (Mittler, 2017). While ROS are important for various signaling and regulatory processes in plants, excessive ROS levels can cause oxidative stress, leading to damage in cellular components such as DNA, proteins, and lipids (Mittler et al., 2022). Some common types of ROS include the superoxide radical (O2*-), hydrogen peroxide (H_2O_2), the hydroxyl radical (*OH), and singlet oxygen ($^1O_2$). Nitric oxide (NO.) and peroxynitrite

(ONOO-) are classified as reactive nitrogen species (RNS) and are capable of exacerbating oxidative stress (del Río and López-Huertas, 2016; Mittler, 2017).

To protect against oxidative stress, plants have evolved elaborate antioxidant defense systems that include thioredoxins (TRX) and enzymes like superoxide dismutase (SOD), catalase, peroxidases, and various non-enzymatic antioxidants like ascorbate and glutathione. These antioxidants help neutralize and regulate ROS levels, maintaining cellular redox balance and protecting the plant from oxidative damage (Mittler et al., 2022). Redox state, short for reduction-oxidation state, refers to the balance of electrons in a chemical reaction. It involves two processes: reduction (gain of electrons) and oxidation (loss of electrons). The connection between redox (reduction-oxidation) reactions and ROS lies in the transfer of electrons during chemical processes. Redox reactions involve the exchange of electrons between molecules, leading to the reduction and oxidation of species. When electrons escape from this redox process, particularly in the electron transport chains of photosynthesis or cellular respiration, they can interact with molecular oxygen, giving rise to ROS (Foyer and Hanke, 2022). Disruptions in redox balance can lead to cellular dysfunction and contribute to diseases (Mittler, 2017) (Mittler et al., 2022).

Each cell in a plant is surrounded by a cellulosic wall which hampers communication between plant cells that involves direct plasma membrane contacts or plasma membrane-facilitated ligand-receptor interactions that are commonly found in animals. Plants have instead evolved intercellular connections called plasmodesmata (PD) that permeate the cell wall to allow direct trafficking of metabolite and signaling molecules between connected cells (Azim and Burch-Smith, 2020). Beyond their roles in trafficking endogenous plant molecules, PD also provide a route for direct cell-to-cell conveyance of viruses during infection (Heinlein, 2015; Reagan and Burch-Smith, 2020). PD are not only routes for direct cell-to-cell signaling, but they also connect individual cells to the vasculature for systemic or long-distance transport in the plant. The last few years have seen an exciting resurgence in interest in the role of PD in long-distance signaling. There is accumulating evidence that many mRNA molecules can travel long distances in the plant from their sites of synthesis (transcription) to their sites of action to regulate developmental processes (Thieme et al., 2015). The role of PD in long-distance signaling via propagation of ROS and calcium signals is also garnering attention (Toyota et al., 2018; Fichman et al., 2021), although the role of PD is these processes may not be as straightforward as thought (Bellandi et al., 2022). This review focuses on local cell-to-cell movement and explore how ROS and redox impacts cell-to-cell communication. It also discusses how redox signaling is modified by plant viruses to manipulate PD for their local cell-to-cell movement during infection as one illustration of how redox mechanisms contribute to plant responses to biotic stress.

Organelles as ROS production sites

ROS are generated within plant cells in response to various stimuli, including biotic and abiotic stresses, hormone signaling, and developmental cues. Chloroplasts, the hub of photosynthesis in plant cells, play a crucial role in the generation of ROS. As part of photosynthesis, electrons can escape from the electron transport chain, leading to the formation of O2·- and H_2O_2. The production of ROS in chloroplasts involves both photosystems I and II, leveraging the surplus of photons harnessed by PSII and directing electrons towards molecular oxygen via PSI, (Foyer and Hanke, 2022; Li and Kim, 2022). In photosynthesizing cells, peroxisomes are required for photorespiration that occurs when ribulose-1,5-bisphosphate carboxylase/oxygenase (RubiscCO) favors O_2 as a substrate instead of CO_2. The phosphoglycolate produced by this reaction is transported from chloroplasts to peroxisomes where it is broken down and H_2O_2 is produced (Tripathy and Oelmuller, 2012). Peroxisomes are also involved in other metabolic processes, including fatty acid and polyamines breakdown and detoxification of harmful substances. Peroxisomal ROS are also produced as by-products during these processes (del Rio and Lopez-Huertas, 2016; Corpas et al., 2020). Thus, in photosynthesizing cells, the chloroplasts and peroxisomes together account for approximately a significant proportion of ROS production. Glyoxysomes, specialized peroxisomes in germinating plant cells, participate in ROS production while converting stored lipids to carbohydrates (Janku et al., 2019; De Bellis et al., 2020). As in all eukaryotes, mitochondria are the respiratory organelles in plant cells. Mitochondrial ROS (mtROS) are produced by electron leakage occurring within the electron transport system, which is influenced by the

inhibition of particular sites within the electron transport chain (ETC) or the reduction state of ETC components, as substrates are metabolized (Moller, 2001; Huang et al., 2016). Together, chloroplasts, peroxisomes and mitochondria account for the bulk of ROS production in plant cells (Tripathy and Oelmuller, 2012).

Apart from these main sources or ROS, the plasma membrane (PM), endoplasmic reticulum (ER) and apoplast also produce ROS. The Respiratory Burst Oxidase Homologs (RBOHs), are transmembrane PM proteins that transfer electrons from cytosolic NADPH to molecular oxygen, leading to superoxide radicals' production and their transformation into other ROS. RBOHs can also be regulated by protein kinases, such as calcium-dependent protein kinases (CDPKs) and mitogen-activated protein kinases (MAPKs), which can phosphorylate and activate RBOHs (Chapman et al., 2019) (Zandalinas and Mittler, 2018). The ER generates ROS during oxidative protein folding, driven by the formation of ROS due to the occasional incorrect formation of disulfide bonds and the NAD(P)H-dependent electron transport system using Cytochrome P450 (Cyt P450) (Sharma et al., 2012; Cao and Kaufman, 2014). The apoplast and cell wall are additional sources of ROS production, involving various factors, such as the respiratory burst, cell wall peroxidases, polyamine oxidases, and processes like cell wall remodeling and lignification. RBOHs release superoxide radicals into the apoplast, notably during stress responses or immune reactions (Schmidt et al., 2018).

3. ROS as a regulator of cell-to-cell communication

There is accumulating evidence for ROS and redox signaling as regulators of cell-to-cell communication via PD. ROS play a dual role in cell-to-cell communication, acting as both messengers and regulators. They are involved in diverse physiological processes, such as growth, development, and responses to abiotic and biotic stresses (Benitez-Alfonso et al., 2011; Pelaez-Vico et al., 2022). ROS function as signaling molecules by modulating the activity of various proteins and enzymes, which in turn influence gene expression and cellular responses (Waszczak et al., 2018) (One example of ROS acting as signaling molecules exerting effects on PD is in organelle-nucleus-plasmodesmata signaling (ONPS, discussed below). In addition, ROS serve as direct regulators by engaging with proteins associated with PD, modulating their activity via various ways, and thereby impacting the exchange of molecules between plant cells through these pores (Waszczak et al., 2018). This finely tuned ROS-mediated communication network allows plants to adapt and coordinate their responses to changing conditions, ensuring their survival and successful growth in their dynamic environments.

3.1 ROS signaling to regulate plasmodesmata

Callose, a β-1,3-glucan, plays a critical role in regulating PD-mediated intercellular trafficking. Accumulation of callose in the cell walls surrounding PD is associated with decreased intercellular trafficking, presumably via the physical occlusion of the PD pore. Conversely, removal or degradation of callose from around PD results in increased intercellular trafficking (Zavaliev et al., 2011). CALLOSE SYNTHASE (CALS) enzymes, (also called GLUCAN SYNTHASE LIKE, GSLs), are responsible for callose deposition at PD while β-1,3-glucanases degrade callose surrounding PD. ROS can influence callose deposition and degradation through the activation of enzymes like callose synthases and β-1,3-glucanases **(Fig. 1)** . Direct application of H_2O_2 shows dose-dependent effects on PD trafficking (Rutschow et al., 2011; Cui and Lee, 2016). One of the best-characterized mechanisms for ROS-induced callose deposition at PD involves PD LOCATED PROTEIN (PDLP)5 and CALS10/GSL8 (Saatian et al., 2023). Several other CALS/GSL proteins associate with PDLPs, and forming complexes that regulate PD trafficking during development or in response to stress. CALS3/GSL12 is involved in regulating intercellular trafficking during root development (Vaten et al., 2011). GSL8/CALS10 is required for guard cell differentiation and stomata development, and loss of GSL8 leads to increased intercellular trafficking CALS1 (Guseman et al., 2010). CALS1 and CALS8 have been implicate in stress-dependent PD regulation, with CALS8's activity in callose deposition being induced in response the H_2O_2 (Cui and Lee, 2016). Further evidence linking ROS to CALS and PD regulation comes from studies with Roxy1 and Roxy2, two Glutaredoxins (GRXs) small oxidoreductases. Mutations in these genes have been shown to affect the expression of CALS5 and two *β-1,3 γλυςανασε* genes, consequently impacting the accumulation of callose in the pollen cell wall (Xing and Zachgo, 2008). (Xing and Zachgo, 2008, The Plant Journal).

ROS can act as secondary messengers, triggering intracellular signaling pathways. For instance, ROS can initiate calcium ion (Ca^{2+}) influx, leading to changes in cytosolic Ca^{2+} concentrations. The rise in Ca2+ levels is recognized as a universal intracellular signal that can initiate a range of cellular responses including changes in PD permeability (Yoneyama et al., 2004; Gilroy et al., 2014; Evans et al., 2016).

Calreticulin is an ER localized Ca^{2+} binding protein important for buffering Ca^{2+} levels, among other functions (Costa et al., 2018). Maize calreticulin was found to be associated with PD (Baluska et al., 1999) as was calreticulin from tobacco (*N. tabacum*) BY2 (Laporte et al., 2003) and Arabidopsis (Bayer et al., 2004) cell cultures. Presumably, PD-associated calreticulin resides in the lumen of the PD-associated ER, the desmotubule. Increased calreticulin levels have been detected when callose has accumulated at PD to limit cell-to-cell trafficking, as in the case of stress due to Aluminium toxicity (Sivaguru et al., 2000). Aluminum stress also induced elevated calreticulin at PD of*Medicago truncatula* roots associated with arbuscular mycorrhizal fungi (AMF) (Sujkowska-Rybkowska and Znojek, 2018). Calreticulin has also been implicated in callose accumulation at PD in response to chilling stress in maize (*Zea mays*) (Bilska and Sowinski, 2010). Calreticulin levels at PD increased as early as four hours after chilling in chilling sensitive maize lines were accompanied by reduced carbon export and physical restriction of PD pores as revealed by transmission electron microscopy. Interestingly, this increase in calreticulin at PD and PD occlusion at early time points during chilling was not associated with callose accumulation, which did not occur until later times. These findings could be interpreted as indicating that calreticulin and its roles in Ca^{2+} regulation may have a distinct functions in regulating PD, physically modulating pore size (Demchenko et al., 2014). This hypothesis is supported by observations of the nitrogen-fixing nodules of *Casuarina glauca,* where PD lost calreticulin as the size of PD apertures decreased during nodule maturation (Demchenko et al., 2014). The PD in these mature cells also do not appear to be associated with callose.

Supporting a role for calreticulins in regulating PD trafficking during stress, a PD-targeted calreticulin was found to interact with the Tobacco mosaic virus (TMV) movement protein, and overexpression of this protein impaired the cell-to-cell trafficking of the movement protein itself and redirects the movement protein from the PD to microtubules (Chen et al., 2005). Concordant with these observations, intercellular viral spread was inhibited when the calreticulin was overexpressed. These findings with TMV contrast with recent findings with Bamboo mosaic virus (BaMV). *N. benthamiana* calreticulin 3 (NbCRT3) along with ER luminal-binding protein 4 (BiP4), both ER proteins, were found to interact with the TBGp3 movement protein of BaMV to facilitate viral cell-to-cell movement (Huang et al., 2023). Increased levels of NbCRT3 resulted in increased viral cell-to-cell spread, possibly due to increased or more efficient targeting of another movement proteinTGBp1 to PD. It remains to be determined whether the effects of NbCRT3 on trafficking of TGBp1 are simply due to increased endomembrane trafficking or if downstream signaling mediated by NbCRT3 is at play.

Besides calreticulin, calmodulins are class of Ca^{2+}sensing proteins that associated with PD (Fernandez-Calvino et al., 2011). Calmodulin-like protein 41 (CML41) from Arabidopsis is involved in responses to bacterial infection where it triggers callose accumulation at PD, resulting in reduced intercellular trafficking of GFP probes (Xu et al., 2017). Indeed, increased PD callose levels and reduced PD trafficking were observed as early as 30 minutes after application of flg22, the elicitor derived from bacterial flagellin, and this response was dependent on Ca^{2+} levels as application of the chelator EGTA abolished PD inhibition in response to flg22. These rapid defenses were absent in plants where *CML41*expression was knocked down, and there was constitutively elevated callose levels at PD in plant overexpressing *CML41* . CML41 interacts with the receptor-like kinase NOVEL CYS-RICH RECEPTOR KINASE (NCRK) as part of a module that causes ROS-induced callose accumulation at PD (Vu et al., 2022). This interaction is crucial because NRCK phosphorylates CML41, which is necessary for the accumulation of callose at the PD mediated by GLS4.

It has been well established that altering redox state in the chloroplast or the mitochondria affects PD permeability and thus cell-to-cell trafficking of biomolecules. The maize mutant *sucrose export defective1 (sxd1)* failed to export photosynthate from sites of photosynthesis and had reduced intercellular trafficking (Russin et al., 1996). It was proposed that was caused by accumulation of callose at PD located at the

bundle-sheath and vascular parenchyma interface (Russin et al., 1996; Botha et al., 2000), although other evidence suggests that this was likely not solely due to altered PD function (Asensi-Fabado et al., 2015). SXD1 is the maize homolog of Arabidopsis *VTE1* which encodes a chloroplast tocopherol cyclase required for production of the antioxidant vitamin E (Provencher et al., 2001; Porfirova et al., 2002). These observations led to the hypothesis that chloroplasts could regulate PD and intercellular trafficking. Subsequent analyses in the Arabidopsis *ise1* and *ise2* mutants with defects in a mitochondrial and chloroplast RNA helicase, respectively, supported this hypothesis (Burch-Smith et al., 2011). These mutants were isolated from a genetic screen of embryonically lethal mutants on the premise that defects in PD would drastically disrupt development leading to unviability (Kim et al., 2002). In *ise1* and *ise2*Arabidopsis embryos and in *N. benthamiana* leaves where*ISE1* or *ISE2* expression was knocked down by virus-induced gene silencing (VIGS) increased intercellular trafficking was correlated with increased numbers of PD (Burch-Smith and Zambryski, 2010). Loss of ISE1 or ISE2 led to massive changes in gene expression, with chloroplast-associated genes representing the largest class of affected genes (Burch-Smith et al., 2011). These findings suggest that defects in organelles resulted in changes in nuclear gene expression, fine tuning expression of not only genes impinging on the defective organelles but also genes affecting organelles that were functionally linked. This hypothesis was described as ONPS when it described effects on PD (Burch-Smith et al., 2011; Brunkard and Burch-Smith, 2018). In particular, signals from chloroplasts may regulate PD-associated nuclear gene expression and thereby intercellular trafficking mediated by PD to control flux of chloroplast metabolites including fixed carbon (Brunkard and Burch-Smith; Ganusova et al., 2020).

A possible clue about the signaling involved in ONPS came using with redox-sensitive GFP (ro-GFP) probes. When expressed in a specific subcellular compartment, ro-GFP can report changes in the redox status of glutathione pools of that compartment (Bombarely et al., 2012) (Goodin et al., 2008). Analyses with these probes revealed that chloroplasts were more reduced in *N. benthamiana* leaves where*ISE1* or *ISE2* was silenced (Stonebloom et al., 2012). In *ISE1* -silenced mitochondria were more oxidized compared to non-silenced leaves. The link between organelle redox status and intercellular trafficking capacity was supported by application of drugs that induced ROS production in specific organelles. Salicylhydroxamic acid (SHAM) induced mitochondrial ROS production and this apparently increased intercellular trafficking, consistent with observations from *ISE1* -silenced leaves. In contrast, paraquat induced chloroplast ROS production and led to decreased intercellular trafficking as expected from results with ISE2-silenced plants. These redox-related findings are also consistent with those from an independent mutant with defective PD function. The Arabidopsis *gfp arrested trafficking (gat)1* mutation also links PD function to chloroplast since it encodes plastid *THIOREDOXIN m3,* and intercellular trafficking was reduced in *gat1* seedlings (Benitez-Alfonso et al., 2009). Analysis of this and related mutants revealed that plastid oxidative stress decreased PD-mediated trafficking (Benitez-Alfonso and Jackson).

Other mutants with defects in chloroplasts and PD-mediated have recently been identified. In the Arabidopsis *dig8* mutant, defects in chloroplasts were associated with increased ROS levels and induction of ROS-related genes, concomitant reduced PD-mediated intercellular trafficking (Zhang et al., 2023). *DIG8* encodes a protein predicted to be a chloroplast peptide release factor and therefore important for chloroplast gene expression. The chloroplast protein Kunitz peptidase inhibitor-like protein (KPILP) is a positive regulator of intercellular trafficking (Ershova et al., 2022). Overexpression led to increased intercellular trafficking of a 2xGFP proteins in *N. benthamiana* leaves. While ROS levels in tissues with increased KPILP levels were not reported, it is interesting that KPILP is induced by prolonged darkness and viral infection, conditions that are known to disturb chloroplast function and ROS production.

Curiously, only a few studies have explicitly examined the relationship between light and intercellular trafficking. Early work found that intercellular trafficking of GFP decreased in leaves kept in the dark (Liarzi and Epel, 2005). In contrast, a more recent study found that intercellular trafficking of GFP was strongly regulated by light, with longer light exposures leading to higher rates of trafficking (Brunkard et al., 2020). Importantly, this study made two other observations relevant to the current discussion. First, it demonstrated that ATP levels are important for intercellular trafficking as plants with reduced chloroplast ATP levels caused by silencing the chloroplast*AtpC* gene has reduced capacity for trafficking GFP. Second, the

difference in trafficking between day and night was not dependent on PD callose levels, hinting at other mechanisms at play in regulating PD function (Brunkard et al., 2020). Further support for ATP (energy) levels as important determinant of PD trafficking capacity come from studies showing that TOR signaling is an important regulator of intercellular trafficking (Brunkard et al., 2020). Independent confirmation of the importance of ATP levels in modulating PD function was supported by work with the Arabidopsis *ch1-3.* Studies with this mutant under varying light regimes revealing that ATP and NADPH levels were important for regulating PD-mediated intercellular trafficking (Dmitrieva et al., 2021). Importantly, one finding of this study is that chloroplast ROS levels and redox states are likely not important regulators of intercellular trafficking. Further investigation of the roles of chloroplast redox state and chloroplast-generated ROS in ONPS is therefore warranted. In response to high light stress in Arabidopsis, the production of reactive oxygen species (ROS) resulted in an augmentation of intercellular carboxyfluorescein transport and an enlargement of plasmodesmata (PD) pores. This effect was contingent on the presence of PDLP1 and PDLP5 {Fichman, 2021}.

3.2 ROS as a direct interactor /regulator

ROS act as direct regulators of plasmodesmata by influencing their permeability, often through mechanisms involving the modulation of callose deposition, actin dynamics, and redox signaling. This regulation is crucial for the coordinated transport of various molecules between plant cells and is a key component of plant growth, development, and stress responses.

Elevated ROS levels influence the phosphorylation status of various proteins, including those associated with plasmodesmata. Phosphorylation modifications can alter the conformation and interactions of these proteins, affecting their ability to regulate plasmodesmal permeability. For example, ROS application intensifies the interaction between PD proteins (CML41) and NCRK [described above; (Vu et al., 2022)]. ROS can also induce redox modifications in proteins, forming disulfide bonds or other covalent modifications. These changes can impact protein-protein interactions, potentially altering the assembly and stability of plasmodesmal structures (Cremers and Jakob, 2013; McDonagh, 2017; Juan et al., 2021).

ROS can modulate actin filament dynamics by activating actin-binding proteins. Alterations in actin organization influence plasmodesmal aperture and transport rates, potentially impacting the movement of molecules through these channels. Actin remodeling is dependent on ROS generated by the defense-associated NADPH oxidase, RBOHD. For example, denser actin arrays were observed in response to pathogen- and damage-associated patterns (PAMP and DAMP) than in mock-treated plants. RBOHD mutant fails to induces in cortical actin arrays in response to PAMP and DAMP (Li et al., 2017).

Lipid peroxidation is a process wherein oxidants, such as free radicals, target lipids containing carbon-carbon double bonds, notably polyunsaturated fatty acids (PUFAs) (Alche, 2019; Juan et al., 2021). Plasma membranes and chloroplasts are particularly susceptible to ROS generation due to their rich content of PUFAs. In PD, there is a higher prevalence of and monounsaturated fatty acids (MUFAs), like sphingolipids, which are essential components of PD, compared to PUFAs (Grison et al., 2015; Zhang et al., 2022). While MUFAs are less susceptible to lipid peroxidation than PUFAs, peroxidation of PUFA in conditions of heightened oxidative stress disrupt lipid bilayer, and has the potential to impact structural integrity and permeability.

4. Roles of redox in virus cell-to-cell movement

Viruses are obligate intercellular pathogens and in plant cells they are restricted to the symplast form by PD connecting host cells. Most plant viruses typically use the phloem for systemic transport, except for a few that can enter the xylem (Kappagantu et al., 2020, Sun et al., 2022). Plant viruses can modulate ROS production and scavenging by targeting enzymes crucial for maintaining redox balance, such as TRXs. TRXs belong to a family of small, highly conserved proteins found in plants and other organisms. Serving as thiol-disulfide oxidoreductases, they play a significant role in plants' antioxidant defense system, contributing to protection against oxidative stress. Typically, they have a positive role in reducing ROS and maintaining cellular redox balance (Sevilla et al., 2015; Mata-Perez and Spoel, 2019). Since most studies report on the roles of TRX in ROS/redox processes related to infection, TRXs will be the focus of this discussion.

Specific members of Trx possess the ability to move from cell to cell and regulate PD permeability. For example, the rice thioredoxin h protein, RPP13-1, can translocate from the initially injected cell into surrounding cells, resulting in an increase in the plasmodesmal permeability (Ishiwatari et al., 1998). In Arabidopsis, when AtTrxh9 is controlled by the tissue-specific promoter SCARECROW (pSCR), which specifically directs downstream gene expression in the single endodermal cell layer of the root, AtTRXh9 migrated from its initial expression site in endodermal cells to other cell layers of the root (Meng et al., 2009). These observations suggests a potential role in regulating PD permeability. It implies that Trx proteins can function as signaling molecules, enhancing intercellular communication by modifying PD-mediated intercellular trafficking.

Certain TRXs are actively involved in plant defense mechanisms against pathogens. They have the capacity to influence the activation of defense genes and the production of antimicrobial compounds in response to pathogen attacks (Mata-Perez and Spoel, 2019). In Arabidopsis, the cytosolic TRXhs AtTRXh3 and AtTRXh5 play critical roles in the salicylic acid (SA)-dependent defense pathway. Their roles revolve around the reduction of disulfide bonds in the NPR1 oligomer complex, thereby generating monomeric NPR1 proteins. These monomeric NPR1 proteins are subsequently translocated to the nucleus, where they function as transcription factors to activate SA-related defense genes [Fig. 1; (Tada et al., 2008; Liu et al., 2020)). A separate study has proposed an SA-independent role for NPR1 in which it exerts a negative influence on the activation of the adaptive unfolded protein response (UPR). This was observed when *npr1* mutants displayed a significant increase in UPR marker genes (Lai et al., 2018). Additionally, it is worth noting that a reduction in roGFP2 activity, a cytosolic ROS sensor, was observed in the cytosol under ER stress conditions. This observation supports the idea that ER stress can lead to a reduction in cytosolic redox potential, akin to the effects of SA accumulation, a condition that is known to induce the nuclear translocation of NPR1 (Lai et al., 2018).

4.1 Cytoplasmic TRX: Regulating ROS and restricting viruses

Plant viruses have developed mechanisms to engage with and manipulate members of the TRX protein family to enhance their own replication and systemic dissemination (Table 1). The exploration of the roles of TRX proteins in plant-virus interactions has uncovered a common theme. In many instances, viruses can repress or counteract the functions of TRX, resulting in favorable conditions for viral cell-to-cell movement. In many cases, viruses can suppress or counteract TRX functions, creating favorable conditions for viral cell-to-cell movement **(Fig. 1)** . This suppression also leads to an increase in ROS levels. However, despite the well-established regulatory roles of ROS in PD permeability and intercellular trafficking, the outcome of virus-redox interactions depends on the organelle where ROS is produced. In some cases, viral control of TRX can help establish a microenvironment rich in ROS that facilitates viral accumulation and spread. In other cases, virus-mediated reduction of ROS hampers SA-related defenses, including those regulating PD permeability **(Table 1)** (Wu et al., 2018; Wang et al., 2021; Vu et al., 2022).

For example, NbTrxh1 hampers the movement of various viruses, including Barley stripe mosaic virus (BSMV), Lychnis ringspot virus, Beet black scorch virus, and Beet necrotic yellow virus (Jiang et al., 2022). However, the interaction between NbTrxh1 and the γb protein of BSMV leads to the reduction of NbTrxh1's reductase activity. This decrease negatively impacts downstream SA-mediated gene expression, thus facilitating viral movement. The study concluded that type-h TRXs play a broad role in defending against both RNA and DNA viruses in plants (Jiang et al., 2022). The enhanced viral movement observed in*NbTrxh1* -silenced *Nicotiana benthamiana* plants suggested an effect of NbTRXh1 on PD permeability although no direct analysis of PD function was made **(Fig. 1)** .

In a similar fashion to *NbTRXh1* , pepper TRXh1 plays a crucial role in defending against Cucumber mosaic virus (CMV) and Euphorbia mosaic virus-Yucatan Peninsula (EuMV-YP). It is worth noting that in*CaTRXh1* -silenced pepper (*Capsicum annum*) plants, the accumulation of SA is higher, yet their susceptibility to EuMV-YP is greater compared to control plants (Luna-Rivero et al., 2016). These findings suggest that cytosolic TRXs negatively affect viral cell-to-cell movement, probably by controlling PD permeability via NPR1-mediated SA signaling.

Like cytosolic TRXs, chloroplast TRXs In *N. benthamiana* play roles in plant-virus interactions. The chloroplast NADPH-dependent thioredoxin C (NTRC) protein has a significant role in resistance against BSMV (Wang et al., 2021). Plants that constitutively expressed NTRC exhibited elevated levels of chloroplast ROS, contributing to their defense against BSMV. Notably, when genes encoding ROS scavengers, such as *2-Cys Prx* which interacts with NTRC, were silenced, BSMV accumulated to higher levels than in control plants (Wang et al., 2021). This phenomenon was attributed to the BSMV-encoded γb protein's capacity to interfere with and subvert NTRC-mediated chloroplast antioxidant defenses, leading to the creation of an oxidative chloroplast microenvironment necessary for BSMV infection. The γb protein was found to interact with NTRC and impair NTRC-2-Cys Prx interactions, thereby facilitating systemic infection. Notably, when the NTRC-γb interaction is disrupted by introducing a mutant γb (H85A), $BSMV_{H85A}$failed to spread systemically and exhibited lower accumulation levels in the inoculated leaves compared to wild-type BSMV (Wang et al., 2021, EMBO Journal). *N. tabacum* transgenic lines constitutively expressing the chloroplast-localized *NtTRXh3* showed increased resistance to TMV and Cucumber mosaic virus (CMV), whereas silencing this gene led to suppressed defense responses, and increased accumulation of both viruses (Sun et al., 2010). There are other examples where viral proteins were reported to interact with different *TRX* members, such as TGBp1 from Pepino mosaic virus with the tomato *TRX SlTXND9* (Mathioudakis et al., 2018), and TRX-like proteins from whitefly with the coat proteins of two begomoviruses (Saurav et al., 2019). However, their exact roles in the context of host-virus interactions remain unexplored.

Recent research has unveiled that virus-derived small interfering RNA (vsiRNA) originating from the wheat yellow mosaic virus (WYMV) not only targets the WYMV genome but also the thioredoxin-like gene within the wheat chloroplast. TaAAED1 was identified as a key player with a negative regulatory role in ROS accumulation (Liu et al., 2021). When*TaAAED1* was overexpressed in wheat protoplast, it promoted susceptibility to WYMV by reducing ROS levels. However, transgenic wheat expressing these vsiRNAs exhibited reduced levels of *TaAAED1* and increased ROS levels, ultimately leading to resistance against various viruses. Nevertheless, it remains unexplored whether this defensive effect is confined to the cellular ROS level or influences the cell-to-cell movement of infecting viruses. Given the established negative impact of TRXs on ROS accumulation and, consequently, PD permeability, it is likely that TaAAED1 exerts similar effects on PD as observed in *N. benthamiana* plants.

While several TRX genes exhibit a common role in reducing ROS levels and governing antiviral resistance (Table 1), an exceptional TRXh variant, predominantly lacking canonical cysteines yet demonstrating chaperone-like attributes, has been unveiled. This distinct maize (*Zea mays)* ZmTRXh variant can suppress the accumulation of sugar cane mosaic virus (SCMV) RNA, thus contributing to resistance against SCMV (Liu et al., 2017). Remarkably, its mechanism of action appears distinct from the conventional SA or JA defense signaling pathways.

4.2 Plasma membrane ROS and viruses

The silencing of *NbTRXh2* , a PM-localized protein, has been associated with increased accumulation of Bamboo mosaic virus (BaMV) in the inoculated leaves, suggesting that *NbTRXh2* acts as a negative regulator of BaMV movement. Further, NbTRXh2 interacts with BaMV's triple gene block protein 2 (TGBp2), although the precise implications for regulating cell-to-cell movement have not been fully explored (Chen et al., 2018). Interestingly, an alteration in the homeostasis of HMG1/2a nucleo-protein in *N. benthamiana* led to reduced levels of*NbTRXh2* and enhanced systemic infection of BaMV (Alazem et al., 2020). This effect, however, was absent in the inoculated leaves. Supporting this notion is the role of *RBOHD* in potato defense against PVY. RBOHD is responsible for ROS production at PM and in the apoplast. In the potato-PVY pathosystem, when *Ny-1* potato plants (Ny-1 is a PVY^N-strain specific *R* -gene) with silenced *RBOHD* (shRBOHD) were infected with the PVY^N strain, the PVY^N accumulated less in the *shRBOHD* -transgenic plants compared to NahG-plants (Lukan et al., 2020). Despite *shRBOHD* plants accumulating high levels of SA, they allowed for more extensive viral trafficking to systemic leaves compared to NahG plants. This led to the conclusion that RBOHD-generated ROS are not involved in the induction of SA biosynthesis in the potato-PVY pathosystem. Instead, it was observed that the*UGT76D1* gene, which plays a role in SA

biosynthesis, was upregulated in *shRBOHD* plants, providing an explanation for the elevated SA content in these plants. The notable presence of high SA levels in the *shRBOHD* plants, coupled with the increased viral trafficking to systemic leaves, raises the question of whether the ROS-mediated effect on cell-to-cell movement relies on the SA pathway, at least within the context of the potato-PVY pathosystem. It should also be noted that SA is reported to induce callose accumulation at PD and reduce intercellular trafficking in a PDLP5-dependet manner (Lee et al., 2011), raising questions about the sites of action of SA with relation to regulating PD.

4.3 Mitochondrial ROS and viruses

Examples of mitochondrial ROS in the context of host-virus interactions indicate varying effects. For example, systemic infection by TMV was exacerbated in SHAM-treated tomato plants (Liao et al., 2012). SHAM is an inhibitor of the mitochondrial alternative oxidase (AOX), resulting in elevated mitochondrial ROS levels in plants (see above). In contrast, treatment with potassium cyanide (KCN), a cytochrome pathway inhibitor, decreased ROS levels and enhanced resistance to TMV (Liao et al., 2012). These results contrast with the effects reported by Stonebloom et al., where increased mtROS led to increased intercellular trafficking (Stonebloom et al., 2012).

While relatively little is known about the involvement of mtROS in plant responses to plant viruses, more is known in animal systems. The NS1 protein from Mink Enteritis Virus (MEV), an autonomous parvovirus causing acute hemorrhagic enteritis in minks, induces apoptosis in HEK293T cells through the mitochondrial pathway. NS1-transfected cells show increased ROS production and activation of p38-MAPK, leading to p53 phosphorylation that mediates the mitochondrial apoptotic process. These findings suggest that MEV pathogenicity depends on disrupting various aspects of mitochondrial function, including disruption of redox status (Lin et al., 2019) (Lin et al., 2019 J. Virology doi: 10.1128/JVI.01249-19.). Mitochondrial *TRXs* can also be involved in viral disruption of host defense systems. For instance, TRX2, which is localized in mitochondria, negatively regulates innate immunity in Hela cells by disrupting the assembly of the virus-induced signaling adaptor (VISA) complex (Li et al., 2020). This complex is crucial for inducing type I interferons and eliciting innate antiviral responses. TRX2 achieves this inhibition by suppressing ROS production. Knockdown of *TRX2* enhances Sendai virus replication by triggering IFN-B induction. It will be interesting to see how commonly plant viruses manipulate mitochondrial ROS during infection.

4.4 TRX's Impact on Plasmodesmata: Dependent on or Independent of SA

The effects of cytosolic TRX on increasing NPR1 monomerization, thereby bolstering the SA-mediated antiviral defenses, are potentially intertwined with the established roles of SA in reducing PD permeability. However, it is worth noting that regardless of their subcellular locations, impairing or silencing most of the TRXs leads to increased PD-mediated intercellular trafficking. This is likely due to the disruption of the redox balance and an increase in ROS, suggesting that the TRX-mediated rise in SA levels and the expression of SA-related genes might not signify heightened resistance to viral threats. Instead, it appears to be a consequence of the fine-tuned equilibrium between cellular defense mechanisms and intracellular mobility via PD (Fig. 1). The established role of SA in regulating callose deposition at PD, which in turn reduces PD permeability (Wang et al., 2013; Huang et al., 2020), along with ROS's function in reducing intercellular trafficking by limiting PD permeability (Rutschow et al., 2011), highlights a crucial point. It is interesting to note that ROS-induced decreases in PD permeability remain consistent, irrespective of the subcellular origin of the ROS (plasma membrane, cytoplasm, or chloroplasts). This collective evidence suggests that the influence of ROS on PD permeability can in some cases be distinguished from that of the SA pathway. This is in harmony with the findings of Cui and Lee, where exogenous application of H_2O_2 decreased PD permeability in SA-mutants and independent of the SA-regulated PDLP5, indicating that there are pathways in which ROS can induce callose at PD independent of SA (Cui and Lee, 2016). Nevertheless, the outcome of virus-redox interactions cannot be generalized and depends on the organelles where a virus influences ROS production to create an environment favoring accumulation and spread (Table 1).

Resistant soybean plants demonstrated a robust upregulation of photosynthesis-related genes in response to soybean mosaic virus (SMV) infection. Upon close examination of two specific genes, namely, the photosystem I (PSI) subunit *PSaC* and the ATP synthase subunit ɑ (*ATΠσψν-ɑ*), it was observed that their overexpression within the SMV genome in a susceptible soybean cultivar resulted in a reduction in SMV accumulation in the inoculated leaves. This was coupled with an increase in the expression of genes associated with the antiviral RNA silencing pathway and defense hormone signaling pathways such as SA and ABA (Bwalya et al., 2022). Interestingly, the C-termini of these two proteins physically interacted with NIb and NIa-Pro proteins encoded in the SMV genome. When SMV chimeras expressing these C-termini infected susceptible soybean plants, the ability of SMV to accumulate locally and spread systemically was diminished (Bwalya et al., 2023). Importantly, the overexpression of these two genes did not lead to an increase in ROS production in soybean leaves. However, given the documented roles of ABA and SA in controlling PD permeability (Alazem, 2017), it can be inferred that the elevated expression of these photosynthesis genes, as well as the ABA and SA genes, impeded aspects of cell-to-cell movement, likely contributing to the limited accumulation of SMV in the inoculated leaves.

Conclusions

Recent years have heralded important advances in understanding the roles of ROS and redox signaling in regulating intercellular trafficking via PD. Yet, many crucial unknowns still exist. How organelle redox state is 'communicated' to PD remains an open question. Current data suggests that other signals besides ROS may be involved and so the role of TRX and other redox systems should be examined. Further, how light affects PD and intercellular trafficking remains mysterious. Given the critical importance of light quantity, quality, and duration to plant development, one can expect that plants would integrate these parameters to optimize not only PD function in trafficking metabolites and signals but also the formation and structure of the PD themselves. The interference of viruses with the mechanisms controlling PD permeability such as ROS and redox remains a complex area. Beyond the TRXs discussed here, other ROS and redox regulators are also implicated in viral infection, although there is room for more exploration. Therefore, using viruses as a tool to understand factors and mechanisms that control PD function could unveil crucial insights into unexplored facets of PD biology, and can help shed the light on the molecular dynamics governing viral spread within plants.

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Table 1. Effects of different enzymes, targeting ROS, on resistance to viruses . VIGS: Virus Induced Gene Silencing. SHAM: salicylhydroxamic acid. AOX: mitochondrial alternative oxidase

Gene/treatment	Subcellular localization	Interacting Virus	Effect on ROS	Effect on virus	References
NbTrxh1	*Cytoplasm*	Barley stripe mosaic virus (BSMV) Lychnis ringspot virus (LRSV) Beet black scorch virus (BBSV) beet necrotic yellow vein virus (BNYVV)	VIGS increased ROS	VIGS increased viral movement	(Jiang et al., 2022)
CaTrxh1	*Cytoplasm*	Cucumber mosaic virus (CMV) Euphorbia mosaic virus-Yucatan Peninsula (EuMV-YP)	VIGS increased ROS	VIGS increased viral accumulation	(Luna-Rivero et al., 2016) et al.
NtTRXh3	*Cytoplasm*	Tobacco mosaic virus (TMV) Cucmber mosaic virus (CMV)	N/A	VIGS increased viral accumulation constitutively expressing increased resistance	(Sun et al., 2010)
ZmTRXh	*Cytoplasm*	Sugar cane mosaic virus (SCMV)	N/A	Overexpression lines showed increased resistance to SCMV	(Liu et al., 2017)
NbNTRC	*Chloroplast*	BSMV	Transient expression increased ROS	Transient expression increased resistance. VIGS increased BSMV accumulation. Silencing ROS scavengers increased BSMV levels.	(Wang et al., 2021)

Gene/treatment	Subcellular localization	Interacting Virus	Effect on ROS	Effect on virus	References
TaAAED1	*Chloroplast*	Wheat yellow mosaic virus (WYMV) Chinese wheat mosaic virus (CWMV) BSMV	Transient expression reduced ROS	*TaAAED1* RNAi lines showed reduced viral accumulation	(Liu et al., 2021)
NbTrxh2	*Plasma membrane*	Bamboo mosaic virus (BaMV)	N/A	VIGS increased viral movement	(Chen et al., 2018)
RBOHD	Plasma membrane	PVY^N	RBOHD-RNAi line Increased ROS	Increase PVY accumulation and movement in potato carrying the *Ny-1 R* gene	(Lukan et al., 2020)
SlTXND9	*Mitochondria*	Pepino mosaic virus (PepMV)	N/A	N/A	(Mathioudakis et al., 2018),
SHAM	Mitochondria	TMV	SHAM inhibits AOX leading to increase in ROS	TMV increased in AOX-inhibited tomato plants	(Liao et al., 2012)

Figure Legend

Fig. 1 Effects of Thioredoxins (TRXs) on Plant-Virus Interactions : Thioredoxins (TRXs), a small family of thiol-disulfide oxidoreductases, play a crucial role in the antioxidant defense system of plants by reducing ROS and maintaining cellular redox balance. Viruses target TRXs to interfere with their function, creating a microenvironment conducive to virus accumulation and intercellular movement. The outcome of the TRX-virus interaction varies based on the organelle associated with TRX: 1- Endoplasmic Reticulum (ER): TRX reductive activity on NPR1 oligomers occurs, leading to NPR-1 monomerization. Monomeric NPR-1 proteins translocate to the nucleus, acting as co-transcription factors for salicylic acid-related genes, such as the *CALLOSE SYNTHASES* (*CALS*). Therefore, cytosolic TRXs regulate cell-to-cell movement in a salicylic acid (SA)-dependent manner, suggesting a potential role in controlling plasmodesmata permeability. 2- Chloroplast: TRX effects on ROS vary depending on the host and the virus. In *N. benthamiana* , NTRC induces ROS, promoting resistance to BSMV. BSMV interferes with NTRC, modifying the ROS environment to favor virus replication and spread. In Wheat, however, *TaAAED1* induces susceptibility to WYMV by reducing ROS levels. 3- Plasma Membrane: ROS production at the plasma membrane via*RBOHD* is involved in PVYN resistance in potatoes. Silencing*RBOHD* increases susceptibility and PVY^Nmovement despite high SA levels in *RBOHD* -silenced plants, indicating an uncoupled effect of *RBOHD* (and ROS) from SA in the PVY-potato pathosystem. 4- Mitochondria: Inhibition of alternative oxidase by salicylhydroxamic acid (SHAM) in tomato plants elevates mitochondrial ROS levels, facilitating fast trafficking of TMV.