

Research

Redox regulation of photosynthetic gene expression

Guillaume Queval and Christine H. Foyer*

Centre for Plant Sciences, Faculty of Biology, University of Leeds, Leeds LS2 9JT, UK

Redox chemistry and redox regulation are central to the operation of photosynthesis and respiration. However, the roles of different oxidants and antioxidants in the regulation of photosynthetic or respiratory gene expression remain poorly understood. Leaf transcriptome profiles of a range of *Arabidopsis thaliana* genotypes that are deficient in either hydrogen peroxide processing enzymes or in low molecular weight antioxidant were therefore compared to determine how different antioxidant systems that process hydrogen peroxide influence transcripts encoding proteins targeted to the chloroplasts or mitochondria. Less than 10 per cent overlap was observed in the transcriptome patterns of leaves that are deficient in either photorespiratory (*catalase (cat)2*) or chloroplastic (*thylakoid ascorbate peroxidase (tapx)*) hydrogen peroxide processing. Transcripts encoding photosystem II (PSII) repair cycle components were lower in glutathione-deficient leaves, as were the thylakoid NAD(P)H (nicotinamide adenine dinucleotide (phosphate)) dehydrogenases (NDH) mRNAs. Some thylakoid NDH mRNAs were also less abundant in tAPX-deficient and ascorbate-deficient leaves. Transcripts encoding the external and internal respiratory NDHs were increased by low glutathione and low ascorbate. Regulation of transcripts encoding specific components of the photosynthetic and respiratory electron transport chains by hydrogen peroxide, ascorbate and glutathione may serve to balance non-cyclic and cyclic electron flow pathways in relation to oxidant production and reductant availability.

Keywords: photosystem II repair cycle; cyclic electron flow; NAD(P)H dehydrogenase (NDH) complex; redox regulation; gene expression; respiration

1. INTRODUCTION

Photosynthesis is regulated by metabolic and environment controls that balance energy supply and demand in order to optimize photosynthetic efficiency while minimizing back reactions [1] that favour the production of reactive oxygen species (ROS). The regulation of the photosynthetic electron transport chain incorporates a range of mechanisms designed to prevent the over-reduction of electron carrier that inevitably leads to ROS production [2]. Flexibility in the production of adenosine triphosphate (ATP) and reductant by the photosynthetic electron transport system is required to match the changing demands of metabolism while accommodating variations in light and CO₂ availability and avoiding over-reduction and uncontrolled oxidation [2,3]. This is achieved through rapid, short-term modifications in post-translational controls and longer term regulation through regulation of gene expression. Photosynthetic control of gene expression is observed in response to state transitions. It also serves to accommodate varying metabolic demands for ATP and reductant

that occur when the rate of photorespiration is varied relative to photosynthesis [2]. In addition, the production and accumulation of different forms of ROS (superoxide (O₂⁻), hydrogen peroxide (H₂O₂) and singlet oxygen (¹O₂)) by photosynthesis and respiration are important regulators of photosynthetic and respiratory gene expression, as are the reductants (reduced glutathione (GSH), ascorbate, thioredoxin (Trx) and peroxiredoxins) that are generated or regenerated by the reduced ferredoxin and NAD(P)H (nicotinamide adenine dinucleotide (phosphate)) produced by photosynthesis [1,2].

Superoxide and hydrogen peroxide are produced by photosynthesis, photorespiration and respiration, as well as other metabolic pathways [4,5]. Genetic evidence has demonstrated unequivocally that ROS are signalling molecules with important and specific roles in the control of gene expression [4,6–10]. Uncontrolled oxidation is prevented by the complex cellular antioxidant network, which limits not only the lifetime of ROS but also facilitates the propagation of oxidative and reductive signals [11].

The majority of proteins with functions in photosynthesis are encoded by the nuclear genome [12]. The coordination of the expression of photosynthetic genes in the nuclear and chloroplast genomes involves a complex network of signalling pathways. Of these, retrograde signals that convey information from the chloroplasts to the nucleus are considered to be essential in tailoring gene expression to match the varying

* Author for correspondence (c.foyer@leeds.ac.uk).

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rstb.2012.0068> or via <http://rstb.royalsocietypublishing.org>.

One contribution of 16 to a Theo Murphy Meeting Issue ‘The plant thylakoid membrane: structure, organization, assembly and dynamic response to the environment’.

requirements of electron transport and metabolism for different components under fluctuating environmental conditions [12,13]. The photosynthetic electron transport chain is an important source of retrograde signals such as singlet oxygen, which is generated by photosystem II (PSII) and superoxide, which is produced abundantly on the reducing side of photosystem I (PSI) [7,9,12,13]. If the hydrogen peroxide generated by photorespiration is not efficiently removed by catalase, salicylic acid (SA)-dependent cell death pathways are activated [8]. Similarly, singlet oxygen can trigger cell death via the activation of a signal transduction sequence involving the two plastid proteins called EXECUTER1 and EXECUTER2 [7,9]. The flexible control of oxidant production and removal is therefore a crucial determinant of cell fate. ROS signals participate in the chloroplast-to-nucleus signalling network, functioning alongside other factors such as the redox state of the plastoquinone pool, intermediates of chlorophyll biosynthesis, genomes uncoupled (GUN) proteins, abscisic acid (ABA) and sugar signalling [14].

2. ANTIOXIDANTS AND REDOX SIGNALLING

The metabolic and signalling action of ROS is 'hormetic' in function because controlled ROS production stimulates growth and stress tolerance. Hormesis describes the biological phenomenon whereby beneficial effects such as improved stress tolerance, growth or longevity are derived from the exposure to low levels (or higher amounts over short periods) of compounds that are toxic at high doses or by exposures to low doses over long periods. Thus, depending on the relative rates of production and removal, singlet oxygen and hydrogen peroxide can either activate the expression of defence genes such as glutathione S-transferases (GST) or trigger genetically programmed cell suicide pathways [7,9,11,13].

Perhaps because of the immense complexity of cellular oxidant/antioxidant interactions, the respective roles of each component in signal transduction pathways remain largely unexplored. However, accumulating evidence suggests that low molecular weight antioxidants such as ascorbate and GSH participate in redox signalling [15–17]. For example, the abundance of ascorbate might convey information concerning the redox buffering capacity of the chloroplast and mitochondria and other cellular compartments. Ascorbate regulates plant growth and defence through the activation of SA- and ABA-dependent pathways that also influence jasmonate signalling [15,17]. In contrast, the GSH pool may contain information concerning the production of γ -glutamyl cysteine and GSH by the chloroplasts, as well as abundance of thiols and the redox state of the cell [16]. GSH controls the cell cycle and is required for the correct establishment of the root meristem [18].

The abundance of ascorbate and GSH in leaves is regulated by the amount and quality of light [19–21]. While the last step of ascorbate synthesis occurs in the mitochondria as indicated in figure 1, the response of ascorbate synthesis to high light is considered to be controlled largely by the regulated expression of *VTC* (*VITAMIN C*)2/5, which encodes guanosine diphosphate-L-galactose phosphorylase, a

cytosolic enzyme that catalyses the first dedicated biosynthetic step of the ascorbate synthesis pathway [21]. The leaves of the *Arabidopsis vtc2* mutants accumulate less (75–85% lower) ascorbate than the wild-type [15,17]. Similarly, *Arabidopsis vtc1* mutants, which carry a point mutation in a gene encoding an enzyme that catalyses an earlier step in the pathway, also accumulate considerably less (about 70% lower) ascorbate than the wild-type [15,17].

Catalases and ascorbate peroxidases (APXs) play important roles in the removal of hydrogen peroxide from the cellular environment (figure 1). While catalases are enzymes that catalyse the disproportionation of hydrogen peroxide to water and oxygen (figure 1), APXs reduce hydrogen peroxide to water, producing monodehydroascorbate and thereafter dehydroascorbate. Ascorbate is rapidly regenerated using photosynthetic reductants as illustrated in figure 1. *Arabidopsis* contains a small family of APX genes that are targeted to the cytosol, peroxisomes, mitochondria and chloroplasts, which has stromal (sAPX) and thylakoid-bound (tAPX) forms. Deficiencies in the sAPX and tAPX forms resulted in increased sensitivity to high light. Moreover, the expression of ROS-responsive genes under high light conditions was repressed in these mutants [22]. Hence, H₂O₂-derived signals produced by the chloroplasts may exert a negative influence on the expression of ROS-responsive genes, particularly under high light [22].

GSH is synthesized in plants from component amino acids by two ATP-dependent steps, the first of which is catalysed by γ -glutamyl cysteine synthetase (γ -ECS) and occurs exclusively in plastids in *Arabidopsis*. The second step is catalysed by glutathione synthetase and is located in the cytosol and chloroplasts [16,23]. Feedback control of GSH synthesis in the chloroplast by GSH [16] can be overcome by high γ -ECS activity [23], which increases the abundance of amino acids that are synthesized in chloroplasts particularly valine, leucine, tyrosine and isoleucine [24]. The *rootmeristemless* (*rml*)1 genotype has a point mutation in the *GSH1* gene that encodes γ -ECS and results in impaired GSH synthesis such that the leaves accumulate less than 5 per cent of the GSH levels observed in the wild-type [18]. GSH is a powerful redox buffer that can prevent the oxidation of surface-exposed cysteines on proteins, which can be oxidized to form sulfenic acid, impairing protein function. Stabilization of the protein sulfenic acid residues can occur by disulfide linkage to another thiol group, or they can be hyper-oxidized to sulfinic and sulfonic acid forms. Along with disulfide reductases, glutaredoxins and Trxs, GSH participates in thiol–disulfide exchange reactions. Thiol–disulfide exchange regulation of proteins can have a profound effect on folding and activity. Crucially, disulfides formed during oxidation function as redox switches. For example, redox modification of transcription factors can alter their DNA-binding properties and activates signal transduction pathways. In addition, there are several examples of transcription factors that are induced by oxidative signalling pathways in plants. For example, the NAC (NAM, ATAF1,2, CUC2) transcription factor *JUNGBRUNNEN* (*JUB*)1 is induced by hydrogen peroxide; *JUB1* expression

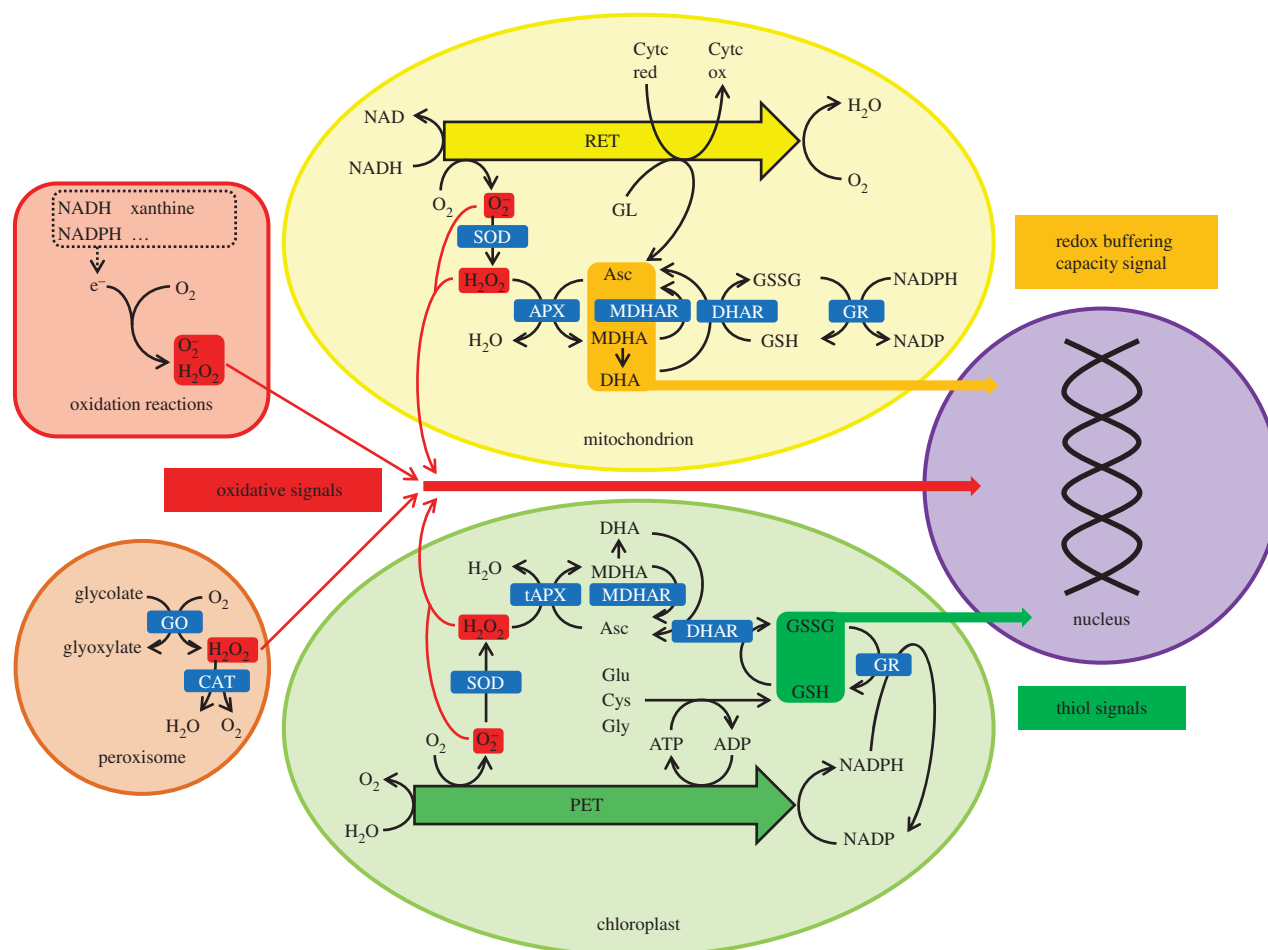


Figure 1. Schematic of possible signalling pathways between organelles and the nucleus triggered by low glutathione synthesis transmitting thiol signals, low ascorbate transmitting signals concerning cellular redox buffering capacity and decreased hydrogen peroxide scavenging transmitting an oxidant signal. APX, ascorbate peroxidase; Asc, ascorbate; CAT, catalase; Cyt c red, reduced cytochrome *c*, ox, oxidized; DHA, dehydroascorbate; DHAR, DHA reductase; GO, glycolate oxidase; GR, glutathione reductase; MDHA, monodehydroascorbate; MDHAR, MDHA reductase; PET, photosynthetic electron transfer chain; RET, respiratory electron transfer chain; SOD, superoxide dismutase; tAPX, thylakoid APX.

serves to delay senescence and extends leaf longevity by promoting enhanced defence and tolerance to abiotic stresses [25].

The ratio of GSH to glutathione disulfide (GSSG) can also modulate protein activity through glutathionylation, which is a reversible post-translational modification consisting of the formation of a mixed disulfide between the free protein thiol and GSH. Glutathionylation of redox-sensitive protein cysteines can be achieved through various reactions, such as thiol–disulfide exchange between protein cysteines and GSSG, by ROS-catalysed production of protein thiol radicals, or through the nitric oxide (NO)-mediated formation of *S*-nitrosothiols [16]. The removal of bound GSH from proteins is catalysed by glutaredoxins and Trxs. Glutathionylation may provide a mechanism for transmitting oxidative signals under stress conditions but the regulation of protein glutathionylation in plants remains largely uncharacterized and poorly understood. The formation of *S*-nitrosothiols particularly *S*-nitroglutathione (GSNO) provides a further mechanism by which GSH make a further contribution to post-translational modifications regulating cell signalling. GSNO acts as a reservoir of NO and it is also important in the transport, storage and delivery

of NO to sites of action. GSH-dependent formaldehyde dehydrogenases (GSNO reductases) are class III alcohol dehydrogenases that catalyse the NADH-dependent reduction of GSNO.

3. COMPARATIVE ANALYSIS OF REDOX TRANSCRIPTOME PROFILES

To provide insights into how gene expression patterns are changed in response to oxidants and antioxidants, we have compared the leaf transcriptome profiles of *A. thaliana* genotypes that are either deficient in either photorespiratory hydrogen peroxide processing (*catalase 2* (*cat2*) [26]), chloroplastic hydrogen peroxide processing (*thylakoid ascorbate peroxidase* (*tapx*) [27]), ascorbate synthesis (*vtc1* and *vtc2* [17]) or GSH synthesis (*rml1* [18]). This comparison might allow for the identification of overlapping patterns or unique transcript changes resulting from variations in hydrogen peroxide processing capacity in different cellular compartments, as well as low GSH (thiol) and low ascorbate availability. Figure 1 provides a very simple schematic model of the transmission of different oxidative and reductive signals from various cellular locations to the nucleus.

The data used in the following analysis of transcript patterns in leaves that are deficient in either ascorbate [17], GSH [18] or catalase [26] were collected by ourselves. The *cat2* mutants and controls (Col0) were grown on soil in controlled environment cabinets, with an 8 h photoperiod ($200 \mu\text{mol m}^{-2} \text{s}^{-1}$), and a 20°C day and 18°C night regime. Plants were grown for five weeks in a CO_2 -enriched atmosphere ($3000 \mu\text{l l}^{-1}$) to suppress photorespiration [26]. Then, two batches of plants were transferred to air ($400 \mu\text{l l}^{-1} \text{CO}_2$; photorespiratory conditions). One batch was grown under an 8 h photoperiod, whereas the second batch was grown under a 16 h photoperiod. Leaf samples were harvested in triplicate, 2 and 4 days after transfer to air. The *vtc1* and *vtc2* mutants and controls (Col0) were grown on soil in controlled environment cabinets, with a 12 h photoperiod ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$), at a constant temperature of 20°C . Whole rosettes were harvested in triplicate per genotype.

For *cat2*, samples were hybridized to AFFYMETRIX GENECHIP *Arabidopsis* ATH1 Genome Arrays (<https://www.affymetrix.com>) at the VIB Microarray Facility (Leuven, Belgium). Raw data processing and normalization were performed with the statistical algorithm robust multiarray average (gcRMA) and the affy package of R/BIOCONDUCTOR (<http://www.bioconductor.org>). The whole microarray data are available at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE27985>. In the following analysis, only data from plants transferred to the 8 h photoperiod have been used. Statistical analyses were performed by a *t*-test comparison using LIMMA algorithm (<http://www.ncbi.nlm.nih.gov/geo/geo2r>); only genes showing false discovery rate (FDR)-corrected *p*-values below 0.05 and a minimum 2-fold change in expression, 2 or 4 days after transfer from high CO_2 growth conditions to air at 8 h photoperiod were used for the analysis.

For *vtc1* and *vtc2*, data were analysed using two methods. Firstly, raw intensity values from the scanned AFFYMETRIX GENECHIP *Arabidopsis* ATH1 Genome Arrays (<https://www.affymetrix.com>) were analysed using the robust multichip average method using RMAEXPRESS (<http://rmaexpress.bmbolstad.com>). A significance test was undertaken by calculating a null hypothesis through a permuted modified *t*-statistic across the replicates for each array comparison. The \log_2 means of the normalized intensity values of the biological replicates were compared as ratios, where a \log_2 ratio of ± 1.0 and with a *p*-value less than 0.01 was selected as worthy of further study. *p*-values were further used to apply significance where the probe \log_2 ratios were more than or equal to 1. Gene expression was also analysed using GENESPRING GX 11.00 and *p*-values were calculated by asymptotic unpaired *t*-test and subjected to multiple testing corrections (Benjamini Hochberg FDR). A cut-off with a *p*-value less than 0.05 and \log_2 expression ratio ± 1 was adopted. The probe targets were as defined by AFFYMETRIX (<https://www.affymetrix.com/analysis/netaffx/index.affx>) and these targets were used to guide annotation of the genes through the use of BLAST against the TAIR (<http://www.arabidopsis.org>) database. All the datasets reported here are deposited at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23331>.

GSH is required for cell proliferation at the root tip after the root primordia have fully formed. The *rml1* seeds germinate and the shoot meristem begins to develop as in the wild-type. However, the growth of the embryonic root meristem arrests after approximately 17 longitudinal cell divisions. The *rml1* mutants are therefore deficient in postembryonic root (but not shoot) development. However, the arrested mutant root cells can carry out normal root functions, such as the production of lateral roots from the pericycle. The deficiency in postembryonic root (but not shoot) development means that plants have to be grown on plates. Mutants were therefore grown in controlled environment cabinets, together with controls (Col0) on vertical plates containing 1 per cent agar in PANG 2 medium for 7 days under a photoperiod of 16 h at a constant temperature of 20°C . The gene expression data used in the present analysis was obtained from shoot samples that were harvested in triplicate. Samples were hybridized to Agronomics1 tiling arrays [28] at the VIB core service (www.nucleomics.be). Quality control and normalization of the data were performed using AFFYMETRIX package and statistical analysis was performed by *t*-test using LIMMA package from the BIOCONDUCTOR software (<http://www.bioconductor.org>). Transcripts were identified based on a minimum 2 fold-change in expression between Col0 and *rml1* and a FDR corrected *p*-value threshold below 0.05 (see <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36893> for full dataset).

The data for the *Arabidopsis* plants in which the tAPX was silenced were taken from Maruta *et al.* [27]. In this study an oestrogen inducible tAPX-silencing line (RNAi, IS-tAPX-19–23) was grown together with control genotype (IS-GUS-2-17) on Murashige and Skoog medium containing 3 per cent sucrose for 7 days under continuous light ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at 25°C . Seedlings were then transferred on soil and grown for a further 10 days under the same conditions. The 17-day-old transgenic plants were then treated with oestrogen for 2 days to induce silencing of the tAPX. Microarray analysis was then performed on two leaf replicates by using Agilent *Arabidopsis* 3 Oligo Microarrays. The data were normalized and analysed using GENESPRING and are available at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE35526>. Genes showing a minimum twofold change in expression ratio in the tAPX-silenced plants were selected for further analysis [27].

4. HYDROGEN PEROXIDE- AND ANTIOXIDANT-TRIGGERED TRANSCRIPTOME PATTERNS

(a) *Transcript responses to low ascorbate, low GSH and enhanced oxidation*

The leaves of the *rml1*, *vtc1* and *vtc2* mutants showed significant transcriptome reprogramming relative to the wild-type leaves, with 1449 genes regulated by low GSH and between 510 (*vtc1*) and 959 (*vtc2*) genes regulated in response to low ascorbate (figure 2a). Of the ascorbate regulated genes only 44 showed GSH-dependent expression changes (figure 2b,c), suggesting that low GSH and low ascorbate have differential effects on gene expression.

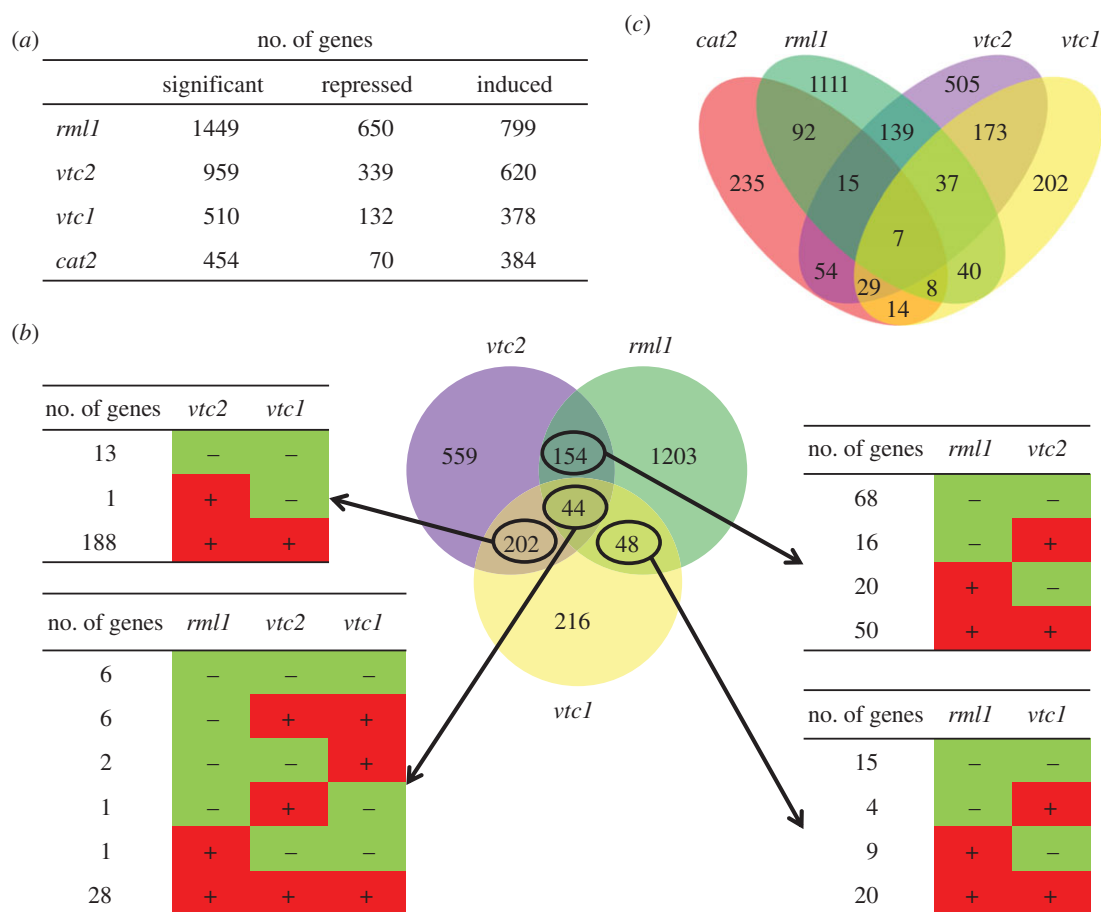


Figure 2. Comparisons of the leaf transcriptome profiles of the *rml1* [18], *vtc2*, *vtc1* [17] and *cat2* [26] mutants. (a) Number of significantly repressed and induced genes in the four genotypes. (b) Venn diagram showing the gene overlaps between the *rml1*, *vtc1* and *vtc2* transcriptomes. Numbers indicate the quantities of repressed and induced genes. (c) Venn diagram showing the numbers of overlapping genes in the four genotypes.

A degree of overlap has been reported in genes regulated by H₂O₂ and by low GSH in plants and animals [13,29]. In human promyelocytic leukemia cells (HL)-60 for example, 2016 genes were regulated by H₂O₂, of which 215 also showed GSH-dependent expression changes [29]. Of the 454 genes that were regulated by H₂O₂ in the *A. thaliana cat2* mutant, 122 showed GSH-dependent expression changes (figure 2c). Of the genes whose expression was similarly regulated by H₂O₂ and by low GSH, only six transcripts were decreased and 95 were increased in abundance (see the electronic supplementary material, table S1). The genes that were similarly modified in expression in the *cat2* and *rml1* genotypes included several transcription factors (*ATAF1*, NAC domain transcription factors *ANAC13*, *ANAC032*, *ANAC052* and *ANAC102*, the zinc finger transcription factors *ZINC TRANSPORTER (ZAT)6* and *ZAT12*, and only one WRKY domain transcription factor, *WRKY 40*), several UDP-glycosyltransferase genes such as *IAGLU*, *UGT73B2*, *UGT73B4*, *UGT73B5*, *UGT73C1*, *UGT75B1*, *UGT84A2* and *UGT84A3*, and GSTs (*GSTU1*, *GSTU4*, *GSTU7*, *GSTU8*, *GSTU24* and *GSTU25*).

Of the 454 genes that were regulated by H₂O₂ in the *A. thaliana cat2* mutant, 47 showed similar ascorbate-dependent expression changes in the *vtc2* mutants (see the electronic supplementary material, table S1). The expression of 58 genes including *WRKY38*,

HEAT SHOCK PROTEIN (HSP)70, *SMALL UBIQUITIN-LIKE MODIFIER (SUMO)3*, *UGT74D1* and *UGT76E11* was reversed in the *cat2* relative to the *vtc2* mutant (see the electronic supplementary material, table S1). Only 26 genes were similarly regulated by H₂O₂ and by low ascorbate in both the *vtc1* and *vtc2* mutants (see the electronic supplementary material, table S1). It is worth noting that relatively few genes such as *WRKY40*, *PINOID-BINDING PROTEIN (PBP)1*, *CYTOCHROME P450 (CYP)81D8* and the *TRANSLOCON AT THE INNER ENVELOPE MEMBRANE OF CHLOROPLASTS (TIC)20-IV* were expressed in a similar manner in response to H₂O₂, low GSH and low ascorbate, whereas *EARLY RESPONSE TO DEHYDRATION (ERD)9*, *CARBONIC ANHYDRASE1* and a *LATE EMBRYOGENESIS ABUNDANT (LEA)3* family protein were induced in response to H₂O₂ and repressed by low GSH and low ascorbate (see the electronic supplementary material, table S1).

(b) Regulation of photosynthetic gene expression by low ascorbate and low GSH

Very few genes encoding chloroplast-localized proteins were regulated in response to low ascorbate or low GSH (figure 3). However, low glutathione increased the abundance of transcripts encoding NADPH:

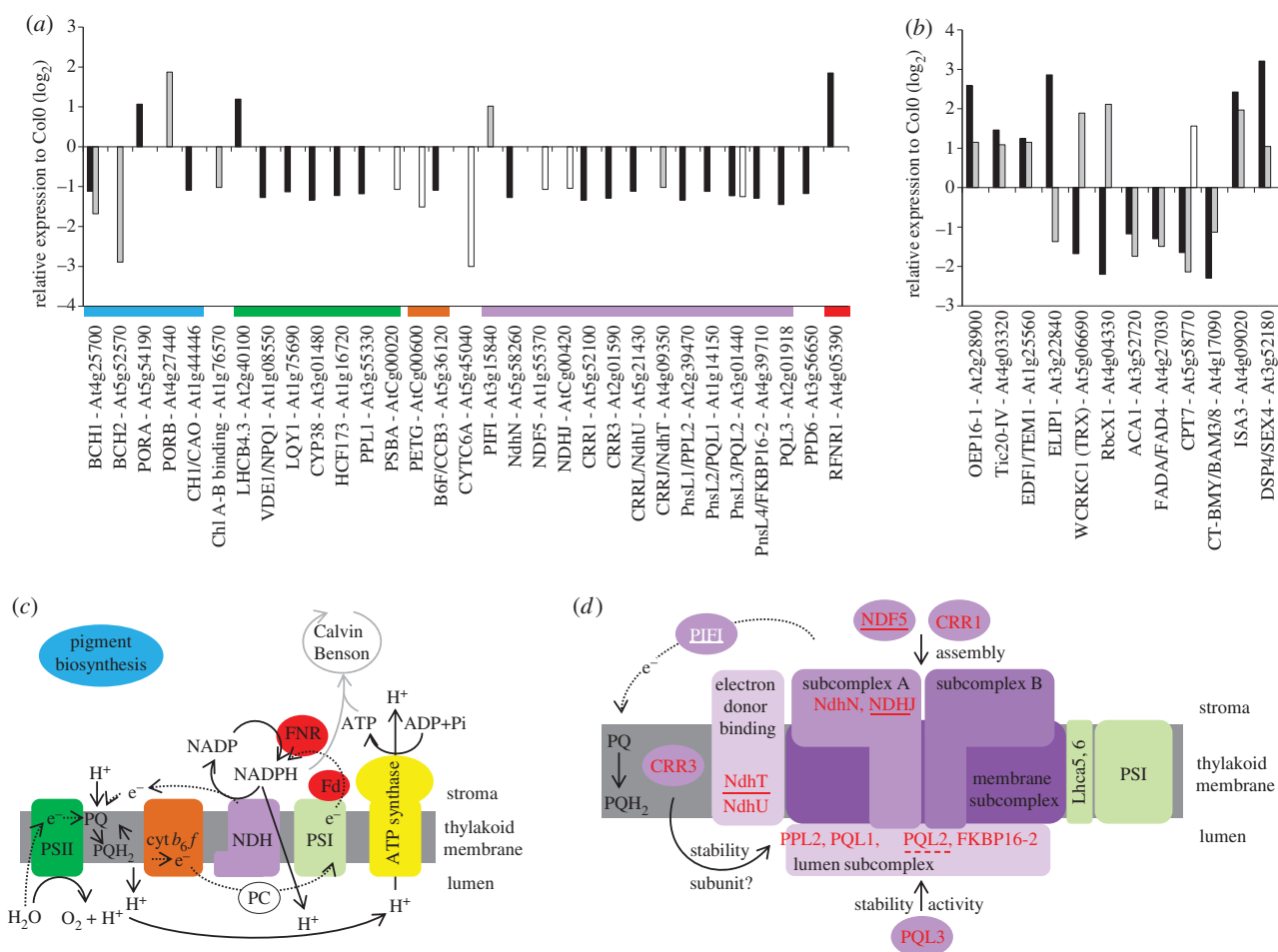


Figure 3. The effects of ascorbate and glutathione on the abundance of transcripts encoding chloroplast proteins. (a) Transcripts encoding components of the photosynthetic electron transfer chain: the colour of the bar below each group indicates the location of the encoded protein as illustrated in diagram (c) or (d). (b) Transcripts encoding chloroplast-associated proteins in the *vtc2* and *rml1* mutants, respectively; for (a) and (b): black bars, low glutathione in *rml1*; grey bars, low ascorbate in *vtc2*; white bars, low ascorbate in *vtc1*. (c) Schematic of the photosynthetic electron transfer chain. (d) Schematic of the NDH complex indicating the localization of proteins encoded by genes identified in (a). The NDH subcomplexes and related proteins are highlighted in purple, the association with PSI is shown in green (white, induced genes; red, repressed genes; not underlined indicates responsive to low GSH; solid underline indicates responsive to low ascorbate; dashed underline indicates responsive to low GSH and low ascorbate).

protochlorophyllide oxidoreductase (POR)A, while low ascorbate increased the expression of *PORB* (figure 3a). Ethylene signalling converges on the *ETHYLENE-INSENSITIVE3 (EIN3)/EIN3-Like (EIL)* transcription factors that bind directly to the specific elements present in the *PORA* and *PORB* promoters. In co-operation with the *PHYTOCHROME-INTERACTING FACTORS* such as *PIF1*, which also shows ascorbate-dependent expression changes, the *EIN3* prevents photo-oxidative damage by repression of the accumulation of protochlorophyllide, a phototoxic intermediate of chlorophyll synthesis [30]. Although the expression of *EIN3* was not induced in the different genotypes studied here, *EIN3-BINDING FOX PROTEIN (EBF)2* was induced by low GSH. These findings suggest that GSH-dependent expression of genes that regulate protochlorophyllide biosynthesis is important in ensuring survival in conditions of low thiol availability.

Low ascorbate decreased the expression of β -CAROTENE HYDROXYLASE (*BCH1*) and *BCH2* (figure 3a). The activities of these β -carotene hydroxylases are required for carotenoid synthesis. Overexpression

of *BCH1* in *Arabidopsis* leads to an increase in the xanthophyll cycle pool (violaxanthin and zeaxanthin) and enhances stress tolerance [31]. Transcripts encoding violaxanthin de-epoxidase, an enzyme that requires ascorbate as a cofactor in zeaxanthin synthesis by the xanthophyll cycle, were decreased by low GSH (figure 3a) but not by low ascorbate. Zeaxanthin is a key component of non-photochemical quenching (NPQ) and thermal energy dissipation in photosynthesis. While photosynthesis in the *vtc1* and *vtc2* mutants is more sensitive to high light stress, the low levels of ascorbate in these mutants have little effect on NPQ even at high light intensities [32,33]. The *vtc2* mutants had lower PSII quantum efficiencies than the wild plants when grown under very high light [33]. Double mutants (such as *vtc2npq4* and *vtc2npq1*) that are defective in NPQ as well as ascorbate synthesis were able to grow under the high-light conditions [33]. However, the PSII quantum efficiencies of the *vtc2npq4* and *vtc2npq1* leaves were similar to those determined in *vtc2*. Taken together, these data suggest that depletion of leaf ascorbate to 20–30% wild-type levels does not severely

impair the protective mechanisms that underpin thermal energy dissipation in photosynthesis.

Other genes that were repressed by low ascorbate or low GSH encode components that are not on the main route of non-cyclic electron flow, particularly those associated with the chloroplastic NAD(P)H dehydrogenase (NDH) complex (figure 3*a*), as well as other genes such as *CHLORORESPIRATORY REDUCTION (CRR)1* and *CRR3* that are classified as co-expressing with *NDH* core complex genes [34,35]. In addition, a small number of other genes associated with photosynthesis are differentially expressed in response to low ascorbate and low GSH (figure 3*b*). One of these, *WCRKC1*, the expression of which is repressed by low GSH but is activated by low ascorbate, encodes a member of the thioredoxin (Trx) superfamily, which fulfil diverse roles in plants ranging from protein disulfide reductases to disulfide isomerases or to disulfide transferases. *WCRKC1* encodes a Trx that has an atypical redox active site and a less reducing redox midpoint potential than that of the classic chloroplast Trxs [36]. This Trx reduces the chloroplast 2-Cys peroxiredoxin (Prx) using electrons derived from non-cyclic photosynthetic electron flow.

The abundance of *WCRKC1* transcripts is specifically decreased by low GSH, together with marked GSH-dependent decreases in the expression of *LOW QUANTUM YIELD OF PHOTOSYSTEM II (LQY)1*, *CYCLOPHILIN (CYP)38* and *HIGH CHLOROPHYLL FLUORESCENCE (HCF)173* (figure 3*a,c*). These genes encode thylakoid accessory proteins that are required for PSII core protein repair and assembly. *HCF173* is involved in the initiation of translation of the *psbA* mRNA and is essential for D1 synthesis [37]. *LQY1* is a Zn-finger protein with disulfide isomerase activity that interacts with the PSII core complex [38]. Although *LQY1* is not absolutely essential for PSII core repair, it participates in the repair and reassembly of photo-damaged PSII proteins. *CYP38*, which has a peptidyl-prolyl *cis-trans* isomerase protein-folding activity, plays an important role in the assembly and stabilization of PSII particularly under high light stress [39]. The half-life of the D1 and D2 proteins in mutants lacking *CYP38* or *LQY1* is shorter under high light than the wild-type [38,39]. Another chloroplast cyclophilin (*CYP20*) has foldase activity, which is enhanced by reduced thioredoxin [40]. In this case, the *CYP20-3* protein interacts with serine acetyltransferase (*SAT1*), which catalyses the rate-limiting enzyme of cysteine biosynthesis (the precursor of GSH biosynthesis). Although *CYP20-3* activity links photosynthetic electron transport processes to the redox-dependent folding of *SAT1*, no such activity has been described for *CYP38* to date. Nevertheless, γ -glutamyl cysteine and GSH biosynthesis in chloroplasts requires ATP produced by photosynthesis. Moreover, the rate of GSH synthesis is dependent on that of cysteine biosynthesis, which is directly regulated by the photosynthetic electron transport system.

Transcripts encoding other extrinsic subunits of PSII that show GSH-dependent regulation include *PPD6*, which encodes a PsbP-domain protein, *PSBP-LIKE*

(*PPL1* and *PPL2* (figure 3*a,c*). While the PsbP protein optimizes the water-splitting reaction, the PsbP-domain-like protein *PPL1* is expressed under stress conditions, and *PPL2* together with subunits of chloroplast NDH complex. Mutants lacking *PPL2* show decreased NDH complex activity while *ppl1* mutants are more sensitive to high-intensity light and show slow recovery from photoinhibition of PSII activity [41]. Of the NDH transcripts that are less abundant in *rml1* leaves, *PPL2*, *PSBQ-LIKE (PQL)1*, *PQL2* and *CCR3* are induced after transfer from enriched CO₂ atmosphere to air [2]. *PQL1* and *PQL2* are necessary for NDH activity and localize together with *PPL2* to the luminal side of the NDH complex (figure 3*d*) [42,43]. While *CRR3* stabilizes the NDH complex and localizes to the membrane fraction of chloroplast, the identity of *CRR3* as an NDH subunit remains to be established (figure 3*d*) [43,44].

(c) Regulation of respiratory gene expression by low ascorbate and low GSH

Ascorbic acid is synthesized from galactono- γ -lactone (GL) in leaves through the activity of galactono- γ -lactone dehydrogenase (GLDH), a mitochondrial enzyme, which uses reduced cytochrome *c* as a cofactor [45]. However, low ascorbate had little effect on the expression of genes encoding mitochondrial proteins (figure 4*a,b*). Transcripts encoding the ALTERNATIVE OXIDASE (AOX)1D and the internal NAD(P)H DEHYDROGENASES (ND)A1 were regulated by low ascorbate in the *vtc2* mutant (figure 4*a*). While *NDA1* showed a similar GSH-dependent expression pattern, *AOX1A* was induced by low GSH compared with *AOX1D* in the low ascorbate mutants (figure 4*a*). *AOX1A* and *AOX1D* are among the most stress responsive of all the known genes encoding mitochondrial proteins.

The AOX is part of the inducible non-phosphorylating alternative pathway of the plant respiratory electron transport chain that also consist of type II NAD(P)H dehydrogenases on both sides of the inner membrane linked through the ubiquinone pool to the AOX. *NDA1*, which is considered to play a key role in integrating metabolic activities of chloroplasts and mitochondria, was decreased in response to low ascorbate and low GSH. However, transcripts (*NDB2* and *NDB4*) encoding external NAD(P)H dehydrogenases forms were increased in abundance in response to low GSH, as were transcripts encoding the flavoprotein subunit (SUCCINATE DEHYDROGENASE (SDH)1 and SDH2) of complex II (figure 4*a,c*). Since a mild reduction in mitochondrial SDH activity had a positive impact on photosynthetic CO₂ assimilation rates [46], the GSH-dependent increase in *SDH1* and *SDH2* might suggest a trend towards a slightly lower photosynthetic performance in plants deficient in GSH.

Complex I (NDH) and Complex III (ubiquinol-cytochrome *bc*₁ reductase) are the major sites for ROS production in the mitochondrial electron transport chain. Mitochondria with reduced respiration are considered to generate a permanent stress condition by producing ROS as a constitutive stress

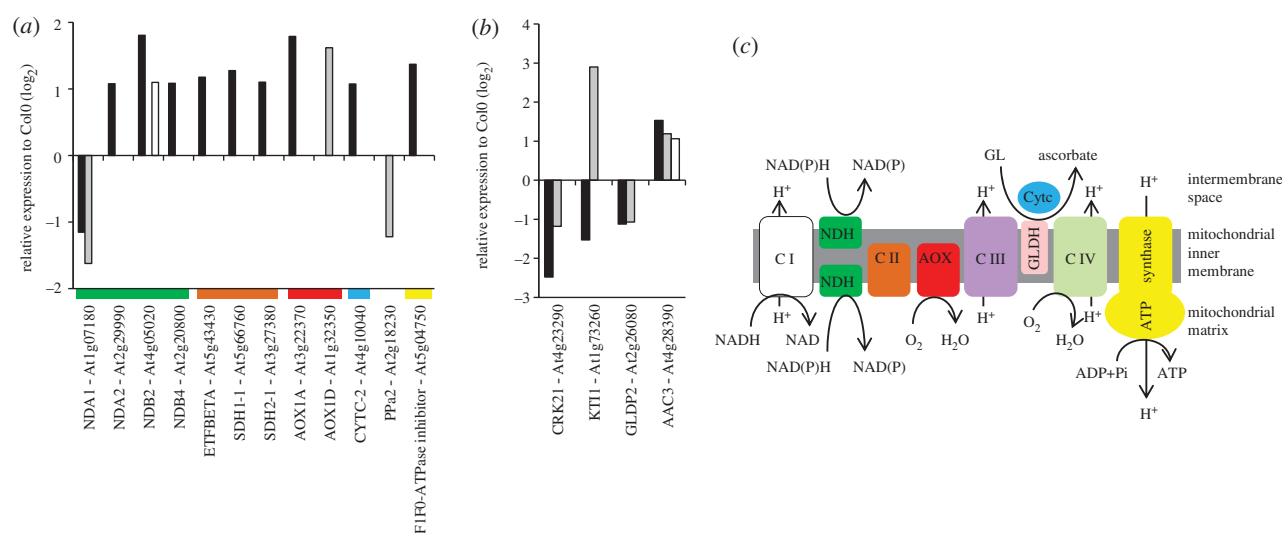


Figure 4. Ascorbate- and glutathione-regulated expression of mitochondrial genes in the *rml1*, *vtc1* and *vtc2* mutants. (a) Expression changes in genes encoding components of the respiratory electron transfer chain. The colour bar below each group indicates the location of the encoded protein as illustrated in diagram (c). (b) Ascorbate- and glutathione-regulated changes in the expression of genes encoding other mitochondrial proteins. (c) Schematic of the respiratory electron transfer chain. (a) and (b): black bars, *rml1*; grey bars, *vtc2*; white bars, *vtc1*.

signal for activation of cellular defense responses. In *Arabidopsis*, this typical stress response is controlled by transcriptional upregulation of *AOX1A* and *AOX1D*. AOX activity bypasses a block in proton pumping Complex III and so maintains electron flux through the respiratory electron transport chain, thus minimizing the possibility of ROS production. By enabling the tricarboxylic acid cycle to continue functioning when the electron transport in the cytochrome *c* pathway is blocked, the induction of the AOX gives essential flexibility to plant respiration as well as contributing to the re-programming of metabolism in response to changing environmental conditions. Taken together, these data suggest that low ascorbate and low GSH induce the expression of key components of the non-phosphorylating alternative pathway of respiration to decrease the possibility of ROS generation (figure 4).

(d) Hydrogen peroxide-derived signals arising from chloroplasts and peroxisomes

Of the transcripts that were regulated by H₂O₂ in the oestrogen inducible tAPX-silencing line, 115 were also changed in a similar manner in *rml1* leaves (figure 5a). Similarly, 87 transcripts were commonly altered in abundance to *tapx* and *vtc2* leaves and 74 transcripts were common to *tapx* and *vtc1* leaves (figure 5a). The transcript profile of the *tapx* leaves was distinct from that of the *cat2* mutants, with only 39 transcripts changed in a similar manner in both genotypes (figure 5a). The absence of a common transcript profile may suggest that hydrogen peroxide-derived signals arising from chloroplasts and peroxisomes have differential effects on gene expression. Of the transcripts that were changed in abundance in the *tapx* leaves, very few encoded proteins that were targeted at the chloroplasts and were associated with photosynthesis (figure 5b,c). Similarly, very few transcripts encoding components of the mitochondrial electron transfer chains were altered in abundance in the *tapx* leaves (figure 5c). However, 10 transcripts encoding proteins associated with the

NDH complex were decreased in abundance in the *tapx* leaves (figure 5b). The location of the encoded proteins within the NDH subcomplexes is shown in figure 5d, which also indicates the location of proteins encoded by transcripts that are decreased in abundance both in *tapx* and *rml1* leaves.

5. SUMMARY AND CONCLUSIONS

Although the conclusions derived from the global transcriptome comparisons reported here must be regarded with a degree of caution as the growth conditions were not the same for all of the mutants, the findings of this study also provide some intriguing new insights into the relationships between different components of the cellular reduction–oxidation (redox) network and photosynthetic gene expression. A common action on leaf transcriptome patterns is demonstrated by the approximate 30 per cent overlap in the transcript changes in *cat2*, *rml1*, *vtc1* and *vtc2* mutants and in *tapx*, *rml1*, *vtc1* and *vtc2* mutants. The common responses in these gene expression patterns must reflect the general role of antioxidants in processing ROS signals. However, less than 10 per cent overlap was observed in the transcriptome patterns of *cat2* and *tapx* even though both enzymes are involved in hydrogen peroxide processing. Although there were marked differences in the growth conditions used for the studies of gene expression in the mutants, it is nevertheless tempting to suggest that at least some of the differences arise from the location of hydrogen peroxide-derived signals, photorespiratory signals in the case of *cat2* and chloroplast signals in the case of *tapx*. The differential transcriptome changes described here suggest that the site of enhanced oxidation (chloroplast or peroxisome) and the nature of the antioxidant exert specific effects on transcript abundance.

The suites of transcripts that encode proteins that can be classified as ROS-regulated are similar to those described previously [4] and they have therefore

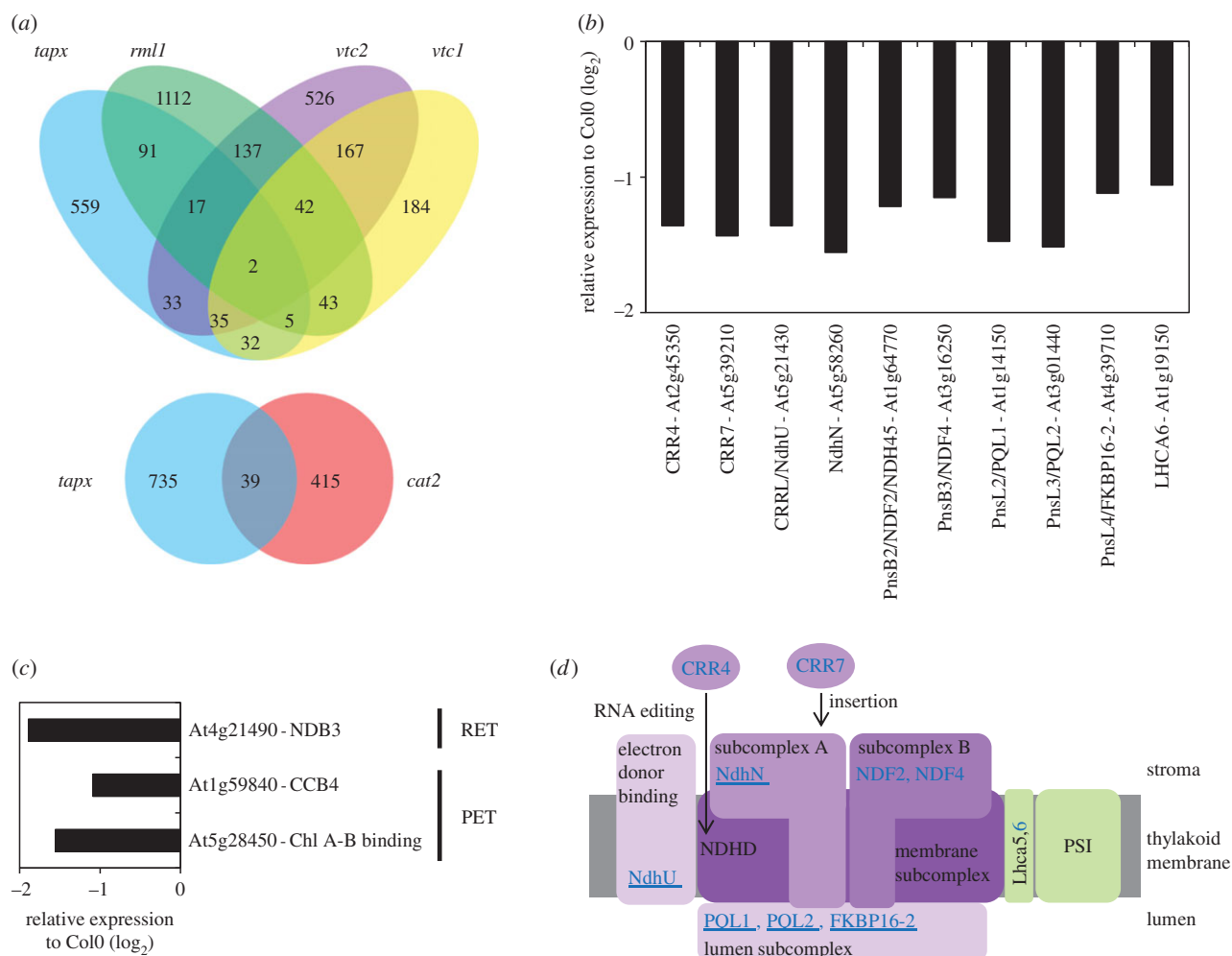


Figure 5. Comparisons of the leaf transcriptome profiles of the *tapx* [27], *rml1* [18], *vtc2* and *vtc1* [17] mutants and expression changes in genes encoding electron transfer chain components in *tapx*. (a) Venn diagram showing the gene overlaps between the *tapx*, *rml1*, *vtc1* and *vtc2* transcriptomes, and between *tapx* and *cat2*. (b) Expression changes in genes encoding components of NDH in *tapx*. (c) Expression changes in genes encoding other components of the photosynthetic (PET) and respiratory (RET) electron transfer chains in *tapx*. (d) Schematic of the NDH complex indicating the localization of proteins encoded by genes identified in (b). The NDH subcomplexes and related proteins are highlighted in purple, the association with PSI is shown in green (blue, repressed genes in *tapx*; underline indicates repression in *rml1*).

not been discussed in detail here. However, it is worthy of note that very few photosynthetic or respiratory pathway genes showed hydrogen peroxide-dependent gene expression. The most striking effect of depletion of several antioxidants, particularly in the *rml1* mutants with low GSH and the *tapx* mutants, was on the abundance of transcripts encoding core and accessory components of the thylakoid NDH complex. The decrease in transcripts encoding NDH complex components by low antioxidant capacity is interesting particularly because of the low abundance of this complex in the thylakoid membranes and its importance in cyclic electron flow and chlororespiration. It is generally considered that the NDH complex associates with PSI to form NDH–PSI supercomplexes that redirect electron flow into a cyclic pathway in which electrons are transported from PSI to the plastoquinone (PQ) pool via NADPH and the NDH complex in order to prevent over-reduction of the stroma. Since the stroma might be perceived as more oxidized when antioxidant capacity is diminished, it is perhaps surprising that low GSH, and to a lower extent low

ascorbate and low tAPX activity, favours a decreased abundance of transcripts encoding NDH complex components that are required to support cyclic electron transport.

Transcripts encoding auxiliary proteins involved in the PSII repair cycle were also specifically decreased in plants with low GSH, as were transcripts encoding proteins that repress protochlorophyllide accumulation. The PSII repair cycle, which involves the replacement of damaged D1 subunits, occurs much more frequently than does de novo PSII biogenesis in mature chloroplasts [47]. This interaction may provide a further link between PSII activity and the redox state of the stroma, in addition to that involving TRX- and cyclophilin-dependent regulation of cysteine synthesis in the chloroplast [40]. Cysteine levels are tightly regulated in plant cells, particularly in relation to GSH synthesis, which is a major sink for cysteine [48]. The reduced demand for cysteine in the chloroplast caused by impaired γ -glutamyl cysteine synthesis in the *rml1* mutant may also have a feedback effect on SAT1 activity [48].

Taken together, these findings indicate that manipulation of the abundance of individual components of the antioxidant network results in specific patterns of transcript changes. Regulated changes in gene expression patterns may hence be tailored to address the perceived imbalances in antioxidant strength and composition relative to oxidant production.

The authors acknowledge funding from the EU Marie-Curie ITN 'COSI' project (Orsay, Leeds). Guillaume Queval is funded by a Marie Curie Individual Fellowship: PIEF-GA-2009-252927: ROXNP.

REFERENCES

- Rutherford, A. W., Osyczka, A. & Rappaport, F. 2012 Back-reactions, short-circuits, leaks and other energy wasteful reactions in biological electron transfer: redox tuning to survive life in O₂. *FEBS Lett.* **586**, 603–616. (doi:10.1016/j.febslet.2011.12.039)
- Foyer, C. H., Neukermans, J., Queval, G., Noctor, G. & Harbinson, J. 2012 Photosynthetic control of electron transport and the regulation of gene expression. *J. Exp. Bot.* **63**, 1637–1661. (doi:10.1093/jxb/ers013)
- Foyer, C. H. & Noctor, G. 2009 Redox regulation in photosynthetic organisms: signaling, acclimation and practical implications. *Antioxid. Redox Signal.* **11**, 862–905. (doi:10.1089/ars.2008.2177)
- Miller, G., Schlauch, K., Tam, R., Cortes, D., Torres, M. A., Shulaev, V., Dangel, J. L. & Mittler, R. 2009 The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Sci. Signal.* **2**, ra45. (doi:10.1126/scisignal.2000448)
- Foyer, C. H., Bloom, A., Queval, G. & Noctor, G. 2009 Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. *Annu. Rev. Plant Biol.* **60**, 455–484. (doi:10.1146/annurev.arplant.043008.091948)
- Queval, G., Issakidis-Bourguet, E., Hoerberichts, F. A., Vandorpe, M., Gakière, B., Vanacker, H., Miginiac-Maslow, M., Van Breusegem, F. & Noctor, G. 2007 Conditional oxidative stress responses in the *Arabidopsis* photorespiratory mutant *cat2* demonstrate that redox state is a key modulator of daylength-dependent gene expression and define photoperiod as a crucial factor in the regulation of H₂O₂-induced cell death. *Plant J.* **52**, 640–657. (doi:10.1111/j.1365-313X.2007.03263.x)
- Wagner, D. *et al.* 2004 The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. *Science* **306**, 1183–1185. (doi:10.1126/science.1103178)
- Chaouch, S., Queval, G., Vanderauwera, S., Mhamdi, A., Vandorpe, M., Langlois-Meurinne, M., Van Breusegem, F., Saindrenan, P. & Noctor, G. 2010 Peroxisomal hydrogen peroxide is coupled to biotic defense responses by ISOCHORISMATE SYNTHASE1 in a daylength-related manner. *Plant Physiol.* **153**, 1692–1705. (doi:10.1104/pp.110.153957)
- Lee, K. P., Kim, C., Landgraf, F. & Apel, K. 2007 EXECUTER1- and EXECUTER2-dependent transfer of stress-related signals from the plastid to the nucleus of *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA.* **104**, 10 270–10 275. (doi:10.1073/pnas.0702061104)
- Foyer, C. H. & Noctor, G. 2005 Redox homeostasis and antioxidant signalling: a metabolic interface between stress perception and physiological responses. *Plant Cell* **17**, 1866–1875. (doi:10.1105/tpc.105.033589)
- Foyer, C. H. & Shigeoka, S. 2011 Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiol.* **155**, 93–100. (doi:10.1104/pp.110.166181)
- Surpin, M., Larkin, R. M. & Chory, J. 2002 Signal transduction between the chloroplast and the nucleus. *Plant Cell* **14**(Suppl.), S327–S338.
- Galvez-Valdivieso, G. & Mullineaux, P. M. 2010 The role of reactive oxygen species in signalling from chloroplasts to nucleus. *Physiol. Plant.* **138**, 430–439. (doi:10.1111/j.1399-3054.2009.01331.x)
- Cottage, C. & Gray, J. C. 2011 Timing the switch to phototrophic growth. *Plant Signal. Behav.* **6**, 578–582. (doi:10.4161/psb.6.4.14900)
- Pastori, G. M., Kiddle, G., Antoniw, J., Bernard, S., Veljovic-Jovanovic, S., Verrier, P. J., Noctor, G. & Foyer, C. H. 2003 Leaf vitamin C contents modulate plant defense transcripts and regulate genes controlling development through hormone signaling. *Plant Cell* **15**, 939–951. (doi:10.1105/tpc.010538)
- Noctor, G., Mhamdi, A., Chaouch, S., Han, Y., Neukermans, J., Marquez-Garcia, B., Queval, G. & Foyer, C. H. 2012 Glutathione functions in plants: an integrated overview. *Plant Cell Environ.* **35**, 454–484. (doi:10.1111/j.1365-3040.2011.02400.x)
- Kerchev, P. I. *et al.* 2011 The transcription factor ABI-4 is required for the ascorbic acid-dependent regulation of growth and regulation of jasmonate-dependent defense signaling pathways in *Arabidopsis*. *Plant Cell* **23**, 3319–3334. (doi:10.1105/tpc.111.090100)
- Vernoux, T. *et al.* 2000 The ROOT MERISTEMLESS1/CADMIUM SENSITIVE2 gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *Plant Cell* **12**, 97–110. (doi:10.1105/tpc.12.1.97)
- Bartoli, C. G., Gómez, F., Fernández, L., Yu, J., McIntosh, L. & Foyer, C. H. 2006 Inter-relationships between light and respiration in the control of ascorbic acid synthesis and accumulation in *Arabidopsis thaliana* leaves. *J. Exp. Bot.* **57**, 1621–1631. (doi:10.1093/jxb/erl005)
- Bartoli, C. G., Tambussi, E. A., Fanello, D. & Foyer, C. H. 2009 Control of ascorbic acid synthesis and accumulation and glutathione by the incident light red/far red ratio in *Phaseolus vulgaris* leaves. *FEBS Lett.* **583**, 118–122. (doi:10.1016/j.febslet.2008.11.034)
- Dowdle, J., Ishikawa, T., Gatzek, S., Rolinski, S. & Smirnoff, N. 2007 Two genes in *Arabidopsis thaliana* encoding GDP-L-galactose phosphorylase are required for ascorbate biosynthesis and seedling viability. *Plant J.* **52**, 673–689. (doi:10.1111/j.1365-313X.2007.03266.x)
- Maruta, T., Tanouchi, A., Tamoi, M., Yabuta, Y., Yoshimura, K., Ishikawa, T. & Shigeoka, S. 2010 *Arabidopsis* chloroplastic ascorbate peroxidase isoenzymes play a dual role in photoprotection and gene regulation under photooxidative stress. *Plant Cell Physiol.* **51**, 190–200. (doi:10.1093/pcp/pcp177)
- Wachter, A., Wolf, S., Steiniger, H., Bogs, J. & Rausch, T. 2005 Differential targeting of *GSH1* and *GSH2* is achieved by multiple transcription initiation: implications for the compartmentation of glutathione biosynthesis in the *Brassicaceae*. *Plant J.* **41**, 15–30. (doi:10.1111/j.1365-313X.2004.02269.x)
- Noctor, G., Arisi, A. C., Jouanin, L. & Foyer, C. H. 1998 Manipulation of glutathione and amino acid biosynthesis in the chloroplast. *Plant Physiol.* **118**, 471–482. (doi:10.1104/pp.118.2.471)
- Wu, A. *et al.* 2012 JUNGBRUNNEN1, a reactive oxygen species responsive NAC transcription factor, regulates longevity in *Arabidopsis*. *Plant Cell* **24**, 482–506. (doi:10.1105/tpc.111.090894)
- Queval, G., Neukermans, J., Vanderauwera, S., Van Breusegem, F. & Noctor, G. 2012 Day length is a key

- regulator of transcriptomic responses to both CO₂ and H₂O₂ in *Arabidopsis*. *Plant Cell Environ.* **35**, 374–387. (doi:10.1111/j.1365-3040.2011.02368.x)
- 27 Maruta, T., Noshi, M., Tanouchi, M., Tamoi, M., Yabuta, Y., Yoshimura, K., Ishikawa, T. & Shigeoka, S. 2012 H₂O₂-triggered retrograde signaling from chloroplasts to nucleus plays specific role in response to stress. *J. Biol. Chem.* **287**, 11 717–11 729. (doi:10.1074/jbc.M111.292847)
- 28 Rehrauer, H. *et al.* 2010 AGRONOMICS1: a new resource for *Arabidopsis* transcriptome profiling. *Plant Physiol.* **152**, 487–499. (doi:10.1104/pp.109.150185)
- 29 Fratelli, M., Goodwin, L. O., Ørom, U. A., Lombardi, S., Tonelli, R., Mengozzi, M. & Ghezzi, P. 2005 Gene expression profiling reveals a signaling role of glutathione in redox regulation. *Proc. Natl Acad. Sci. USA* **102**, 13 998–14 003. (doi:10.1073/pnas.0504398102)
- 30 Zhong, S., Zhao, M., Shi, T., Shi, H., An, F., Zhao, Q. & Guo, H. 2009 EIN3/EIL1 cooperate with PIF1 to prevent photo-oxidation and to promote greening of *Arabidopsis* seedlings. *Proc. Natl Acad. Sci. USA* **106**, 21 431–21 436. (doi:10.1073/pnas.0907670106)
- 31 Davison, P. A., Hunter, C. N. & Horton, P. 2002 Overexpression of β-carotene hydroxylase enhances stress tolerance in *Arabidopsis*. *Nature* **418**, 203–206. (doi:10.1038/nature00861)
- 32 Veljovic-Jovanovic, S., Pignocchi, C., Noctor, G. & Foyer, C. H. 2001 Low vitamin C in the *vtc 1* mutant of *Arabidopsis thaliana* is associated with decreased growth and intracellular redistribution of the antioxidant system. *Plant Physiol.* **127**, 426–435. (doi:10.1104/pp.010141)
- 33 Muller-Moulé, P., Golan, T. & Niyogi, K. K. 2004 Ascorbate-deficient mutants of *Arabidopsis* grow in high light despite chronic photooxidative stress. *Plant Physiol.* **134**, 1163–1172. (doi:10.1104/pp.103.032375)
- 34 Takabayashi, A., Ishikawa, N., Obayashi, T., Ishida, S., Obokata, T., Endo, J. & Sato, S. 2009 Three novel subunits of *Arabidopsis* chloroplastic NAD(P)H dehydrogenase identified by bioinformatic and reverse genetic approaches. *Plant J.* **57**, 207–219. (doi:10.1111/j.1365-313X.2008.03680.x)
- 35 Shimizu, H. & Shikanai, T. 2007 Dihydrodipicolinate reductase-like protein, CRR1, is essential for chloroplast NAD(P)H dehydrogenase in *Arabidopsis*. *Plant J.* **52**, 539–547. (doi:10.1111/j.1365-313X.2007.03256.x)
- 36 Dangoor, I., Peled-Zehavi, H., Levitan, A., Pasand, O. & Danon, A. 2009 A small family of chloroplast atypical thioredoxins. *Plant Physiol.* **149**, 1240–1250. (doi:10.1104/pp.108.128314)
- 37 Schult, K., Meierhoff, K., Paradies, S., Töller, T., Wolff, P. & Westhoff, P. 2007 The nuclear-encoded factor HCF173 is involved in the initiation of translation of the *psbA* mRNA in *Arabidopsis thaliana*. *Plant Cell* **19**, 1329–1346. (doi:10.1105/tpc.106.042895)
- 38 Lu, Y., Hall, D. A. & Last, R. L. 2012 A small zinc finger thylakoid protein plays a role in maintenance of photosystem II in *Arabidopsis thaliana*. *Plant Cell* **23**, 1861–1875. (doi:10.1105/tpc.111.085456)
- 39 Fu, A., He, Z., Cho, H. S., Lima, A., Buchanan, B. B. & Luan, S. 2007 A chloroplast cyclophilin and maintenance of functions in the assembly and maintenance of photosystem II in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **104**, 15 947–15 952. (doi:10.1073/pnas.0707851104)
- 40 Dominguez-Solis, J. R., He, Z. Y., Lima, A., Ting, J., Buchanan, B. B. & Luan, S. 2008 A cyclophilin links redox and light signals to cysteine biosynthesis and stress responses in chloroplasts. *Proc. Natl Acad. Sci. USA* **105**, 16 386–16 391. (doi:10.1073/pnas.0808204105)
- 41 Ishihara, S., Takabayashi, A., Ido, K., Endo, T., Ifuku, K. & Sato, F. 2007 Distinct functions for the two PsbP-like proteins PPL1 and PPL2 in the chloroplast thylakoid lumen of *Arabidopsis*. *Plant Physiol.* **145**, 668–679. (doi:10.1104/pp.107.105866)
- 42 Yabuta, S., Ifuku, K., Takabayashi, A., Ishihara, S., Ido, K., Ishikawa, N., Endo, T. & Sato, F. 2010 Three PsbQ-like proteins are required for the function of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. *Plant Cell Physiol.* **51**, 866–876. (doi:10.1093/pcp/pcq060)
- 43 Ifuku, K., Endo, T., Shikanai, T. & Aro, E. M. 2011 Structure of the chloroplast NADH dehydrogenase-like complex: nomenclature for nuclear-encoded subunits. *Plant Cell Physiol.* **52**, 1560–1568. (doi:10.1093/pcp/pcr098)
- 44 Muraoka, R., Okuda, K., Kobayashi, Y. & Shikanai, T. 2006 A eukaryotic factor required for accumulation of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. *Plant Physiol.* **142**, 1683–1689. (doi:10.1104/pp.106.088682)
- 45 Bartoli, C., Pastori, G. & Foyer, C. H. 2000 Ascorbate biosynthesis in mitochondria is linked to the electron transport chain between complexes III and IV. *Plant Physiol.* **123**, 335–343. (doi:10.1104/pp.123.1.335)
- 46 Fuentes, D. *et al.* 2011 A deficiency in the flavoprotein of *Arabidopsis* mitochondrial complex II results in elevated photosynthesis and better growth in nitrogen-limiting conditions. *Plant Physiol.* **157**, 1114–1127. (doi:10.1104/pp.111.183939)
- 47 Nixon, P. J., Michoux, F., Yu, J., Boehm, M. & Komenda, J. 2010 Recent advances in understanding the assembly and repair of photosystem II. *Ann. Bot.* **106**, 1–16. (doi:10.1093/aob/mcq059)
- 48 Noctor, G., Gomez, L., Vanacker, H. & Foyer, C. H. 2002 Glutathione homeostasis and signalling: the influence of biosynthesis, compartmentation and transport. *J. Exp. Bot.* **53**, 1283–1304. (doi:10.1093/jexbot/53.372.1283)