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Strategies to maintain redox homeostasis during photosynthesis under changing conditions

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Abstract

Plants perform photosynthesis and assimilatory processes in a continuously changing environment. Energy production in the various cell compartments and energy consumption in endergonic processes have to be well adjusted to the varying conditions. In addition, dissipatory pathways are required to avoid any detrimental effects caused by over-reduction. A large number of short-term and long-term mechanisms interact with each other in a flexible way, depending on intensity and the type of impact. Therefore, all levels of regulation are involved, starting from energy absorption and electron flow events through to post-transcriptional control. The simultaneous presence of strong oxidants and strong reductants during oxygenic photosynthesis is the basis for regulation. However, redox-dependent control also interacts with other signal transduction pathways in order to adapt metabolic processes and redox-control to the developmental state. Examples are given here for short-term and long-term control following changes of light intensity and photoperiod, focusing on the dynamic nature of the plant regulatory systems. An integrating network of all these mechanisms exists at all levels of control. Cellular homeostasis will be maintained as long as the mechanisms for acclimation are present in sufficiently high capacities. If an impact is too rapid, and acclimation on the level of gene expression cannot occur, cellular damage and cell death are initiated.

Key words: Light acclimation, malate valve, over-reduction, oxidative stress, photosynthesis, poisoning mechanisms, redox control, redox homeostasis, regulatory networks.

Introduction

Plants operate well between the extreme situations of over-oxidation and over-reduction, as caused by the presence of oxygen, and the simultaneous generation of strong reductants in the photosynthetic electron transport chain. There is a close relationship between over-reduction of the electron transport chain and generation of oxygen radicals when the Mehler reaction is occurring during active photosynthesis. Antioxidants and redox buffers are therefore present in order to minimize the risk of detrimental effects. Energy-dissipating pathways are also functioning at the level of photosystem II to reduce energy input.

Since electron transport is coupled with ATP production, further reactions are required to adjust the ratio between NADPH and ATP to the actual demand. D1 turnover, state transitions, non-photochemical energy quenching, xanthophyll cycle, chlororespiration, cyclic electron transport, and the Mehler reaction are some of the poising systems of the chloroplast, frequently described as flexible ways to maintain a balanced electron flow and the required rate of ATP production under changing conditions. Further pathways, involving cell compartments, such as the malate valve, alternative oxidase (AOX), Q cycles in the electron transport chains, and photorespiration, also contribute and give rise to flexible ATP/e\(^{-}\) ratios.

Living cells as open systems require a constant flux of energy for continuous biomass production and consumption, and depend on cellular homeostasis to maintain all functions. This, in turn, can only be achieved when the relatively small pools of ATP/ADP, NAD(P)H/NAD(P) and other redox carriers, as well as cellular pH, remain at balanced ratios. This will allow the input and withdrawal of energy and reducing equivalents at the required rates and keep the system at homeostasis.

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Abbreviations: AOX, alternative oxidase; Fd, ferredoxin, GSH/GSSG, reduced/oxidized glutathione; LHC, light-harvesting complex; NADP-MDH, NADP-dependent malate dehydrogenase; PS, photosystem; ROS, reactive oxygen species.

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Apart from the need for homeostasis of the redox systems, there is, however, a need for redox signals that induce the acclimation of metabolism to sustained changes, i.e. during development and upon stress situations. Various factors have already been suggested to initiate such signal transduction pathways leading to the changed expression of certain genes. Reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂), oxygen radicals (O₂•−), nitric oxide (NO), or the redox-state of any of the intersystem or soluble redox components could act as signals (Foyer and Noctor, 2003; Laloi et al., 2004). Therefore, although balanced pools of reductants/oxidants are essential as redox buffers (ascorbate/dehydroascorbate and GSH/GSSG) in order to accommodate (or dampen) very rapid changes without affecting homeostasis, glutathione, ascorbate, and other redox components have also been implicated in redox signalling (May et al., 1998; Dietz, 2003; De Gara, 2004).

In this paper, the hypothesis put forward is that a large number of factors are able to induce acclimation reactions well before any damage becomes apparent, and that the major redox pools are kept relatively constant over a wide range of conditions. Therefore, it is assumed that over-reduction is sensed by the plant before any major imbalances of the redox components occur and, moreover, before any oxidative damage occurs. This is achieved by the presence of flexible mechanisms that interact in multidimensional networks of regulation at many levels of cellular activities. On the other hand, once a certain limit has been reached or the developmental fate has been programmed previously, cell death will occur within a short time (Fig. 1).

**Fig. 1.** Flexible system of redox-control in plants during photosynthesis. Depending on the metabolic, environmental, and developmental situation as well as on the type of change of conditions (light, temperature, CO₂) redox-signals lead to readjustment of the components of photosynthesis, poising, and antioxidant systems. Both short-term (by direct effects) and long-term (at the level of gene expression) control interact with further metabolic and hormonal signals. When pre-acclimation has failed to occur in time or senescence has started already, irreversible breakdown processes will start (cell death).

### Short-term adjustment of the redox state in the electron transport chain

Photosynthesis takes place under continuously changing conditions, as light, temperature, internal CO₂, and nutrient supply are concerned. Plants are therefore adapted to a dynamic environment by multiple regulatory systems that provide high flexibility. Transient short-term changes can be balanced by a set of protection systems which stabilize the redox poise in the electron transport chains and modulate light-use efficiency. Mechanisms that demonstrate the high flexibility of electron transfer and the concomitant formation of a proton motive force have been summarized recently, showing the multiple ways that each of these processes can be adjusted in a varying environment (Kramer et al., 2004; Nedbal et al., 2003; Holt et al., 2004; Avenson et al., 2005). For dissipation of excess excitation energy, various mechanisms have been described that are able either to avoid the generation of reductants giving rise to the production of ROS, or to serve as alternative electron acceptors in order to avoid over-reduction and, potentially, the formation of toxic intermediates (Mullineaux and Karpinski, 2002; Niyogi, 1999). Imbalance between the light energy distribution of PSII and PSI can be regulated and controlled by state transitions (Allen, 2002). There is strong experimental evidence that under increasing light intensities the LHCII kinase is inactivated by reduction via a membrane-bound thioredoxin-like protein, which itself is controlled by the thiol-redox potential of the chloroplast stroma (Martinsuo et al., 2003). The mode of action of the state transitions indicates that they act primarily to compensate for differential excitation of the two photosystems, as occurs when the spectral composition of the light changes, for example, during dawn and sunset or shading by other leaves during the day.

Light energy is primarily converted into electron flow that concomittantly results in the generation of the pH gradient which again is the driving force for ATP synthesis. Since the demand for reducing equivalents and ATP is varying, uncoupling of this primary process is required to provide flexibility. Poising mechanisms act principally in three different ways: (i) by transfer of electrons back into the photosynthetic electron transport chains (cyclic electron transport), (ii) by reduction of O₂ and subsequent metabolism of ROS (pseudocyclic electron flow or water–water cycle), and (iii) by indirect export of reducing equivalents (malate valve, described in more detail below). Although the underlying physiological and biochemical mechanisms are completely different, all three pathways are regulated in such a way that they do not compete for electrons required for reductive assimilatory reactions in the chloroplasts (Backhausen et al., 2000).

From many *in vitro* measurements, it can deduced that C₃ plants are capable of performing cyclic electron flow, but the problem is to estimate the flux through this cyclic process in...
intact leaves (Heber, 2002). Using different techniques, contradictory results were obtained. Thus the discussion is ongoing as to whether C3 plants are, in fact, using this pathway in vivo (discussed in detail by Johnson, 2005). Cyclic electron flow around PSI in the presence of ‘active’ PSII can only be measured under stress conditions, when the linear electron transport is saturated either in high light, at low temperature, or under conditions when carbon fixation is limited. Under these conditions a bulk of previously inactive PSI seems to be activated (Golding et al., 2004), and there is experimental evidence that this activation is linked to a decreasing stromal NADP/NADPH ratio (Rajagopal et al., 2003). In addition, it has been proposed that the cytochrome b6/f complex is regulated by the stromal redox potential of the chloroplast stroma via a thioredoxin-mediated mechanism (Johnson, 2003). Nevertheless, one important piece of evidence concerning the in vivo relevance of cyclic electron transport came from Arabidopsis double mutants impaired in NDH (NDH dehydrogenase) and PGR5 (proton gradient regulation) as part of theFd-dependent pathway (Munekage et al., 2004). These authors suggested that the PGR5 pathway contributes to the generation of a proton gradient inducing thermal dissipation when Calvin-cycle activity is reduced. An additional advantage might be to limit the over-reduction of the acceptor side of PSI, thus preventing PSI inhibition. In conclusion, down-regulation of linear electron flow and activation of cyclic electron flow seem to respond to a common signal: the stromal redox poise (Johnson, 2003).

Various environmental conditions, i.e. light, cold, and drought stress, as well as pathogen attack, lead to a limitation of linear electron transport due to an over-reduction of stromal electron acceptors. Under these conditions the formation of different kinds of ROS is accelerated either by transfer of electrons to O2, generating superoxide radicals (O2-), hydrogen peroxide (H2O2) or hydroxyl radicals (HO•), respectively. In addition, singlet oxygen (O1) can be formed by energy transfer from triplet P680. The stroma-exposed centres of PSI act as electron donors (Asada, 2000), as long as none of the physiological electron acceptors are available in the oxidized state. Additional sites such as plastosemiquinone, where O2 reduction may occur, are still under debate (Ivanov and Khorobrykh, 2003). The subsequent reactions (disproportionation of O2 into H2O2, its detoxification by ascorbate, and ascorbate regeneration by GSH at the expense of NADPH) have been reviewed in much detail (Foyer and Noctor, 2000). The two scavenging systems, one micro-compartmentalized with the PSI complex and one present in the stroma, exhibit high affinities, and all enzymes are present with high activities (Asada, 2000). Thus, the concentration of H2O2, the most stable intermediate, is kept below 1 μM under non-stressing conditions. Even under photon-stress conditions, the flux through the water–water cycle increases, but no accumulation of O2 and H2O2 is detectable when the supply with reductant is sufficient (Polle, 1996; Asada, 2000). It is interesting to note that peroxiredoxins have been found to have a regulatory function in this process (König et al., 2002).

As a third option for balancing the stromal NADPH/ATP ratio, the malate valve was put forward as a system to export excess reducing equivalents as malate while continuing ATP production (Scheibe, 2004). This highly flexible mechanism results in strong crosstalk with cytosol, mitochondria, and peroxisomes, and will be discussed later in more detail.

**Ferredoxin-thioredoxin system for regulation of the stromal redox-state**

During photosynthesis, electrons become available as reduced ferredoxin, and NADPH and ATP are generated. Various chloroplast enzymes are regulated by the ferredoxin-thioredoxin system (Buchanan, 1984; Scheibe, 1991; Schürmann and Jacquot, 2000). Electrons are transferred from ferredoxin to the thioredoxins which are present in the chloroplast in various isoforms. Finally, electrons are transferred to the target proteins (Dai et al., 2000; Marchand et al., 2004). Among the many targets identified and proposed to date, proteins with different functions such as Calvin-cycle enzymes, and other metabolic enzymes, as well as proteins involved in the stress response, can be found.

Four steps in the Calvin cycle are subject to such regulation. Unique regulatory sequences containing disulfide bridges are reduced in the target enzymes in the light. Continuous oxidation by O2 reverts the reducing step, thus leading to a ‘futile cycle’ for the sake of regulation, consuming electrons for the continuous reduction of the re-oxidized regulatory cysteine residues. Light/dark-modulated enzymes are unique with their very negative redox potentials of their regulatory cysteines (Hirasawa et al., 2000). A differential fine-regulation of the activation of the target enzymes ensures that all steps of triose-phosphate production and ribulose 1,5-bisphosphate regeneration are adjusted to the required actual fluxes (Faske et al., 1995). So, during photosynthesis, fluxes are adjusted continuously at each step depending on the metabolic situation. While redox-cycles are the basis, fine-tuning by metabolites is acting individually in each case as a feed-forward or a feed-back mechanism. Thus there is a common principle in the basis of these fast and flexibly responding regulatory systems (Scheibe, 1991).

The ferredoxin-thioredoxin system is also involved in the regulation of the malate valve (see below) and ATP synthase, as well as in the light-induced inactivation of the plastidic isomerase of glucose 6-phosphate dehydrogenase (Scheibe, 1991). It is interesting to note that chloroplasts possess a large number of thioredoxin isoforms
Crosstalk between chloroplast and cytosol: the malate valve

One of the chloroplast enzymes which is also reduced via the ferredoxin-thioredoxin system in the light is NADP-malate dehydrogenase (NADP-MDH) which serves as a redox valve. It uses excess NADPH to convert OAA to malate in order to regenerate the electron acceptor NADP. NADP-MDH activation is inhibited by its product NADP, so NADP-MDH switches off its own activity when NADPH is consumed for assimilatory processes in the chloroplast, and therefore no reducing equivalents should be exported as malate (Scheibe, 1991). Since ATP production continues while electrons are transferred to malate, the malate valve as an indirect export system for reducing equivalents is a useful means to balance the ATP/NADPH ratio in the chloroplast (Backhausen et al., 1998).

Malate can easily be transported across shuttle systems of the cellular membranes. Plastidic dicarboxylate transporters have recently been identified which could fulfill the malate-oxaloacetate shuttle function (Taniguchi et al., 2002; Renne et al., 2003). In the cytosol, malate can serve (i) to provide NADH for nitrate reduction, (ii) to generate ATP in the mitochondria, (iii) to support photospiration, or (iv) to be stored in the vacuole. Recently, the vacuolar malate transporter from Arabidopsis (ArtDT) has been identified (Emmerlich et al., 2003).

Malate is a very common organic acid found in every plant tissue that plays a central role in plant metabolism (Lance and Rustin, 1984) since it is intermediate in the Krebs and glyoxylate cycles and serves as a mobile storage (Lance and Rustin, 1984) since it is intermediate in the plant tissue that plays a central role in plant metabolism.

Mitochondrial and peroxisomal contributions to photosynthetic efficiency and redox homeostasis

Apart from the mechanisms outlined above, there are also strong contributions to redox homeostasis coming from mitochondrial and peroxisomal reactions in the light. Malate exported from the chloroplast can serve as a substrate for light-enhanced dark respiration (LEDR) (Padmasree et al., 2002), and AOX was shown to function as an ‘antioxidant enzyme’ by dissipating electrons without the generation of ATP, thus preventing the formation of ROS due to over-reduction of the mitochondrial electron transport chain (Vanlerbergh et al., 1992). Interestingly, AOX is a target of redox-modification by the mitochondrial thioredoxin system (Gray et al., 2004), and induction of AOX transcription and translation is caused by multiple stress factors such as cold (Vanlerbergh et al., 1992), or over-reduction (Zhang et al., 2003). The role of leaf mitochondria for cellular redox homeostasis was shown when the cytoplasmic male-sterile mutant CMS II of tobacco was used (Dutilleul et al., 2003). These plants do not possess a functional complex I, but due to multiple adjustments of their redox-balancing systems no oxidative stress becomes apparent, and an increased tolerance to ozone and pathogens was observed (Noctor et al., 2004). In this context, it is of interest to mention the recent findings showing that knock-out plants lacking the mitochondrial type II peroxiredoxin F possess a strong phenotype, especially during stress and when AOX was inhibited (Finkemeier et al., 2005). Finally, disruption of the TCA cycle by decreasing the amount of mitochondrial MDH had dramatic effects on photosynthesis and growth (Nunes-Nesi et al., 2005).

Most importantly, photosynthetic plant cells are unique in possessing various pathways of energy production that are flexibly linked and interact in different ways in order to maintain redox homeostasis. The essential contribution of mitochondria for efficient photosynthesis has been stressed repeatedly. The supporting role of photosynthesis for redox balancing during photosynthesis, especially under stress conditions should be also mentioned.
Long-term acclimation to changed light intensities

Considering the various changes imposed on the cells performing photosynthesis under changing conditions, it is obvious that sustained imbalances in the redox situation can also cause long-term acclimation, i.e. restructuring of the cellular systems at the level of gene expression. Therefore, it has to be assumed that a small shift in the redox balance should be sensed in the nucleus, after translocation of a redox signal to the nucleus, possibly mediated by phosphorylation cascades, has occurred. As a well studied example, acclimation to increased light intensities is described. In contrast to ‘poising’ or short-term acclimation, as described earlier, that is characterized by reaction times of seconds and minutes, long-term acclimation includes changed gene transcription and responds in the range of hours or days.

Moderate, but longer lasting changes of light intensity and temperature cause large alterations in leaf ultrastructure, and of protein and pigment composition (Anderson and Osmond, 1987; Chow et al., 1990). Chloroplasts of leaves acclimated to low light are characterized by a high content of grana thylakoids, and a relatively high number of photosystems and light-harvesting complexes (LHCII), but they possess only low quantities of stromal proteins. Such low-light plants respond to an increase in light intensity with a very characteristic alteration of gene expression. As a consequence of high-light acclimation, the light saturation of photosynthesis is reached at higher light intensities, the Chl a/b ratio decreases due to a loss of LHCII, and the content of ATPase and of Calvin-cycle enzymes increases. The relative amount of stroma thylakoids is also increased, while the portion of grana thylakoids is lowered (Anderson and Osmond, 1987). In the case of Arabidopsis, it was shown that the response towards different light intensities is not linear, but follows a complex pattern with separate low-light and high-light responses (Bailey et al., 2001).

There is evidence that, in the case of fully developed plants, the alterations in gene expression occurring during light acclimation are strongly influenced by redox signals that are released by the chloroplasts. Imbalances between the input of light energy and the capacities to use this energy for metabolism are immediately detected in the photosynthetic apparatus (Fujita et al., 1987; Huner et al., 1998), although it is not yet clear from where such redox signals originate. Inside the thylakoid membranes, the cyt b6f complex, the plastoquinone pool, and the phosphorylation status of PSI are possible sources and soluble, redox-active components, such as thioredoxins or glutathione, are also considered as signals in the current discussions.

The influence of photoreceptors (phytochromes, cryptochromes, and phototropins), which control the expression of many target genes of redox regulation in the early stages of development or during greening (e.g. LHCII, Rubisco), seems to disappear with increasing plant age. This is experimentally difficult to confirm with wild-type plants, since an increase in light intensity will not only increase the redox state of the chloroplasts, but will also lead to an increased input of photons into the photoreceptors. However, there is some evidence for this assumption: At first, transgenic potato plants underexpressing Fd1, the major ferredoxin isoform for photosynthetic electron distribution inside the chloroplasts, when grown under high light intensities, suffer permanently from elevated redox states of plastoquinone and other intersystem carriers. Results obtained with the mutant plants indicate that they display an enhanced light-acclimation response (Holtgreve et al., 2003). Arabidopsis plants which lack functional photoreceptors are able to acclimate to a changed light intensity. Only the det1-signal transduction mutant showed some differences (Walters et al., 1999), and it may be possible that the final steps during redox-mediated light acclimation use the same mechanisms that are used by the photoreceptors in seedlings during greening, with only the signal input having switched from phytochromes and cryptochromes towards the photosynthetic apparatus.

Finally, changes of gene expression as they occur during light acclimation cannot be regarded as simple events, with one signal leading directly to expression changes of only one gene or of a group of genes. Firstly, the signal is delayed by short-term acting mechanisms inside the chloroplasts which buffer imbalances as much as possible. Secondly, type and extent of the response are further influenced in many ways by factors which link it to the actual status of the cell (e.g. sugars, pH, redox-state, and phytohormones).

Acclimation of metabolism to photoperiod and developmental needs

The interrelationship between the various metabolic needs during distinct phases of development and the control of the expression of components required for energy metabolism will be demonstrated using another example. Not only light quality and quantity, but also the duration of the photoperiod is decisive for many responses in plants. Unfortunately, few data concerning the influence of daylength on metabolic processes are available, because the majority of the published data deals with the morphogenic aspect of flowering induction, i.e. the shift in the primordia from the vegetative state to the formation of flower organs.

Arabidopsis is a facultative long-day plant, i.e. flowering is promoted in long days (in the ecotype Columbia),
especially when the light period lasts 16 h or longer (Koornneef et al., 1998). Arabidopsis will also form flowers in shorter days, but then the vegetative phase lasts longer, with the consequence that these plants accumulate more biomass before flowering. Even in the extreme case of complete darkness, when enough energy is supplied as sucrose, Arabidopsis plants will finally form flowers. As far as the metabolic strategies are concerned, there are big differences between short-day and long-day-grown plants, long before flower formation is started. During vegetative growth, biomass production is of prime importance. Flowering redirects the assimilates from the leaves to inflorescence growth and seed formation. Although photoperiod and temperature act as strong inducers of flowering, other factors have been identified which are also involved in flower induction. Light, temperature, sucrose, glutamine, as well as gibberellins and cytokinins, act together to induce floral morphogenesis (for a recent review see Corbesier and Coupland, 2005).

The need for light acclimation and for optimization of the assimilatory processes is apparent in the vegetative stage of the plant and in young leaves. There are already various examples of a changed level of NADP-MDH in situations such as growth under cold stress or drought at high light, when photosynthesis and carbon metabolism are affected to such as growth under cold stress or drought at high light, when photosynthesis and carbon metabolism are affected to a different extent (Huner et al., 1996; Savitch et al., 2000, 2001). Under elevated CO2, expression of NADP-MDH in tobacco is decreased (Backhausen and Scheibe, 1999). In short-day grown Arabidopsis plants, a treatment with low temperature and high light induces expression of NADP-MDH, while this is not the case in leaves of long-day-grown plants which apparently start to become source organs due to floral induction, and simply increase their antioxidant systems (B Becker, S Holtgrefe, JE Backhausen, R Scheibe, unpublished results).

In tobacco, the function of the individual leaves changes with age and developmental stage. Young leaves are sink organs initially, then they develop into source leaves. In later stages, tobacco leaves are often used as storage tissues, mostly for starch. The capacity of NADP-MDH is highest in young leaves, and starts to decrease after sink leaves have turned into source leaves (Faske et al., 1997). The ability of the leaf cells to change the capacity of NADP-MDH after acclimation to altered CO2 concentrations is also much higher in young leaves, while in sink leaves, it remains more or less uninfluenced by the CO2 level (Backhausen and Scheibe, 1999). It has been suggested by Walters (2005) that a difference in the carbohydrate status of the leaves may influence light acclimation. The overall pattern of gene expression changes dramatically in senescent leaves, including the induction of senescence-specific transcription factors (Zentgraf et al., 2004). Again, the natural ageing process appears to be correlated with a shift of the cellular redox state to more oxidized conditions, and is subject to a combination of redox and hormone signalling as shown recently for legume nodules (Puppo et al., 2004).

**Networks of signal transduction pathways**

When acclimation to long-term changes of the environment is induced at the level of gene expression, a plethora of different signal molecules has been implicated. Beyond the harmful action of ROS, these molecules play a central role in many signalling pathways (for reviews see Mullineaux and Karpinski, 2002; Apel and Hirt, 2004). For redox-related effects, ROS (H2O2, O2•−), NO, ascorbate, glutathione, and many others were shown to induce gene expression of many more-or-less specific enzyme systems (Wingate et al., 1988; Arrigoni and De Tullio, 2002; Neill et al., 2002; Mittler, 2002; Wendehenne et al., 2004). An interesting question is the source of the signal, the transduction pathway across cellular compartments and the integration of various types of information. Finally, the localisation of a messenger molecule to the nucleus has to be assumed. In this respect, the large gene family of thioredoxins and glutaredoxins could be important, since more functions and targets of the large number of isoforms present in plants are yet to be discovered (Meyer et al., 1999; Marchand et al., 2004). Most metabolic pathways are closely linked with primary energy production or consumption. Since enzymes and cofactors involved in these processes can apparently also function in modifying gene expression, an interesting hypothesis has been put forward for their role as a direct link between metabolism and transcription (Shi and Shi, 2004).

From the large number of studies presenting genome-wide expression analyses and the availability of mutants for each gene, it now becomes clear that upon one type of imposed stress there is no single pathway or any isolated response, but a sophisticated network of signal transduction pathways. These are connected in multiple ways, as can be assumed from the large overlap of genes expressed upon different kinds of stress (Takahashi et al., 2004). The expression profiles of transcription factors involved in stress responses also suggest a complex network for signalling pathways and co-ordinated transcriptional regulation (for review see Chen and Zhu, 2004). Again, the crosstalk with phytohormone-induced processes becomes apparent, as was shown recently for the det-2 mutants with a gene defect of brassinosteroid biosynthesis that causes an early acclimation to overcome this deficiency, resulting in an increased resistance to oxidative stress (Cao et al., 2005).

However, even with the same stress applied to a plant, there must still be differential sets of responses depending on the superimposed developmental programme that is active at a certain time or in a particular part of the plant. Therefore, genes involved in energy metabolism and redox homeostasis would be expected to respond in different ways, depending on the developmental state of the plant.
(B Becker, S Holtgreve, JE Backhausen, R Scheibe, unpublished results). In a dynamic light environment, the temporal pattern of the changes (in terms of length and direction either up or down in intensity) will influence the type and extent of the responses at the different levels of regulation. This means that a specific response can be differently modulated depending on the additional factors acting on the system. This is also true when the individual history of each plant is considered. Different types of responses can be expected if, in the past, vernalization or pathogen attack, for example, have occurred.

The role of the large number of small, thiol-containing redox-active proteins in plants, such as thioredoxins, glutaredoxins, peroxiredoxins, and cyclophilins as well as protein-disulphide isomerases might well be within this network, interconnecting the different parts of the hierarchical system, acting at a borderline between redox poise, redox signalling, and repair. Due to the unique situation in plant cells, oxidants are easily generated when reductants accumulate as can happen during photosynthesis in the absence of electron acceptors. When the impact on the system is too strong and too rapid, the poised systems might not be sufficient for short-term acclimation. If then long-term acclimation cannot take place in time, oxidative damage will occur, although the redox-balance is buffered by various systems as described above. S-glutathionylation could serve as a protective mechanism for protein thiols (Ito et al., 2003), as shown earlier for vertebrates where it had been suggested as a link between stress protection and regulation of cell cycle (Cotgreave and Gerdes, 1998). A typical pattern of response for a living organism, possessing all the mechanisms of control as discussed previously, is a damped oscillation using transiently the full capacity of the respective system. This is true for the photosynthetic electron transport system upon rapid changes of light (Scheibe and Stitt, 1988). In the gene expression pattern observed a short time after a stress condition has been applied, such oscillation patterns in the kinetics for different gene clusters might reflect the flexible adjustment to a new homeostatic state.

Recent studies of protein–protein interactions have started to draw a picture of microcompartmentation as an additional level of control. Such transient associations of enzymes to membranes and cytoskeleton might help to channel certain pathways to subcellular sites. Such a concept has been put forward for the glycolytic pathway of animals (Masters et al., 1987) and the synthesis of several secondary products in plants (Winkel, 2004), and might well turn out to be of importance for complex signal transduction pathways.

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