The complex intertwined regulation of redox and metabolism in plant cells

Redox control of plant energy metabolism

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Maintenance of the cellular redox status is crucial both to keep metabolic processes running and to prevent oxidation of cellular components by reactive oxygen species under fluctuating environments. The plastid is a plant-specific organelle in which considerable redox-active reactions occur and therefore the redox status in this energy organelle, as well as that of the mitochondria, must be tightly regulated. Plants employ multiple mechanisms to actively regulate energy metabolism in response to the redox status and to integrate subcellular redox signals to orchestrate redox status at the cellular level. In this article, we describe the redox regulation of the major flux bearing reactions in these two energy organelles and survey recent advances concerning interorganellar redox communication. The sum action of this complex regulatory network allows both the fine-tuning of metabolic activities for cellular redox homeostasis and that of redox to allow optimal metabolic function.

Redox regulation of metabolism leads to alteration of the redox status itself

The cellular redox status is the balance between oxidation and reduction of various redox-active molecules. NAD(P)H is commonly distributed in cellular organelles and is involved in the majority of central metabolic processes including glycolysis and the oxidative pentose phosphate pathway in the cytosol (and plastid) and the tricarboxylic acid (TCA) cycle and respiratory electron transport in mitochondria. In addition, light energy is converted into reducing power during the process of photosynthesis in chloroplast. Reductants such as ferredoxin (Fdx) and NADPH are reduced by the photosynthetic electron transport chain (ETC) and used for assimilation and many other cellular processes. These reductants are not only an intrinsic part of metabolism, but also play active roles in metabolic regulation. There are various ways in which redox regulation exerts effects on metabolic enzymes however they can generally be classified into five mechanisms (Figure 1). The plastidial Fdx/thioredoxin (Trx) system is the most intensively examined mechanism of redox regulation in plants. Fdx acquires electrons from the photosynthetic ETC and further transfers to Trxs via Fdx/Trx reductase (FTR). Trxs are small regulatory proteins containing a redox-active disulfide group that controls the thiol-disulfide exchange of target proteins.

Key words: Calvin–Benson cycle, plastid, reactive oxygen species, redox, tricarboxylic acid cycle

Abbreviations: ACCase, acetyl-CoA carboxylase; AGPase, ADP-glucose pyrophosphorylase; AOX, alternative oxidase; ETC, electron transport chain; Fdx, ferredoxin; FTR, ferredoxin-thioredoxin reductase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MDH, malate dehydrogenase; NTR, NADPH-dependent thioredoxin reductase; ROS, reactive oxygen species; TCA, tricarboxylic acid; Trx, thioredoxin; UCP, uncoupling protein
in order to regulate their properties. Trx can also be reduced by NADPH-dependent Trx reductase (NTR). This NTR/Trx system does not require light energy and can function also in cellular compartments other than the plastid and in non-photosynthetic organs. NAD(P)H can also directly regulate the enzymatic activity as an allosteric regulator. Another post-translational enzyme modification is mostly the inhibition via the oxidation by reactive oxygen species (ROS) which accumulate under particular conditions. In addition to these post-translational regulations, redox status is involved in the reprogramming of gene expression and even in the underlying signalling pathways responsible for this.

Since many pathways which act as electron and/or ATP sinks are targets of redox regulation, redox status regulates itself by metabolic processes and at the same time operates as a major integrator of cellular metabolism. This allows readjustment of global metabolic pathways and energy homeostasis in response to fluctuating environmental conditions. Despite the considerable amount of knowledge of light activation of photosynthetic processes and recent evidence for the existence of redox signals co-ordinating metabolism and gene expression between different organelles, little is known concerning the redox regulation of global metabolic processes in plants.

**Plastidial redox biology**

Oxygenc photosynthesis is one of the most important energy-producing processes on Earth as well as a reaction responsible for the production of reducing equivalents in illuminated plant leaves. During this process, light energy is captured by photosystems in the thylakoid membrane of the chloroplast and activates the photosynthetic electron transport from water, resulting in reduction of Fdx. Reduced Fdx functions as a mobile electron carrier distributing electrons to NADP⁺ via Fdx/NADP reductase to produce NADPH or directly to specific processes such as sulfur and nitrogen assimilation and the synthesis of chlorophyll and fatty acids (Figure 2). Since the reductants produced in this process are highly reactive and can destroy cellular molecules by oxidation, sensitive regulation of photosynthesis and recycling of cofactors are essential to ensure redox and energy homeostasis. The light-dependent control of the Calvin–Benson cycle, which consumes ATP and NADPH produced by light reaction and serves as the primary carbon fixation pathway in plants, is the most well described redox regulation of plant metabolism. Several enzymes of the Calvin–Benson cycle are activated via the Fdx/Trx system, which is directly linked to light-driven photosynthetic electron transport, rather than to stromal metabolism.

**Figure 1.** Redox regulation of enzymatic proteins. Enzymes (Enzyme_ox, orange circle) which are activated under reduced conditions (Enzyme_red, red circle) are assumed. (a) Ferredoxin (Fdx)/thioredoxin (Trx) system. Fdx is reduced by electrons deriving from photosynthetic electron transport chain. Then Fdx/Trx reductase (FTR) reduces Trx by using reduced Fdx. Trx activates target enzymes by reducing either intra- or inter-molecular disulfide bonds. (b) NAD(P)H/Trx reductase (NTR)/Trx system. Here Trx is reduced by the action of NTR using reducing equivalent from NAD(P)H. (c) Allosteric regulation by NAD(P)H. Reductants can also act as allosteric regulators of enzymes. (d) Oxidative inactivation of enzymes by reactive oxygen species (ROS) produced under oxidative conditions. (e) Transcriptional regulation of enzyme abundance by cytosolic and retrograde signals.
The Calvin–Benson cycle intermediates are used to synthesize sucrose and starch as major end-products, which serve as the ultimate source of carbon for plant growth. Starch biosynthesis is localized in the plastid stroma and largely controlled by the redox regulation of ADP-glucose pyrophosphorylase (AGPase) enzyme activity. Light rapidly activates AGPase by cleaving the intermolecular disulfide bond between the two small subunits of the heterotetrameric enzyme via the Fdx/Trx system (Figure 2). Similarly, reactions of de novo fatty acid biosynthesis occur also exclusively in the plastid and are a strong sink of ATP and NADPH. The regulation of this pathway is quite similar to starch biosynthesis and the key regulatory enzyme, acetyl-CoA carboxylase (ACCase), is regulated by the Fdx/Trx system (Figure 2). Both AGPase and ACCase are also redox-regulated in a sucrose-dependent manner and this could involve plastid NTR carrying a Trx domain in its C-terminus as a dark-operative redox system.

**Mitochondrial redox biology**

Mitochondrial respiration and the glycine decarboxylation reactions of photorespiration represent the two major pathways determining redox status in this organelle (Figure 3). Mitochondrial redox biology affects cellular redox status not only under heterotrophic conditions, but also under photosynthetic conditions, despite the fact that cellular redox activity is considerably down-regulated in the light. Therefore the activities of these pathways must be flexibly regulated in response to light conditions as well as to tissue types and stresses. The respiratory pathway produces NADH by the reaction of four dehydrogenases of the TCA cycle and pyruvate oxidation by the pyruvate dehydrogenase complex at the entry of this pathway. It has long been demonstrated that these enzymes display product inhibition by NADH in vitro. Such regulation allows the organelle to balance the rate of pyruvate oxidation and subsequent TCA cycle activity with that of oxidative phosphorylation. In addition to the allosteric effects of NADH, redox status probably regulates TCA cycle enzyme activity by thiol modification via the NTR/Trx system, since many of the enzymes have been demonstrated to bind Trx (Figure 3).

Another major NADH-producing reaction in the mitochondria is photorespiratory amino acid metabolism. Two photorespiratory enzymes, glycine decarboxylase and serine hydroxymethyltransferase, are among the most prominent proteins of the mitochondrial matrix in illuminated plant leaves. Little is known about the regulation of these enzymes, but recent analysis of an Arabidopsis mutant lacking mitochondrial uncoupling protein (UCP) provided strong support for a functional link between the demand for NADH oxidation and photorespiratory glycine oxidation (Figure 3).

Oxidation of NADH to recycle NAD⁺ is performed by the NADH dehydrogenases in mitochondria (Figure 3). In addition to the common NADH dehydrogenase (complex I) functioning in cytochrome-dependent ETC, the plant mitochondrial ETC is highly branched and employs several alternative NAD(P)H dehydrogenases as well as the alternative oxidase (AOX), which bypass cytochrome pathway and oxidative phosphorylation respectively. Owing to the low affinity for NADH, alternative dehydrogenases are considered to oxidize NADH under high matrix NADH concentrations, achieved, for example, under conditions of high photorespiratory flux, and thereby prevent over-reduction of NAD⁺. AOX, like UCP, provides a means to relax the coupling between respiration and ATP production. AOX activity is regulated by redox.
modulation of intermolecular disulfide bond between the two monomers of a homodimer. AOX is activated by reduction of this bond by a mitochondrial Trx and then becomes sensitive to further activation by specific organic acids, most notably pyruvate (Figure 3).

From the evidence described above, the mitochondrial redox status, especially the NADH/NAD⁺ ratio, seems to have great influence on mitochondrial metabolism via a combination of allosteric regulation and the NTR/Trx system. However, to date, there are limited studies dealing with this topic, and considerably more research will be required to elucidate the regulatory network of plant mitochondrial redox biology.

Integration of redox signals at the cellular level

The above-mentioned redox regulatory mechanisms function in redox homoeostasis in individual organelles. The multiple regulatory mechanisms for each metabolic pathway allow fine-tuning of energy metabolism under fluctuating environment. However, the reducing equivalent from a cellular compartment can dissipate to the others and therefore the redox metabolism must be orchestrated also at the cellular level. Photosynthetic organisms uniquely have two major organelles responsible for the production of reducing equivalent and their interorganelle communication is particularly important. One way to achieve this is reprogramming of the gene expression network by redox signals from each cellular compartment. Plastid signals play important roles in various cellular processes vital to the plant by influencing nuclear gene expression during different stress conditions. This includes different signal components related to photosynthetic electron transport, changes of the chloroplast redox state, accumulation of ROS and metabolic intermediates (Figure 4). Retrograde signalling from mitochondria is far less well characterized than its plastidial counterpart, with no single pathway being completely elucidated to date. Several candidate redox related signals have, however, been proposed including superoxide, NO, H₂O₂, ascorbate, glutathione, lipid peroxide, peroxynitrite, lipoic acid, cysteine, NAD⁺/NADH and oxidized proteins, as well as TCA cycle organic acids (Figure 4).

Exchange of reducing equivalent between organelles is another way to keep redox homoeostasis at the cellular level. Generally reductants are not directly transported across biomembranes and the redox status in energy organelles is transmitted to other cellular compartments by the exchange of surrogates such as organic acids. The malate–oxaloacetate shuttle, sometimes referred to as the ‘malate valve’ of the plastid, is the most well recognized systems participating to the exchange of reducing power among the plastid and cytosol. These two organic acids are transported by antiporters across plastidial and mitochondrial membranes and can serve as a source and a sink of NAD(P)H respectively via reactions of malate dehydrogenases (MDHs) (Figure 4). One of the functions of the malate valve is considered to release excess reducing equivalent from illuminated chloroplast to mitochondria. Malate transported into mitochondria is oxidized by respiration and allows the recycling of NAD⁺ in chloroplasts. However, Arabidopsis mutants lacking plastidal NAD⁺-dependent MDH, essential for the malate valve, can still be protected from photoinhibition, suggesting the employment of additional mechanism (Figure 3).

Analysis of tomato plants deficient in mitochondrial MDH allows us to postulate another signalling pathway of interorganelle communication. These transgenic lines showed an enhanced use of L-galactono-1,4-lactone as an alternative substrate for ETC resulting in an increase in ascorbate content. This phenotype is linked to enhanced photosynthesis and growth, suggesting a regulatory role of ascorbate on photosynthesis, although the exact mechanism is still unclear (Figure 3).

Another example of interorganellar communication has been described in the ripening process of tomato fruits. Here the malate valve transmits the mitochondrial redox signal to the plastid and affect its metabolism. Fruit-specific suppression of mitochondrial MDH or fumarase in tomato led to mild changes in ripening, but dramatic ones on the accumulation of transitory starch and soluble sugars at harvest. The levels of malate were inversely correlated with the accumulation of transitory starch and soluble sugars at harvest. The levels of malate were inversely correlated with the accumulation of transitory starch and soluble sugars at harvest.
In this article, we have described the most important reactions affecting plant cellular redox status and how they are controlled by redox status in energy organelles. It is becoming clear that the redox signals are integrated at the cellular level and play a central role in sensing physiological and environmental situations. This is crucial for fine-tuning of the metabolic network and for metabolic homeostasis under fluctuating environmental conditions. Further work is, however, required to resolve the network of intra- and inter-organellar redox regulations, including an identification of players in these almost unknown signalling pathways.

Figure 4. Pathways of interorganellar redox communication. Plastidial redox status affects nuclear gene expression via reactive oxygen species and GUN1-AB4-mediated pathways. Mitochondrial signals as such as nitric oxide (NO) and ascorbic acid (ASA) have been proposed to regulate photosynthesis and gene expression, although any molecular mechanisms are unclear. Plastidial redox status can be transmitted into the cytosol by the exchange of 3-phosphoglycerate (PGA) and glyceraldehyde-3-phosphate (GAP)/dihydroxyacetone phosphate (DHAP) via the phosphate translocator (PT) in inner plastid membrane. Reducing equivalent can also be exchanged among the plastid, cytosol and the mitochondria by malate (Mal)/oxaloacetate (OAA) shuttle, also known as the malate valve on the chloroplast. Here Mal/OAA exchange is mediated by dicarboxylate translocator (DT) and malate translocator (MT) in plastidial and mitochondrial inner membranes respectively. These compounds serve as substrates of reactions of phosphoglycerate kinase (PGK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and malate dehydrogenase (MDH) to produce/consume reductants and ATP in the desired compartments. Mg-proto, Mg$^{2+}$-protoporphyrin IX; GL, l-galactono-1,4-lactone; GLDH, GL dehydrogenase; ROS, reactive oxygen species; ETC, electron transport chain.

References