COMMENTARY

Dark deactivation of chloroplast enzymes finally comes to light

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From the pioneering work that the Buchanan group started in the late 1960s, we know that the division of photosynthesis into light and dark reactions is inadequate because the activity of a number of chloroplast enzymes, many involved in the Calvin–Benson cycle, is strictly controlled by light; that is, they are activated in the light and deactivated in the dark. An exception is glucose-6-phosphate dehydrogenase, which is regulated in an opposite manner; that is, activation in the dark and deactivation in the light. It took a decade to biochemically dissect the newly identifed redox regulatory pathway responsible for this regulation. Known as the ferredoxin-thioredoxin system, the pathway is composed of three components: ferredoxin, ferredoxin-thioredoxin reductase (FTR), and thioredoxin (Trx) that relay the reducing power generated at photosystem 1 (PSI) to target regulatory enzymes (1, 2).

Over the last four decades there have been a number of milestones in the field of redox regulation in plants. The first step was to identify ferredoxin, FTR, and Trx as key components of the regulatory system. We then observed that there were multiple Trxs with differential selectivities in plant eukaryotic cells, and although this seems of little significance now, in the genomic era it was the subject of intense debate at the time. A very important contribution was the elucidation of the 3D structures and catalytic mechanisms of FTRs and Trxs. A lot of effort has also been dedicated to structure/function studies of redox-regulated enzymes, leading to the understanding at the molecular level of how they are constrained in the oxidized inactive state and how their reduction leads to opening of selected active sites and binding of substrates. In line with nonphotosynthetic organisms, plants were also shown to contain NADPH thioredoxin reductases (NTR and NTRC), which constitute alternate reduction systems. As a consequence of the latter observation, redox regulation has been extended to other cellular territories of plant eukaryotic photosynthetic cells, especially to mitochondria, the cytosol, the thylakoid lumen, and possibly the nucleus. A very interesting development was the description of plant thiol peroxidases (also called peroxiredoxins) in all plant cell compartments and the demonstration of their dependence on either Trx or glutaredoxin (Grx) as source of reducing power. Unexpectedly, it was found that so-called glutathione peroxidases are in fact also Trx/Grx-dependent enzymes in plants and thus function as thiol peroxidases. Overall, these lines of evidence have connected the degradation of peroxides to the Trx and Grx systems, which is a very important notion with respect to the Yoshida et al. (3) paper. Of course genomic studies have demonstrated that the Trx and Grx gene families are extremely developed in eukaryotic plant cells (on average they contain 5–10 times more genes than nonphotosynthetic counterparts). An unexpected discovery based on the capacity of some plant Grx to link iron-sulfur centers (ISCs) was that Grx are involved in iron-sulfur assembly both in chloroplasts and mitochondria, reactions that deviate considerably from the traditional redox function. Nevertheless they are crucial for the cell functioning in particular for the buildup of the electron transfer chains of mitochondria and chloroplasts, which comprise several

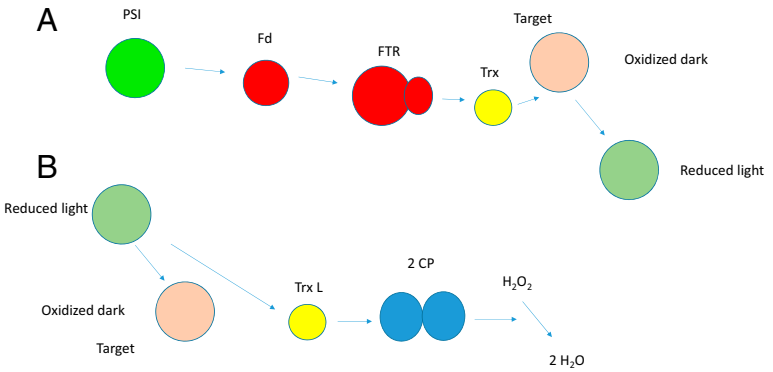

Fig. 1. Simplified working model of chloroplast redox regulation. (*A*) Light-linked reduction chain of target enzymes. (*B*) Dark-induced oxidation chain of target enzymes. Fd, ferredoxin; FTR, ferredoxin-thioredoxin reductase; PSI, photosystem 1; Trx, thioredoxin; 2CP, 2 Cys peroxiredoxin. NTRC was not included in this simplified model.

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proteins containing ISCs. Of course many soluble proteins also contain ISCs and any defect on ISC assembly has profound consequences on the cell metabolism. An interesting development was the observation that some Trxs classified as such on a phylogenetic basis actually behave kinetically more like Grxs, leading to the concept of interconnections between the Trx and Grx systems. Finally, a large effort is currently being devoted to study the evolution of redox regulatory systems.

The original references concerning these advances can be found in several review articles (1, 2, 4–6).

One area that has been neglected is the dark deactivation of redox-controlled chloroplast enzymes. Much effort has been devoted to understanding the activation of enzymes by light, but this goes with the corollary that the reduced enzymes must be oxidized and deactivated in the dark. NADP-malate dehydrogenase is a classic example of the cycling behavior; see for example figure 1 in Vidal et al. (7).

Many of us have observed over the years that reduced Trx, indeed, is the preferred reductive activator of redox-regulated enzymes. Conversely, oxidized Trx can be a very good deactivator/oxidant. The hypothesis that in light/dark transition Trx becomes quickly oxidized because no electrons are fed from PSI any more in the dark was kind of seducing, but identifying the ultimate oxidant was the key to the problem. This is exactly what Yoshida et al. (3) have done in the paper presently analyzed. Using technology allowing the identification of the redox status of the enzymes based on mass increase and SDS/PAGE, the authors beautifully demonstrate that an efficient chain of oxidation, and thus deactivation of enzymes in the dark, is constituted by Trx, a 2 Cys peroxiredoxin, and H_2O_2. Using that combination Yoshida et al. are able to produce oxidation rates that are fully compatible with the in vivo kinetics, and they have done that with an impressive number of redox-regulated enzymes. In addition, Yoshida et al. boost the significance of this paper by studying *Arabidopsis* mutants of 2-Cys peroxiredoxin in which dark deactivation considerably slows down. A comprehensive scheme outlining the light-activation and dark-deactivation schemes inspired from figure 7 of the Yoshida et al. (3) paper is shown in Fig. 1.

I believe that the Yoshida et al. (3) paper has all of the qualities to become a landmark paper in the redox field. It brings new knowledge in an elegant and technically remarkably well-mastered set of experiments characteristic of the Hisabori team. This paper opens new avenues of research, as the Trx that they have found as the best deactivator has been previously described as behaving kinetically more like a Grx (8, 9). Yoshida et al. (3) hint that Trx L2 is perhaps a better deactivator because it has a less electronegative redox potential than the more classic Trx *f* and Trx *m*, for example. In the present study the authors do not describe the properties of a mutant lacking Trx L2, as they are not available yet. It will be very interesting to see the properties of this mutant when it becomes available. In addition, it is a general property of Grxs to possess disulfides with higher redox potentials than Trxs. Because Trx L2 behaves as a Grx, and because several Grxs are good donors to peroxiredoxins, this opens the possibility that Grxs could also be involved in the dark deactivation/oxidation of the chloroplast enzymes—or not. Needless to say, we are waiting eagerly for the next episodes of that story!

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