Regulation by Redox Poise in Chloroplasts

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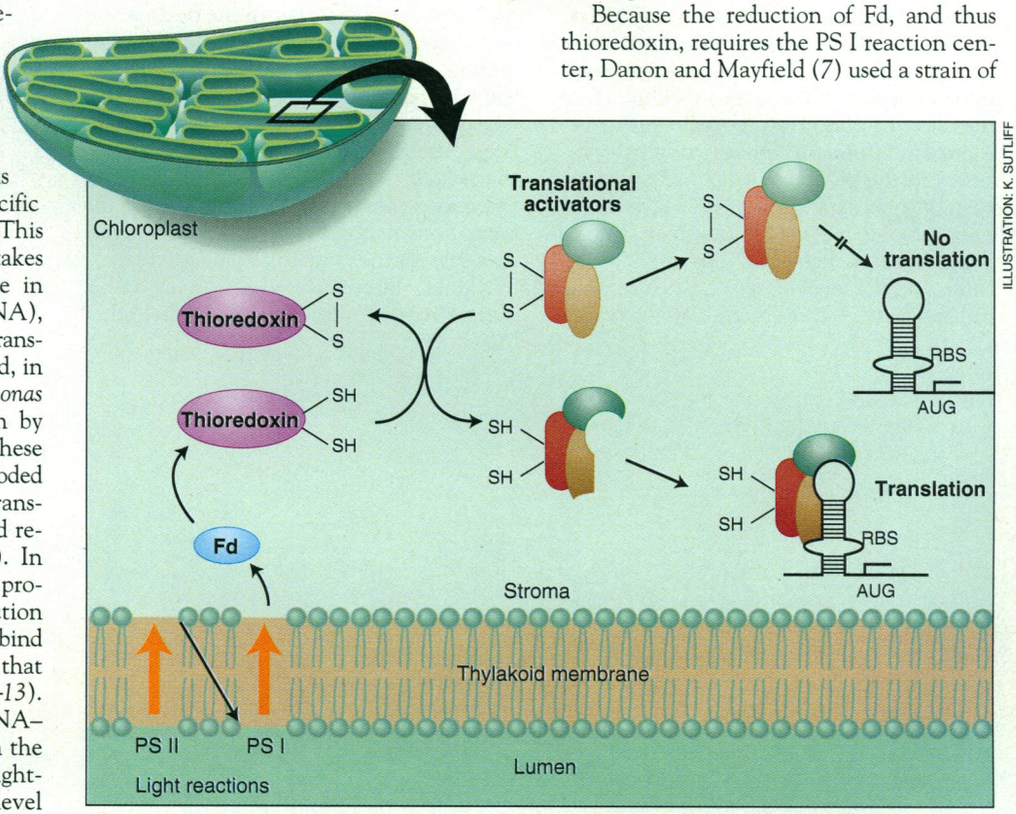
The protein thioredoxin controls the redox state of regulatory sulfhydryl-disulfide bonds in many eukaryotic and prokaryotic enzymes (*1*). Indeed, light exerts its regulatory effect on photosynthesis through the action of reduced thioredoxin. Reduction of regulatory sulfhydryl-disulfide systems by reduced thioredoxins in the chloroplast stroma accelerates several key enzymes of the reductive pentose phosphate pathway, as well as the adenosine 5′-triphosphate (ATP)–synthase complex (*2*). The reduction of thioredoxin is linked directly to the functioning of the photosynthetic electron transfer chain through the electron transfer protein ferredoxin (Fd). As Fd becomes reduced by photosystem I (PS I) in the light, it poises the reduction state of the chloroplast thioredoxins by reducing ferredoxin-thioredoxin reductase, which in turn reduces the thioredoxins. This elegant system is now shown to regulate more than the enzymes of photosynthesis.

Illumination of plant and algae cells (*3*–*6*) increases the synthesis rate of specific chloroplast proteins 50 to 100 times. This large increase in protein synthesis takes place without a corresponding change in the amounts of messenger RNA (mRNA), strongly suggesting that light regulates translation of these chloroplast mRNAs. And, in fact, in the unicellular alga *Chlamydomonas reinhardtii*, light modulates translation by redox potential (*7*). For translation, these chloroplast mRNAs recruit nuclear-encoded translational activators that promote translation by binding to the 5′-untranslated region (5′-UTR) of the mRNA (*8*–*11*). In *psbA* mRNA, which encodes the D1 protein of the photosystem II (PS II) reaction center, several mRNA-binding proteins bind to a stem-loop structure in the 5′-UTR that includes the ribosome binding site (*12*–*13*). Because the abundance of *psbA* mRNA–protein complex (RNP) correlates with the amount of translation of *psbA* in both light- and dark-grown *C. reinhardtii* cells, the level of RNP is thought to regulate the translation of *psbA* mRNA.

Binding of the protein complex to *psbA* mRNA and the resulting translation of *psbA* mRNA are modulated by redox potential through an Fd-thioredoxin system (*7*). This regulatory scheme has great appeal, because it provides a direct link between the quantity of light absorbed by photosynthesis and the synthesis of the PS II reaction center proteins by coupling the reducing power generated during photosynthetic electron transfer to the regulation of translation.

To determine whether redox potential could affect the assembly of the *psbA* mRNA–binding protein complex, Danon and Mayfield (*7*) conducted in vitro binding assays in which purified *psbA* RNP complex was treated with either reducing or oxidizing reagents. After abolishing RNA binding to the protein complex with an oxidizing reagent, they showed that reduction of the protein complex with dithiothreitol (DTT), a vicinal dithiol reductant, restores binding, but β-mercaptoethanol, a monothiol reductant, does not. The reactivation of RNP formation with DTT demonstrated that *psbA* mRNA binding responds to the redox state, probably because a redox-responsive sulfhydryl-disulfide regulatory site exists in the protein complex. Accordingly, the regulatory site promotes RNA-binding activity when reduced but inhibits binding to RNA when oxidized. In addition, they tested the effect of thioredoxin in redox-responsive regulation by including it in the in vitro binding assay. DTT-reduced thioredoxin from *Escherichia coli* activates RNA-binding activity better than DTT alone; however, oxidized thioredoxin and thioredoxin reduced with β-mercaptoethanol do not restore RNA binding. These data point to reduced thioredoxin as the in vivo factor responsible for reducing a regulatory disulfide bond in the *psbA* mRNA–binding complex to cause binding to the mRNA.

Because the reduction of Fd, and thus thioredoxin, requires the PS I reaction center, Danon and Mayfield (*7*) used a strain of



Light modulates the translation of *psbA* mRNA by changing the redox state of thioredoxin. The increased reducing power generated by photosynthesis in the light reduces ferredoxin (Fd), which in turn reduces thioredoxin. Two reducing equivalents are needed for the conversion of the disulfide bond of thioredoxin to dithiols. The reduced thioredoxin activates the RNA binding of the translational activators by reducing the regulatory disulfide bond of the RNA-binding protein complex. Only the reduced translational activator protein complex can bind to the 5′-UTR of the *psbA* mRNA. RBS, ribosome-binding site; AUG, initiator codon. The binding of the translational activator protein complex to the mRNA allows for translation of the D1 protein. [Prepared by A. Danon and S. P. Mayfield]

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C. reinhardtii (*cc703*) lacking the PS I reaction center to demonstrate that redox potential modulates *psbA* mRNA binding and translation in vivo. They reasoned that if reduced thioredoxin participates in the activation of *psbA* mRNA binding and translation, strain *cc703* should have less RNP-complexed *psbA* and, therefore, less translation of *psbA* mRNA than the wild-type strain when grown in light. Indeed, proteins from *cc703* cells form less RNP complex than proteins from wild-type cells. As expected, *cc703* cells synthesize less D1 protein than wild-type cells. *cc703* cells also synthesize less of the other light-regulated proteins (D2, CP43, and CP47) than wild-type cells; however, *cc703* and wild-type cells synthesize similar amounts of two other proteins, the α and β subunits of the ATP-synthase complex, which are not regulated by light. Differences in the synthesis of D1 and the other proteins are attributed to the rate of translation rather than differences in mRNA amounts, because *cc703* and wild-type cells contain similar amounts of mRNA. These data support the notion that the reducing power produced by PS I activates the translation of the light-regulated chloroplast mRNAs.

Reduced thioredoxin, a transducer of the redox potential produced by the light reactions of photosynthesis, furnishes the chloroplast with a means to coordinate the activities of various photosynthetic processes in the presence of light (*2*). On the basis of their data, Danon and Mayfield (*7*) have proposed a model in which thioredoxin links the light reactions of photosynthesis and the level of *psbA* mRNA binding to *psbA* mRNA translation (see figure). In this model, light activates the translation of *psbA* and other light-regulated chloroplast proteins by changes in the redox potential generated during photosynthesis. In the light, reducing power generated by photosynthesis reduces thioredoxin through a series of electron transfer reactions that begin with reduced Fd. Reduced thioredoxin then reduces a regulatory disulfide bond on the *psbA* mRNA–binding complex; this leads to an increased binding of the complex to the 5′-UTR of *psbA* mRNA and increased translation of several chloroplast-encoded proteins. A critical feature of this model is that it provides for the replacement of photo-oxidized reaction center proteins, such as D1, at a rate coordinated with light and photosynthesis.

In addition to the thioredoxin-mediated regulation of the translation of chloroplast-encoded genes, chloroplast redox poise also regulates the transcription of a nuclear-encoded chloroplast gene (*14*). In this case, the redox state of the plastoquinone pool senses light input and couples it to the transcriptional regulation of the nuclear-encoded *cab* gene. Regulation likely acts through a signal transduction pathway that runs from the chloroplast to the nucleus and is initiated by the action of a chloroplast protein kinase that becomes activated as the plastoquinone pool becomes more reduced. The chloroplast has evolved ways of coupling organelle redox state not only to the activity of functional components of the photosynthetic process but also to the regulation of photosynthetic gene expression in both the chloroplast and nucleus.

Could thioredoxin also convey redox signals in the mitochondrion—the other semiautonomous organelle of plants? Several labs have shown that plant mitochondria contain thioredoxin (*15*, *16*). Although no direct activation of respiratory-linked enzymes by mitochondrial thioredoxin has been demonstrated to date, the activity of the unique cyanide-resistant oxidase of plant mitochondria shows sensitivity to the redox poise of a sulfhydryl-disulfide system in the enzyme (*17*). By analogy to the chloroplast, redox poise may also regulate plant mitochondrial gene expression.

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