Photosynthesis Research **73:** 215–222, 2002.

Minireview

The ferredoxin/thioredoxin system: from discovery to molecular structures and beyond

Bob B. Buchanan[1,*], P. Schürmann[2], Ricardo A. Wolosiuk[3] & Jean-Pierre Jacquot[4]
[1]*Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA;* [2]*Laboratoire de Biochimie Végétale, Université de Neuchâtel, CH-2007 Neuchâtel, Switzerland;* [3]*Fundacion Campomar, 1405 Buenos Aires, Argentina;* [4]*Unité Mixte de Recherche Interactions Arbres Microorganismes, Biochimie et Biologie Moléculaire Végétale, Université de Nancy I BP 239, 54506 Vandoeuvre Cedex, France;* [*]*Author for correspondence (e-mail: view@nature.berkeley.edu; fax: +1-510-642-7356)*

Received 4 July 2001; accepted in revised form 14 November 2001

Key words: B.B. Buchanan, ferredoxin, ferredoxin/thioredoxin system, J.-P. Jacquot, redox regulation, P. Schürmann, thioredoxin, R.A. Wolosiuk

Abstract

Experiments initiated in the early 1960s on fermentative bacteria led to the discovery of ferredoxin-dependent alpha-ketocarboxylation reactions that were later found to be key to a new cycle for the assimilation of carbon dioxide in photosynthetic bacteria (the reductive carboxylic acid or reverse citric cycle). The latter finding set the stage for the discovery of a regulatory system, the ferredoxin/thioredoxin system, functional in photosynthesis in chloroplasts and oxygen-evolving photosynthetic prokaryotes. The chloroplast research led to a description of the extraplastidic NADP/thioredoxin system that is now known to function in heterotrophic plant processes such as seed germination and self-incompatibility. Extensions of the fundamental research have begun to open doors to the broad application of thioredoxin in technology and medicine.

Background

In the early 1960s, the discovery of ferredoxin-linked carboxylation reactions in fermentative bacteria (Bachofen et al. 1964) led to the elucidation of a cycle for the assimilation of carbon dioxide that was independent of the Calvin–Benson cycle – the mechanism that was then considered essential for the growth of all autotrophic cells. The new cycle, named the reductive carboxylic acid or reverse citric acid cycle (Evans et al. 1966), was found in photosynthetic bacteria and was especially prominent in the green sulfur bacterium *Chlorobium limicola* (formerly *Chlorobium thiosulfatophilum*). The cycle depended on reduced ferredoxin and specific enzymes to promote the synthesis of the alpha-keto acids pyruvate and alpha-ketoglutarate (from CO_2 and acetyl-CoA and succinyl CoA, respectively) and on citrate lyase to drive the citric acid cycle in the reverse direction. After many years of controversy, the reverse citric acid cycle is now accepted by the scientific community (Buchanan and Arnon 1990; Madigan et al. 1997). One of the lingering points of contention was whether *Chlorobium* contains the carboxylation enzyme of the Calvin–Benson cycle, ribulose 1, 5-biphosphatase carboxylase/oxygenase (Rubisco). Recent evidence has shown that *Chlorobium* lacks a functional Rubisco gene and hence cannot operate the Calvin-Benson cycle (Hanson and Tabita 2001). Interestingly, the organism has a Rubisco-like enzyme that functions in another capacity, perhaps in the oxidation of a sulfur derivative during growth.

The question arose as to whether the new cycle occurs in oxygenic photosynthetic organisms, i.e., chloroplasts of higher plants. This question prompted a search for the two ferredoxin-linked carboxylation reactions that serve as markers of the cycle. Although the experiments were unsuccessful, they prompted an

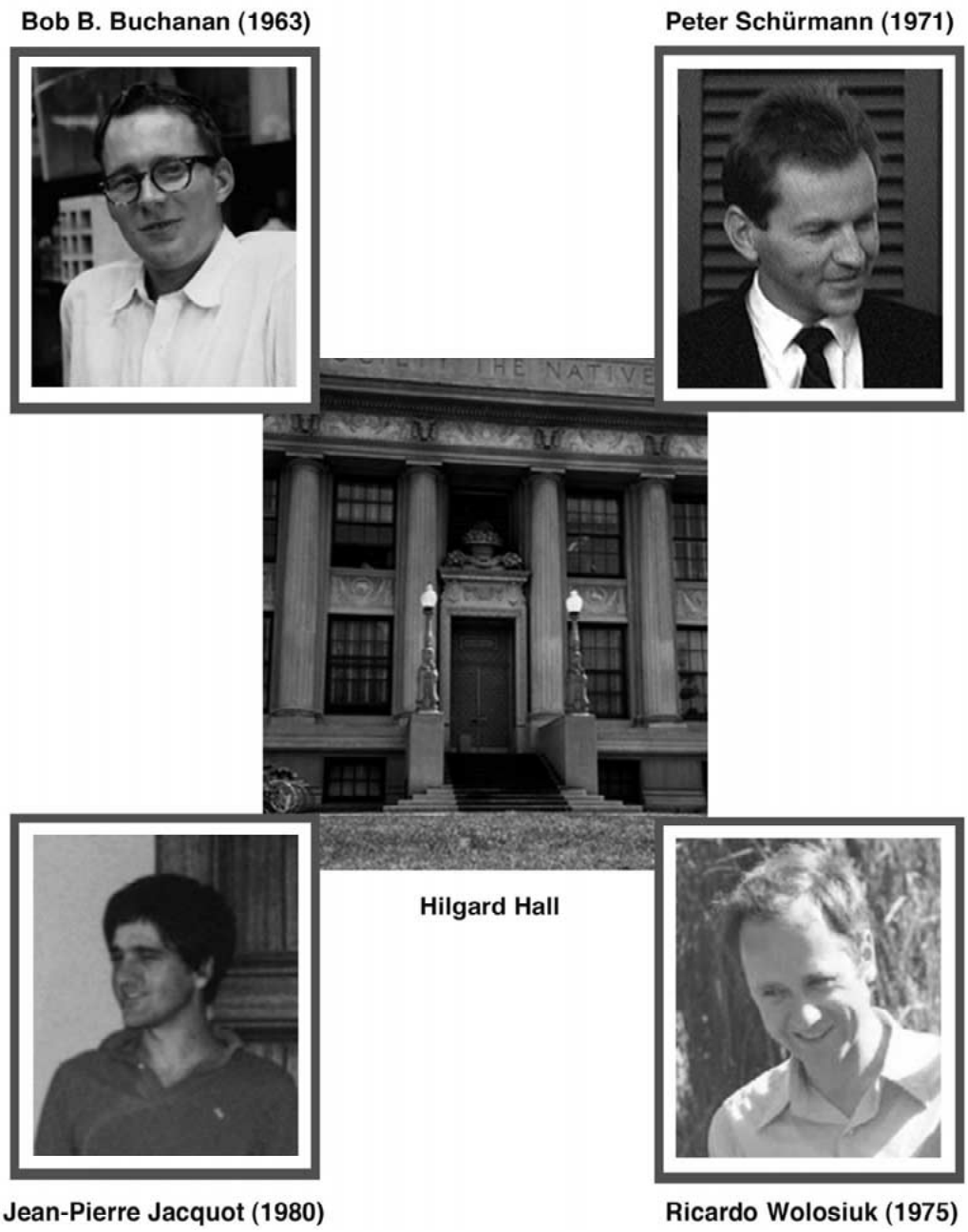

Figure 1. Early investigators who studied the ferredoxin-thioredoxin system in Hilgard Hall at Berkeley during the 1960s, 1970s and 1980s.

observation which, it turns out, led to experiments that shaped the future careers of each of us (Figure 1).

Discovery

The search for ferredoxin-linked carboxylation reactions in chloroplasts led to the observation that CO_2 fixation in the presence of fructose 1,6-bisphosphate was stimulated by reduced ferredoxin (Figure 2). An analysis of this phenomenon revealed that ferredoxin activated fructose 1,6-bisphosphatase (then called fructose diphosphatase) in preparations from isolated chloroplasts (Buchanan et al. 1967). The ferredoxin-linked activation of this enzyme, a member of the Calvin–Benson cycle, was an unexpected finding,

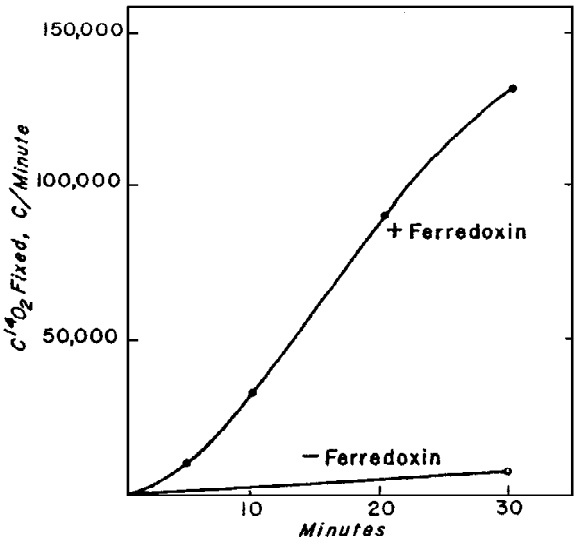

Figure 2. Early experiment showing the role of thioredoxin in enzyme regulation. The ferredoxin-dependent fixation of $^{14}CO_2$ was measured in an illuminated reconstituted chloroplast fraction containing stroma plus thylakoid membranes. The reaction mixture contained Tris buffer, pH 8.0, fructose 1,6-bisphosphate, ATP, Mg^{++} and ferredoxin. At the indicated times, aliquots were removed and fixed (acid-nonvolatile) radioactivity was measured. Paper chromatography analyses showed that radioactivity was recovered primarily in 3-phosphoglycerate. The results were interpreted as showing (1) an activation of chloroplast fructose 1,6-bisphosphatase by ferredoxin; (2) recombination of the newly generated fructose 6-phosphate to form ribulose 1,5-bisphosphate in the presence of ATP; and (3) fixation of $^{14}CO_2$ into ribulose 1,5-bisphosphate to form ^{14}C-3-phosphoglycerate. Enzymes of the Calvin–Benson cycle present in the stromal fraction catalyzed the formation and carboxylation of ribulose 1-5-bisphosphate. Note that the Mg^{++} was added only to 1 mM; the effect of ferredoxin was less pronounced at higher Mg^{++} concentrations owing to its ability to activate partially the oxidized (less active) form of fructose 1,6-bisphosphatase independently of reduction (unpublished data of B. B. Buchanan, P. P. Kalberer and D. I. Arnon, 1967).

in that the reaction does not involve oxidation or reduction (Equation (1)).

$$\text{Fructose 1,6-bisphosphate} + H_2O \rightarrow \text{Fructose 6-phosphate} + P_i \quad (1)$$

This property provided the first clue that ferredoxin might function in a new type of enzyme regulation – a possibility consistent with the findings that fructose 1,6-bisphosphatase was activated by light (Pedersen et al. 1966). During this period, NADP-glyceraldehyde 3-phosphate dehydrogenase was also shown to be activated by light via noncyclic electron transport (Müller et al. 1969). This conclusion superseded the earlier interpretation that the observed effect of light was to promote the synthesis of this enzyme (Ziegler and Ziegler 1965).

Table 1. Demonstration of the requirement for assimilation regulatory proteins, ARP_a and ARP_b, for activation of fructose 1,6-bisphosphatase by ferredoxin. In this experiment, the ferredoxin-dependent activation of fructose 1,6-bisphosphatase was measured directly (Schürmann et al. 1976). Conditions were as in Figure 2 except that $^{14}CO_2$ was omitted and released Pi was measured. ARP_a was later identified as FTR and ARP_b as thioredoxin *f* as described in the text

Treatment	P_i released (nmol per min)
Control	0
ARP_a	14
ARP_b	0
ARP_a + ARP_b	73

Experiments on the purification of crude protein preparations revealed that ferredoxin did not react with the fructose 1,6-bisphosphatase enzyme directly but that an additional protein fraction (termed the protein factor) was required (Buchanan et al. 1971). Efforts to purify the protein factor resulted in a loss of activity when partially purified preparations were passed through a size exclusion column. However, the activity could be restored when fractions representing proteins of two different sizes were combined. It turned out that the protein factor consisted of two types of proteins, a larger one with an apparent molecular mass of about 30 kDa, named 'assimilation regulatory protein A' (ARP_a), and a smaller protein of about 12 kDa, 'assimilation regulatory protein B' (ARP_b), both necessary for the activation of FBPase (Table 1; Schürmann et al. 1976). It was eventually shown that the assimilation regulatory protein B is a thioredoxin, and the protein A counterpart is the enzyme catalyzing its reduction by reduced ferredoxin, ferredoxin:thioredoxin reductase (FTR; Buchanan and Wolosiuk 1976; Wolosiuk and Buchanan 1977; Buchanan 1980). At the time, thioredoxin was known to occur in bacteria and animals but was not known to have a regulatory function (Holmgren 1985). The original view that oxidized glutathione was involved in deactivation (oxidation) of thioredoxin-activated enzymes (Wolosiuk and Buchanan 1977) has yielded to evidence that oxygen serves primarily in this capacity (Schürmann 1983; Schürmann and Kobayashi 1984). It became clear that these three chloroplast proteins functioned in what is now known as the ferredoxin/thioredoxin system – a reductive mechanism that links light to the regulation of enzymes of photosynthetic CO_2 as-

similation (Buchanan 1980) (Equations (2–4)) [Fd – ferredoxin; Trx – thioredoxin; E – target enzyme].

$$H_2O + 2Fd_{ox} \xrightarrow[\text{thylakoids}]{\text{light}} > 2Fd^-_{red} + 2H^+ + 1/2O_2 \quad (2)$$

$$2Fd^-_{red} + 2H^+ + \underset{-S-S-}{Trx_{ox}} \xrightarrow{FTR} 2Fd_{ox} + \underset{-SH\ HS-}{Trx_{red}} \quad (3)$$

$$\underset{-S-S}{E_{ox}} + \underset{-SH\ HS-}{Trx_{red}} \longrightarrow \underset{-SH\ HS-}{E_{red}} + \underset{-S-S-}{Trx_{ox}} \quad (4)$$

INACTIVE/ ACTIVE — ACTIVE/ INACTIVE

Taken together, these two reactions (Equations (3) and (4)) constituted the first evidence for a cascade of thiol/disulfide exchanges involved in enzyme regulation. The new mechanism also raised the possibility, later documented (Hertig and Wolosiuk 1980), to modulate enzyme activation independently of catalysis by the concerted action of reduced thioredoxin, metabolites, and Mg^{++} or H^+, all of which change in light-dark transition (Buchanan 1980, 1991). Functioning together, these light-induced changes regulate synthetic and opposing degradatory pathways so that the cell can optimize available resources. The elucidation of this and other regulatory systems that link light to enzyme activity during photosynthesis (Buchanan 1980) changed the long-held view that the sole contribution of light was to provide the reductant (NADPH) and energy (ATP) for the CO_2 assimilation reactions (Trebst et al. 1958). Accordingly, the terminology separating photosynthesis into 'light' and 'dark' reactions that stems from the work of F.F. Blackman at the beginning of the last century (Blackman 1905) was slowly reevaluated. As a result, the term 'dark reactions' has to a large extent disappeared from textbooks and been replaced by 'carbon reactions.'

Progress

Following recognition of the importance of the ferredoxin/thioredoxin system, decisive progress has been made in elucidating the structural and physical properties of its components as well as its target enzymes. Early work showed that chloroplasts contain two different thioredoxins, each showing selectivity toward different enzymes: thioredoxin *f* (named for its capability to activate fructose 1,6-bisphosphatase) and thioredoxin *m* (named for its capability to activate NADP-malate dehydrogenase) (Jacquot et al. 1976, 1978; Kagawa and Hatch 1977; Buchanan et al. 1978; Wolosiuk et al. 1979). A recent illustration describing the structures of the members of the system (Dai et al. 2000a) is shown in Figure 3 (ferredoxin, FTR and thioredoxins *f* and *m*). In addition to ferredoxin, FTR, and the thioredoxins, the structures of two target enzymes have also been determined, NADP-malate dehydrogenase (Carr et al. 1999; Johansson et al. 1999) and fructose 1,6-bisphosphatase (Chiadmi et al. 1999), and the mechanism by which they are activated is understood (Ruelland and Miginiac-Maslow et al. 1999; Dai et al. 2000a, b). The two thioredoxins have also been found to have different evolutionary histories: thioredoxin *m* is of prokaryotic and thioredoxin *f* of eukaryotic origin (Hartman et al. 1990; Meyer et al. 1999; Schürmann and Jacquot 2000).

The ferredoxin/thioredoxin system has been shown to function similarly in the different types of plant photosynthesis (C_3, C_4, CAM) and to control, either directly or indirectly, the activity of each of the five regulatory enzymes of the Calvin–Benson cycle: fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase (an enzyme discovered in connection with studies on thioredoxin; Breazeale et al. 1978), phosphoribulokinase, NADP-glyceraldehyde 3-phosphate dehydrogenase, and Rubisco. Each of these enzymes is specific for thioredoxin *f* under certain conditions (Buchanan et al. 1980; Schürmann and Jacquot 2000).

Regulatory enzymes of a number of other fundamental chloroplast processes were also found to be linked to thioredoxin: oxidative pentose phosphate cycle (glucose 6-phosphate dehydrogenase), ATP synthesis (ATP synthase), C_4 photosynthesis (NADP-malate dehydrogenase), malate valve (NADP-malate dehydrogenase), and fatty acid synthesis (acetyl-CoA carboxylase; Buchanan 1980, 1991; Scheibe 1991; Jacquot et al. 1997; Dai et al. 2000a, b; Schürmann and Jacquot 2000; Schürmann and Buchanan 2001). Except for glucose 6-phosphate dehydrogenase and NADP-malate dehydrogenase, which are active with thioredoxin *m*, these enzymes show a strong preference for thioredoxin *f*. In addition, aside from glucose 6-phosphate dehydrogenase, which is reductively deactivated by thioredoxin, each of the above enzymes resembles those of the Calvin–Benson cycle in undergoing an activation on reduction by thioredoxin (Buchanan 1980; Schürmann and Jacquot 2000). Fi-

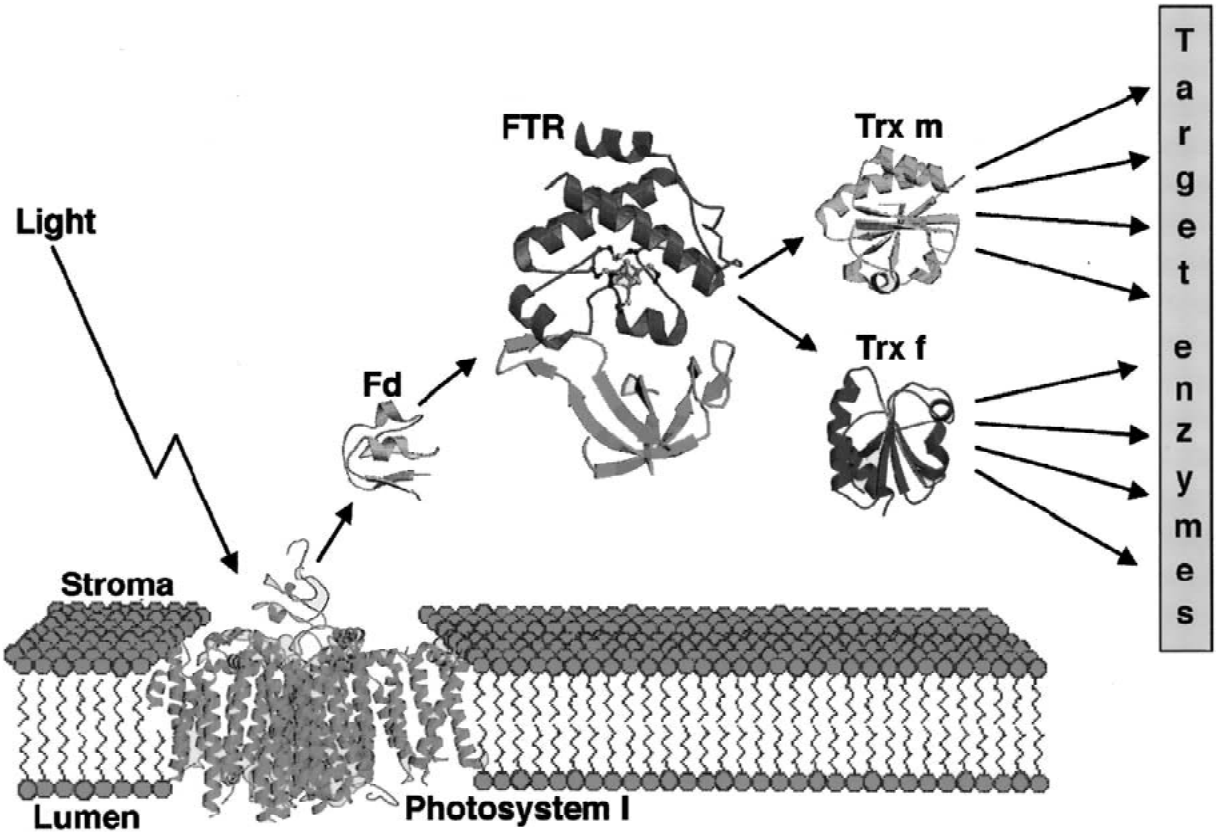

Figure 3. The photosynthetic ferredoxin/thioredoxin system. Electrons from the photooxidation of water are transported via the photosynthetic electron transport chain to the iron–sulfur protein ferredoxin (Fd). The enzyme FTR combines the electrons from ferredoxin with protons from the medium to reduce the disulfide group of thioredoxin (Trx) *f* or *m*. Finally, the reduced thioredoxins, in turn, reduce specific disulfide group on the target enzymes, converting them, in the case of biosynthetic enzymes, from an inactive to an active form. Enzymes of carbohydrate degradation behave in the opposite manner, i.e., they are inactive in the reduced state (in the light) and active when oxidized (in the dark) (Dai et al. 2000a). For a color version of this figure, see section in the front of the issue.

nally, while most of the work has been done with chloroplasts, oxygenic prokaryotes such as cyanobacteria use the ferredoxin/thioredoxin system for linking light to the regulation of key processes (Buchanan 1980; Schürmann and Jacquot 2000). It is anticipated that emerging techniques aimed at identifying new thioredoxin target proteins will facilitate work in photosynthesis as well as other processes (Meyer et al. 1999; Motohashi et al. 2001; Yano et al. 2001).

Typical of new fields, all paths exploring protein-mediated activation of chloroplast enzymes by light were not successful and, in some cases, were culs-de-sac. For example, the 'Light Effect Mediator' or LEM system, originally thought to represent a purely membrane-based regulatory system that was independent of ferredoxin (Anderson and Avron 1976), was later ascribed to the binding of the components of the ferredoxin/thioredoxin system to thylakoids (Droux et al. 1987; Ford et al. 1987). Similarly, membrane-bound ferredoxin and thioredoxin led to the description of a soluble protein named 'ferralterin' that was believed to function independently of ferredoxin and thioredoxin in the light activation of enzymes (Lara et al. 1980; de la Torre et al. 1982; Ford et al. 1987). By using thoroughly washed thylakoid membranes, ferralterin was later shown to be identical with ferredoxin:thioredoxin reductase (Droux et al. 1987). Although not establishing lasting new directions, the work on these problems was profitable in that it clarified certain properties of components of the ferredoxin/thioredoxin system (de la Torre et al. 1982) and stimulated research, thereby facilitating arrival at the present picture of the ferredoxin/thioredoxin system.

Despite the strides made in our understanding of the ferredoxin/thioredoxin system, several outstanding problems remain. For example, structural and functional studies are needed to establish the mechanism

by which thioredoxin-activated enzymes become inactive in the dark. In addition, our understanding of the noncovalent interactions of the protein components of the system with one another and with thylakoid membranes remains limited (Hirasawa et al. 1988).

Beyond chloroplasts

The research with the chloroplast ferredoxin/thioredoxin system led one of us (BBB) to investigate the NADP-linked thioredoxin system that had been extensively studied in bacterial and animals cells. While known to occur in plants (Wagner et al. 1978; Berstermann et al. 1983; Follmann and Häberlein 1995–1996), neither the cellular location nor the properties of the protein components of the plant system were known at the time. Experiments showed that the participating thioredoxin was of a new type [originally called thioredoxin *c* (Wolosiuk et al. 1979) and later renamed thioredoxin *h*] that, as in bacterial and animal cells, was linked to the flavoprotein NADP-thioredoxin reductase (NTR; Johnson et al. 1987; Florencio et al. 1988; Marcus et al. 1991). The plant NADP/thioredoxin system can be summarized as shown in Equation (5).

$$\text{NADPH} + \text{thioredoxin } h_{\text{ox}} \xrightarrow{\text{NTR}} \text{NADP} + \text{thioredoxin} h_{\text{red}} \quad (5)$$

The three-dimensional structures of both thioredoxin *h* (Mittard et al. 1997) and NTR (Jacquot et al. 1994; Dai et al. 1996) have been determined.

Cell fractionation experiments revealed that thioredoxin *h*, which like thioredoxin *f* is related to eukaryotic counterparts (Meyer et al. 1999), was extraplastidic and occurred in the cytosol, endoplasmic reticulum, and mitochondrion (Marcus et al. 1991). Current evidence suggests that the NADP/thioredoxin system is present in all plant cells, photosynthetic as well as nonphotosynthetic, and that it functions in a range of processes, including the regulation of seed germination (Besse et al. 1996; Lozano et al. 1996; Besse and Buchanan 1997; Buchanan 2001) and self-incompatibility (Bower et al. 1996; Li et al. 1997; Cabrillac et al. 2001). The seed work has opened the door to emerging new technologies, including ones applicable to the improvement of major foods (Buchanan et al. 1994, 1998; Buchanan 2001). Parallel progress is being made on the application of thioredoxin (Nakamura et al. 1997) and NTR (Mustacich and Powis 2000) to medical problems (Buchanan et al. 1994).

Concluding comments

Research initiated with chloroplasts in the mid-1960s established a function for thioredoxin in enzyme regulation. Since that time, thioredoxin has been shown to represent a family of proteins and to play a regulatory role in all types of cells. In plants, thioredoxins regulate CO_2 assimilation and related processes in photosynthesis and a growing number of heterotrophic processes whose identity is just beginning to unfold. Ongoing research in a number of laboratories is taking thioredoxin to a new level in showing its potential as a tool in technology and medicine. In addition to documenting the importance of thioredoxin, the results during the past four decades demonstrate that we still have much to learn about the catalytic capability of this protein.

Note added in proof

Recent work has demonstrated the occurrence of a new type of thioredoxin (designated '*o*') and an associated NTR in plant mitochondria, thus opening the door to elucidating thioredoxin function in that organelle (Laloi et al. 2001).

References

Anderson LE and Avron M (1976) Light modulation of enzyme activity in chloroplasts. Generation of membrane-bound vicinal dithiol groups by photosynthetic electron transport. Plant Physiol 57: 209–213

Bachofen R, Buchanan BB and Arnon DI (1964) Ferredoxin as a reductant in pyruvate synthesis by a bacterial extract. Proc Natl Acad Sci USA 51: 690–694

Berstermann A, Vogt K and Follmann H (1983) Plant seeds contain several thioredoxins of regular size. Eur J Biochem 131: 339–44

Besse I and Buchanan BB (1997) Thioredoxin-linked plant and animal processes: the new generation. Bot Bull Acad Sin (Taipei) 38: 1–11

Besse I, Wong JH, Kobrehel K and Buchanan BB (1996) Thiocalsin: a thioredoxin-linked substrate-specific protease dependent on calcium. Proc Natl Acad Sci USA 93: 3169–3175

Blackman FF (1905) Optima and limiting factors. Ann Bot 19:281–295

Bower MS, Matias DD, Fernandes-Carvalho E, Mazzurco M, Gu T, Rothstein SJ and Goring DR (1996) Two members of the thioredoxin-h family interact with the kinase domain of a *Brassica* S locus receptor kinase. Plant Cell 8: 1641–1650

Breazeale VD, Buchanan BB and Wolosiuk RA (1978) Chloroplast sedoheptulose 1,7-bisphosphatase: evidence for regulation by the ferredoxin/thioredoxin system. Z Naturforsch 33c: 521–528

Buchanan BB (1980) Role of light in the regulation of chloroplast enzymes Annu Rev Plant Physiol 31: 341–374

Buchanan BB (1991) Regulation of CO_2 assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. Perspective

on its discovery, present status, and future development. Arch Biochem Biophys 288: 1–9

Buchanan BB (2001) Thioredoxin: a photosynthetic regulatory protein finds application in food improvement. J Sci Food Agric 81: 1–8

Buchanan BB and Arnon DI (1990) A reverse KREBS cycle in photosynthesis: consensus at last. Photosynth Res 24: 47–53

Buchanan BB and Wolosiuk RA (1976) Photosynthetic regulatory protein found in animal and bacterial cells. Nature 264: 669–670

Buchanan BB, Kalberer PP and Arnon DI (1967) Ferredoxin-activated fructose diphosphatase in isolated chloroplasts. Biochem Biophys Res Commun 29: 74–79

Buchanan BB, Schürmann P and Kalberer PP (1971) Ferredoxin-activated fructose diphosphatase of spinach chloroplasts: resolution of the system, properties of the alkaline fructose diphosphatase component, and physiological significance of the ferredoxin-linked activation. J Biol Chem 246: 5952–5959

Buchanan BB, Wolosiuk RA, Crawford NA and Yee BC (1978) Evidence for three thioredoxins in leaves. Plant Physiol 61: 38s

Buchanan BB, Schürmann P, Decottignies P and Lozano RM (1994) Thioredoxin: a multifunctional regulatory protein with a bright future in technology and medicine. Arch Biochem Biophys 314: 257–260

Buchanan BB, del Val G and Frick OL (1998) Alleviation of food allergies using a thioredoxin-based technology. Leatherhead Food RA Food Ind J 1: 97–105

Cabrillac D, Cock JM, Dumas C and Gaude T. (2001) The S-locus receptor kinase is inhibited by thioredoxins and activated by pollen coat proteins. Nature 410: 220–223

Carr PD, Verger D, Ashton AR, and Ollis DL (1999) Chloroplast NADP-malate dehydrogenase: structural basis of light-dependent regulation of activity by thiol oxidation and reduction. Structure 7: 461–475

Chiadmi M, Navaza A, Miginiac-Maslow M, Jacquot J-P and Cherfils J (1999) Redox signalling in the chloroplast: structure of oxidized pea fructose-1,6-bisphosphate phosphatase. EMBO J 18: 6809–6815

Dai S, Saarinen M, Ramaswamy S, Meyer Y, Jacquot J-P and Eklund H (1996) Crystal structure of *Arabidopsis thaliana* NADPH dependent thioredoxin reductase at 2.5 Å resolution. J Mol Biol 264: 1044–1057

Dai S, Schwendtmayer C, Johansson K, Ramaswamy S, Schürmann P and Eklund H (2000a) How does light regulate chloroplast enzymes? Structure-function studies of the ferredoxin/thioredoxin system. Q Rev Biophys 33: 67–108

Dai S, Schwendtmayer C, Schürmann P, Ramaswamy S and Eklund H (2000b) Redox signaling in chloroplasts: cleavage of disulfides by an iron–sulfur cluster. Science 287: 655–658

de la Torre A, Lara C, Yee BC, Malkin R and Buchanan BB (1982) Physiochemical properties of ferralterin, a regulatory iron–sulfur protein functional in oxygenic photosynthesis. Arch Biochem Biophys 213: 545–550

Droux M, Jacquot J-P, Miginiac-Maslow M, Gadal P, Huet JC, Crawford NA, Yee BC and Buchanan BB (1987) Ferredoxin-thioredoxin reductase (FTR): an iron-sulfur enzyme linking light to enzyme regulation in oxygenic photosynthesis. Purification and properties of the enzyme from C_3, C_4 and cyanobacterial species. Arch Biochem Biophys 252: 426–439

Evans MCW, Buchanan BB and Arnon DI (1966) A new ferredoxin-dependent carbon reduction cycle in a photosynthetic bacterium. Proc Natl Acad Sci USA 53: 1420–1425

Florencio FJ, Yee BC and Buchanan BB (1988) A NADP/thioredoxin system in green leaves: purification and characterization of NADP/thioredoxin reductase and thioredoxin *h* from spinach. Arch Biochem Biophys 266: 491–507

Follmann H and Häberlein I (1995–1996) Thioredoxins: universal, yet specific thiol-disulfide redox cofactors. Biofactors 5: 147–156

Ford DM, Jablonski PP, Mohamed AH and Anderson LE (1987) Protein modulase appears to be a complex of ferredoxin, ferredoxin/thioredoxin reductase and thioredoxin. Plant Physiol 83: 628–632

Hanson TE and Tabita FR (2001) A rubisco-like protein from *Chlorobium tepidum* that is involved with sulfur metabolism and the response to oxidative stress. Proc Natl Acad Sci USA 98: 4397–4402

Hartman H, Syvanen M and Buchanan BB (1990) Contrasting evolutionary histories of chloroplast thioredoxins *f* and *m*. Mol Biol Evol 7: 247–254

Hertig C and Wolosiuk RA (1980) A dual effect of Ca^{2+} on chloroplast fructose-1,6-bisphosphatase. Biochem Biophys Res Commun 97: 325–333

Hirasawa M, Droux M, Gray KA, Boyer JM, Davis DJ, Buchanan BB and Knaff D (1988) Ferredoxin-thioredoxin reductased: properties of its complex with ferredoxin. Biochim Biophys Acta 935: 1–8

Holmgren A (1985) Thioredoxin. Annu Rev Biochem 54: 237–271

Jacquot J-P, Lancelin JM and Meyer Y (1997) Thioredoxin: structure and function in plant cells. New Phytol 136: 543–570

Jacquot J-P, Rivera-Madrid R, Marinho P, Kollarova M, Le Maréchal P, Miginiac-Maslow M and Meyer Y (1994) *Arabidopsis thaliana* NADPH thioredoxin reductase cDNA characterization and expression of the recombinant protein in *Escherichia coli*. J Mol Biol 235: 1357–1763

Jacquot J-P, Vidal J and Gadal P (1976) Identification of a protein factor involved in dithiothreitol activation of NADP-malate dehydrogenase. FEBS Lett 71: 223–227

Jacquot J-P, Vidal J, Gadal P and Schürmann P (1978) Evidence for the existence of several enzyme specific thioredoxins in plants FEBS Lett 96: 243–246

Johansson K, Ramaswamy S, Saarinen M, Lemaire-Chamley M, Issakidis-Bourguet E, Miginiac-Maslow M and Eklund H (1999) Structural basis for light activation of a chloroplast enzyme: the structure of sorghum NADP-malate dehydrogenase in its oxidized form. Biochemistry 38: 4319–4326

Johnson TC, Cao RQ, Kung JE and Buchanan BB (1987) Thioredoxin and NADP-thioredoxin reductase from cultured carrot cells. Planta 171: 321–331

Kagawa T and Hatch MD (1977) Regulation of C4 photosynthesis: characterization of a protein factor mediating the activation and inactivation of NADP-malate dehydrogenase. Arch Biochem Biophys 184: 290–297

Laloi C, Rayapuram N, Chartier Y, Grienberger J-M, Bonnard G and Meyer Y (2001) Identification and characterization of a mitochondrial thioredoxin system in plants. Proc Natl Acad Sci 98: 14144–14149

Lara C, de la Torre A and Buchanan BB (1980) Ferralterin: an iron–sulfur protein functional in enzyme regulation in photosynthesis. Biochem Biophys Res Commun 94: 1337–1344

Li X, Paech N, Nield J, Hayman D and Langridge P (1997) Self-incompatibility in the grasses: evolutionary relationship of the S gene from *Phalaris coerulescens* to homologous sequences in other grasses. Plant Mol Biol 34: 223–232

Lozano RM, Wong JH, Yee BC, Peters A, Kobrehel K and Buchanan BB (1996) New evidence for a role for thioredoxin *h* in germination and seedling development. Planta 200: 100–106

Madigan MT, Martinko JM and Parker J (1997) Brock Biology of Micro-Organisms, 8th edition, pp 650–651. Prentice Hall, Upper Saddle River, New Jersey

Marcus F, Chamberlin SH, Chu C, Masiarz FR, Shin S, Yee BC and Buchanan BB (1991) Plant thioredoxin *h*: an animal like thioredoxin occurring in multiple cell compartments. Arch Biochem Biophys 287: 195–198

Meyer Y, Verdoucq L and Vignols F (1999) Plant thioredoxins and glutaredoxins: identity and putative roles. Trends Plant Sci 4: 388–394

Mittard V, Blackledge MJ, Stein M, Jacquot JP, Marion D and Lancelin JM (1997) NMR solution structure of an oxidised thioredoxin *h* from the eukaryotic green alga *Chlamydomonas reinhardtii*. Eur J Biochem 243: 374–383

Müller B, Ziegler I and Ziegler H (1969) Light-induced, reversible increase in the activity of NADP-glycerealdehyde-3-phosphate dehydrogenase in chloroplasts. On the mechanism of the reaction. Eur J Biochem 9: 101–106

Motohashi K, Kondoh A, Stumpp MT and Hisabori T (2001) Comprehensive survey of proteins targeted by chloroplast thioredoxin. Proc Natl Acad Sci USA 98: 11224–11229

Mustacich D and Powis G. (2000) Thioredoxin reductase. Biochem J 346: 1–8

Nakamura H, Nakamura K and Yodoi J (1997) Redox regulation of cellular activation. Annu Rev Immunol 15: 351–369

Pedersen TA, Kirk M and Bassham JA (1966) Light-dark transients in levels of intermediate compounds during photosynthesis in air adapted *Chlorella*. Physiol Plant 19: 219–231

Ruelland E and Miginiac-Maslow M (1999) Regulation of chloroplast enzyme activities by thioredoxins: activation or relief from inhibition? Trends Plant Sci 4: 136–141

Scheibe R (1991) Redox-modulation of chloroplast enzymes – a common principle for individual control. Plant Physiol 26: 1–3

Schürmann P (1983) The light activation of chloroplast enzymes through the ferredoxin/thioredoxin system. In: Metzner H (ed) Photosynthesis and Plant Productivity, pp 255–258. Wissenschaftliche Verlagsgesellschaft, Stuttgart

Schürmann P and Buchanan BB (2001) The structure and function of the ferredoxin/thioredoxin system. In: Andersson B and Aro EM (eds) Regulatory Aspects of Photosynthesis. Advances in Photosynthesis, Vol 11, pp 331–361. Kluwer Academic Publishers, Dordrecht, The Netherlands

Schürmann P and Jacquot JP (2000) Plant thioredoxin system revisited. Annu Rev Plant Physiol Plant Mol Biol 51: 371–400

Schürmann P and Kobayashi Y (1984) Regulation of chloroplast fructose 1,6-bisphosphatase activity by the ferredoxin/thioredoxin system. In: Sybesma C (ed) Advances in Photosynthesis Research, Vol 3, pp 629–632. Martinus Nijhoff/Dr W Junk Publishers, The Hague

Schürmann P, Wolosiuk RA, Breazeale VD and Buchanan BB (1976) Two proteins function in the regulation of photosynthetic CO_2 assimilation in chloroplasts. Nature 263: 257–258

Trebst AV, Tsujimoto HY and Arnon DI (1958) Separation of light and dark phases in the photosynthesis of isolated chloroplasts. Nature 182: 351–355

Wagner W, Follmann H and Schmidt A (1978) Multiple forms of thioredoxins. Z Naturforsch Teil C 33: 517–520

Wolosiuk RA and Buchanan BB (1977) Thioredoxin and glutathione regulate photosynthesis in chloroplasts. Nature 266: 565–567

Wolosiuk RA, Crawford NA, Yee BC and Buchanan BB (1979) Isolation of three thioredoxins from spinach leaves. J Biol Chem 254: 1627–1632

Yano H, Wong JH, Lee YM, Cho MJ and Buchanan BB (2001) A strategy for the identification of proteins targeted by thioredoxin. Proc Natl Acad Sci USA 98: 4794–4799

Ziegler H and Ziegler I (1965) The influence of light on the $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase. Planta 65: 369–380