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Thioredoxin-linked plant and animal processes: the new generation

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Abstract. The renaissance in the study of thioredoxin in heterotrophic cells that began just prior to this decade has provided new insight into the function and significance of this ubiquitous regulatory disulfide protein. Thioredoxin, reduced enzymically with NADPH via NADP-thioredoxin reductase, has been found to regulate a range of biochemical processes in plants as well as animals. In so doing, thioredoxin targets intramolecular disulfide bonds of proteins such as enzyme inhibitors, seed storage proteins and enzymes. As may be seen, the target proteins have not been identified for a number of thioredoxin functions uncovered in complex systems, such as those involving transcription factors or gene inactivation. In a series of ongoing studies stemming from the fundamental work, thioredoxin has emerged as a new tool in technology and medicine. It now seems clear that we stand at a frontier in which the rapidly expanding knowledge of thioredoxin will lead to applications in industry and health.

Keywords: Thioredoxin; Thioredoxin reductase; Redox regulation.

Contents

Introduction

Thioredoxins are 12 kDa proteins with a catalytically active disulfide group that function in a spectrum of biochemical pathways. They have been found in virtually all organisms and were first identified as one of the hydrogen donors for ribonucleotide reductase in *Escherichia coli* over thirty years ago (Laurent et al., 1964; Holmgren, 1981, 1985). The active site of thioredoxin has two redox-active cysteine residues in a highly conserved sequence [-Trp-Cys-Gly(Ala)-Pro-Cys-]. The oxidized (-S-S-) form of thioredoxin contains a disulfide bridge that is reduced to the sulfhydryl (-SH) level by either reduced ferredoxin or NADPH via one of two specific enzymes. The reduced form is an excellent catalyst for the reduction of disulfide bonds that are, at best, sluggishingly reduced by glutathione (Holmgren, 1985, 1989; Eklund et al., 1991).

While only one type of thioredoxin has been detected in *E. coli* or animal cells, three well characterized variants exist in photosynthetic cells. Two of the three (*m* and *f*) are located in chloroplasts and can be distinguished from one another on the basis of their primary structure and specificity for target enzymes. The two chloroplast thioredoxins are members of the ferredoxin/thioredoxin system, a regulatory system in oxygenic photosynthesis. Electrons provided by the excitation of chlorophyll are transferred via ferredoxin and an iron-sulfur enzyme, ferredoxin-thioredoxin reductase (FTR) to either of the two

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types of plastid thioredoxins, which, in turn, selectively activate photosynthetic enzymes by reduction of well defined regulatory sites (see Buchanan, 1980, 1991, 1992; Buchanan et al., 1994a; Scheibe, 1991 and Wolosiuk et al., 1993 for reviews on the ferredoxin/thioredoxin system). Studies with the unicellular alga *Chlamydomonas reinhardtii* have extended the role of chloroplast thioredoxins to the control of mRNA translation (Danon and Mayfield, 1994).

Plants contain a second thioredoxin system composed of NADPH, a flavin enzyme called NADP-thioredoxin reductase (NTR), and an associated thioredoxin of yet another type (Suske et al., 1979; Berstermann et al., 1983). Named thioredoxin *h* (for heterotrophic) as it was first identified in cultured cells, seeds and roots (Johnson et al., 1987a, 1987b), *h*-type thioredoxins are also present in leaves and eukaryotic algae (Wagner et al., 1978; Wolosiuk et al., 1979; Florencio et al., 1988; Marcus et al., 1991; Schürmann, 1993). The NADP/thioredoxin system is widely distributed among organisms and is thought to be ubiquitous in aerobes. The elucidation of the biological role of NADP-linked thioredoxin is currently an area of extensive investigation in plants as well as animals, where it functions in a growing array of critical processes.

A review on thioredoxin is timely. An impressive amount of new information has appeared on the regulatory function of thioredoxin since the field was last surveyed. The scope of this article (the first on thioredoxin in this journal) encompasses the current knowledge of thiol redox control in plant and animal systems, and the associated potential technological and medical applications.

Plant Thioredoxin *h*

Plants have three types of thioredoxin whereas animals have only one. The two chloroplast thioredoxins (*f* and *m*) have been intensively studied since their identification (Wolosiuk and Buchanan, 1977; Buchanan et al., 1978; Jacquot et al., 1978) because of their importance in regulating photosynthetic activity. The third class of plant thioredoxins, the *h*-type is located in the cytosol and endoplasmic reticulum (Marcus et al., 1991). A thioredoxin, perhaps the *h*-type, has also been described for mitochondria (Bodenstein-Lang et al., 1989; Marcus et al., 1991). Thioredoxin *h* differs in both structure and activity from the *f* and *m*-type thioredoxins of chloroplasts (Florencio et al., 1988; Marcus et al., 1991; Buchanan et al., 1994b). There is a growing body of evidence that thioredoxin *m* is of prokaryotic origin, wheras both thioredoxins *f* and *h* first developed in eukaryotes (Hartman et al., 1990; Sahrawy et al., 1996). Thioredoxin *f* is an excellent example of an organelle protein which appears to be of eukaryotic origin. As such, thioredoxin *f* would have been a "foreign" member of the prokaryotic ferredoxin/thioredoxin system as photosynthesis evolved in eukaryotes. Such a change would have introduced a degree of enzyme specificity that was possibly advantageous as the cell became compartmentalized.

Occurrence

Thioredoxin *h* is generally assumed to be cytosolic—a conclusion supported by the absence of a transit peptide domain in the genes cloned for the isoforms from tobacco (Marty and Meyer, 1991; Brugidou et al., 1993), and *Arabidopsis* (Rivera-Madrid et al., 1993, 1995). Moreover, the several forms of thioredoxin *h* detected in spinach leaves (Florencio et al., 1988), wheat flour (Johnson et al., 1987b), and rice sieve tubes (Ishiwatari et al., 1995), support the view that most higher plants possess multiple and divergent thioredoxin genes (Rivera-Madrid et al., 1995). The identification of multiple *h*-type thioredoxin genes, including five in *Arabidopsis*, raises questions relating to the specificity and physiological role of the protein. While much remains to be documented, we describe below the target proteins identified so far, and then proceed to summarize evidence for the function of thioredoxin *h*.

Proteins Targeted

The first evidence that the NADP/thioredoxin system specifically reduces intramolecular disulfide bonds of small proteins was obtained fifteen years ago with purothionin—a 5 kDa disulfide rich protein of wheat endosperm (Wada and Buchanan, 1981; Johnson et al., 1987b). Currently considered a member of the thionin protein family of cereals (Bohlmann and Apel, 1991), purothionin has not been ascribed a function other than that of a storage or defense protein (Garcia-Olmedo et al., 1989). As there is no direct evidence to support these functions, the consequences of reduction by thioredoxin remain to be elucidated.

More recent studies have shown that the NADP/thioredoxin system specifically reduces a variety of target proteins that, like purothionin, contain intramolecular disulfide bonds. Included are: wheat α-amylase/trypsin inhibitors, (also known as CM-proteins) (Kobrehel et al., 1991); wheat storage proteins, gliadins and glutenins (Kobrehel et al., 1992); soybean Kunitz and Bowman-Birk trypsin inhibitors (Jiao et al., 1992); castor seed 2S protein (Shin et al., 1993); barley α-amylase/subtilisin inhibitor (Jiao et al., 1993); barley limit-dextrinase (pullulanase) inhibitors (Wong et al., 1996).

Thioredoxin was also found to reduce and thereby activate a new type of serine protease named "thiocalsin" (Besse et al., 1996). Purified from germinating wheat endosperm, thiocalsin also requires calcium for activity. A regulatory role for thioredoxin was also suggested for an amylolytic enzyme purified from leaves of mature poplar (Witt and Sauter, 1996). Here, thioredoxin in combination with calcium, was the most effective reducing agent for reactivation of the enzyme that had been oxidized *in vitro*. A similar reactivating effect of thioredoxin has been observed with oxidized cysteine proteases such as papain—enzymes long known to be dependent on mono- or dithiol reagents for activity (Stephen et al., 1993).

Proteins found to be reduced by thioredoxin thus range from different types of seed storage proteins to enzymes

and enzyme inhibitors. Reduction of these target proteins is accompanied by either a loss (enzyme inhibitors) or a gain of biochemical activity (enzymes) as well as by an increase in susceptibility to heat and proteolysis (all proteins tested).

Function

Germination and post-germination events constitute critical transitions in the life cycle of higher plants. During this period, dramatic physiological changes occur in concert with establishment of the basic architecture of the mature plant from the pattern formed during embryogenesis. Starch and protein reserves are mobilized in the endosperm of germinating seed to provide carbon and nitrogen for the seedling prior to the initiation of photosynthesis. Several lines of evidence support the conclusion that thioredoxin promotes the mobilization of carbon and nitrogen in the early period of seedling development during germination.

- The major representatives of the storage proteins in wheat—gliadins and glutenins—are specifically reduced by thioredoxin (Kobrehel et al., 1992; Wong et al., 1993). Reduction of the gliadins and glutenins in vivo has been found to take place early, reaching a peak two to three days after imbibition (Kobrehel et al., 1992). Parallel experiments show that the endosperm fraction contains the enzyme necessary to reduce NADP by the oxidative pentose phosphate pathway (hexokinase, glucose-6 phosphate dehydrogenase, 6-phosphogluconate dehydrogenase). The NADPH so formed can reduce indigenous thioredoxin *h* owing to the presence of NTR in the endosperm (Lozano et al., 1996).
- Thioredoxin *h* is converted from an oxidized to a partially reduced state one day after imbibition. Although the level of thioredoxin later declined, the available reducing equivalents of thioredoxin increased in accord with its function in the reduction of storage proteins. In addition, the abundance of thioredoxin *h* in wheat endosperm appeared to be controlled by the embryo via the hormones gibberellic and abscisic acid (Lozano et al., 1996; Wong et al., 1996).
- The new type of serine protease, thiocalsin, dependent both on thioredoxin and calcium, catalyzes the degradation of the reduced forms of gliadins and glutenins (Besse et al., 1996).
- Thioredoxin reduces several inhibitors of enzymes participating in the mobilization of carbon. Among them is the α-amylase/subtilisin inhibitor of barley, which, once reduced, loses its ability to inhibit indigenous type II α-amylase and becomes more susceptible to proteases (Jiao et al., 1993), and the related specific inhibitors, which, after reduction by thioredoxin, lose their ability to inhibit limit-dextrinase (Wong et al., 1996).

In conclusion, an extensive body of evidence suggests that thioredoxin plays a central role in the coordination of multiple regulatory steps during the germination of cereals. In this way the stored starch and proteins are stabilized until they are needed to provide the growing seedling with sufficient nitrogen and carbon.

Certain plants have the ability to recognize and reject self-pollen, thereby preventing self-fertilization. In *Phalaris coerulescens*, gametophytic self-incompatibility is controlled by two unlinked genes, S and Z (Li et al., 1994). Two S alleles encode proteins that are highly conserved at the C-terminal region and show significant homology to thioredoxin *h*. These domains have apparent biochemical properties in common with thioredoxin, including serving as a substrate for *E. coli* thioredoxin reductase, and as an effective protein disulfide reductase (Li et al., 1995, 1996). Furthermore, the estimation of the positions and the sequences of the introns within the genes confirm that, although having diverged in their amino acid sequences, the *Phalaris* protein domains most probably share a common ancestor with cytosolic thioredoxin *h* (Sahrawy et al., 1996). The results suggest that self-incompatibility in *Phalaris* consists of a complex series of post-translational modifications triggered by the S-protein, such as folding and disulfide bond formation or other thioredoxin related functions.

In *Arabidopsis thaliana*, a *cis*-acting promoter element named *tef-1* box has been implicated in the transcriptional activation of a class of genes related to cell division (Curie et al., 1991). In addition, *tef-1* boxes were found within the promoters of genes encoding for the tobacco thioredoxin *h*2 or the rice thioredoxin *h* as well as other plant genes involved in redox processes or protection against oxidative damage, such as glutathione S-transferase (Regad et al., 1995). It is suggested that a *tef-1* dependent regulation of genes encoding proteins involved in the redox control would occur in cells at the transition period from quiescence to growth. This study is of particular interest as it provides the first evidence for a potential regulatory role for thioredoxin in plant cell division.

New Technologies

An extension of the studies showing its effectiveness in reducing disulfide bonds of cereal proteins has led to the application of thioredoxin to long-standing practical problems. The results obtained so far are promising.

First, when added as a supplement to wheat flour of poor cooking quality, thioredoxin improves dough strength and bread quality (Wong et al., 1993; Kobrehel et al., 1994). The results suggest that thioredoxin selectively reduces intramolecular disulfide bonds of flour proteins (gliadins and glutenins) which, through a series of sulfhydryl/disulfide exchange reactions, result in the formation of new intermolecular disulfide bonds. In this manner, thioredoxin appears to promote the formation of a protein network that improves the final baked product. In parallel studies, thioredoxin has also been shown to promote the formation of a dough-like product when applied to flour of nonglutenous cereals such as rice, maize, and sorghum (Kobrehel et al., 1994). Finally, the application of thioredoxin to foods, such as wheat, decreases their allergenicity by mitigating the IgE-mediated type I hyper-

sensitivity response in sensitized dogs (Buchanan et al., 1997). The results suggest that thioredoxin, reduced by NADPH and NTR, may be useful for the production of new and improved foods. Included would be foods with lowered allergenicity (wheat) and enhanced nutritional value (soy).

Animal Thioredoxin

Animal cells contain a classical thioredoxin system including a single type of thioredoxin and a thioredoxin reductase which uses NADPH as an electron donor. Several primary structures of animal thioredoxins have been determined, including representatives from rat liver (Luthman and Holmgren, 1982), chicken (Jones and Luk, 1988), rabbit bone marrow (Johnson et al., 1988), calf thymus (Eklund et al., 1991), mouse (Matsui et al., 1995) and humans (Wollman et al., 1988; Tonissen and Wells, 1991; Kaghad et al., 1994). In addition, a number of mammalian proteins of high molecular weight have been found to contain thioredoxin domains. For example, protein disulfide isomerase (PDI) contains two regions that exhibit internal sequence homology to thioredoxin (Edman et al., 1985). PDI is a substrate for thioredoxin reductase, indicating that the domains are folded and recognized as thioredoxins (Lundstrom and Holmgren, 1990). Similar structures have been reported for proteins isolated from the endoplasmic reticulum on the basis of their calcium binding and stress response properties (Fullekrug et al., 1994; Lundstrom-Ljung et al., 1995) and for phospholipase C enzymes (Bennett et al., 1988).

Although mammalian thioredoxins appear to target proteins and display functions that differ from one tissue to another, most, if not all, are involved in co- and/or post-translational processes. This prominent feature suggests that thioredoxin targets proteins of general importance to cell growth and multiplication.

Occurrence

Thioredoxin can act as a radical scavenger. Under oxidative treatments known to generate reactive oxygen intermediates intracellularly (UV, X-ray irradiation or exposure to hydrogen peroxide), thioredoxin is effective in facilitating the regeneration of oxidatively damaged proteins (Schallreuter and Wood, 1986; Fernando et al., 1992). A series of deletion analyses show that thioredoxin expression is enhanced through a *cis*-acting promoter element responsive for the oxidative stress (Tanigushi et al., 1996). Recently, thioredoxin has been localized in the pregnant human uterus where it may protect the fertilized egg and placental trophoblasts from the cytotoxic effects of oxygen radicals (Kobayashi et al., 1995). The effectiveness of thioredoxin as a radical scavenger has also been recognized through its ability to lower reperfusion injury in animal lung transplantation model in vivo (Yagi et al., 1994; Yokomise et al., 1994; Fukuse et al., 1995). In a related role, thioredoxin is believed to be responsible for the development of cellular resistance to anticancer chemotherapeutic drugs like *cis*-diamminedichloroplatinum II (CDDP), possibly by scavenging intracellular toxic oxidants generated by this anticancer agent (Sasada et al., 1996). Recent studies suggest that thioltransferase (also known as glutaredoxin), together with glutathione, glutathione reductase and NADPH, also repair oxidatively damaged proteins (Yoshitake et al., 1994). The evidence indicates that thioredoxin and glutaredoxin show different specificity in their interactions with protein substrates. It will be interesting to determine the relative importance of the two proteins in radical scavenging in different animal tissues. According to present evidence, it may depend on the mechanisms by which the -SH groups are oxidized.

Thioredoxin can also be secreted and taken up rapidly by various cells, suggesting a putative function in intercellular signaling. Indeed, the protein has been purified from supernatants of T- and B-cell lines following infection with human T-lymphotropic virus type I (HTLV-I) and Epstein-Barr virus (EBV), respectively. Thioredoxin has also been found to act as an autocrine growth factor for both types of cells (Tagaya et al., 1989; Wakasugi et al., 1990). Furthermore, the expression of thioredoxin and its reducing activity have been shown to be dependent on the cell cycle of these virally transformed cells (Ueda-Tanigushi et al., 1995).

Clarke et al. (1991) have described an early pregnancy factor (EPF) which has the same sequence as human thioredoxin at the cDNA level. Similarly, a thioredoxin homolog in *Drosophila* has been ascribed a function in female meiosis and early embryonic development (Salz et al., 1994). Moreover, a targeted disruption of the mouse thioredoxin single gene is lethal for the embryo (Matsui et al., 1996). It is worth noting that the homozygous mouse embryos die immediately after implantation. It is suggested that such early lethalities may be caused by impaired DNA replication resulting from thioredoxin depletion. This finding together with earlier work in yeast (Muller, 1995) and *Xenopus* (Hartman et al., 1993) strongly suggests that thioredoxin plays an as yet undefined role in DNA replication.

Proteins Targeted

In mammalian cells, thioredoxin can serve as a substrate for several enzymes, including methionine sulfoxide reductase (Nagamine et al., 1992), protein disulfide isomerase (Lundstrom and Holmgren, 1990), a novel type of peroxide reductase (Chae et al., 1994) and, as noted above, ribonucleotide reductase (although here glutaredoxin appears to be the physiological cofactor) (Holmgren, 1981). In addition to a substrate function, thioredoxin reductively regulates different processes involving protein/receptor interaction, toxin inactivation or DNA binding activity of transcription factors. Thioredoxin is an activating factor for the cytosolic glucocorticoid receptor (Grippo et al., 1983) and the extracellular domain of the interferon-γ receptor (Fountoulakis, 1992). It can also specifically reduce the neurotoxins of different venoms (snake, scorpion

and bee) and the principal allergen of bee venom, a non-neurotoxic phospholipase A (Lozano et al., 1994).

Gene expression is regulated by *cis* elements which in turn, are recognized by transcription factors whose activity is modulated by incoming signals. Two immunologically important transcription factors, NF-κB and activator protein-1 (AP-1), are both activated by redox-dependent processes (Meyer et al., 1993; Schenk et al., 1994). The redox regulation of NF-κB has received increased attention because of its importance in the activation of gene required for the immune response and for the replication of the human immunodeficiency virus (HIV) (Droge et al., 1992). In the cytosol, NF-κB is in an inactive form and cannot bind DNA owing to its own binding to an inhibitory molecule, IκB (Baeuerle and Baltimore, 1988). Activation of cells with appropriate stimuli results in dissociation of NF-κB from IκB and translocation to the nucleus (Miyamoto and Verma, 1995). According to current evidence, thioredoxin seems to play two different roles in the redox control of the NF-κB/DNA interaction, one in the cytosol and the other in the nucleus. According to one group, thioredoxin appears to inhibit the release of IκB as one of the regulatory steps of NF-κB activation, via a pathway involving a reactive oxygen intermediate (Schenk et al., 1994). Then, free NF-κB would act in the nucleus to bind DNA. According to a second group, the main control exerted by thioredoxin is located inside the nucleus (Hayashi et al., 1993). In vitro observations suggest that the free NF-κB requires further reduction to acquire its full DNA binding activity. Among the reductants tested, either physiological or artificial, thioredoxin is the most effective in stimulating the DNA binding activity of NF-κB, which, in turn, increases the expression of NF-κB regulated-genes such as those involved in HIV replication (Matthews et al., 1992). The resolution of the contradictory views of these two groups awaits further work.

The transcription factor AP-1, another broad mediator of immediate early gene expression, couples extracellular signals to gene-activating events associated with growth, differentiation, and cellular stress (Angel and Karin, 1991). An important mediator of tumor proliferation, AP-1 is composed of the *jun* and *fos* gene products, which form homodimeric (*jun*/*jun*) or heterodimeric (*jun*/*fos*) complexes (Abate et al., 1990). The activity of AP-1 is regulated by complex mechanisms consisting of posttranslational events centering on pre-existing AP-1 molecules and on transcriptional activation resulting in increased amounts of AP-1 binding proteins (Xanthoudakis et al., 1992). In contrast to NK-κB, AP-1 activity is markedly increased upon transient expression or addition of thioredoxin (Schenk et al., 1994), suggesting that redox modification in the DNA-binding domain of *fos* and *jun* may be another mechanism of controlling DNA binding of AP-1. As seen for NF-κB, a number of potential targets have been identified which may be involved in the activation of AP-1 by thioredoxin. Similarly, an increasing number of studies show that other types of transcription factors are also under redox control by thioredoxin (Kambe et al., 1996; Wu et al., 1996), including ones responsible for the stimulation of expression of cytokine genes (Schenk et al., 1996).

Function

During the last five years, numerous lines of evidence point to an autoregulatory role for thioredoxin in cell proliferation. A factor originally named adult T-cell leukemia derived factor (ADF) (Tagaya et al., 1989), and later shown to be identical to thioredoxin (Gasdaska et al., 1994), is constitutively produced and released as an autocrine growth factor by human lymphoid cell lines transformed by HTLV-I. HTLV-I is a retrovirus, similar in some ways to HIV, that causes a peculiar form of acute lymphoblastic leukemia. Transformation of cells by the herpes EBV, closely associated with Burkitt's lymphoma in Africa and nasopharyngeal carcinoma in Southeast Asia, results in similar changes (Wakasugi et al., 1990; Yodoi and Tursz, 1991). Thioredoxin is reported to have growth promoting activity on the HTLV-I infected T-cell line ATL-2 and the EBV infected B-cell line 3B6. It also induces the expression of growth factor receptors (such as interleukin-2, IL-2R) and synergizes activity with suboptimal amounts of several lymphokines. It is suggested therefore, that thioredoxin may act as a competence factor to "sensitize" a cell and make it responsive to minimal amounts of a series of growth factors at certain steps of the virus transformation process. Thioredoxin thus has co-cytokine activity in enhancing growth of transformed lymphocytes and the expression of IL-2R (Tagaya et al., 1989). This effect is dependent on its disulfide reducing activity (Yamauchi et al., 1992). The use of immunosuppressants demonstrates that thioredoxin induction in T cell activation depends on calcineurin-related events in the early phase (Furuke et al., 1995).

The importance of thioredoxin in the immune system is indicated by studies revealing that it has cytokine-like activity in a number of systems (Schenk et al., 1996). Thioredoxin can promote B cell differentiation (Rosen et al., 1986; Rosen et al., 1995). Endogenous thioredoxin expression is induced in both normal B lymphocytes and in leukemic B-CLL cells (chronic lymphocyte leukemia) after growth stimulation. These results suggest that induction of thioredoxin synthesis and secretion may be a normal stimulus of B lymphocyte activation and not necessarily linked to cellular malignancy (Ericson et al., 1992). This is a strong argument in favor of a *de novo* synthesis of thioredoxin for secretion during cell proliferation as opposed to a mobilization of an intracellular form (Rubartelli et al., 1992).

The biochemical mechanism by which thioredoxin affects the growth of B cells, however remains obscure. Recent data have shown that thioredoxin is able to induce critical early signals essential for the initiation of cell division. As an example, thioredoxin efficiently increases intracellular calcium and activates protein kinase C (PKC) through its translocation to the membrane (Biguet et al., 1994).

While several mechanisms have been proposed, key questions remain unanswered regarding the mode of action of thioredoxin in cell division. Thioredoxin may facilitate protein-nucleotide interactions as suggested by gel retardation experiments showing that thioredoxin enhances the binding of NF-κB to the target sequence in IL-2Rα chain promoter (Okamoto et al., 1992). Through a chaperone-like activity, thioredoxin may also be involved in the translocation of molecules from the cytosol to the nucleus (Yodoi and Uchiyama, 1992). The identification of the exact cellular targets of extracellular thioredoxin, however, awaits future investigation.

Relation to Human Disorders

One of the most exciting developments in the past five years has been the demonstration that thioredoxin controls central functions in the body defense system. There is, as mentioned above, evidence that thioredoxin functions in the activation, differentiation, and proliferation of lymphocytes. Independent experiments suggest a role in enhancing the non-specific defense system, through increasing the migration and cytotoxicity of eosinophils (Balcewicz-Sablinka et al., 1991; Hori et al., 1993). The latter is apparently accomplished via a thioredoxin-dependent induction of the release of the major basic proteins (MBP) responsible for phagocytic activity of mature eosinophils (Koyanagi et al., 1995). It is worth noting that the disulfide bond seems important in both the stimulation (e. g., food allergens) and response of the immune system (e. g., B cell production). Through its ability to reduce disulfide bonds of this nature, thioredoxin can intervene at both ends.

It is well known that radiation of cells or tissues with UV (especially UVB) generates intracellular reactive oxygen intermediates (ROI) which subsequently induce various cytotoxic effects, including DNA damage and immune suppression. Thioredoxin secretion is enhanced in lymphoid cells as well as epidermal cells in sun-exposed skin (Wakita et al., 1992; Sachi et al., 1995). The results suggest that thioredoxin is induced by oxidative reactions and acts as an anti-stress protein to repair cellular damage. Recent results thus raise the possibility that oxidation of thioredoxin could, at least in part, be responsible for the severe damage that radiation has long been known to have on the immune system.

By using immunohistologic analyses, Saito et al. (1996) have shown that thioredoxin expression is also induced in B cells and epithelial cells of the salivary glands of patients with Sjögren's syndrome. This condition is an autoimmune disease in which both T and B cells progressively destroy the salivary and lacrimal glands by infiltration, thereby leading to dryness of the mouth and the eyes.

Similarly, the expression of thioredoxin mRNA and secretion of the protein are elevated in cancerous cells of the lung (Gasdaska et al., 1994), the liver (Rubartelli et al., 1995), the breast (Gasdaska et al., 1995) as well as in those tumor cells that have been analyzed. The changes in thioredoxin observed in these conditions complement the earlier work on HTLV-I and the Epstein-Barr virus described above.

There remains the interesting question of the relation of thioredoxin to AIDS. Nakamura et al. (1996) have shown that certain HIV-infected patients display elevated levels of plasma thioredoxin at a late stage of the disease. Earlier studies, however, contradict these results in demonstrating a down-regulation of thioredoxin production in HIV-infection (Masutani et al., 1992). While a resolution of these differences awaits further work, one can explain the discrepancy by proposing that thioredoxin levels are related to the intensity or state of progression of the disease. One report possibly supports this hypothesis through demonstrating that HIV replication can be strongly suppressed by micromolar concentrations of thioredoxin (Newman et al., 1994).

A growing body of evidence has related a number of human disorders to a change in the level of thioredoxin or its mRNA. This work not only highlights the importance of thioredoxin in health maintenance but also raises the possibility that thioredoxin may be useful for diagnosing and monitoring the progression of diseases.

Summary and Perspectives

Although thioredoxin was considered an enzyme substrate for about fifteen years after its discovery, research in the ensuing two decades has revealed that its primary

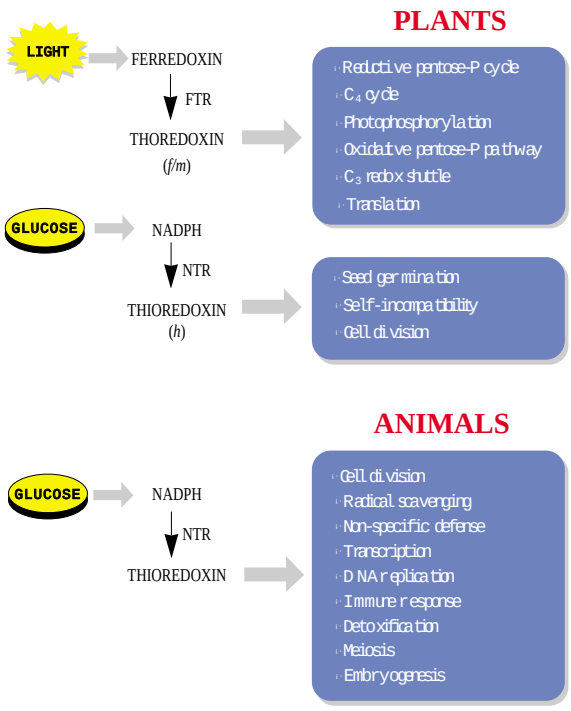

Figure 1. Plant and animal processes regulated by thioredoxin. The individual processes are presented, from top to bottom in each box, in the order in which they were first shown to be regulated by thioredoxin.

function lies in regulation. First demonstrated for enzymes of photosynthesis, the regulatory role of thioredoxin now extends to a range of other processes in plant and animal cells. Seed germination, self-incompatibility, cell division and translation have recently been found to be under thioredoxin control in plants. For animals, there is evidence that thioredoxin regulates an increasing number of processes: cell division, radical scavenging, non-specific defense, transcription, DNA replication, immune response, detoxification, meiosis, and embryogenesis. In fulfilling these functions, thioredoxin has emerged as a regulatory protein of fundamental importance to plants as well as animals (Figure 1). One of the exciting questions that remains in many cases is the physiological basis for thioredoxin-linked redox regulation. While clear for certain processes (photosynthesis and related reactions, seed germination, radical scavenging), the link between thioredoxin and a redox signal is not obvious for others. It may be that a driving force in certain reactions is the level of thioredoxin that may change with the needs of the cell.

Extensions of the biochemical and molecular studies have led to the potential application of thioredoxin in technology and medicine. Work currently in progress indicates that thioredoxin may be used to improve foods through, among other changes, lowering allergenicity and increasing digestibility. Ongoing studies have uncovered the potential use of thioredoxin as a marker in diagnosing and monitoring human diseases (Sjögren's syndrome, certain malignancies and viral infections), in neutralizing venom neurotoxins, and in improving the success of organ transplants. When viewing the evidence as a whole, it seems that we stand on the threshold of advances in our understanding of thioredoxin that could improve the lives of all of us.

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