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Review article

Nuclear thiol redox systems in plants

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ABSTRACT

Thiol-disulfide redox regulation is essential for many cellular functions in plants. It has major roles in defense mechanisms, maintains the redox status of the cell and plays structural, with regulatory roles for many proteins. Although thiol-based redox regulation has been extensively studied in subcellular organelles such as chloroplasts, it has been much less studied in the nucleus. Thiol-disulfide redox regulation is dependent on the conserved redox proteins, glutathione/glutaredoxin (GRX) and thioredoxin (TRX) systems. We first focus on the functions of glutathione in the nucleus and discuss recent data concerning accumulation of glutathione in the nucleus. We also provide evidence that glutathione reduction is potentially active in the nucleus. Recent data suggests that the nucleus is enriched in specific GRX and TRX isoforms. We discuss the biochemical and molecular characteristics of these isoforms and focus on genetic evidences for their potential nuclear functions. Finally, we make an overview of the different thiol-based redox regulated proteins in the nucleus. These proteins are involved in various pathways including transcriptional regulation, metabolism and signaling.

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1. Introduction

Aerobic organisms are continuously exposed to oxygen which renders them prone to damage generated by oxygen-derived free radicals. Oxidative stress is mediated by the reactive oxygen species (ROS), including the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\bullet}$) and reactive nitrogen species (RNS) which include the free radical nitric oxide (NO) and peroxynitrite (ONOO), a product of the reaction between NO and $O_2^{\bullet -}$ [1,2]. Generation of ROS in non-stressed cells is principally restricted to organelles such as chloroplasts, mitochondria and peroxisomes in which they are produced as by-products of electron chains and metabolic reactions. ROS are also produced by NADPH oxidases, peroxidases and amine oxidases at the plasma membrane, cell wall or the apoplast as well as by FAD-containing enzymes of the oxidative protein folding machinery in the secretory pathway [3,4]. Exposure to various environmental factors is also leading to oxidative stress which can cause oxidative damage to proteins, DNA, and lipids [2]. To scavenge excessive ROS/RNS, plants exhibit a large battery of enzymes like peroxidases, catalases, superoxide dismutases and of antioxidant molecules like ascorbate, glutathione, tocopherol, carotenoides. ROS and RNS act also as signaling molecules in important physiological processes. In plants, they are involved in developmental programs like root development, stomatal closure or programmed cell death. They are also key signaling molecules for the responses to abiotic and biotic stresses, activating transcriptional, post-transcriptional and post-translational responses [5,6].

The nuclear compartment that orchestrates genetic programs of cell life is particularly sensitive to the deleterious effects of oxidation. ROS, and particularly H_2O_2, probably diffuse through membranes and invade neighboring compartments, including the nucleus [6]. Experiments performed with isolated tobacco BY-2 nuclei also suggest that plant nuclei are an active source of production of ROS, in particular of H_2O_2 [7].

Increasing evidence has highlighted the role of redox signaling in the regulation of many nuclear proteins like transcription factors, kinases and chromatin-modifying enzymes [8]. The redox regulation generally occurs through modification of the redox state of thiol residues. Solvant exposed thiols are prone to oxidation especially in a basic environment since thiol deprotonation leads to formation of a thiolate residue (R-S-), a nucleophilic residue sensitive to oxidation. In the presence of ROS, such as H_2O_2, oxidation leads to successive oxidation to sulfenic (R-SOH), sulfinic ($R\text{-}SO_2H$) and sulfonic ($R\text{-}SO_2H$) acids [9]. Thiol groups can also be oxidized by reactive nitrogen species (RNS) or oxidized glutathione (GSSG) resulting in *S*-nitrosylation (R-SNO) or *S*-glutathionylation (R-S-SG) [10,11,12]. Two closely situated cysteine residues can also get oxidized to form a disulfide bridge (S—S). All these modifications can alter the structure and/or the activity of many proteins in all the cellular compartments, including the nucleus.

Thiol are reduced by two major redox systems, glutathione/glutaredoxin (GRX) and thioredoxin (TRX), which have been extensively studied in plants [13–15]. Glutathione, a thiol-containing tripeptide, exists in a reduced (GSH) or an oxidized form (GSSG). It has a major role as a redox buffer. The dynamics of its metabolism and compartmentation is the subject of many studies. Still, its functions in the nuclear compartment are poorly understood [16,17]. Oxidized glutathione is reduced by glutathione reductases in mitochondria, chloroplasts, the cytosol and peroxisomes in plants [18–20]. Glutathione also serves as a powerful electron donor for many enzyme activities, including glutaredoxins, for which different functions in the nucleus have been recently identified (see below).

The second major thiol reduction system is based on thioredoxins, which are reduced by NADPH-dependent thioredoxin reductase or by a ferredoxin-dependent thioredoxin reductase in chloroplasts [15]. A functional thioredoxin system in the plant nucleus has been described [21,22]. Various biochemical and proteomic approaches have been developed to identify target proteins of glutaredoxin and thioredoxins (reviewed in [23]). Some of the 400 potential targets of thioredoxins and glutaredoxins are nuclear proteins. Only a few of these candidate proteins have been experimentally validated. We review the data currently available on major nuclear thiol reduction systems in plants and their associated functions.

2. Nuclear glutaredoxin system

2.1. Glutathione homeostasis in the nucleus

Glutathione (γ-glutamyl-cysteinyl-glycine) is the most abundant low-molecular weight thiol of the cell. It is widely distributed in all subcellular compartments with concentrations in the low millimolar range (1–10 mM) [24]. The detection of glutathione at the subcellular level is technically challenging. Biochemical measurements after organelle isolation or non-aqueous fractionation can lead to contamination among fractions. Microscopic methods using fluorescent dyes often suffer from the inability of the stain to equally access all cellular compartments (*e.g.*, [25]), as well as from insufficient retention of the dyes in the respective compartments. This is most evident from vacuolar sequestration of fluorescent glutathione conjugates, which additionally makes quantitative analysis rather challenging [26]. An immunocytochemistry approach coupled to high resolution electron microscopy has recently been established that enables simultaneous detection of ascorbate and glutathione in all cell compartments [27,28]. The major limitation of such techniques is that they are not able to measure the redox state of the respective redox couples. Genetically encoded probes such as the reduction–oxidation-sensitive green fluorescent proteins (roGFPs) have the promise of overcoming these limitations. They have been extensively used to measure the standard redox potential of glutathione (E_{GSH}) in different cellular compartments, including the nucleus, in wild type and different mutant backgrounds as well as under non-stress and stress conditions [29–33]. Glutathione biosynthesis is restricted to cytosol and plastids. All other cellular compartments import glutathione from the cytosol, either by free diffusion, or by an uptake system. An ATP-dependent transport exports GSSG from the cytosol to the tonoplast in plants and yeast [34,35] and from plant mitochondria [36]. The only GSH-transporters identified in *Arabidopsis* so far are members of the CLT-protein family located on the plastid envelope, where they mediate bidirectional transport of the GSH precursor gamma-glutamyl–cysteine (γ-EC) and GSH between the plastid stroma and the cytosol [32–37]. The multidrug resistance-associated protein (MRP) family, now denoted subclass C of the ATP-binding cassette (ABC) translocators are involved in the sequestration of glutathione conjugates in the vacuole but there is no evidence that they also transport GSH itself [38] . Nuclear pores are generally assumed to allow for unrestricted bidirectional diffusion of GSH across the nuclear envelope, in contrast to plastids and mitochondria (Fig. 3). GSH gradients between the nucleus and the cytosol have been reported during the cell cycle progression by using the thiol-labelling dye 5-chloromethylfluorescein diacetate (CMFDA). A massive glutathione oxidation has been observed early in the G1 phase of the cell cycle in both mammalian and plant cells, which appears to have a critical regulatory step in the progression to the S-phase [39,40] . GSH is then assumed to be recruited into the nucleus from the cytoplasm where it accumulates and co-localizes with nuclear DNA. This led to the development of a model for a redox