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Review

Nuclear glutathione ☆

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ABSTRACT

Glutathione (GSH) is a linchpin of cellular defences in plants and animals with physiologically-important roles in the protection of cells from biotic and abiotic stresses. Moreover, glutathione participates in numerous metabolic and cell signalling processes including protein synthesis and amino acid transport, DNA repair and the control of cell division and cell suicide programmes. While it is has long been appreciated that cellular glutathione homeostasis is regulated by factors such as synthesis, degradation, transport, and redox turnover, relatively little attention has been paid to the influence of the intracellular partitioning on glutathione and its implications for the regulation of cell functions and signalling. We focus here on the functions of glutathione in the nucleus, particularly in relation to physiological processes such as the cell cycle and cell death. The sequestration of GSH in the nucleus of proliferating animal and plant cells suggests that common redox mechanisms exist for DNA regulation in G1 and mitosis in all eukaryotes. We propose that glutathione acts as "redox sensor" at the onset of DNA synthesis with roles in maintaining the nuclear architecture by providing the appropriate redox environment for the DNA replication and safeguarding DNA integrity. In addition, nuclear GSH may be involved in epigenetic phenomena and in the control of nuclear protein degradation by nuclear proteasome. Moreover, by increasing the nuclear GSH pool and reducing disulfide bonds on nuclear proteins at the onset of cell proliferation, an appropriate redox environment is generated for the stimulation of chromatin decompaction. This article is part of a Special Issue entitled Cellular functions of glutathione.

1. Introduction. Metabolism and changes in nuclear glutathione in different cell models

Glutathione is an essential metabolite in plants and animals. For example, glutathione depletion in knockout mutants of the model plant species *Arabidopsis thaliana* that lack the first enzyme of GSH synthesis causes embryo lethality [1]. Similarly, knockout mutants that are defective in the second enzyme of the GSH synthesis pathway have a seedling-lethal phenotype [2]. The absolute requirement for glutathione is not surprising given that this redox metabolite is at the heart of the cellular antioxidant network with multiple functions in metabolism and redox signaling [3]. Although many glutathione-dependent reactions are involved in oxidative stress-mediated cellular processes, the simple concept of glutathione as a ROS-scavenging antioxidant is inaccurate and misleading. Increasing evidence suggests key roles for glutathione-mediated redox signaling pathways, particularly through components such as glutaredoxins (GRX), glutathionylation and nitrosoglutathione (GSNO) [4–7]. Moreover, through GS-conjugate formation and the synthesis of *S*-containing and secondary metabolites, glutathione regulates the biological activity of metabolic intermediates and active products. In many cases however the precise roles of different glutathione-dependent enzymes in cell signaling remain to be fully characterized, as does the interplay between thioredoxin (TRX) and glutathione/GRX systems. In particular, the precise functions of the nuclear glutathione pool are poorly characterized and largely unexplored.

Until recently, the role of glutathione in the cell nucleus was not thought to be important because of the traditionally held concept that glutathione can diffuse freely between the nucleus and cytoplasm through the nuclear pores. This view had arisen largely because of a lack of effective techniques and methods for the accurate determination of the pool of glutathione in the nucleus. For example, many early reports concerning the nuclear glutathione pool were based largely on cell-fractionation techniques that had proved to be useful for studies on mitochondria. A comprehensive review by Söderdahl et al. [8] suggested that it was impossible to determine the nuclear concentration of glutathione using standard cell fractionation techniques or similar analytical methods. Thus, the value of these approaches in the analysis of the nuclear glutathione pool and

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that of other cellular compartments such as the endoplasmic reticulum has always been debatable. It wasn't until the development of improved *in vivo* methods such as redox-sensitive GFP and YFP sensors that it was possible to monitor the dynamic pools of nuclear and cytosolic glutathione, together with accompanying redox changes. Recent studies have revealed that under specific circumstances such as when mammalian, plant or yeast cells are in the active phases of cell proliferation, the nucleus accumulates GSH to much greater concentrations than those present in the cytoplasm [9–11]. Moreover, a recent analysis in yeast has shown that the redox states of the nucleus and cytosol are largely independent and subject subtle distinct patterns of redox change that reflect both active and passive mechanisms of regulation [12].

We consider that the accurate measurement of the redox state of thiols and disulfides in organisms, cells and tissue extracts is possible and accurate methodologies are available. However, the analysis of redox potentials within individual subcellular compartments is more difficult and available methods are more subject to error, particularly because of the length and complexity of the extraction and purification procedures required to obtain subcellular fractions. This is especially true for nuclear fractions. The use of fluorescent proteins can partially overcome the problem, as discussed previously by Jones and Go [13]. The measurement of the NAD/NADH or NADP/NADPH ratios in nuclei from isolated cells is not possible using most methodologies that currently available. Unfortunately, non-aqueous fractionation procedures that can overcome some of these problems by limiting the diffusion of metabolites from organelles during isolation are rarely used for such studies.

A further contributing factor underpinning earlier conflicting views that are prevalent in the literature on nuclear glutathione is that most, if not all, of the reports were based on the widely held concept that the distribution of GSH between the cytosol and nucleus was not controlled. This situation arose largely because in most cases, cells have been studied under steady state conditions *i.e.* when they are confluent at the G_0/G_1 phase of the cell cycle. A large body of evidence supports a key role for GSH in cell proliferation in animals and plants. Here, we discuss recent advances in the field and consider how alterations in nuclear glutathione status, such as those observed during cell proliferation may participate in genetic and epigenetic regulation. We highlight some of the issues surrounding the recruitment of GSH into the nucleus, the regulation of nuclear glutathione contents, and how the functions of nuclear glutathione and other thiols are integrated to fine-tune nuclear processes and gene expression through appropriate modification of sensitive protein cysteine residues and epigenetic events.

1.1. Synthesis

Reduced glutathione (GSH) is the major non-protein thiol in animal and plant cells. It is synthesized from glutamate, cysteine, and glycine by two ATP-dependent enzymes in a metabolic pathway that is similar in all organisms [14]. The production of γ-glutamylcysteine (γ-EC) in first step of the pathway, which is catalyzed by glutamate cysteine ligase (GCL; also known as γ-EC synthetase) is considered to be rate-limiting because this intermediate is present at very low concentrations [15].

While GCL is a cytosolic enzyme in animal cells, the enzyme which is encoded by the *GSH1* gene is restricted to plastids in plants such as *Arabidopsis thaliana* [16]. Moreover, while the animal GCL is composed of catalytic (GCLC) and modifier (GCLM) subunits only the catalytic protein has been found to date in plants. The second step of GSH synthesis is catalyzed by GSH synthetase (GS, also known as GSH synthetase). In animals, this enzyme is located in the cytosol but in plants it is found in both the plastids and cytosol [16]. Thus, in plants the first step of GSH synthesis is located in plastids (mainly chloroplasts) and the second step occurs in both plastids and the cytosol. The export of γ-EC across the chloroplast envelope is facilitated by a family of three plastid thiol transporters that are homologous to the *Plasmodium falciparum* chloroquine-resistance transporter, PfCRT [17].

The availability of cysteine and GCL activity are considered to be the most important factors regulating the rate of glutathione synthesis, [18]. Other factors such as glycine and ATP availability may also affect GSH contents under certain conditions [19–21]. A number of studies have convincingly shown that the glutathione pool in plant tissues can be increased by overexpression of the biosynthetic enzymes or the enzymes involved in cysteine synthesis [15,22–27]. Transcriptional and post-transcriptional controls regulate GCL activity. Of these, post-transcriptional controls may be the most important in plants as relatively few conditions have been shown to cause marked increases in *GSH1* or *GSH2* transcripts [28,29]. It has long been recognized that feedback control of GCL activity by GSH could make an important contribution to the overall regulation of glutathione homeostasis [30]. The plant GCL protein forms a homodimer linked by two disulfide bonds [31], one of which is involved in redox regulation and the up-regulation of GSH synthesis in response to oxidative stress [32,33]. Alleviation of feedback inhibition is likely to be an important mechanism driving accelerated rates of GSH synthesis under conditions in which glutathione is being consumed or when it is recruited into the nucleus. Evidence for the overall importance of these mechanisms in the regulation of glutathione accumulation in plants is provided by studies involving ectopic expression of a bifunctional GCL/GSH-S enzyme from *Streptococcus thermophilus*, which is neither redox regulated or sensitive to feedback inhibition [34]. The leaves of the transgenic tobacco plants showed dramatic increases in GSH accumulation (up to 30-fold increases) without any adverse affects [34]. While higher glutathione accumulation has clear benefits in terms of enhanced abiotic stress resistance [34,35], the dynamic changes that occur in nuclear/cytosol GSH distribution early in the cell cycle in both mammalian and plant cells are likely to impair stress resistance responses [9,11].

1.2. In vivo measurements of glutathione in the nucleus

GSH is differentially distributed between the various subcellular compartments of cytosol, mitochondria, endoplasmic reticulum, and nucleus in plant and animal cells, giving rise to distinct redox pools. Under most circumstances the cytosolic pool is considered to account for over 70% of the total cellular glutathione, while the nuclear and cytoplasmic (e.g. mitochondrial compartments) account for the remaining 30% [36,37]. The cytosolic GSH pool, which is traditionally considered to be the most abundant and the easiest to measure, is highly reduced. Under physiological conditions cytosolic GSH concentrations are generally considered to be in the range of 1 to 11 mM, levels that are far in excess of free cysteine. A key characteristic of the glutathione pool is its high reduction state. GSH is continuously oxidized to a disulfide form (GSSG) but this is rapidly recycled to GSH by NADPH-dependent glutathione reductases (GR) in key organelles and the cytosol such that the glutathione pool is largely reduced with little GSSG being present. In the absence of stress, tissues such as leaves typically maintain measurable GSH: GSSG ratios of 20:1 to 10:1 [38]. *In vivo* determinations using ro-GFP fluorescence methods (illustrated in Fig. 1) suggest that ratio may be even higher in cytosol [39,40].

The glutathione content of tissues is generally determined in a spectrophotometer using 5′5′-dithiobis-(2-nitrobenzoic acid; DTNB otherwise known as Ellman's reagent) or using HPLC techniques based on fluorescent labeling, as discussed by Noctor et al. [41]. These assay methods provide a useful gross estimate of tissue GSH contents. The spectrophotometric method measures all DTNB-reactive thiols present in tissue extracts but it does not detect GSSG. If GR and NADPH are included in the reaction mixture, then the technique can be used to provide a much more sensitive and specific assay system for glutathione [41]. Tissue GSH contents can be also analyzed in enzyme-catalyzed procedures that incorporate glutathione-S-transferases such as the diazo coupling