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REVIEW ARTICLE

Role of glutathione in cell nucleus

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Abstract
Cells with high proliferation rate have high glutathione levels. This typical feature of cancer cells is viewed usually as a defence mechanism against ionizing radiation or chemotherapy. Efforts have been made in order to decrease cellular glutathione levels in tumours as a necessary pre-treatment for cancer therapy. However, very few reports have considered cellular glutathione as a physiological tool for cells to proliferate and that most of this high glutathione levels were located in the nucleus. The role of nuclear glutathione in cell physiology has become more important in the last years. This review summarizes new findings that point to the nuclear reduced status as an environment that induces heterochromatin formation. Glutathionylation and oxidation of nuclear proteins appear as a reversible physiological mechanism able to regulate DNA compaction, cell cycle and DNA repair.

Keywords: *Chromatin, histones, genetic instability, cell cycle, glutathione*

Introduction

The tripeptide gamma-glutamyl-cysteinyl-glycine (glutathione) has been considered essential for survival in eukaryotic, but not in prokaryotic cells [1–4]. The reasons for this difference between nuclear and anucleated cells are unknown.

Traditionally the role of glutathione (GSH) in the cell nucleus has been related mainly to the protection of DNA against oxidative stress [5], the protection of DNA binding proteins [6] or against ionizing radiation [7]. The role of glutathione defending the genome due to its antioxidant capabilities was an obvious and expected function. However, glutathione has also been reported to play a seminal role in the synthesis of DNA [8] and in the reduction of Cys residues on zinc-finger DNA binding motifs [9]. However, all these reports describe the action of cellular, but not nuclear, glutathione concentration in cells and its relation to DNA or DNA binding proteins. These functions and related reports do not explain why GSH is so important in nucleated cells but not in prokaryotic cells. It is possible that the antioxidant (protective) functions of GSH on DNA could be one but not the only important function of nuclear GSH.

Although not many, several reports point to GSH as a key factor in nuclear homeostasis. Dijkwel and Wenink [10] reported almost 15 years ago that GSH was necessary for the organization of the nuclear matrix. It was also reported long ago that GSH was important for chromosome consolidation [11]. In the 1990s, Atzori et al. [12] showed that variations in the amount and redox state of cellular thiols, particularly reduced glutathione, regulates growth and squamous differentiation of human bronchial epithelial cells. Sometime later it was shown that depletion of nuclear GSH to 50–60% of initial values prior to irradiation (400 cGy) resulted in nuclear DNA fragmentation and apoptosis [13]. Thalidomide is a drug that was used in the 1950s and 1960s as a painkiller and antiemetic for pregnant women. Most unfortunately it had devastating effects on the newborns.

Reports by Hansen et al. [14] underscore the role of nuclear glutathione in the deleterious effects of thalidomide. Rat thalidomide-resistant and a rabbit thalidomide-sensitive species were used to compare

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potential differences among limb bud cells. Confocal microscopy studies revealed that glutathione distribution was different in these cell types. Thalidomide induced cytosolic GSH depletion in both cell lines; however, nuclear GSH levels remained high in the rat thalidomide resistant cells but not in the rabbit thalidomide sensitive cells. The authors suggested that a redox shift in the nucleus may result in the misregulation of interactions between transcription factors and DNA, causing defective growth and development [14]. Thalidomide is still used nowadays as an effective drug for the treatment of leprosy among other diseases.

We also reported that GSH plays an important role on telomerase activity. This enzyme, necessary for the elongation of telomeres, is over-expressed in cancer. High telomerase activity is a marker of malignancy and bad prognosis. We found that telomerase activity correlates with cellular GSH, being maximal during cell proliferation. Experiments performed *in vitro* showed that the presence of a reduced environment increases telomerase activity to values as high as in cancer cells [15].

Despite the reports mentioned above and the importance of the functions described and its basic role in cell function, the role of nuclear glutathione has remained elusive. This is due in part to the fact that it is impossible to determine the nuclear concentration of GSH using standard cell fractionation and analytical approaches (for a review, see [16]). Opposite to what happens with glutaredoxin or thioredoxin, where both the oxidized and the reduced form of these antioxidants can be determined in the cell nucleus. These methods have been reviewed by Hansen et al. [17]. Two forms of the redox blot have been developed to separate, on the basis of different charge or mass, oxidized nuclear proteins. Other techniques include antibodies that bind only to the oxidized form of the protein [18] and mass spectrometry [19]. Another recent approach that could be used in the near future to determine the nuclear redox state is the use of redox-sensitive green fluorescent proteins (roGFP). Dick and his group have been able to determine the glutathione redox status both in the cytosol and in mitochondria [20], but as far as we know this method has not been used in the cell nucleus.

However, to determine the oxidized form of glutathione in the nucleus is at the moment not possible. Even determination of total glutathione or its reduced form in the cell nucleus offers conflicting results. Mercury orange, monochlorobimane (BmCl) and 5-chloromethylfluorescein diacetate (CMFDA) were the most commonly-used probes for GSH determination, but the results obtained by these methods have been conflicting. Bellomo et al. [21] using monochlorobimane-GSH conjugation showed a 3:1 nucleus: cytoplasm ratio. However, more recent reports by Briviba et al. [22] showed that the high nuclear fluorescence was due to an influx of the fluorescent bimane-GSH adduct into the nucleus. Thomas et al. [23] used fractionation techniques and flow cytometry with mercury orange, as this probe readily forms fluorescent adducts with GSH and other non-protein sulphydryls, reacting much more slowly with protein sulphydryls. Contrary to the previous reports, they found lower GSH levels in the nucleus than in the cytoplasm. The mean nucleus-cytoplasm ratio they found was 0.57 ± 0.05. They suggested that there is a distinct pool of GSH in the nucleus since it was partially resistant to BSO depletion compared with the cytoplasm. More recently, Söderdahl et al. [16] showed the highest GSH staining in a perinuclear mitochondrial-rich compartment and low nuclear GSH staining using mercury orange and a specific GSH antibody. Finally, Voehringer et al. [24] using CMFDA (which binds in 95% to GSH) [25] showed that GSH was mainly distributed in the cytoplasm, although Bcl-2 over-expression was able to increase nuclear GSH levels.

Aside of the methodological problems already mentioned, another source of common misleading results is the fact that most, if not all, the reports share the common view of nuclear GSH distribution in a static situation. Cells are usually studied under steady state conditions, i.e. when cells are confluent (G_0/G_1 phase of the cell cycle). There are very few reports showing how GSH is distributed within the cell during the different stages of the cell cycle. It is obvious, but probably not considered fully when investigating GSH distribution, that the nucleus as the whole itself passes through extremely dramatic changes during the cell cycle and its study must be followed during the different phases of its cycle.

In view of the conflicting results using cell fractionation and analytical techniques we used confocal microscopy. Selecting 5-chloromethylfluorescein diacetate (CMFDA) as fluorescent probe since, as discussed previously, other probes have shown misleading results. CMFDA is a vital fluorochrome that with a specificity of 95% binds reduced glutathione, while 5% is bonded to SH groups of proteins [25]. It permits the visualization of reduced glutathione in living cells, and the distribution of the fluorescence defines the GSH distribution in the cell compartments. The method that we have used to quantify the nuclear compartmentation of GSH along the cell cycle is a combination of confocal microscopy and image analysis and is described in detail in our publications [26,27].

We described that glutathione is recruited into the nucleus in early phases of cell proliferation when most of the cells are in an active division phase and it redistributes uniformly between the nucleus and cytoplasm when cells reach confluence [26].

Our findings are in line with several other studies aimed to elucidate the fine redox regulatory mechanisms that lie behind the correct cell cycle progression. Conour et al. [28] suggested that the reduction of the intracellular environment as cells progress from quiescence to proliferation, as shown in our study, may protect genomic DNA from oxidative damage upon

breakdown of the nuclear envelope during mitosis [28]. Indeed, one of the assertions in support of this premise derives from the study of oxidative stress related to genotoxicity, recently published by Green [29]; oxidative DNA modifications displayed a negative linear correlation with nuclear GSH. This is of special importance considering the report of Menon et al. [30] on the necessity of the oxidative event in early G1 phase to allow G1-S transition. Even more, it has recently been postulated that the restriction of DNA synthesis to the reductive phase of the cycle in yeast may be an evolutionarily important mechanism for reducing oxidative damage to DNA during replication [31], which implies the common mechanism of the DNA protection during S phase in all eukaryotes.

Modifications of nuclear proteins along the cell cycle

One possible way in which nuclear GSH could influence cell proliferation is mediating the protein modifications dependent on the redox environment, like oxidation or glutathionylation. Glutathionylation is a protein modification which consists in the covalent union of the tripeptide glutathione to one SH group of Cysteine residue. Experimental evidence started to accumulate recently, documenting that S-glutathionylation occurs in a number of physiologically relevant situations, where it can produce discrete modulatory effects on protein function [21]. Besides the modulatory effects, glutathionylation may also provide protection for protein-SH against irreversible modifications and protein damage in response to higher levels of oxidative stress [22]. Nonetheless, we have observed that S-glutathionylation is a post-translational modification that occurs not only during oxidative stress, but also under basal conditions during the cell cycle.

Consequent with our finding that nuclear GSH is high during the early phases of cell cycle and low when cells are confluent, nuclear oxidized protein levels are higher during cell cycle arrest than before the proliferation. On the contrary, the level of glutathionylated proteins was higher before the proliferation, remained high during cell proliferation and decreased when cell growth stopped (see Figure 1).

Various studies have demonstrated that the nucleus is more reduced than the cytosol (15 mM GSH vs 11 mM, respectively) [21,32,33]. An important number of nuclear proteins, including transcription factors, require a reduced environment to bind to DNA. More than 62 proteins are involved directly in transcription, nucleotide metabolism, (de)phosphorylation or (de) ubiquitinylation, which are all essential processes for cell cycle progression [28]. For instance, it appears that, at the onset of cell proliferation in the early G1 phase, an increase of ROS in the cytoplasm is necessary for the initiation of the phosphorylation cascade mediated by epidermal growth factor (EGF) that, subsequently, activates DNA replication and the cell division [34]. According to Jang and Surh [35] nuclear GSH may act as a transcriptional regulator of NF-κB, AP-1 and p53 by altering their nuclear redox state. The transcription factor NF-κB is an example of distinct redox-sensitive activation and DNA binding [17]; it is activated by various physiological stimuli known to produce ROS; on the contrary, to permit DNA binding, similar to Fos, Jun and, as suggested also to Nrf2 [36,37], cysteine residue within DNA binding domain must be reduced. Both processes are guaranteed by the adequate redox state of the cytosolic and nuclear environment, respectively.

In addition, the reduced nuclear environment could protect oxidant sensitive proteins from oxidation [28]. Indeed, our study showed lower levels of protein oxidation in nuclear extracts at 6 and 48 h of culture, when the nuclear GSH was high, than at confluence, when the nuclear GSH level was lower and equal to cytoplasmic.

Interestingly, the nuclear proteins underwent stronger glutathionylation before and at the onset of cell proliferation than at quiescence. It is not surprising if we bear in mind that a high level of GSH in the nucleus could provide protection to the proteins against the oxidative threat coming from the cytoplasm at the early phase of cell proliferation and that glutathionylation, as it is a reversible modification, could be just the way. On the other hand, based on the simplicity of the redox transition from thiol to disulphide and on the fact that the reversibility was energetically favourable, Cotgrave and Gerdes [38] more than 10 years ago proposed glutathionylation as a post-transcriptional modification with the regulatory finality. They state that it offers a strong possibility for transducing 'oxidative information' from intracellular oxidants via the GSH redox buffer to individual proteins containing 'regulatory thiols'. Also, recently, this post-translational modification was proposed as a likely molecular mechanism for redox dependent signalling mediated by GSH [39]. Thus, a high level of GSH in the nucleus, observed before and at the onset of cell proliferation, could provide the 'GSH redox buffer' necessary for the progressing of oxidant stimulated mitogenesis.

The occurrence of the glutathione in the nucleus; active transport, *de novo* synthesis, diffusion or something else

How GSH enters the nucleus and how it is regulated during the different phases of the cell cycle is still a matter of debate. The regulation of such interactions is also unclear. According to Smith et al. [40], the possible biochemical mechanisms responsible for the turnover of nuclear GSH are the following: (1) GSH may be taken up from the cytoplasm into the nuclei either passively or through energy-dependent processes; (2)

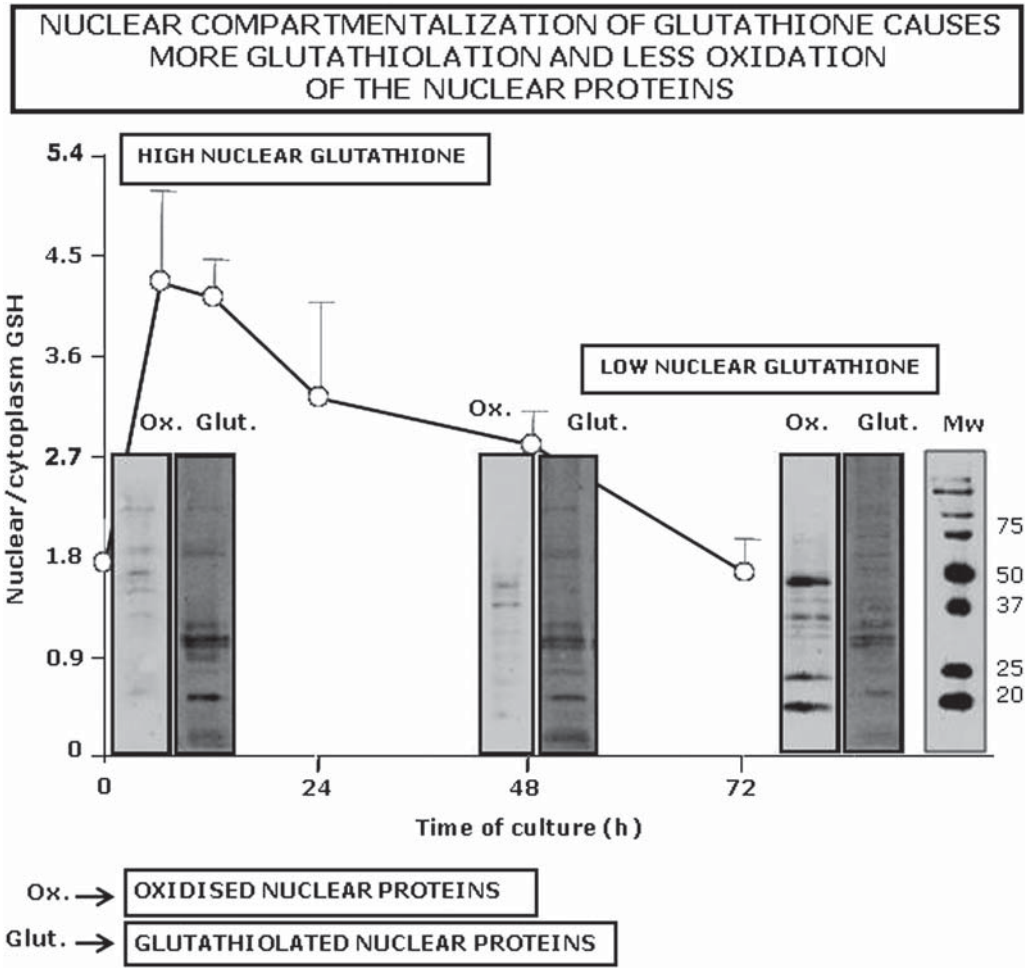

Figure 1. Modifications of nuclear proteins along the cell cycle. Nuclear compartimentalization of GSH causes more glutathionylation and less oxidation of nuclear proteins. Nuclear:cytoplasm GSH relationship was measured using green 5-choromethyl-fluorescein diacetate (CMFDA), by confocal microscopy and quantification of the fluorescence emission in the nuclear area. To measure the level of nuclear protein oxidation, western blotting was performed. Nuclear lysate cells were derivatized to 2,4-dinitrophenilhydrazone by its reaction with 2,4-dinitrophenilhidrazine. The derivatized samples were separated by electrophoresis in a acrilamide gel followed by western blotting and inmunodetection of carbonylated proteins, using the Oxy Blot protein Oxidation Detection Kit (Intergen Company, Burlington, MA). To determine glutathionylated proteins western blotting of nuclear extract were performed without addition of any reducing agents. Anti-glutathione antibody (Virogen, Grater Boston, MA) was used to detect glutathionylated modifications in the proteins. One possible way in which nuclear GSH could influence cell proliferation is mediating the protein modifications dependent on the redox environment, like oxidation or glutathionylation. Consequent with the finding that nuclear GSH is high during the early phases of cell cycle and low when cells are confluent, nuclear oxidized protein levels are higher during cell cycle arrest than during the proliferation. On the contrary, the level of glutathionylated proteins was higher before the proliferation, maintained high during the exponential phase and decreased when cell growth stopped.

GSH may be synthesized *de novo* in the nucleus by the enzymes glutamate cysteine ligase and GSH synthetase; and (3) GSH may function to transport γ-glu-cys-cys.

The role of ATP-dependent mechanisms in maintaining the nuclear:cytoplasmic GSH concentration in hepatocytes was demonstrated by Bellomo et al. [21]. Despite its high specificity for glutathione, monochlorobimane (BmCl) was found to be of no value in the study of cellular GSH distribution; once GSH-BmCl conjugate is formed it demonstrates an increased tendency of nuclear compartmentalization [22]. Indeed, in our study using CMFDA, we have not found an ATP-dependent mechanism of nuclear GSH compartmentalization in 3T3 fibroblasts [26]. Ho and Guenthner [41], using nuclear fractions, concluded that GSH is taken up by the nucleus by passive diffusion and no evidence for an ATP-dependent mechanism for GSH concentration was observed.

Glutamate cysteine ligase (GCL) and GSH synthetase activities have been reported in nuclei and a portion of 4–8% of cell GSH synthetic activity is considered capable of maintaining nuclear GSH levels [41]. However, we could not find GCL expression in nuclei of 3T3 fibroblasts [26]. In addition, as previously reported, BSO, a specific inhibitor of GCL, was unable to decrease nuclear glutathione levels. Thus, at least under our experimental conditions, the possible '*de novo*' synthesis of nuclear glutathione seems improbable in 3T3 fibroblasts.

The nuclear pore complex is the biochemical machinery that controls the molecular traffic across the nuclear

envelope [42,43]. Ions and small hydrophilic molecules, like glutathione, are considered to move by free and fast diffusion across the nuclear pore [44]; nevertheless ion gradients and transnuclear ATP-dependent membrane potential have also been reported [43]. In a series of creative experiments published in the early 1990s, Feldherr and Akin [45,46] showed that permeability of nuclear envelope and nuclear transport were higher in proliferating than in quiescent cells. One pore forming protein that has been brought into the connection to nuclear glutathione content is bcl-2. Voehringer et al. [24] showed that over-expression of Bcl-2 recruits GSH to the nucleus. The presence of this protein at the nuclear envelope was demonstrated [47] and the association with the nuclear pore complexes was suggested. Moreover, Zimmermann et al. [48] demonstrated that GSH binds to Bcl-2 in mitochondria, providing a molecular basis for its antioxidant function. Thus, bcl-2 located in the mitochondrial membrane introduces GSH in the mitochondrial space. We have found that the presence of bcl-2 in the nucleus is higher in proliferating than in quiescent 3T3 fibroblasts, i.e. it coincides with the high level of glutathione in the nucleus as well as with the intense nuclear transport regulated by the nuclear pores [26].

Thus, we suggested that during the changes in the nuclear membrane that precede the cell division, nuclear Bcl-2 could facilitate the translocation of glutathione to the cell nucleus.

The depletion of nuclear GSH severely interferes with the cell cycle progression

In view of the striking differences between the nucleus and the cytosol when 3T3 fibroblasts were proliferating we decided to deplete GSH levels in order to see the effect of nuclear glutathione during cell proliferation. GSH concentration can be selectively decreased *in vivo* by various methods, e.g. by buthionine sulphoximine (BSO) which is a transition state inactivator of glutamate cysteine ligase (GCL) that catalyses the first limiting step of GSH synthesis. Alternatively, it can be inhibited by non-specific agents: diamide (a thiol-oxidizing agent), N-ethylmaleimide (a thiol-alkylating compound) and butylhydroperoxide [49]. Diethylmaleate (DEM) also decreases intracellular GSH concentration through a reaction catalysed by the enzyme glutathione-S-transferase [50].

However, to decrease nuclear GSH level is not so easy, especially if the cell must remain alive. A great deal of work has been done previously to decrease GSH levels in the whole cell and decrease cell proliferation. However, no information has been provided in order to correlate nuclear GSH levels and cell proliferation. Early studies showed GSH depletion effects on cell proliferation [51]. In the following years a number of reports focused on the consequences of the depletion of cellular glutathione levels on changes in cellular proliferation [52,53]. However, all those reports were performed measuring cellular or total glutathione levels. As mentioned before a number of studies have indicated the existence of a nuclear GSH pool that resists depletion after exposure of cells to BSO (for a review see [54]). However, Thomas et al. [23] showed that depletion of GSH with N-ethylmaleimide or DEM decreased mercury orange fluorescence in the nucleus and cytoplasm to a similar extent. In contrast, mercury orange fluorescence in the nucleus was much more resistant to BSO depletion than that in the cytoplasm. Spyrou and Holmgren [55] showed that inhibition of glutathione synthesis by 0.1 mM BSO was able to decrease GSH synthesis after treatment for 12 h, but GSH-depleted cells grew as well as control 3T6 cells with no decrease in DNA synthesis. Thus, incubation of cells with low concentration of BSO, although it decreases glutathione levels, does not change cell proliferation. Esposito et al. [56] showed that DEM treatment induces cell cycle arrest that is accompanied by several redox-dependent changes in cell-cycle related proteins. Precisely, the p53-independent accumulation of p21 was detected. These authors demonstrated that DEM treatment strongly activates p21, showing a clear inhibition of cell proliferation.

Thus, after reviewing literature we decided to compare the effects of BSO (10 μM) and DEM (100 μM) on cell proliferation. These agents decrease GSH levels by two different means; BSO decreases GSH synthesis and DEM, a weak electrophile, forms DEM-GSH adducts, although DEM may not be absolutely specific to GSH [57]. We found that DEM was able to decrease both nuclear and cytoplasmic GSH. However, BSO at the concentration used only decreased cytoplasmic GSH and, although total GSH levels were lower in the BSO-treated 3T3 fibroblasts, only DEM was able to impair cell growth. When cells were simultaneously treated with DEM and glutathione ethyl ester, cell growth was similar to the control group. We showed for the first time that cellular proliferation specifically relates with nuclear, but not with total cellular GSH levels. The results underscored the importance of maintaining a reduced nuclear environment in order to maintain normal cell cycle progression.

Hansen et al. [17] suggested that a redox shift in the nucleus may result in the misregulation of interactions between transcription factors and DNA, causing defective growth and development. We reported that GSH level increases in 3T3 fibroblasts before exponential cell growth. Chen et al. [58] reported the specific role of nuclear GSH preventing apoptosis and its importance in oestrogen action.

Thus, as a preliminary conclusion nuclear glutathione plays an important role in cells proliferation, however how GSH exerts its action is not clear. Interaction of GSH with chromatin structure can explain, at least in part, the reported actions.

Role of glutathione in chromatin structure

Studies developed during the last two decades have shown the importance of GSH during the process of mammalian chromatin sperm decondensation [59–61].

The chromatin and nuclear architecture differences between embryonic stem (ES) cells and differentiated or somatic cells have been explained [62–64]. In this way, in undifferentiated mouse ES cells, which have high levels of GSH, the chromatin structure itself is more homogeneous and has less frequent condensed chromatin foci [65]. However, when differentiation of ES cells is induced by retinoic acid, a strong condensation of heterochromatin can be observed by electron microscopy [66].

As a general rule, poorly differentiated cells have high levels of GSH, while well differentiated cells have a lower concentration of GSH [67,68].

The alteration of nuclear redox conditions modulates chromatin conformation and stability. The use of oxidants such as hydrogen peroxide produce the degradation of higher ordered chromatin structures [69]. Oxidation of chromatin affects interaction between nucleosomes and the addition of reducing agents increases the susceptibility of chromatin to digestion by nucleases. This suggests that changes in the levels of reducing agents in the nucleus modify the packing of the chromatin [70]. The overall redox mechanisms that may regulate all these events remain unknown, although it has been speculated that ROS generation induced by a shift in the redox status of cells could affect gene expression by altering chromatin conformation [71]. Furthermore, in this review we summarize some redox-related post-translational modifications that may be implied in chromatin structure changes.

Elegant studies developed by Torres et al. [72] show some chromatin remodelling events occur after GSH depletion. First, the repressor complex Id2/Sin3A is released from c-myc promoter. After that, the transcription factor STAT3 associates with its coactivator CBP/p300, which contains intrinsic histone acetyltransferase activity. Then, STAT3 and histone H3 are acetylated, producing the aperture of chromatin needed for transcription of c-myc.

Torres et al. [72] show how they can modulate the expression of c-myc by altering GSH levels in BSO-treated mice and they propose a physiological role for GSH depletion in human chromatin remodelling events.

Glutathione is closely connected with epigenetic mechanisms. Indeed, synthesis of S-Adenosylmethionine (SAM) by SAM synthetases is a redox regulated process that depends on the GSH/GSSG ratio. When cells are depleted of glutathione by chemical means, methyl donors become deficient, leading to genome-wide DNA hypomethylation [73,74]. This could occur because the pro-oxidant cellular state requires restoration of GSH levels and this is at the expense of depleting SAM via the transulphuration pathway [75].

These previous observations led us to suggest that chromatin structure and glutathione levels seem to be strongly related.

Connecting histones and redox-related post-translational modifications

Alterations in chromatin structure affect the accessibility of the DNA by proteins and can generate specific domains of chromatin with a particular function. Recently it has been published that peroxynitrite can mediated oxidation and nitration of histone H3 [76]. These *in vitro* studies show that histone H3 is highly sensitive to peroxynitrite oxidation, producing carbonylation and nitration of this histone. These modifications induce changes in H3 tertiary structure and in turn alter the package of the DNA around the nucleosome [76].

Although carbonyl modification of total proteins increases during ageing and several age-related diseases as a result of high oxidative stress, Sharma et al. [77] observe the apparent paradoxical finding of reduced carbonylation of histones in the liver of old animals. Surprisingly, higher levels of this modification in histones obtained from young and dietary restricted animals are detected [77].

What could be the physiological function of carbonylation of histones? It is known that histone protein protects DNA from insults [78]. Nuclear polyamines like spermine or spermidine are effective antioxidants protecting DNA from attack by reactive oxygen species [79,80] and a spermine-carbonyl adduct has been identified as a result of this protection mechanism [81]. In a similar manner, histone carbonylation can be regarded as a 'sacrifice' in order to protect the integrity of the DNA.

However, other theories are plausible. Since carbonylation of histones can mask positive charges at Lys and Arg residues, it may act as acetylation of histones, affecting DNA and core histones interaction and relaxing chromatin compaction. This theory would be defendable, due to younger chromatin being known to be more relaxed than older one [82].

There is the possibility that proteins are oxidized to generate carbonyl groups, directly or indirectly, as a consequence of glycation reaction of poly-(ADP)-ribose, as discussed by Wondrak et al. [83]. These authors studied the carbonylation of histones in PC12 cells following alkylating stress and showed a mechanism for histone modification by ADP-ribose, indicating that carbonylation involves formation of a stable acyclic ketoamine, after a reaction of glycation of ADP-ribose.

This modification of histones has been related with the NAD^+/NADH levels into the cell. The constitutive levels of poly-(ADP)ribose are usually low in unstimulated cells. However, in response to mitogenic stimuli

or genotoxic stress, the PARP activity and the levels of poly-(ADP)ribose increases, while cellular NAD^+ levels are correspondingly decreased due to the requirement of NAD^+ as precursor for the poly-(ADP)ribose synthesis [84].

Poly ADP-ribosylation occurs during DNA replication [85,86] and during DNA damage and oxidative stress [87–89]. After DNA damage, poly-(ADP)ribose synthesized by PARP-1 and to a lesser extent by PARP-2 could dissociate histones/nucleosomes from DNA, thus granting the DNA repair machinery accessibility to damaged DNA [90]. In this way, poly-ADP-ribosylation of histones appears to have an interesting mission. Theoretically, poly-(ADP) ribosylation produces the increases of the negative charge in the histones and probably produces unsettling effects on nucleosome structure. Like histone acetyltransferases (HATs) and ATP-dependent chromatin remodellers, PARP-1 can affect multiple genomic DNA-dependent processes including transcription, replication, repair and recombination [91] and it can participate directly in the assembly of transcription complexes at enhancers and promoters [92].

Poly-(ADP) ribosylation has been described to occur through NH in Lys, Arg and through SH group in Cys [93], offering the Cys residue as a target for post-translational modification (PTM).

These previous reports prompted us to hypothesize other possible redox-related modifications of histone H3 through Cys residues.

Histone H3 is the only nucleosomal protein with a cysteine residue in their sequence. In mammalian, four variants of H3 have been described (H3.1, H3.2, H3.3 and H3.1t). These four variants have cysteine residue in position 110. In addition, H3.1 and H3.1t have a second cysteine in position 96. This sulphydryl-containing residue may play a specialized role in the function of proteins that contain it and the redox behaviour (disulphide bond, sulphenic acid formation, etc…) can be modulated by the environment [94]. For this reason, redox mechanisms appear to play important roles in the nucleus [75]. Hake and Allis [95] suggest that it is necessary to determine the extension of H3 oxidation/reduction *in vivo* to understand the role of the Cys110 and Cys96, during the process of chromatin compaction. In that case, cysteines in H3 variants might be important for nucleosome and chromatin higher-order structures, but this challenging point remains to be determined. At the moment, H3.3 histone variant has been associated with transcriptionally active gene loci and is enriched in covalent modifications associated with gene activation [96,97].

Cysteine appears as an important component in the regulation of protein's function [98,99]. Although Cysteines, in the histone core, are apparently buried, Cys110 has been shown to be accessible [100]. On the other hand, Bode and Standt [101] showed that it is possible to induce conformational heterogeneity changes, causing a partial exposure of both Cys side chains. Furthermore, core particles prepared in oxidizing conditions have the H3-C110 side chains linked through a disulphide bond, and these particles have somewhat different properties from those kept in fully reducing conditions [102], suggesting that redox balance could modulate the accessibility of Cys in order to induce disulphide bonds formation.

Hypothesis about the implication of Cys present in histone H3 variants in chromatin conformation events are a matter of debate. For example, H3 variants H3.1 and H3.3 are incorporated into the chromatin by separate chaperones (CAF-1 and HIRA, respectively) [103]. In the particular case of chaperone CAF-1, it may specifically recognize the region containing Cys96 in H3.1 because CAF-1 can incorporate H3.1 in the correct loci, but not H3.2. Histone variant H3.1 has the same sequence of H3.2, with the exception of position 96, occupied by a Cys in the former and a Ser in the last one, suggesting a direct implication of the Cys96 in the recognition process. Glutathionylation of H3' Cys, during the entrance of GSH into the nucleus, could affect the recognition of H3 variants by specific chaperones acting as a selective step for histone H3 deposition into the chromatin.

On the other hand, it has been proposed that H3.1 variant might bind to other H3.1 from a different nucleosome, forming a disulphide bond between both Cys96, bringing two contiguous nucleosomes and producing heterochromatin by chromatin condensation [95].

Nuclear proteins such as lamin B receptor (LBR), a protein located in the inner nuclear envelope, can interact with histone H3 variants through disulphide bond. It has been suggested that, in an oxidizing environment, the Cys226 at the end of the transmembrane segment of LBR could potentially form a disulphide bond with a second LBR [104]. Makatsori et al. [105] found silencing PTM associated to histone H3 co-purified with LBR. Disulphide bond between Cys from H3.1 and one of the seven Cys present in the LBR could be formed, producing chromatin condensation in the inner nuclear membrane [105], adding more evidence for the importance of Cys in histones in the regulation of chromatin compaction and the possibility that modification of these critical Cys by glutathionylation could have some consequences.

Another interesting protein is HP-1 (heterochromatin protein 1), also called Chromobox protein homologue 5, involved in the mechanism of chromatin silencing. This protein has three Cys residues and the high level of Lys residues provides the ability of the SH groups to form an ionizable sulphydryl group. A study by Polioudaki et al. [106] suggests the formation of higher order complex structures including H3, LBR and HP-1 in order to produce compacted chromatin, but it is unresolved whether LBR located in the inner nuclear envelope contains only the H3.1

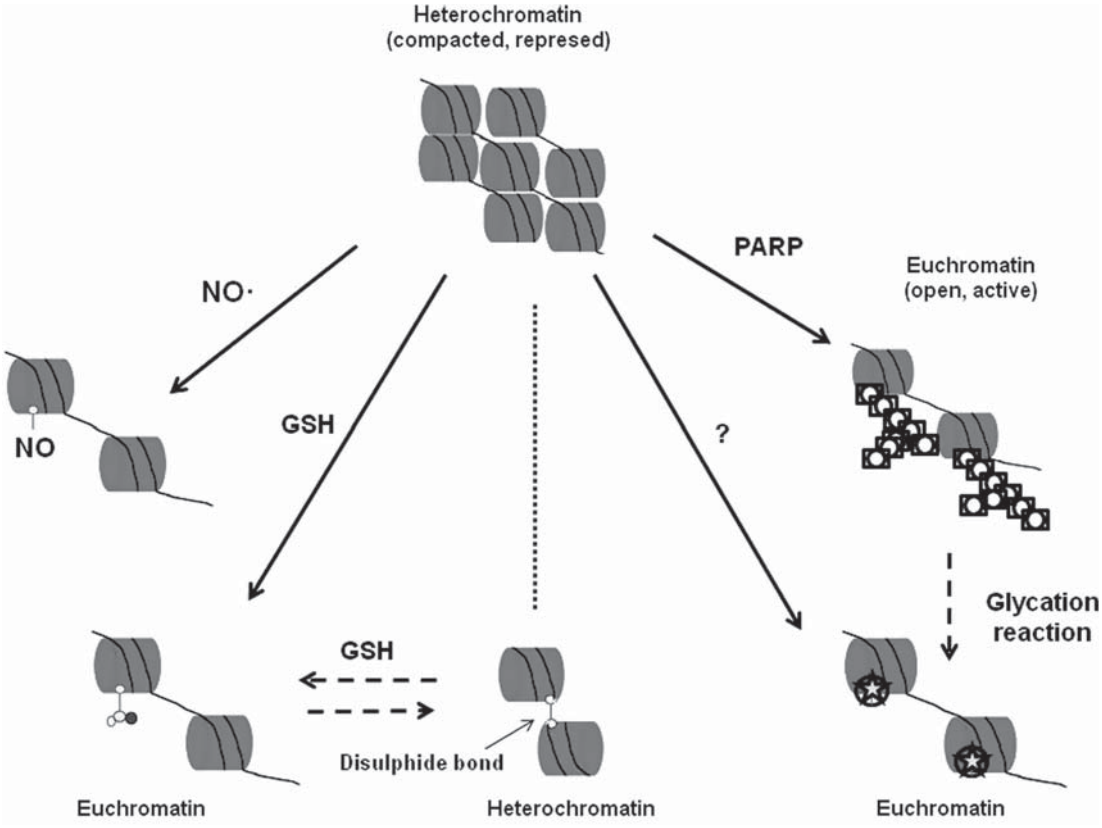

Figure 2. Redox related post-translational modifications in histones. Changes in the redox environment and cellular signals induce histone post-translational modifications that in turn, produce the alteration of nucleosome structure and for extension changes in chromatin compaction. Poly-(ADP)ribosylation, catalysed by PARP, has shown the ability to open the chromatin. In the same way, carbonylation of histones, a direct consequence of glycation reaction from ADP molecules or oxidative process from peroxynitrite, produces relaxation of chromatin due to can mask Lys and Arg positive charges. As these modifications, *in vitro* experiments have shown the ability of peroxynitrite to produce histone nitration. The consequence of this modification is the alteration of nucleosome tertiary structure and relaxation of the DNA around the histone core particle. Finally, histone H3 glutathionylation can alter nucleosome compaction affecting histone H3-H3 interaction or (H3-H4)2 tetramer accommodation into the histone core.

variant and, if so, whether the cysteine 96 is implied in the binding between the nuclear envelope and heterochromatin.

Although scarce literature appears mentioning the importance of Cys in histone H3 in chromatin remodelling events, there are other classical studies that point to the ability of Cys to react with chemical agents producing chromatin structure modifications and changes in nucleosomal DNA stability. Early studies developed by Prior et al. [107] and Lewis and Chiu [108] using calf and chicken histones showed the distinct reactivity of H3 cysteines against sulphydryl reagents in oxidative conditions. Cysteines were exposed in euchromatin regions and could form disulphide bonds with these reagents. In heterochromatin, cysteine residues did not react with the reagents, suggesting that these cysteines are deeply buried in the macromolecule [107]. Other studies developed by Lewis [109,110] showed that sulphydryl oxidation and modification in both Cys96 and Cys110 destabilized the calf thymus histone H3-H4 dimer, indicating the importance of sulphydryl groups in H3 for maintaining the tri-dimensional structure of the nucleosome.

Other classical studies based in electrophoretic mobility in TAU gels (Triton X-100, acetic acid and urea) have shown the ability to identify histone H3, with their sulphydryl groups modified, forming sulphoxides, sulphones or disulphide bonds. The author explains the differences in the histone mobility in terms of the decrease of the helical contents of the histones by the oxidation of methionine and cysteine residues [111].

These antecedents show the importance of cysteines present in H3 variants to establish interaction with other histone H3 and other nuclear proteins and offer to nuclear Cys an important role in chromatin package and organization. That this points to the variation of glutathionylation levels of nuclear proteins, during cell proliferation, could have a relevant mission in this aspect.

Histone H3 glutathionylation. Is it a possible event for chromatin regulation?

As we have mentioned, some histone variants can be incorporated in a dependent or independent DNA replication manner using different machineries (chaperones

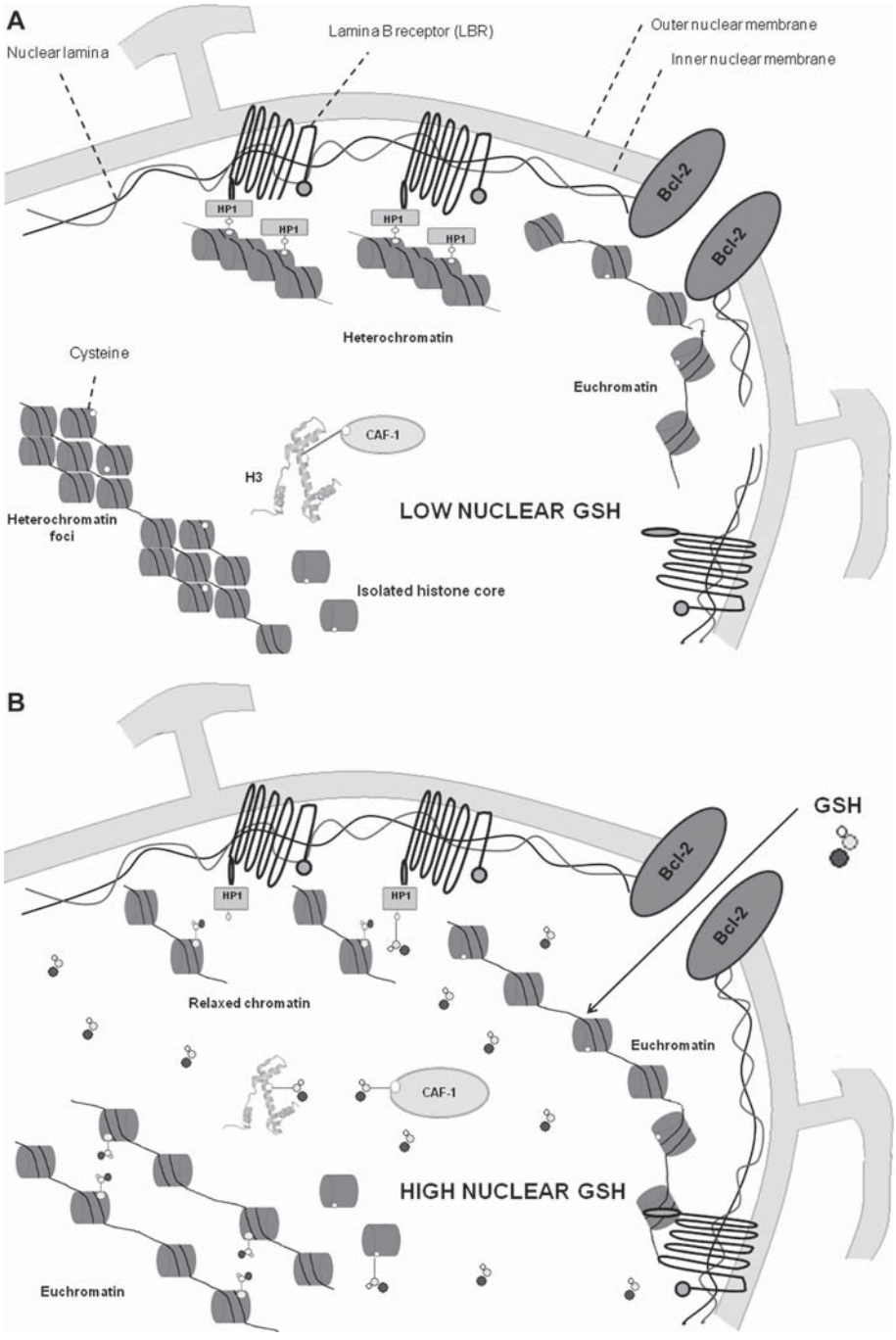

Figure 3. Schematic representation of the possible role of nuclear GSH. Our hypothesis points to the fact that GSH may modify different nuclear proteins affecting chromatin compaction and distinct nuclear events. (A) When nuclear GSH is low (quiescent cells), chromatin is compacted because disulphide bonds could be formed through Cys96 between two different nucleosomes. Furthermore, chaperone CAF-1 may recognize Cys96 of histone variant H3.1 (heterochromatin constitutive histone) and locate this histone into the specific position. Moreover, a disulphide bond could be formed between HP-1 (heterochromatin protein 1) and histone H3, locating the heterochromating along the inner nuclear membrane. (B) When GSH is high (proliferating cells) some events may occur: glutathionylation of histone H3 may relax the chromatin compaction by affecting the formation of disulphide bonds between two H3. On the other hand, histone variant H3.1 glutathionylation may affect the recognition process by CAF-1, immobilizing this histone variant almost through the CAF-1 catalysed process. In the same way, heterochromatin around the inner nuclear membrane may be free from HP1 and LBR. The consequences of all these events are that chromatin is more accessible to replication machineries and chaperones. Furthermore, the high nuclear GSH may protect the DNA and other nuclear proteins during these processes take place.

CAF-1 and HIRA and probably other chaperones that recognize histone variants) that recognize Cys positions in histone H3. These machineries are responsible for the deposition of histones in specific chromatin loci (active or silent genes, centromeres and telomeres). As we have shown in studies developed by Tagami et al. [112], Cys96 were important in the chaperone-H3.1 recognition process. This chaperone has shown the ability to locate the H3.1 in heterochromatin foci through a replication dependent mechanism. The

changes in nuclear GSH during cell cycle and our observation that glutathionylated protein levels increase during the cellular replication phase (Figure 1) may make us think about the possibility of H3 glutathionylation and their implication in the chaperone recognition phenomena. For this reason, we suggest that Cys glutathionylation of histone H3 variants during cell cycle or during different cellular events, such as oxidative stress, would have consequences on the recognition of proteins that recognize specifically H3-cys loci. Glutathionylation of Cys can modulate the recognition process and help the chaperones to locate the histone H3 variants in the specific place of the chromatin (euchromatin or heterochromatin). The changes in nuclear glutathione concentration and glutathionylation of nuclear proteins, during cell cycle, appears then as a regulatory event. One of these proteins may be the histone H3 that in turn may regulate nucleosome and chromatin conformation.

It was suggested by Camerini-Otero and Felsenfeld [113] that the formation of disulphide bond between chicken histone H3 molecules permits the nucleosome reconstitution. As we have mentioned above, chemical modifications on sulphydryl residues of H3 produce a decrease of the yield of reconstituted nucleosome and its thermal stability [108]. In the same way, histone H3 glutathionylation might regulate nucleosome stability and control the precise higher order folding of chromatin by changing the tridimensional structure and the total volume of the nucleosome. Moreover, H3 glutathionylation may affect the intermolecular interactions between H3 variants and HP-1 and/or LBR protein by mask Cys residues. The direct consequences would be chromatin decondensation around the inner nuclear membrane or in specific loci (see Figure 2), facilitating the interaction of the DNA with the replicative or transcriptional machineries.

Our observations concerning the entry of GSH in the nucleus suggest that the glutathionylation of histone H3 during the entrance of GSH into the nucleus during proliferation phase could be an important event to control the recognition of the histone H3 variants by the chaperones, in order to introduce it in the specific place at the concrete time. Moreover, glutathionylation of H3 can modulate the chromatin availability to replicative or transcriptional machineries by affecting interactions between H3 and proteins such as LBR and HP-1. Moreover, histone H3 glutathionylation may affect the H3.1 recognition process by chaperone CAF-1, a possible consequence is that this constitutive heterochromatin H3 variant could be immobilized during the replication process and reincorporated into the chromatin just when GSH levels return to being low (see Figure 3).

Furthermore, the glutathionylation would convert the H3 variants in suitable substrates for the appropriate post-translational histone modifying enzymes, contributing to the histone code and activating or silencing transcriptional mechanisms.

Nowadays, it is known that disruption of the balance of epigenetic networks can cause several major pathologies, including cancer, syndromes involving chromosomal instabilities and mental retardation [114]. Clearly, many questions remain unanswered. What is the driving force that moves GSH to the nucleus only when cells are ready to proliferate? What is the role of nuclear GSH during apoptosis? Is it the regulation of the epigenetic process and its association with the gene expression during cell cycle related to GSH? Could glutathionylation of nuclear proteins play a role in the physiopathology of epigenetic-related diseases? Probably in the near future we will be able to answer these and other intriguing questions.

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References

[1] Zheng-Zheng Shi Z-Z, Osei-Frimpong J, Kala G, Kala SV, Barrios RJ, Habib GM, Lukin DJ, Danney CM, Matzuk MM, Lieberman MW. Glutathione synthesis is essential for mouse development but not for cell growth in culture. PNAS 2000;97:5101–5106.

[2] Wu AL, Moye-Rowley WS. GSH1, which encodes gammaglutamylcysteine synthetase, is a target gene for yAP-1 transcriptional regulation. Mol Cell Biol 1994;14:5832–5839.

[3] Grant CM, MacIver FH, Dawes IW. Glutathione is an essential metabolite required for resistance to oxidative stress in the yeast Saccharomyces cerevisiae. Curr Genet 1996;29:511–515.

[4] Stephen DWS, Jamieson DJ. Glutathione is an important antioxidant molecule in the yeast Saccharomyces cerevisiae. FEMS Microbiol. Lett 1996;141:207–212.

[5] Sandström BAM. Effects of variation in glutathione peroxidase activity on DNA damage and cell survival in human cells exposed to hydrogen peroxide and t-butyl hydroperoxide. Biochem J 1990;271:17–23.

[6] Sen CK, Packer L. Antioxidant and redox regulation of gene transcription. Faseb J 1996;10:709–720.

[7] Biaglow JE, Varnes ME, Clark EP, Epp ER. The role of thiols in cellular response to radiation and drugs. Radiat Res 1983;95:437–455.

[8] Reichard P, Thelander L. Reduction of ribonucleotides. Ann Rev Biochem 1979;48:133–158.

[9] Klug A, Rhodes. A novel protein motif for nucleic acid recognition. Cold Spring Harbor Symp Quant Biol 1987;52:473–482.

[10] Dijkwel PA, Wenink PW. Structural integrity of the nuclear matrix: differential effects of thiol agents and metal chelators. J Cell Sci 1986;84:53–67.

[11] De Capo A, Ferraro M, Lavia P, Pelliccia F, Finazzi Agro A. Silver staining of the nucleolus organizer regions (NOR) requires clusters of sulfhydril groups. J. Hystochem Cytochem 1982;30:908–911.

[12] Atzori L, Dypbukt JM, Hybbinette SS, Moldeus P, Grafstrom RC. Modifications of cellular thiols during growth and squamous differentiation of cultured human bronchial epithelial cells. Exp Cell Res 1994;211:115–20.

[13] Morales A, Miranda M, Sanchez-Reyes A, Biete A, Fernandez-Checa JC. Oxidative damage of mitochondrial and nuclear DNA induced by ionizing radiation in human hepatoblastoma cells. Int J Radiat Oncol Biol Phys 1998;42:191–203.

[14] Hansen JM, Harris KK, Philbert MA, Harris C. Thalidomide modulates nuclear redox status and preferentially depletes glutathione in rabbit limb versus rat limb. J Pharmacol Exp Ther 2002;300:768–776.
[15] Borras C, Esteve JM, Vina JR, Sastre J, Vina J, Pallardo FV. Glutathione regulates telomerase activity in 3T3 fibroblasts. J Biol Chem 2004;279:34332–34335.
[16] Soderdahl T, Enoksson M, Lundberg M, Holmgren A, Ottersen OP, Orrenius S, Bolcsfoldi G, Cotgreave IA. Visualization of the compartmentalization of glutathione and protein-glutathione mixed disulfides in cultured cells. Faseb J 2003;17:124–1266.
[17] Hansen JM, Go YM, Jones DP. Nuclear and mitochondrial compartmentation of oxidative stress and redox signaling. Annu Rev Pharmacol Toxicol 2006;46:215–234.
[18] Woo HA, Chae HZ, Hwang SC, Yang KS, Kang SW, Kim K, Rhee SG. Reversing the inactivation of peroxiredoxins caused by cysteine sulfinic acid formation. Science 2003;300: 653–656.
[19] Kim JR, Lee SM, Cho SH, Kim JH, Kim BH, Kwon J, Choi CY, Kim YD, Lee SR. Oxidation of thioredoxin reductase in HeLa cells stimulated with tumor necrosis factor-alpha. FEBS Lett 2004;567:189–196.
[20] Gutscher M, Pauleau AL, Marty L, Brach T, Wabnitz GH, Samstag Y, Meyer AJ, Dick TP. Real-time imaging of the intracellular glutathione redox potential. Nat Methods 2008; 5:553–559.
[21] Bellomo G, Palladini G, Vairetti M. Intranuclear distribution, function and fate of glutathione and glutathione-S-conjugate in living rat hepatocytes studied by fluorescence microscopy. Microsc Res Tech 1997;36:243–252.
[22] Briviba K, Fraser G, Sies H, Ketterer B. Distribution of the monochlorobimane-glutathione conjugate between nucleus and cytosol in isolated hepatocytes. Biochem J 1993;294: 631–633.
[23] Thomas M, Nicklee T, Hedley DW. Differential effects of depleting agents on cytoplasmic and nuclear non-protein sulphydryls: a fluorescence image cytometry study. Br J Cancer 1995;72:45–50.
[24] Voehringer DW, McConkey DJ, McDonnell TJ, Brisbay S, Meyn RE. Bcl-2 expression causes redistribution of glutathione to the nucleus. Proc Natl Acad Sci USA 1998;95: 2956–2960.
[25] Hedley DW, Chow S. Evaluation of methods for measuring cellular glutathione content using flow cytometry. Cytometry 1994;15:349–358.
[26] Markovic J, Borras C, Ortega A, Sastre J, Vina J, Pallardo FV. Glutathione is recruited into the nucleus in early phases of cell. Proliferation. J Biol Chem 2007;282:20416–20424.
[27] Markovic J, Mora NJ, Broseta AM, Gimeno A, de la Concepción N, Viña J, Pallardó FV. The depletion of nuclear glutathione impairs cell proliferation in 3T3 fibroblasts. PloS ONE 4:e6413.
[28] Conour JE, Graham WV, Gaskins HR. A combined in vitro/bioinformatic investigation of redox regulatory mechanisms governing cell cycle progression. Physiol Genomics 2004;18: 196–205.
[29] Green RM, Graham M, O'Donovan MR, Chipman JK, Hodges NJ. Subcellular compartmentalization of glutathione: correlations with parameters of oxidative stress related to genotoxicity. Mutagenesis 2006;21:383–390.
[30] Menon SG, Sarsour EH, Spitz DR, Higashikubo R, Sturm M, Zhang H, Goswami PC. Redox regulation of the G1 to S phase transition in the mouse embryo fibroblast cell cycle. Cancer Res 2003;63:2109–2117.
[31] Klevecz RR, Bolen J, Forrest G, Murray DB. A genomewide oscillation in transcription gates DNA replication and cell cycle. Proc Natl Acad Sci USA 2004;101:1200–1205.
[32] Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 2001;30: 1191–1212.
[33] Soboll S, Grundel S, Harris J, Kolb-Bachofen V, Ketterer B, Sies H. The content of glutathione and glutathione S-transferases and the glutathione peroxidase activity in rat liver nuclei determined by a non-aqueous technique of cell fractionation. Biochem J 1995;311:889–894.
[34] Carpenter G, Cohen S. Epidermal growth factor. J Biol Chem 1990;265:7709–7712.
[35] Jang JH, Surh YJ. Potentiation of cellular antioxidant capacity by Bcl-2: implications for its antiapoptotic function. Biochem Pharmacol 2003;66:1371–1379.
[36] Kim Y-C, Yamaguchi Y, Kondo N, Masutani H, Yodoi J. Thioredoxin-dependent redox regulation of the antioxidant responsive element (ARE) in electrophile response. Oncogene 2003;22:1860–1865.
[37] Bloom D, Dhakshinamoorthy S, Jaiswal AK. Site-directed mutagenesis of cysteine to serine in the DNA binding region of Nrf2 decreases its capacity to upregulate antioxidant response element-mediated expression and antioxidant induction of NAD(P)H:quinone oxidoreductase1 gene. Oncogene 2002;21:2191–2200.
[38] Cotgreave IA, Gerdes RG. Recent trends in glutathione biochemistry-glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? Biochem Biophys Res Commun 1998;242:1–9.
[39] Fratelli M, Goodwin LO, Orom UA, Lombardi S, Tonelli R, Mengozzi M, Ghezzi P. Gene expression profiling reveals a signaling role of glutathione in redox regulation. Proc Natl Acad Sci USA 2005;102:13998–14003.
[40] Smith CV, Jones DP, Guenthner TM, Lash LH, Lauterburg BH. Compartmentation of glutathione: implications for the study of toxicity and disease. Toxicol Appl Pharmacol 1996; 140:1–12.
[41] Ho YF, Guenthner TM. Isolation of liver nuclei that retain functional trans-membrane transport. J Pharmacol Toxicol Methods 1997;38:163–168.
[42] Feldherr CM, Akin D. EM visualization of nucleocytoplasmic transport processes. Electron Microsc Rev 1990;3: 73–86.
[43] Nigg EA. Nucleocytoplasmic transport: signals, mechanisms and regulation. Nature 1997;386:779–787.
[44] Ribbeck K, Gorlich D. Kinetic analysis of translocation through nuclear pore complexes. Embo J 2001;20: 1320–1330.
[45] Feldherr CM, Akin D. The permeability of the nuclear envelope in dividing and nondividing cell cultures. J Cell Biol 1990;111:1–8.
[46] Feldherr CM, Akin D. Regulation of nuclear transport in proliferating and quiescent cells. Exp Cell Res 1993;205: 179–186.
[47] Krajewski S, Tanaka S, Takayama S, Schibler MJ, Fenton W, Reed JC. Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes. Cancer Res 1993;53:4701–4714.
[48] Zimmermann AK, Loucks FA, Schroeder EK, Bouchard RJ, Tyler KL, Linseman DA. Glutathione binding to the Bcl-2 homology-3 domaingroove: a molecular basis for Bcl-2 antioxidant function at mitochondria. J Biol Chem 2007;282: 29296–29304.
[49] Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. Pharmacol Ther 1991;51:155–194.
[50] Boyland E, Chasseaud LF. Enzyme-catalysed conjugations of glutathione with unsaturated compounds. Biochem J 1967;104:95–102.
[51] Kosower NS, Kosower EM. The glutathione status of cells. Int Rev Cytol 1978;54:109–160.

[52] Li N, Oberley TD. Modulation of antioxidant enzymes, reactive oxygen species, and glutathione levels in manganese superoxide dismutaseoverexpressing NIH/3T3 fibroblasts during the cell cycle. J Cell Physiol 1998;177:148–160.
[53] Rahman I, Biswas SK, Jimenez LA, Torres M, Forman HJ. Glutathione, stress responses, and redox signaling in lung inflammation. Antioxid Redox Signal 2005;7:42–59.
[54] Borroz KI, Buetler TM, Eaton DL. Modulation of gamma-glutamylcysteine synthetase large subunit mRNA expression by butylated hydroxyanisole. Toxicol Appl Pharmacol 1994; 126:150–155.
[55] Spyrou G, Holmgren A. Deoxyribonucleoside triphosphate pools and growth of glutathione-depleted 3T6 mouse fibroblasts. Biochem Biophys Res Commun 1996;220:42–46.
[56] Esposito F, Russo T, Cimino F. Generation of prooxidant conditions in intact cells to induce modifications of cell cycle regulatory proteins. Methods Enzymol 2002;352: 258–268.
[57] Mirkovic N, Voehringer DW, Story MD, McConkey DJ, McDonnell TJ, Meyn RE. Resistance to radiation-induced apoptosis in Bcl-2-expressing cells is reversed by depleting cellular thiols. Oncogene 1997;15:1461–1470.
[58] Chen JDM, Odwin S, He P, Trush MA, Yager JD. Enhanced mitochondrial gene ranscript, ATP, bcl-2 protein levels, and altered glutathione distribution in ethynil estradiol cultured female rat hepatocytes. Toxicol Sci 2003;75: 271–278.
[59] Perreault SD, Wolff RA and Zirkin BR. The role of disulphide bond reduction during mammalian sperm nuclear decondensation in vitro. Dev Biol 1984;101:160–167.
[60] Maeda Y, Yanagimachi H, Tateno H, Usui N, Yanagimachi R. Decondensation of the mouse sperm nucleus within the interphase nucleus. Zygote 1998;6:39–45.
[61] Romanato M, Regueira E, Cameo MS, Baldini C, Calvo L, Calvo JC. Further evidences on the role of heparan sulfate as protamine acceptor during the decondensation of human spermatozoa. Human Repro 2005;20:2784–2789.
[62] Meshorer E. Chromatin in embryonic stem cell neuronal differentiation. Histol Histopathol 2007;22:311–319.
[63] Meshorer E, Misteli T. Chromatin in pluripotent embryonic stem cells and differentiation. Nat Rev Mol Cell Biol 2006;7: 540–546.
[64] Meshorer E, Yellajoshula D, George E, Scambler PJ, Brown DT, Misteli T. Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. Dev Cell 2006;10: 105–116.
[65] Efroni S, Duttagupta R, Cheng J, Dehghani H, Hoeppner DJ, Dash C, Bazett-Jones DP, Le Grice S, MacKay RD, Buetow KH. Global transcription in pluripotent embryonic stem cells. Cell Stem Cell 2008;2:437–447.
[66] Park SH, Kook MC, Kim EY, Park S, Lim JH. Ultrastructure of human embryonic stem cells and spontaneous and retinoic acid-induced differentiating cells. Ultrastruct Pathol 2004;28:229–238.
[67] Takahashi S, Zeydel M. Gamma-Glutamyl transpeptidase and glutathione in aging IMR-90 fibroblasts and in differentiating 3T3 L1 preadipocytes. Arch Biochem Biophys 1982;214:260–267.
[68] Warshaw JB, Wilson CW, Saito K, Prough RA. The responses of glutathione and antioxidant enzymes to hyperoxia in developing lung. Pediatr Res 1985;19:819–823.
[69] Bai H, Konat GW. Hydrogen peroxide mediates higher order chromatin degradation. Neurochem Int 2003;42:123–129.
[70] Tas S, Walford RL. Influence of disulfide-reducing agents on fractionation of the chromatin complex by endogenous nucleases and deoxyribonuclease I in aging mice. J Gerontol 1982;37:673–679.
[71] Hitchler MJ, Domann FE. An epigenetic perspective on the free radical theory development. Free Radic Biol Med 2007; 43:1023–1036.
[72] Torres L, Sandoval J, Penella E, Zaragozá R, García C, Rodríguez JL, Viña JR, García-Trevijano ER. *In vivo* GSH depletion induces c-myc expression by modulation of chromatin protein complexes. Free Radic Biol Med 2009; 46:1534–1542.
[73] Lertratanangkoon K, Savaraj N, Scimeca JM, Thomas ML. Glutathione depletion-induced thymidylate insufficiency for DNA repair synthesis. Biochem Biophys Res Commun 1997; 234:470–475.
[74] Lertratanangkoon KOR, Scimeca JM. Methyl-donor deficiency due to chemically induced glutathione depletion. Cancer Res 1996;56:995–1005.
[75] Hitchler MJ, Domann FE. Metabolic defects provide a spark for the epigenetic switch in cancer. Free Radic Biol Med 2009;47:115–127.
[76] Dixit K, Asad Khan M, Sharma YD, Uddin M., Alam K. Physicochemical studies on peroxynitrite-modified H3 histone. Int J Biol Macromol 2010;46:20–26.
[77] Sharma R, Nakamura A, Takahashi R, Nakamoto H, Goto S. Carbonyl modification in rat liver histones: Decrease with age and increase by dietary restriction. Free Radic Biol Med. 2006;40:1179–1184.
[78] Enright HU, Miller WJ, Hebbel RP. Nucleosomal histone protein protects DNA from iron-mediated damage. Nucleic Acids Res 1992;20:3341–3346.
[79] Khan AU, Mei YH, Wilson T. A proposed function for spermine and spermidine: protection of replicating DNA against damage by singlet oxygen. Proc Natl Acad Sci USA 1992;89: 11426–11427.
[80] Khan AU, Di Mascio P, Medeiros MGG, Wilson T. Spermine and spermidine protection of plasmid DNA agains single-strand breaks induced by singlet oxygen. Proc Natl Acad Sci USA 1992;89:11428–11439.
[81] Ha HC, Sirisoma NS, Kuppusamy P, Zweier JL, Woster PM, Casero RA Jr. The natural polyamine spermine functions directly as radical scavenger. Proc Natl Acad Sci USA 1998;95:11140–11145.
[82] Oberdoerffer P, Sinclair DA. The role of nuclear architecture in genomic instability and ageing. Nature Reviews Mol Cell Biol 2007;8:692–702.
[83] Wondrak GT, Cervantes-Laurean D, Jacobson EL, Jacobson MK. Histone carbonylation in vivo and in vitro. Biochem J 2000;351:769–777.
[84] Hassa PO, Haenni SS, Elser M, Hottiger MO. Nuclear ADP-ribosylation reactions in Mammalian cells: where are we today and where are we going? Microbiol Mol Biol Rev 2006;70:789–829.
[85] Boulikas T. Poly(ADP-ribosylated) Histones in chromatin replication 1990;265:14638–14647.
[86] Meyer-Ficca ML, Scherthan H, Bürkle A, Meyer R. Poly (ADP-ribosyl)ation during chromatin remodeling steps in rat spermiogenesis. Chromosoma 2005;114:67–74.
[87] Lindahl T, Wood RD. Quality control by DNA repair. Science 1999;286:1897–1905.
[88] Decker P, Muller S. Modulating poly(ADP-ribose) polymerase activity: potential for the prevention and therapy of pathogenic situations involving DNA damage and oxidative stress. Curr Pharm Biotechnol 2002;3:275–283.
[89] Szabó E, Virág L, Bakondi E, Gyüre L, Haskó G, Bai P, Huyadi J, Gergel P, Sabó C. Peroxynitreite production, DNA breakage and poly(ADP-ribose) polymerase activation in a mouse model of oxazolone-induced contact hypersensitivity. J Invest Dermatol 2001;117:74–80.
[90] Realini CA, Althaus FR. Histone shutting by poly-(ADP) ribosylation. J Biol Chem 1992;267:18858–18865.
[91] Kraus WL, Wong J. Nuclear receptor-dependent transcription with chromatin. Is it all about enzymes? Eur J Biochem 2002;269:2275–2283.
[92] Kraus WL, Lis JT. PARP goes transcription. Cell 2003;113: 677–683.

[93] Tanuma S, Endo H. Mono (ADP-ribosy)ation of Gi by eukaryotic cystein-specific mono (ADP-ribosyl) transferase attenuates inhibition of adenylate cyclase by epinephrine. Biochim Biophys Acta 1989;1010:246–249.
[94] Netto LE, de Oliveira MA, Monteiro G, Demasi AP, Cussiol JR, Discola KF, Demasi M, Silva GM, Alves SV, Faria VG, Horta BB. Reactive cysteine in proteins: protein folding, antioxidant defense, redox signaling and more. Comp Biochem Physiol C Toxicol Pharmacol 2007;146:180–193.
[95] Hake SB, Allis CD. Histone H3 variants and their potential role in indexing mammalian genomes: the "H3 barcode hypothesis". Proc Natl Acad Sci USA 2006;103:6428–6435.
[96] Workman JL, Abmayr SM. Histone H3 variants and modifications on transcribed genes. Proc Natl Acad Sci USA 2004;101:1429–1430.
[97] McKittrick E, Gafken PR, Ahmad K, Henikoff S. Histone H3.3 is enriched in covalent modifications associated with active chromatin. Proc Natl Acad Sci USA 2004;101:1525–1530.
[98] Gergel D, Cederbaum AI. Inhibition of the catalytic activity of alcohol dehydrogenase by nitric oxide is associated with S-nitrosylation and the release of zinc. Biochemistry 1996;35:16186–16194.
[99] Lipton SA, Choi Y-B, Takahashi H, Zhang D, Li W, Godzik A, Bankston LA. Cysteine regulation of protein function- as exemplified by NMDA receptor modulation. Trends Neurosci 2002;25:474–480.
[100] Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8A resolution. Nature 1997;389:231–233.
[101] Bode J, Standt UD. Structural aspects of histone complexes and nucleosomes revealed by the accessibility of cysteine side chains. Z Naturforsch C 1978;33:884–890.
[102] Camerini-Otero RD, Felsenfeld G. Histone H3 disulfide dimers and nucleosome structure. Proc Natl Acad Sci USA 1977;74:5519–5523.
[103] Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y. Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell 2004;116:51–61.

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[104] Smith S, Blobel G. The first membrane spanning region of the Lamin B receptor is sufficient for sorting to the inner nuclear membrane. J Cell Biol 1993;120:631–637.
[105] Makatsori D, Kourmouli N, Polioudaki H, Shultz LD, McLean K, Theodoropoulos PA, Singh PB, Georgatos SD. The inner nuclear membrane protein lamin B receptor forms distinct microdomains and links epigenetically marked chromatin to the nuclear envelope. J Biol Chem 2004;279:25567–25573.
[106] Polioudaki H, Kourmouli N, Drosou V, Bakou A, Theodoropoulos PA, Singh PB, Giannakouros T, Georgatos SD. Histones H3/H4 form a thight complex with the inner nuclear membrane protein LBR and heterochromatin protein 1. EMBO Rep 2001;2:920–925.
[107] Prior CP, Cantor CR, Johnson EM, Littau VC, Allfrey VG. Reversible changes in nucleosome structure and histone H3 accessibillity in transcriptionally active and inactive states of rDNA chromatin. Cell 1983;34:1033–1042.
[108] Lewis PN, Chiu SS. Effect of histone H3 sulphydryl modifications on Histone-Histone interactions and nucleosoma formation and structure. Eur J Biochem 1980;109:369–376.
[109] Lewis PN. On the native structure of the histone H3-H4 complex. Biochem. Biophys Res Commun 1976;68:329–335.
[110] Lewis PN. Histone-histone interactions. II. Structural stability of the histone H3-H4 complex. Can J Biochem 1976;54:963–970.
[111] Hamana K. Triton-Gel electrophoresis of histones containing oxidized methionine and cysteine residues. Ann Rep Med Care Technol 1983;4:115–127.
[112] Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y, Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell 2004;116:51–61.
[113] Camerini-Otero RD, Felsenfeld G. Histone H3 disulphide dimmers and nucleosome structure. Proc Natl Acad Sci USA 1977;74:5519–5523.
[114] Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature 2004;429:457–463.