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Review

Selenium and the Regulation of Cell Signaling, Growth, and Survival: Molecular and Mechanistic Aspects

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ABSTRACT

In the past 30 years, it has been recognized that dietary selenium (Se) is essential for the normal function of many of the systems of the body. Furthermore, low Se intake can have deleterious effects on several aspects of human and animal health. The importance of Se is characterized in its role as a constituent of several key antioxidant and redox enzyme families. Most of the effects of Se are probably mediated by selenoproteins, which have the micronutrient covalently incorporated into the protein. The purpose of this review is to examine basic mechanisms by which Se regulates cell growth, gene transcription, cell signaling, and cell death. We start with the historical background to Se. The synthesis and function of selenoproteins are described, followed by details of the dietary sources of Se and Se status in different parts of the world, together with the clinical effects of Se deficiency and toxicity. We consider some aspects of the molecular mechanisms by which Se modulates cell growth, intracellular signaling, and gene transcription. Antioxid. Redox Signal. 4, 339–351.

SELENIUM—AN INTRODUCTION

SELENIUM (Se) was discovered in 1817 by the Swedish chemist, Jöns Jacob Berzelius, who named the element after Selene the Greek goddess of the moon. The element is widely used in industry and can be highly toxic. In the 1950s, it was demonstrated that Se was an essential trace element as it could prevent liver necrosis in vitamin E-deficient rats (76). Initially, Se was considered to have an antioxidant role with the discovery that it was a constituent of the antioxidant enzyme, cytosolic glutathione peroxidase (cyGPX) (72). It is now clear, however, that Se is essential to human nutrition, acting through the expression of a wide range of selenoproteins that have diverse roles. Thus, Se can act as a growth factor, has powerful antioxidant and anticancer properties, and is involved in thyroid hormone homeostasis, immunity, and fertility. Many selenoproteins have now been identified by sodium dodecyl sulphate–polyacrylamide gel electrophoresis of ^{75}Se-labelled tissue, but only up to 21 have been characterized by purification and cloning (6, 32, 51). Se is classed as a metalloid by virtue of sharing properties of both metals and nonmetals and lies between sulphur and tellurium in Group VI of the Periodic Table of Elements. Chemical similarities exist between Se and sulphur, but despite sharing some physical and chemical properties, Se and sulphur are not interchangeable in biological systems (26). Se exists naturally as inorganic selenides, selenites, and selenates and as organic selenoamino acids, which include selenocysteine and selenomethionine (SM). Humans and animals receive dietary Se as organic selenoamino acids through ingestion of plant and animal proteins, and inorganic forms, such as sodium selenite and (SS) sodium selenate, are often used in dietary supplements (animal and human) and in experimental diets.

SELENOPROTEIN SYNTHESIS

Se is incorporated specifically into selenoproteins as selenocysteine residues through a cotranslational event directed by the UGA codon (40). Although the UGA codon was originally identified as a termination codon, in selenoprotein synthesis the cell is required to recognise the UGA as a code to

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insert a selenocysteine residue rather than terminate protein synthesis.

The mechanism for the synthesis of selenoproteins was first characterized in *E. coli*. The synthesis of selenocysteine and its insertion into specific selenoproteins in prokaryotes involve the products of four genes (*selA, selB, selC,* and *selD*). The gene products are as follows: a selenocysteine-specific tRNA species ($tRNA^{Sec}$) (*selC*), which carries the anticodon for UGA; the enzymes, selenocysteine synthase (*selA*) and selenophosphate synthetase (*selD*), which are essential for the formation of selenocysteine-$tRNA^{Sec}$ from seryl-$tRNA^{Sec}$; and the elongation factor that specifically recognises the selenocysteine-tRNA (*selB*) (for reviews, see 1, 13, 40).

In eukaryotes, the process of selenoprotein synthesis has similarities to the mechanism in *E. coli,* but also differs in many crucial features. At least two forms of the $tRNA^{Sec}$ have been isolated in eukaryotes (59). Like bacterial $tRNA^{Sec,}$ in eukaryotes $tRNA^{Sec}$ is esterified with serine and is subsequently converted to seryl-$tRNA^{sec}$. However, the nature of the mRNA selenocysteine insertion sequence (SECIS) elements, which are responsible for the recognition of the UGA as a selenocysteine insertion codon, differs between prokaryotes and eukaryotes (49). In prokaryotic selenoproteins, these sequences form a stem-loop structure immediately downstream (3′) from the UGA codon in the open reading frame. In contrast, eukaryotic SECIS elements are located in the 3′-untranslated region of the mRNA (36). Two selenophosphate synthetases (Sel D in prokaryotes) have been identified in eukaryotes and named *Sps 1* and *Sps 2*. *Sps2* is a Se-containing protein; thus, if its activity decreases when Se supplies are limited, this would provide a sensitive autoregulation of selenoprotein synthesis. Such a mechanism would be particularly important if *Sps 2* is specific for individual selenoproteins. Additionally, two proteins have been identified in eukaryotes that carry out the functions equivalent to Sel B. These proteins, one that binds to the SECIS sequence (SBP2) and a second that is a selenocyseyl-$tRNA^{[Ser]Sec}$-specific elongation factor (eEFSec), allow the translation of UGA as selenocysteine instead of a termination codon. The interactions between SBP2, eEFSec, and the termination release factors eRF1 and eRF3 may provide yet another level of control of selenoprotein synthesis that ensures the protein and tissue-specific expression that occurs with differing Se intake *in vivo* (36, 59).

SELENOPROTEINS

The many biochemical roles of Se and the large number of diseases with which Se deficiency has been associated are reflected in the number of selenoproteins that have been identified. Approximately 30–40 selenoproteins can be demonstrated by ^{75}Se labelling of mammals *in vivo* or in cells in culture. Many of these proteins have been further characterized by purification and/or cloning. Bioinformatic techniques have also been used to search published nucleotide sequences for SECIS insertion elements that are characteristic of selenoprotein synthesis and are thus used to identify potential sequences for selenoproteins. Some potential selenoproteins identified in this manner have been expressed, although in all cases their enzyme activities or functions have not been identified.

Of the selenoproteins that have been characterized (Table 1), four are glutathione peroxidases (GPXs), three are iodothyronine deiodinases, and three are thioredoxin reductases (TRs). Thus, Se can influence at least three broad areas of cell biochemistry, namely, antioxidant function, thyroid hormone metabolism, and redox status. There may be some overlap between antioxidant and redox functions. For example, if the antioxidant function of peroxide metabolism is not effi-

TABLE 1. MAMMALIAN SELENOPROTEINS AND THEIR POSTULATED FUNCTIONS

Selenoprotein	*Postulated functions*
Glutathione peroxidase (GPX)	
Cytosolic GPX (GPX I)	Intracellular antioxidant, Se store?
Plasma GPX (GPX III)	Plasma antioxidant
Gastrointestinal GPX (GPX II)	Gastrointestinal tract, antioxidant
Phospholipid hydroperoxide GPX (GPX IV)	Intracellular antioxidant, structural role in spermatozoa
Iodothyronine deiodinase	
Types I and II	Catalyzes the conversion of thyroxine (T4) to 3,5,3′-triiodothyronine
Types I and III	Catalyzes the conversion of T4 to 3,3′,5′ reverse triiodothyronine
Selenoprotein P	
10 or 12 selenocysteines	Transport? Antioxidant role?
Thioredoxin reductase	
TrxR1, TrxR2, and TRβ	Multiple roles associated with its role as part of a dithiol-disulphide oxidoreductase system
Selenoprotein W	Antioxidant role?
Selenophosphate synthetase	Catalyses the production of selenophosphate, required for selenoprotein synthesis
Selenoproteins (R, X), T, N	Unknown (R and X same protein)
15-kDa selenoprotein	T cells
15-kDa selenoprotein	Linked with prostate cancer?
18-kDa mitochondrial selenoprotein	Unknown?
34-kDa nuclear selenoprotein	Antioxidant?

cient, this can have indirect redox effects through peroxides, causing oxidation of cell components. The different "families" of selenoproteins, such as the iodothyronine deiodinases, have many homologies in the structure of their isoforms, and like the SECIS elements, these similarities may be used to identify other proteins of a similar function.

All the selenoproteins that have been characterized contain selenocysteine at the active site. The Se in selenocysteine is fully ionized at physiological pH and acts as a very efficient redox catalyst. Clearly, however, the very different active sites of the characterized selenoproteins confer a wide range of biological activities, thus exploiting the efficient catalysis for many biochemical processes. Detailed studies on the structure and function of many selenoproteins have been reviewed elsewhere (see reference 32 and accompanying chapters therein) and will not be considered in detail in this review. The intracellular compartments in which the principal selenoenzymes are located is illustrated in Fig. 1. TR is discussed in detail as this selenoprotein has the potential to act on many different cellular processes.

THIOREDOXIN REDUCTASE

TR can affect many cellular processes. The enzyme is a member of the pyridine nucleotide-disulphide family of proteins, which includes lipoamide dehydrogenase, mercuric reductase, trypanothione reductase and glutathione reductase (42, 54, 85). However, of these only TR is a selenoprotein (31). TR is a FAD-containing homodimeric selenoenzyme that, together with thioredoxin (Trx) as a substrate and NADPH as a cofactor, forms a powerful dithiol-disulphide oxidoreductase system, referred to as the TR/Trx system. Each TR subunit has a single selenocysteine as the penultimate C-terminal residue (31), and this is essential for enzyme function (64).

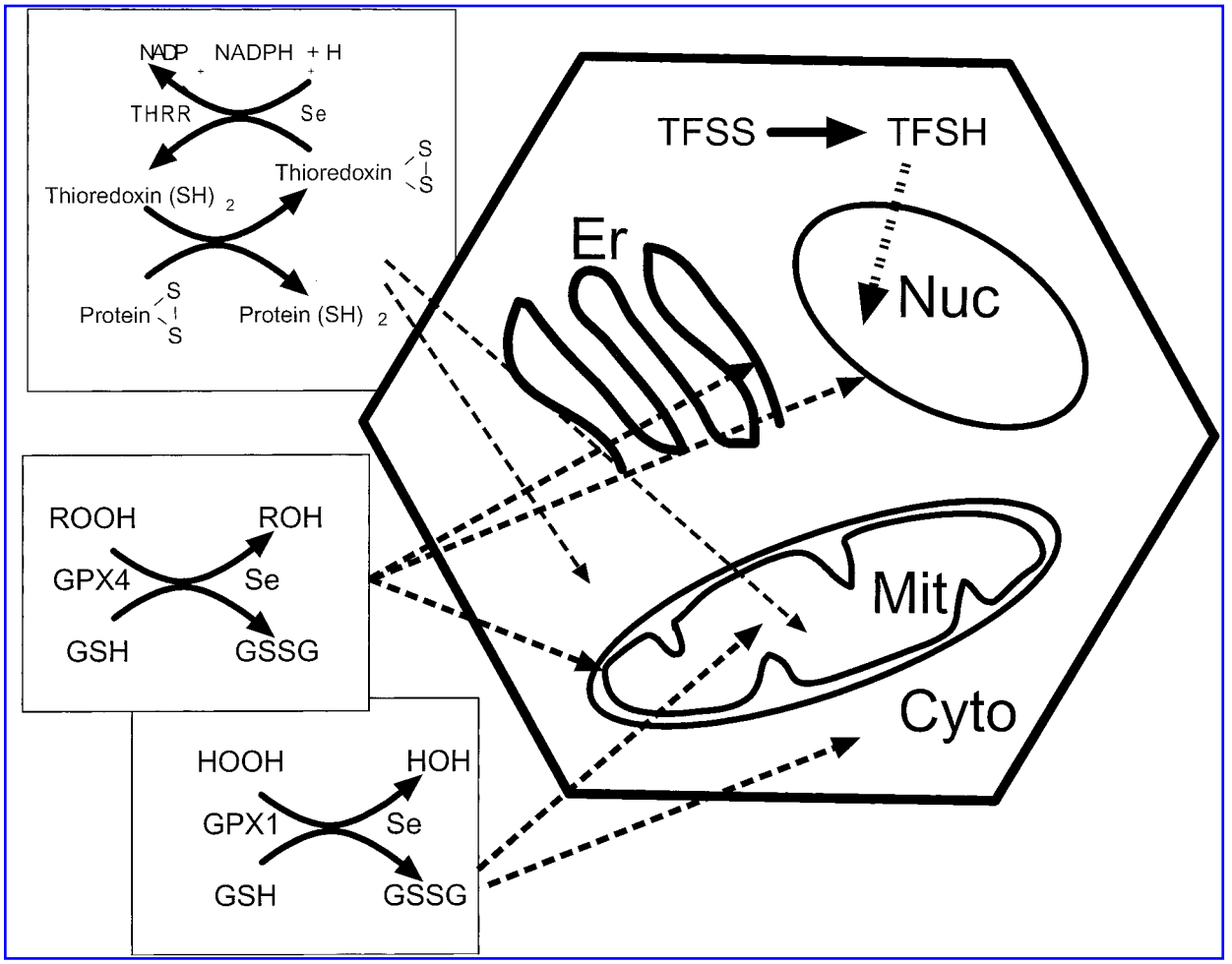

FIG. 1. The location of Se-dependent redox reactions in the cell. The selenoenzymes thioredoxin reductase (THRR, cytosolic, mitochondrial), GPX1 (cytosolic glutathione peroxidase), and GPX4 (phospholipid hydroperoxide glutathione peroxidase; membrane associated) → function in different cell compartments to carry out reductive reactions on peroxides and dithiols. These reactions all favour the reduction of thiol groups on transcription factors (TFSS TFSH). These will then bind to DNA in the nucleus (Nuc) regulating transcription. GPX1 and GPX4 are also essential for regulation of eicosanoid metabolism (not shown). Er, endoplasmic reticulum; Mit, mitochondrion; Cyto, cytoplasm.

Isoenzymes of TR exist, including the cytosolic (TR1) and mitochondrial (TR2) isoforms. The postulated function of mitochondrial TR2 is to provide a mitochondria-specific defense against reactive oxygen species (ROS) produced by the respiratory chain, thus maintaining a redox balance critical for cell survival (54). A third human TR (TR-β) has recently been found that has a distinct pattern of tissue expression, with high levels found in the prostate, testis, liver, uterus, and small intestine (3).

Functions of TR

Mammalian TR can catalyze the reduction of a variety of substrates, including Trx, protein disulphide-isomerase, low molecular weight disulphides, and nondisulphides such as selenite, selenodiglutathione, vitamin K, alloxan, and lipid hydroperoxides (42). TR can also reduce dehydroascorbic acid to ascorbic acid (42). The reduction of Trx (Trx-S_2) to Trx-$(SH)_2$ catalyzed by TR (Eq. 1) provides a powerful protein disulphide reductase (Eq. 2), which has multiple roles.

$$\text{NADPH} + \text{H}^+ + \text{Trx-S}_2 \xrightarrow{\text{TR}} \text{NADP}^+ + \text{Trx-(SH)}_2 \quad (1)$$

$$\text{Trx-(SH)}_2 + \text{protein-S}_2 \xrightarrow{\text{spontaneous}} \text{Trx-S}_2 + \text{protein-(SH)}_2 \quad (2)$$

Trx serves as a hydrogen donor for ribonucleotide reductase in the initial and rate-limiting step in DNA synthesis. The TR/Trx system has also been associated with many diverse cellular functions, including regulation of cell growth, inhibition of apoptosis via the binding of oxidised Trx to the apoptosis signaling kinase-1 (74), and the regeneration of proteins inactivated by oxidative stress, such as nitric oxide synthase (67). TR also regulates gene expression through the activation of DNA-binding activity of transcription factors (11, 28). These include nuclear factor-κB (NF-κB) (60), the glucocorticoid receptor (56), TFIIIC (22), and BZLF1 (7), and the modulation of activator protein-1 (AP-1; Fos/Jun heterodimer) indirectly through a nuclear redox factor ref-1/HAPE (94) is also thought to involve the TR/Trx system. The TR/Trx system regulates the cellular redox state of cells and may also protect against oxidative stress. In a cell-free system, TR/Trx can directly reduce and detoxify hydrogen peroxide, organic hydroperoxides, and lipid hydroperoxides (42) and serve as an electron donor to plasma GPX (14) and type I iodothyronine deiodinase.

As well as the TRs, GPXs, and iodothyronine deiodinases discussed above, there is a Se-containing selenophosphate synthetase that is involved in the synthesis of selenophosphate as a precursor of selenocysteine. The autoregulation of this enzyme where Se supply is limiting has been discussed above.

OTHER SELENOPROTEINS

Selenoprotein P is the major form (>70%) of Se in human plasma. This is an intriguing protein with multiple selenocysteines in its structure; having between 10 and 12 molecules of the amino acids dependent on species. In addition, it contains large numbers of histidine and cysteine molecules, which should confer strong metal-binding properties. The function of selenoprotein P is not known, but may be associated with antioxidant activity and protection of endothelial cells to which it binds on the surface membranes.

Selenoprotein W is found in muscle and many other tissues. Although its function is also uncertain, it may be involved in protection of muscle against the process that leads to myopathy in Se and vitamin E deficiencies.

The other selenoproteins S, T, (R or X), Z, a 15-kDa protein from prostate, a more ubiquitous 15-kDa protein, a 34-kDa nuclear protein, and a further 18-kDa mitochondrial protein have all been either purified or identified by bioinformatic methods (32, 51). However, their true functions have yet to be described. Nonetheless, their existence as specific proteins further emphasizes the wide range of metabolic processes that can potentially be influenced by changes in Se status.

The demonstration of the essentiality for normal health in mammals of the selenoproteins that have been identified comes from many studies in which low dietary Se intake has been associated with disease. Some diseases, particularly in humans, are considered further in this review. However, Se deficiency can have very different effects in different species. Even within a single species, the effects of low Se in the diet can vary, dependent on whether there is concurrent vitamin E deficiency or oxidative stress. Part of the reason for these different responses is the very specific mechanisms whereby there is a selective retention of Se in different organs when dietary supplies are inadequate. Thus, a prioritization of Se supply exists that may help protect crucial biochemical processes and organs (10, 12). Tissues, such as muscle in ruminants, which lose Se most readily and thus have low selenoprotein activities in Se deficiency, are those in which the clinical signs of deficiency are most clearly manifested.

Regulation of selenoprotein expression

Selenoprotein expression is regulated by Se supply, and there is a strict hierarchy of selenoprotein expression (10, 12, 13, 18, 90). Se status does not appear to affect the transcription rate of genes for any of the selenoproteins studied (12, 18, 91). However, changes in the expression of selenoproteins in Se deficiency are accompanied by changes in mRNA levels that appear to arise from alterations in mRNA translation and/or stability (12, 13, 28, 73, 86). Factors other than Se supply influence the expression of specific selenoproteins. For example, oxidative stress leads to induction of TR expression and GPX. Activation of second-messenger pathways also modifies the expression of specific selenoproteins in a tissue-specific manner (2, 9, 43, 44).

Dietary sources of selenium and determination of selenium status

Se can be present in almost any foodstuff at different levels. In the case of plants, the Se content is very dependent on the levels of Se available in the soil. Thus, both humans and animals eating only locally produced foodstuffs will have

a Se status that reflects the Se content of the foods. Daily Se intakes of humans can vary from <5 μg/day up to 3,000 μg/day. However, these intakes represent extremes. In most cases, Se intakes are between 30 and 200 μg/day, and intakes officially recommended by health authorities in different countries vary usually between 50 and 70 μg/day (71). These values are based on the Se intake that has been shown to cause a plateauing of plasma GPX activity or blood GPX activity. Thus, the Se requirements are based on biochemical parameters, and there is no consensus as to other biochemical or physiological effects of Se status that may be used to determine optimal intake. The reliance on the saturation of plasma GPX activity as an indicator of optimal Se intake is due to its accessibility for measurement and its discovery before many of the other selenoproteins described above. The numerous studies in animals that show both selenoprotein-specific and organ-specific Se metabolism indicate the need for investigation of indicators of Se status other than the GPXs. Thus, in particular circumstances, e.g., when thyroid hormone metabolism may be compromised, some direct or indirect measure of deiodinase activity as influenced by Se may provide a suitable indicator of optimal Se intake. The wide range of dietary Se levels that are found throughout the world imply that inadequate supplies of the micronutrient could be involved in disease processes specific to different populations. In many cases, selenoprotein activities will be below the maximum that can be achieved with higher dietary Se intakes, and the consequent biochemical changes need to be investigated.

EFFECT OF LOW SELENIUM STATUS OR SELENIUM DEFICIENCY IN HUMANS

There is still debate, however, concerning the validity of using maximal GPX expression as a nutritional goal because the anticancer effects of Se occur at intakes in excess of 70 μg/day (21, 71). Clear clinical manifestations of Se deficiency in humans occur when intake falls to <12 μg/day. However, many studies have suggested that Se intakes of >12 μg/day, but less than the recommended daily intake, are associated with an increased risk of developing common health problems. These include cancer, cardiovascular disease, malaria, altered immune function, male infertility, rheumatoid arthritis, and AIDS and have been reviewed elsewhere (8, 26, 27, 29, 55, 95).

THE ROLE OF SELENIUM IN THE CONTROL OF CELL GROWTH, CELL SIGNALING, AND GENE EXPRESSION

Selenoproteins indirectly affect cell growth by the effects that TR has on ribonucleotide reductase (42, 88). Trx is reduced by TR and reduced Trx leads to activation of ribonucleotide reductase, an essential enzyme for nucleotide synthesis, leading to DNA synthesis. Also, Trx seems to increase the response of the cell to other growth factors and is commonly secreted in large amounts by tumor cells (70). However, different selenocompounds have effects on growth depending on whether the cells are normal or arise from tumors. Thus, selenocompounds can promote cell death via apoptosis or promote cell survival against death induced by oxidative stress such as wrought by ultraviolet radiation (UVR) or compounds that generate free radicals. Prevention of damage in healthy cells and induction of death by apoptosis in tumor cells may be behind the anticarcinogenic effects of Se. Some of these Se effects are direct, and others appear to be mediated by augmented expression of selenoproteins. The protective effects can be broadly classified into: (a) prevention of oxidative damage to proteins; (b) selective regulation of gene expression; (c) decreased oxidative damage to DNA; and (d) diminished lipid peroxidation and damage to cell membranes.

EFFECTS OF SELENIUM ON CELL SIGNALING AND GENE EXPRESSION

As already indicated, some of the biological effects of Se are a result of antioxidant selenoprotein expression and subsequent removal of ROS and organic peroxides. Free radicals have been recognized as part of the intracellular signaling pathways involved in signal transduction from the cell membranes across the cytoplasm to the nucleus (for reviews, see 24, 52). It is important to appreciate that this is a normal process and only becomes pathological when excessive concentrations of these species are produced, as may occur in inflammatory diseases. The fact that the GPXs and TR can break down these radical species suggests that selenoproteins play a part in the regulation of intracellular signaling. Processes in which Se has been found to exert regulatory effects include the following: (a) ROS-mediated stimulation of protein kinases in the cytoplasm and the nucleus; (b) ROS-mediated covalent modification of thiol, cysteine, and tyrosine groups of proteins; (c) alterations in cellular redox state due to ROS production causing activation of transcription factors leading to *de novo* gene expression; (d) regulation of cell surface and nuclear receptor expression leading to alteration in cell growth, responsiveness, and behavior; and (e) regulation of cell death/survival signals.

Examples of each of these mechanisms will be discussed in the following paragraphs and are summarized in Fig. 2.

SELENIUM EFFECTS ON KINASES AND CELL SURFACE RECEPTORS

The effect of Se compounds on the expression and function of cell-surface receptors has not been studied in depth, but perhaps further research in this area would uncover other mechanisms by which Se controls cell growth and survival.

Fibronectin is an extracellular component important in cell–matrix interactions of cells; thus, this molecule has an important role in the survival of adherent cells and in the metastatic process. Exposure of cells to selenite (but not selenocysteine, selenate, or SM) decreased the number of cell-surface fibronectin receptors. Preincubation with selenite was necessary, indicating that the selenite was not acting directly

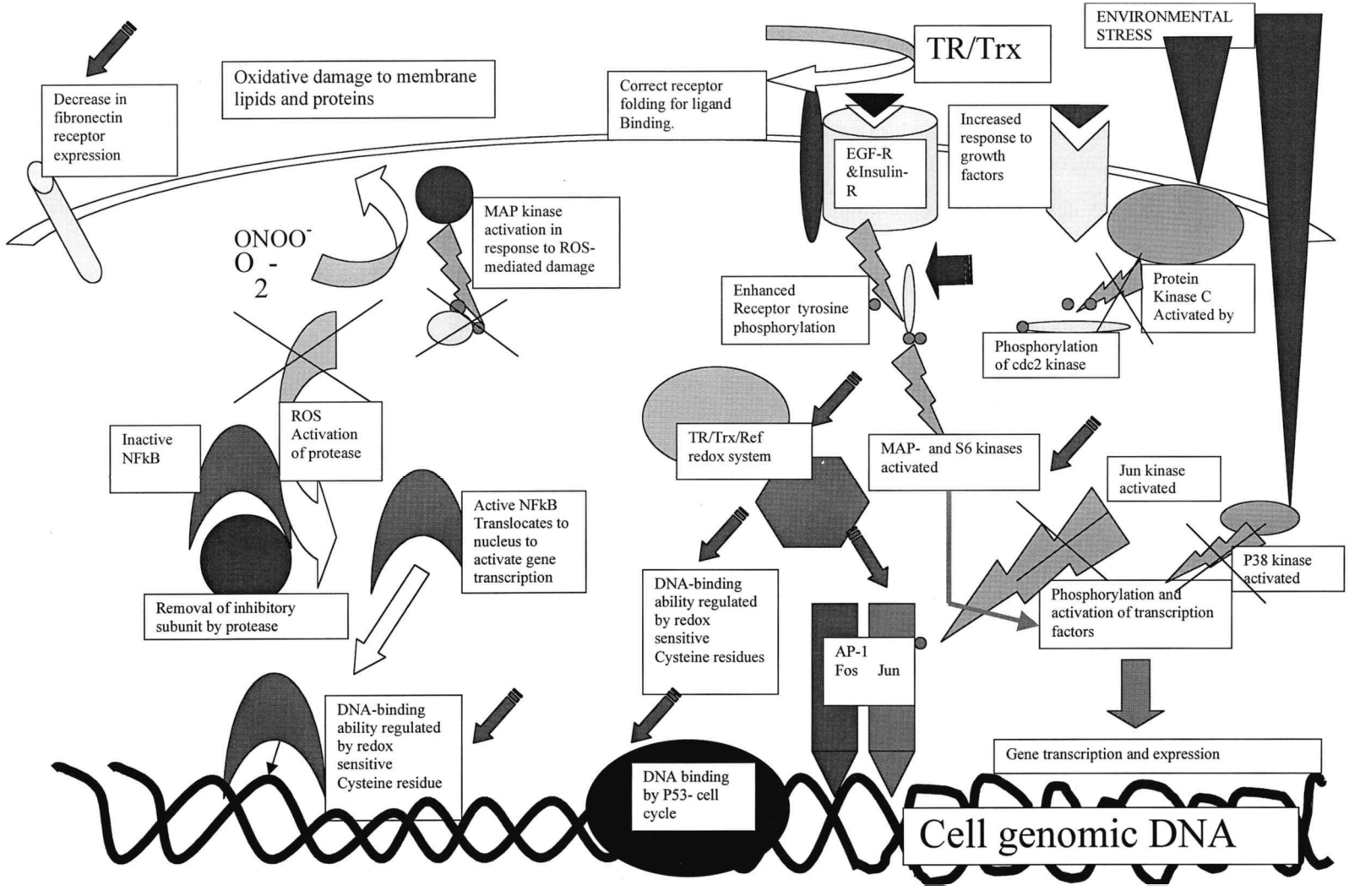

FIG. 2. Regulation of intracellular cell signalling, gene expression and growth by Se compounds. At the top of the figure, the plasma membrane is shown with growth factor receptors and associated kinases. The effects of Se on the transcription factors NFκB, AP-1, and p53 are shown along with inhibitory and stimulatory effects of Se on signaling cascades. Abbreviations are as used in the text. For full details, please refer to the text. = processes stimulated by Se; = translocation; = processes inhibited by Se; = phosphorylation; = reactions catalyzed or initiated; = phosphate groups; = growth factor receptor.

on the receptors (96), but protein synthesis was not necessary either, suggesting that the effect was not mediated by selenoproteins (97). The TR system plays an important role in the folding of the glucocorticoid receptor into a stable conformation for binding glucocorticoids (35).

Se compounds also have potent effects on the intracellular signaling protein kinases. These pathways include the mitogen-activated (MAP) kinases, the p38 kinase, and the c-jun/stress-activated kinase (JNK/SAPK). These are involved in the growth responses of cells to stressful and inflammatory stimuli, including nitric oxide, UVR, hydroperoxides, and superoxide (75 and references therein), and lead to phosphorylation and activation of c-jun, a component of the transcription factor, AP-1 (see below), which is involved in cell growth. Several studies have identified Se (as selenate and methyl selenocysteine) as a stimulator of the tyrosine kinases; particularly in the distal signaling of the insulin signalling cascade. The tyrosine phosphorylation activity of proteins associated with the insulin signaling cascade (the P-subunit of the insulin receptor, IRS-1, and MAP-kinase) was stimulated by the presence of selenate (39, 69, 83). In adipocytes and hepatocytes, both the p42 and p44 MAP kinases were stimulated by selenate, the latter by sixfold (69). There was no effect of selenate on ligand binding for either the insulin receptor or the epidermal growth factor receptor, (EGFR), indicating that the mechanism involved some intracellular component. Pillay and Makgoba proposed that selenate could be causing oxidation of sulphydryl groups, as the stimulatory effects could be reversed by mercaptoethanol treatment (69). The findings were also inconsistent with stimulation of protein phosphatase activity by selenate, and this has been ruled out in adipocytes (23). Stimulation of kinases by Se may explain the insulin mimetic activity of Se. However, stimulation of the tyrosine phosphorylation activity of MAP kinase has deeper implications, as this is a key enzyme on the signaling pathway utilized by many growth factors. Conversely, stimulation of the p38 MAP kinase and protein tyrosine nitrosylation by the oxidant peroxynitrite ($ONOO^-$) is inhibited by selenate (75). This suggests that selenate can inhibit the activation of gene transcription in response to oxidants; these authors also found that hydrogen peroxide could stimulate p38 kinase. An alternative mechanism for p38 kinase inhibition by Se could be through destruction of peroxides by GPX.

Two further simulatory effects of Se on mechanisms that promote cell division have been recorded. Selenate stimulates the S6 kinase (involved in cell growth), which is also activated by insulin (23, 39). The epidermal growth factor receptor tyrosine kinase activity is also stimulated by selenate in A431 cells, leading to autophosphorylation of the receptor (69).

Se compounds with anticancer properties have inhibitory effects on protein kinase Cε, through effects on redox-sensitive cysteine residues within the catalytic domain of the protein (34, 79). These downstream effects were evident as a 72% decrease in the phosphorylation of the cdk2 kinase and a 2.5–7-fold increase in the expression of GADD, a cell senescence gene. This was associated with decreased cdc kinase activity, cessation of DNA synthesis, and apoptosis (79). Recently, Gopalakrishna and Jaken have reviewed the regulation of protein kinase C by oxidants (33). The N-terminal cysteine of protein kinase C binds zinc and mediates protein binding. It is oxidized by $ONOO^-$, thus stimulating protein kinase C activity. However, antioxidants modulate the redox state of the cysteines in the catalytic domain, so inhibiting the enzyme and accounting for some of the anticancer effects of antioxidants. The reduced form of Trx, modulated by TR, also appears to regulate apoptosis by acting as a negative regulator of the apoptosis signaling kinase (74), which promotes apoptosis. Thus, the redox state of the cell influences the propensity of the cells to die by apoptosis, with oxidized Trx, promoting cell death.

Further downstream in the signaling cascade, selenite suppresses the activity of the JNK/SAPK, through a thiol-dependent mechanism, as well as the p38 kinase. Furthermore, selenite suppressed the activity of a luciferase reporter gene dependent on c-jun in the same cells, suggesting inhibition of protooncogene expression (65).

SELENIUM EFFECTS ON TRANSCRIPTION FACTORS

As mentioned earlier, TR can regulate the DNA-binding ability of the transcription factors, including NFκB, AP-1, and the glucorticoid receptor, TFIIIC, and BZLF1 (7, 11, 22, 28, 56, 60, 94). The mechanisms will be examined in more detail. Se can affect transcription factor activation by either affecting DNA-binding strength (regulating the redox state of cysteine residues on the factors that are necessary for binding to DNA) or changing activation of the transcription factor by modulation of regulatory subunits, *e.g.,* by phosphorylation. The DNA repair protein Ref-1 facilitates the binding of several transcription factors, which depend on a redox-sensitive cysteine residue. Cysteine-65 of Ref-1 is the active redox site, and this is reduced by Trx (41). This probably implicates TR indirectly in the redox regulation of several transcription factors. Exactly how Se regulates transcription factors is an area of research that is still being clarified, and the effects of Se on AP-1, NKκB, and p53 are discussed below. The redox regulation of transcription factors has been extensively reviewed (4, 25).

Selenium and AP-1

The AP-1 factor binds to the phorbol ester (TPA) response element present in many stress-induced genes. In turn, AP-1 is stimulated by a variety of stimuli, including UVR, growth factors, osmotic stress, and activators of protein kinase C (for review, see 46). AP-1 is a heterodimeric complex composed of either c-jun family (including c-jun, JunB, and Jun D) homodimers or c-jun–c-fos heterodimers. The c-fos family, which cannot form homodimers, includes c-fos, fosB, Fra-1, and Fra-2. The proteins are joined by a leucine zipper domain that allows binding to the TRE motif on the DNA duplex. The fos–jun complex utilises a conserved cysteine in the DNA-binding domain of both the monomers, consistent with the structure of other transcription factors subject to redox control. Constitutive activation of AP-1 occurs in malignant cells and may be a fundamental route to neoplasia (4, 46). In normal cells, activation of AP-1 induces the expression of a battery of genes associated with inflammation (adhesion molecules, cytokines, and metalloproteinases).

The induction of AP-1 is rapid (within minutes of stimulation) and consists of dephosphorylation of c-jun homodimers that bind to a TRE-like site in the c-jun promoter, inducing *de novo* mRNA and protein synthesis. Transcription of the c-fos gene depends on activation of a serum response factor, which binds to the serum response element site in the c-fos promoter. This reaction is redox-sensitive. The regulation of AP-1 is complex. Both antioxidants and oxidants can activate it under different circumstances (62). The binding of fos–jun complexes to DNA is stimulated by Ref-1 (94). Gold and selenite (5 and 1 μM, respectively) reduced AP-1 binding to DNA by 50% in gel shift assays and decreased expression of an AP-1 (but not an AP-2) coupled reporter gene (37). The authors speculated that this could be the therapeutic mechanism of gold in arthritis. Blocking AP-1 may also be the basis of the anticancer effect of selenite and selenodiglutathione (82). It is probably relevant that 5 μM gold would completely inhibit TR, although it would not affect GPX. Nuclear extracts of lymphocytes exposed to 10 μM selenite for 4 h showed a 50% decrease in AP-1 binding. This was proposed to occur due to oxidation, by selenite or selenodiglutathione, of reduced Trx. This may explain the inhibitory effects of selenite and selenodiglutathione on cell growth, by diminishing the reduced Trx pool. This would also affect other redox-sensitive transcription factors, but it is likely at these high concentrations of selenite that other processes (such as prooxidant DNA damage) are being induced (84).

Selenium and NFκB activity

NFκB is a generic name for a family of transcription factors made up of hetero- or homodimers of related members of the Rel family of DNA-binding proteins. There are five known members of the Rel family, which all contain an ankyrin repeat domain that allows the proteins to bind the inhibitory protein IκB. The NFκB/IκB complex resides in the cytoplasm. Activation requires release of IκB from the core complex, normally by phosphorylation and subsequent activation of the p50 and p65 subunits of NFκB. The NFκB core complex is then free to migrate to the nucleus, where it is able to bind DNA and activate gene expression. Many of the proinflammatory cytokines have NFκB-responsive sites in their promoters. Recently, the inhibition of NFκB for therapeutic purposes has become of great interest, because inappropriate activation of the complex may be important in chronic inflammatory conditions (for review, see 58). Evidence is accumulating that lipoxygenase products may be an important stimulus for NFκB activation (25). Detailed descriptions of the NFκB family are given in several reviews (25, 38, 58).

The NFκB complex can be activated by various stimuli that cause oxidative damage, including UVR, cytokines, and hydrogen peroxide (for reviews, see 25, 38, 58). The simplest evidence to support a role for selenoenzymes in the modulation of NFκB are the observations that both addition of Se compounds to cells in culture and overexpression of either cyGPX or phospholipid GPX decrease the activation of NFκB (15, 50). The literature is complex, because some investigators have added Se to the cells during incubations in culture and others have assessed the effects of the presence of selenite on the ability of NFκB to bind to DNA *in vitro*. Excess levels of selenite *in vitro* probably lead to formation of thiol–selenite adducts (47), which inhibit both the essential cysteine residues on both selenoenzymes and NFκB. If, as has been suggested, transcription factors must be in a reduced state to bind DNA, then high levels of selenite (5–10 μM) as used in some of the experiments (47) would naturally prevent DNA binding and NFκB (and AP-1) activation. However, free selenite does not reach such levels inside cells. Thus, the suggestion that selenoproteins prevent NFκB activation by breaking down excess hydrogen peroxides produced by oxidative damage seems more physiologically relevant, particularly as normal transcription factor operation requires that the cysteine residues be in a reduced state.

In this respect, the following studies suggest that enrichment of selenoenzyme activity by Se supplementation prevents activation of NFκB. Cells overexpressing a cyGPX had lower constitutive ROS levels and lower ROS levels in response to tumor necrosis factor-α stimulation (50). The amounts of the NFκB complex components were unaffected by overexpression, suggesting an abrogation of transcription factor activation rather than interference with synthesis. Furthermore, phosphorylation of IκB was inhibited in the cells overexpressing GPX (50). Similarly, overexpression of phospholipid GPX in a cell line led to decreased activation of NFκB in response to interleukin-1 stimulation (15). The activation of NFκB in HIV-infected patients by tumor necrosis factor-α leads to activation of the long terminal repeat of the virus, which has an NFκB site, and viral replication. Supplementation of T-cell lines with Se increased GPX activity and decreased the activation of a luciferase reporter gene linked to the HIV long terminal repeat (57). These authors claimed that the effect was specific to NFκB, as the activity of AP-1 was not suppressed. It would appear that one explanation of the confusing and often contradictory nature of the literature reflects that the experiments were carried out in different cell types with different Se concentrations and forms. The effects that Se has on transcription factor activation are likely to be dose- and cell type-dependent.

Selenium and p53

The p53 protein has been called the "guardian of the genome" (79). DNA damage leads to phosphorylation of p53 protein and loss of Mdm2 binding and results in stabilization, activation, and accumulation of the protein. Binding of active, phosphorylated p53 to DNA induces either cell-cycle arrest in G1 through induction of the cell cyclin-dependent kinase inhibitor p21, or apoptosis. The p53 protein also induces Fas and Bax genes, as well as other p53-responsive genes (PIGS), (for review, see 19). If DNA damage is severe, then these gene products will initiate cell death through apoptosis. Some of the PIGS result in the generation of ROS that damage mitochondria, leading to release of cytochrome *c* and the initiation of caspase activation and apoptosis (for review, see 19). Expression of the antiapoptotic protein Bcl-2 is decreased by p53; thus, it is the ratio of Bcl-2 to Bax protein, in part, that determines whether damaged cells live or die. Induction of apoptosis by selenodiglutathione in tumor cells was preceded by induction of p53 (93). Cellular redox state

appears to be a critical regulator of p53 activity (66). Specificity of p53-DNA binding is lost by thiol oxidation of the nine cysteines in the DNA-binding domain (92) and a decrease in its transactivation ability (84). Moreover, inactivation of TR in cells leads to a loss of p53 activity, suggesting regulation of p53 cysteine redox state by Trx (68). A possible feedback loop exists between selenoproteins and p53; activation of p53 protein, caused by DNA damage, stimulates transcription of the GPX gene (68).

Effects of selenium on other transcription factors

The MAZ transcription factor is a zinc-finger protein that negatively regulates the protooncogene, c-myc. Screening of adenocarcinoma cell lines grown in the presence or absence of selenite (SS) showed that expression of MAZ was enhanced by Se (63), suggesting a mechanism by which Se could protect against colon cancer. Evidence has been provided to suggest that SS (but not selenate) prevents the binding of 3,5,3′-triiodothyronine (T3) to the rat liver nuclear T3 receptor (17).

Thus, Se can modulate intracellular signaling and transcription factor activity by both direct and indirect methods.

Selenium in cell death and survival

As we have seen, Se can regulate intracellular signaling and transcription factor activation. It is therefore possible that it may also influence the transmission of "death signals" or molecular events leading to their production, which could influence whether a cell survives or embarks or initiates a programmed death or apoptotic programme (19). Some types of apoptosis are p53-dependent to initiate the death pathway and some are not, however, all cases of apoptosis require the activation of caspases (19). The ability of certain Se forms to trigger apoptosis (for review, see 81) may be one basis for their anticancer effects (29). The situation is complicated, because depending on the circumstances Se can promote cell death or protect cells from death due to oxidative damage to DNA, proteins, and lipid peroxidation (for review, see 61). More bewildering is the fact that SS itself can act as a prooxidant under certain conditions and actually cause oxidative damage to the cell (see Fig. 3 and 84). The precise effect of Se on cell survival will depend on the following variables: (a) The type of cell, neoplastic or normal. (b) The type of Se compound (organic or inorganic) and its possible metabolites. Inorganic forms (*e.g.,* SS) are usually more toxic. (c) The dose of Se compound. High levels are cytostatic or toxic, particularly with SS. (d) The levels of other antioxidants (GSH, vitamins C and E) or other metal ions (particularly copper or heavy metals) in the cell. Examples of these effects and possible mechanisms by which Se protects cells from oxidative stress are discussed below.

At low doses in cell culture (nanomolar), both SS and SM protect normal cells from oxidative damage. A good example is the protection of skin cells from the oxidative stress caused by UVR. Skin cells are exposed to oxidative stress from both UVR in the environment and the activities of skin commensal organisms. SS (1–100 n*M*) and SM (50–200 n*M*) prevent UVR-induced necrosis, apoptosis, lipid peroxidation, and oxidative DNA damage, as well as oxidative stress-induced cell death caused by menadione (for review, see 61). Protection by SS from UVR-induced apoptosis may be mediated by inhibition of caspase-3, a key protease for the initiation of apoptosis (61). The protection also appears to require selenoprotein synthesis. However, with both SS and SM, these protective effects are lost at higher doses (>200 n*M*), doses that are nontoxic and not cytostatic (61).

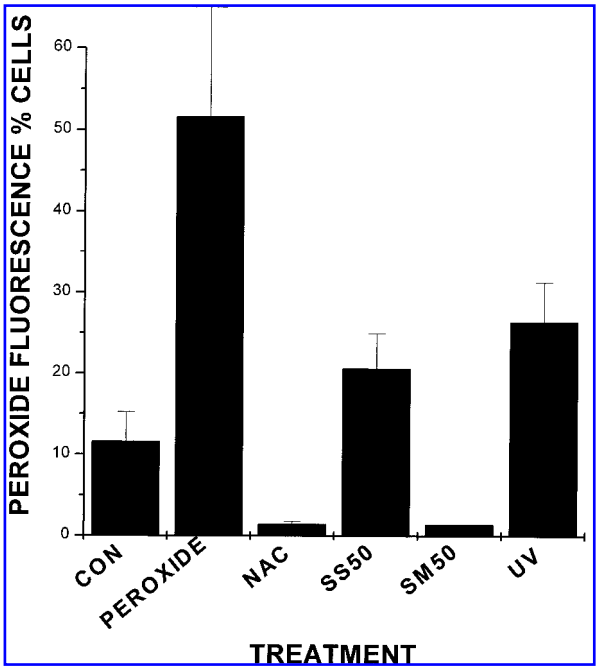

FIG. 3. Relative oxidising activity of Se compounds. HaCaT cells, a keratinocyte cell line, were treated with the following compounds for 24 h prior to incubation with a dye, 2′, 7′-dihydrochlorofluorescein (50 μ*M* in the media), which is sensitive to the production of peroxides. Fluorescence due to hydrogen peroxide and $ONOO^-$ was measured by flow cytometry and is indicated on the ordinate. Control cells (CON) had no additions. UV, 200 J/m^2 ultraviolet B immediately before flow cytometry; SS50, sodium selenite (50 n*M*); SM50, selenomethionine (50 n*M*); Peroxide, hydrogen peroxide (10 m*M*); NAC, *N*-acetylcysteine (30 m*M*).

Se can also protect cells from the damaging effects of reactive nitrogen species. Host cells use nitric oxide to kill bacteria and viruses, and nitric oxide is produced by immune cells, yet under oxidative conditions in which superoxide (O_2^-) is produced concomitantly (usually by neutrophils), the injurious oxidant $ONOO^-$ is produced. If not metabolized, this reactive anion causes damage to proteins (through tyrosine nitrosylation), lipid peroxidation, and single-strand breaks in DNA (89, 90). Selenocysteine and SM protect DNA from $ONOO^-$-mediated damage more effectively than the GPX mimic ebselen or SS (16, 61). Selenoenzymes also protect from $ONOO^-$-mediated damage, *in vitro* studies showed that GPX protects against nitrotyrosine formation in fibroblast lysates (16, 61), and TR can also break down $ONOO^-$ (5).

Tumor cells are killed by incubation with Se compounds by DNA damage-inducing pathways that can be p53-dependent or -independent (61, 89). In human hepatoma cells, sele-

nium dioxide induces p53 and down-regulates Bcl-2 (89). Colon cancer cells, incubated with SM, are induced to die by apoptosis through prolonged cell-cycle arrest due to inhibition of cyclin B and cdc2 kinase (20). Ganther and Spallholz have reviewed the anticancer effectiveness and mechanisms of different classes of Se compounds (29, 81). The methylated selenols and selenocysteines seem to be the most potent Se anticancer compounds. Selenite appears to kill by necrosis caused by its prooxidant and toxic effects at high doses (80). Cell death induced by SS involves activation of p38 MAP kinase, and the c-jun N-terminal kinase (45, 48) and oxidative damage to DNA, evident from the accumulation of 8-hydroxydeoxyguanosine adducts. SM is much less toxic than SS, which is toxic at lower doses than SM or other organic selenocompounds for reasons given above, but even SM can be toxic to cells, but only at levels of >50 μM (77, 80).

The presence of other antioxidants modulates the effects of Se compounds. For example, GSH can act either as a prooxidant, facilitating Se-induced oxidative stress and apoptosis, or as an antioxidant in HepG(2) hepatoma cells (77). A complex relationship appears to exist between the levels of Se and GSH and cell health. Pence's group has recently shown that normal human keratinocytes incubated with vitamin C or copper sulphate were protected from SS oxidative DNA damage and apoptosis (78). However, treatments with SM and Trolox (an antioxidant mixture containing vitamin E) or copper sulphate elevated the level of 8-hydroxydeoxyguanosine. Copper ions appeared to protect cells from oxidative damage caused by SS.

PERSPECTIVE

The redox state of the cell is a critical regulator of intracellular signaling and gene transcription, which in turn regulates the ability of the cell to survive, proliferate, or die. The dietary trace element Se is an important antioxidant for the maintenance of an optimum cellular redox state and to prevent oxidative damage to cellular components. Most of the protective activity of Se can be ascribed to it being incorporated into Se-containing enzymes or selenoproteins. We have described the principal selenoproteins and the vitally important selenoprotein families, the GPXs, and the TRs that confer protection from oxidative damage. From the discussion presented, it is clear that Se plays an important role in the regulation of cell signaling, cell growth, and death. We point out that Se dietary supplementation may be therapeutic against many of the most damaging of human diseases.

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ABBREVIATIONS

AP-1, activator protein-1; cyGPX, cytosolic glutathione peroxidase; GPX, glutathione peroxidase; HIV, human immunodeficie ncy virus; IκB, inhibitor nuclear factor-κB; JNK/SAPK, c-Jun/stress-activated kinase; MAP kinase, mitogen activated protein kinase; NFκB, nuclear factor-κB; $ONOO^-$, peroxynitrite; ROS, reactive oxygen species; Se, selenium; SECIS, selenocysteine insertion sequence; SM, selenomethionine; SS, sodium selenite; TR, thioredoxin reductase; Trx, thioredoxin; UVR, ultraviolet radiation.

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