Review

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Recent advances in the mechanism of selenoamino acids toxicity in eukaryotic cells

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Abstract: Selenium is an essential trace element due to its incorporation into selenoproteins with important biological functions. However, at high doses it is toxic. Selenium toxicity is generally attributed to the induction of oxidative stress. However, it has become apparent that the mode of action of seleno-compounds varies, depending on its chemical form and speciation. Recent studies in various eukaryotic systems, in particular the model organism *Saccharomyces cerevisiae*, provide new insights on the cytotoxic mechanisms of selenomethionine and selenocysteine. This review first summarizes current knowledge on reactive oxygen species (ROS)-induced genotoxicity of inorganic selenium species. Then, we discuss recent advances on our understanding of the molecular mechanisms of selenocysteine and selenomethionine cytotoxicity. We present evidences indicating that both oxidative stress and ROS-independent mechanisms contribute to selenoamino acids cytotoxicity. These latter mechanisms include disruption of protein homeostasis by selenocysteine misincorporation in proteins and/or reaction of selenols with protein thiols.

Keywords: protein aggregation; reactive oxygen species; selenium toxicity; selenocysteine; selenomethionine.

Introduction

Selenium is an essential micronutrient for humans, nearly all animals and some bacteria and archaea, because it is a component of selenoproteins with essential biological functions. In these organisms, selenium is specifically incorporated as the amino acid selenocysteine (SeCys), the so-called 21st amino acid. A complex translational machinery is devoted to this task (1, 2). So far, 25 selenoproteins have been identified in humans (3). Many of them possess redox properties and function as antioxidants in which SeCys is the catalytic residue (4). The last decades have witnessed a growing interest in selenium biology because of its reported beneficial effects in prevention against cancer and other diseases at supranutritional intake levels (5, 6). Selenium is thus becoming a widely used dietary supplement for humans and livestock (7). Potential benefits are, however, not without risk because of the relatively narrow window between intakes that result in efficacy or toxicity (8).

Although the mechanistic bases of selenium toxicity are still not fully understood, toxicity is generally attributed to the ability of seleno-compounds to induce oxidative stress and to generate reactive oxygen species (ROS) (9, 10). However, it has become apparent that selenium compounds differ in their metabolic routes and biological activities and that toxicity of this metalloid, depends on the chemical species under consideration (11).

Because of the chemical similarity between selenium and sulfur, most enzymes involved in sulfur metabolism do not discriminate between the two chalcogen elements (12). As a result, in yeasts and higher plants, inorganic selenium (selenate, selenite) can use the sulfur assimilation pathway (13) to form selenoamino acids [selenomethionine (SeMet), selenohomocysteine (SeHCys) and selenocysteine (SeCys)]. SeMet and SeCys can be incorporated into proteins in the place of methionine and cysteine, generating proteins containing non-genetically encoded selenoamino acids (14). Mammalian organisms do not synthetize SeMet from inorganic Se precursors (15). Instead, SeMet is obtained from the diet and has been estimated to account for more than 50% of human dietary selenium (16).

This review focuses on recent advances on selenoamino acid toxicity in eukaryotic cells, with a particular emphasis on the use of the *Saccharomyces cerevisiae* model to elucidate at the molecular level, the toxic effects of SeMet and its metabolic derivatives. For the sake of comparison, current knowledge on the mechanism of inorganic selenium toxicity will also be briefly considered, although readers are invited to consult recent reviews on the subject (17–20).

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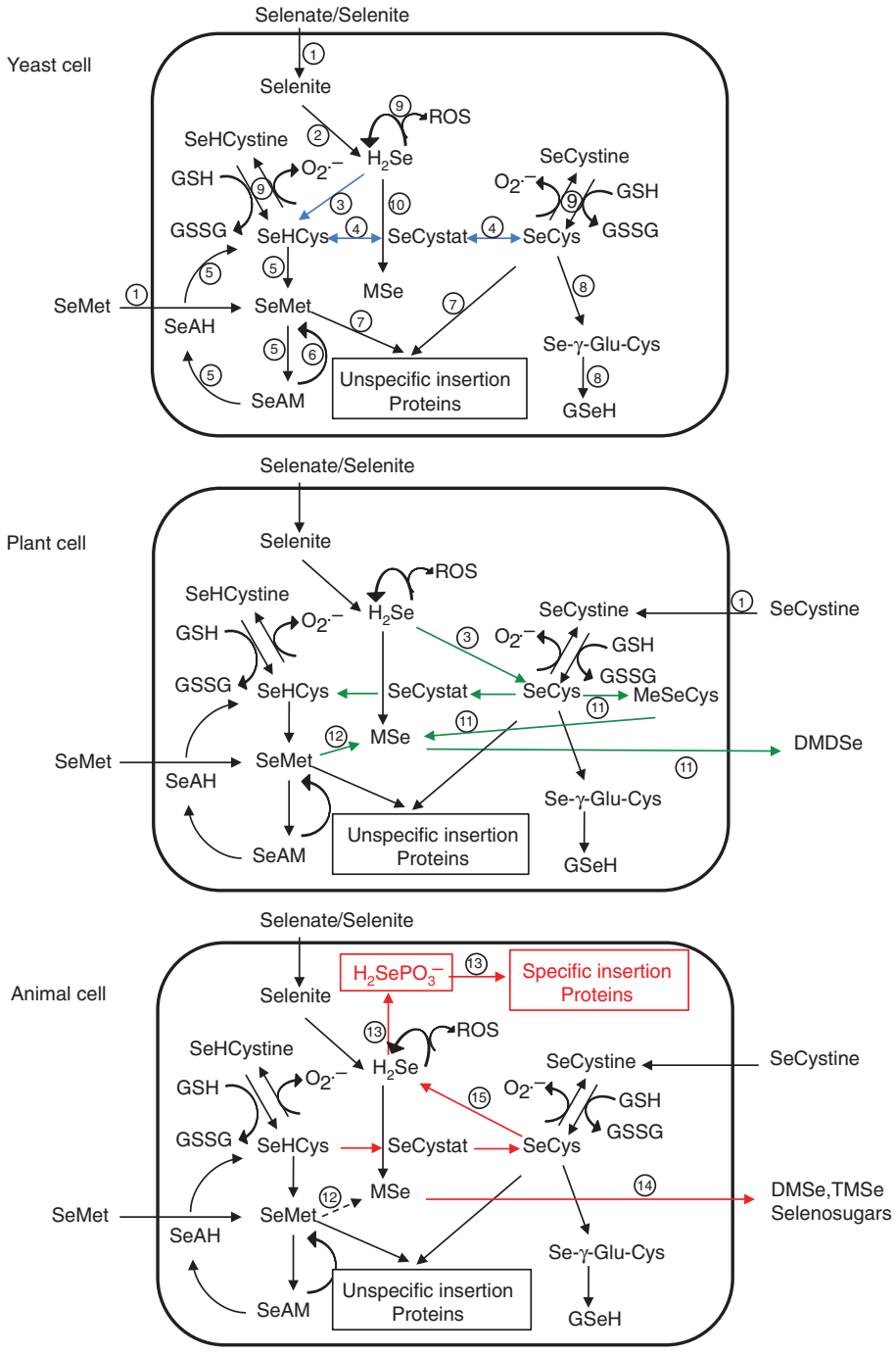

Saccharomyces cerevisiae as an experimental model for the study of selenoamino acid biology and toxicity

Saccharomyces cerevisiae was the first eukaryote to have its complete genome sequenced in 1996 (21). Since then, because of its ease of manipulation and amenability to genetic modifications, yeast has become an extremely powerful model system, to study eukaryotic cell biology (22, 23). The development of high-throughput methodologies combined to a high-level of functional conservation from yeast to humans (24, 25), has proven especially useful in studying the basic biology that underlies cell functioning and even human diseases (26).

Since the 1950s, yeast has been extensively used to study the molecular mechanisms of selenium toxicity (27–30). Although the sulfur amino acid pathway is well

Figure 1: Schematic representation of the main selenium metabolic pathways in eukaryotic cells.
From top to bottom, a *Saccharomyces cerevisiae*, higher plant and mammalian cell are represented with specific pathways highlighted in blue [yeast (31, 32)], green [plant (33–35)], and red [mammalian (36)] arrows. (1) Selenate is taken up by sulfate transporters and reduced to selenite by the sulfate reduction pathway. In yeast and plant cells, selenite is imported by phosphate transporters (37, 38) and by the monocarboxylate transporter Jen1p in yeast (39). The mammalian proteins involved in selenite absorption are anion transporters not yet precisely identified (40). Selenoamino acids are taken up by specific and general amino acid permeases (41). Selenocystine (SeCystine) is not transported across *S. cerevisiae* plasma membrane. (2) Selenite is reduced to selenide (see (Figure 2D). (3) H_2Se is condensed with O-acetylhomoserine to form SeHCys in yeast or with O-acetylserine to generate SeCys in plants. (4) The enzymes of the trans-sulfuration (cystathionine γ-synthase and cystathionine β-lyase) and reverse trans-sulfuration (cystathionine β-synthase and cystathionine γ-lyase) pathways interconvert cysteine and homocysteine. The four enzymes are present in *S. cerevisiae*. Therefore, SeMet and SeCys can each be synthetized from the other. In plants, only the reverse trans-sulfuration pathway functions. In animals, only the direct pathway exists. (5) The methionine cycle is common to all forms of life. SeMet is converted to Se-adenosylmethionine (SeAM) by S-adenosylmethionine (SAM) synthases. SeAM is used in methylation reactions, the product of which is Se-adenosylhomocysteine (SeAH). SeAH is hydrolyzed to SeHCys by S-adenosylhomocysteine hydrolase. SeAH is methylated back to SeMet by methionine synthase, or by betaine-homocyteine methyltransferase in mammals. (6) When SeAM is used in polyamine synthesis (or in plants, in ethylene synthesis), the selenium atom is recycled to SeMet by the Met salvage pathway. (7) SeMet and SeCys may be non-specifically incorporated in proteins by the translation machinery when methionine- or cysteine- tRNA synthetases activate and transfer onto tRNA the seleno-analogue to the cognate sulfur-amino acid. (8) SeCys is used in a two enzymes pathway to synthetize the tripeptide selenoglutathione (GSeH). (9) Selenide and selenols redox cycle with oxygen and glutathione (GSH) (or other thiols) with concomitant generation of ROS (see (Figure 2A and B). (10) Selenide may spontaneously react with SAM to generate methylselenol (MeSe) (42). (11) In several species of plants, SeCys methyltransferase catalyzes the methylation of SeCys to methyl-selenocysteine, which can be further metabolized to volatile MeSe and dimethyldiselenide (DMDSe) (43). (12) Methionine γ-lyase activity catalyzing the conversion of methionine to methylthiol has been characterized in various plants (44). Its occurrence in mammalian cells is doubtful. (13) In animal cells, selenophosphate synthetase generates selenophosphate from H_2Se and ATP (45), which is the precursor for selenoprotein synthesis. (14) Excess selenium can be eliminated by successive methylation to form volatile dimethylselenide and trimethylselenonium or conversion to a selenosugar before excretion (46, 47). (15) Higher eukaryotes SeCys β-lyase catalyzes the conversion of SeCys into alanine and elemental selenium, which is further reduced to H_2Se non-enzymatically (48).

conserved across genus evolution (Figure 1), several differences exist from yeast to humans that should be kept in mind. The major difference lies in the absence, in yeast and higher plant cells, of a specific SeCys incorporation pathway and consequently of genetically encoded selenoproteins. Actually, this specificity can translate into an advantage when studying mechanisms of toxicity, because it allows to ignore the effects related to the function of selenium in the active site of selenoenzymes. Another difference is that trans-sulfuration is reversible in *S. cerevisiae*, whereas it is unidirectional, from methionine to cysteine, in mammals and from cysteine to methionine in higher plants. In mammalian tissues, SeCys can be further transformed by specific selenocysteine β-lyases (48), that decompose SeCys into alanine and an enzyme-bound selenylsulfide intermediate (49) which can be further reduced to H_2Se. In plants, cleavage of SeCys can be performed by cysteine desulfurases (50). These proteins provide the sulfur necessary for iron-sulfur cluster biogenesis and thiol-modification of tRNAs. A cysteine desulfurase also exists in yeast (51). The role of these enzymes in selenium metabolism in yeast and plants is unknown. In several species of plants, SeCys is converted to the less reactive methylselenocysteine by selenocysteine methyltransferases (52). Lastly, methionine γ-lyase (or methioninase) that cleaves selenomethionine to MeSe, α-ketobutyrate and ammonia, exists in bacteria and plants (53). The presence of such an activity has been reported in mammalian and fish cells but is questionable.

Despite these differences, the yeast model offers several advantages to address issues on selenium toxicity. For instance, it is admitted that the effects of selenium are strongly dependent upon its chemical form (54). Metabolization of seleno-compounds *in vivo* gives rise to multiple different metabolites. For example, more than 60 low-molecular-weight different selenium-metabolites were found in Se-enriched yeast grown in a medium containing selenite (55). Therefore, deciphering the effects of a given metabolite is a major difficulty in selenium studies, which can be overcome by using yeast strains deleted for individual genes in a particular pathway. Another advantage of yeast is the availability of a full genome deletion collection (56). In these mutant strains, a cassette that contains a selectable marker flanked on each side by a different molecular barcode or 'tag' replaces each of the approximately 6000 known open reading frames (ORF). The tags for each ORF deletion being unique, the relative abundance of each strain in a pool can be determined by DNA microarray hybridization, allowing genome-wide fitness assays under various environmental stress conditions (57). Yeast toxicogenomic studies have been applied to hundreds of growth-inhibitory chemicals

with the aim of uncovering their mechanisms of action (58–60). They have helped to identify biological functions involved in the toxicity of several compounds including metals, pesticides and pharmaceutical drugs (61–63).

Selenium and oxidative stress

Today, it is generally admitted that oxidative stress is a determinant factor of selenium cytotoxicity as well as anticarcinogenic properties (17, 64). Pro-oxidant properties of selenium originate from the *in vivo* conversion of seleno-compounds into H_2Se or into selenols (RSeH: SeCys, MeSe, GSeH,…), which are readily oxidized by oxygen with concomitant generation of ROS (Figure 2A and B) (9, 65–68). The products of these oxidation reactions ($Se^{(0)}$ or RSeSeR) can be reduced by low molecular-weight thiols (cysteine, GSH) or by the thioredoxin (Trx) and glutaredoxin (Grx) systems (69, 70), with regeneration of H_2Se or RSeH that will initiate a new cycle of oxidation/reduction (71). These redox cycles consume intracellular antioxidants such as GSH and, consequently, the reducing cofactor NADPH. They lead to massive production of ROS, which can damage nucleic acids, proteins and lipids. In addition, oxidized selenols react with protein-thiols, to form selenylsulfide bridges (Figure 2C) (72–75) and can also catalyze the formation of disulfide bridges between low molecular-weight thiols and proteins (70) or between proteins (76–78), potentially leading to protein inactivation or aggregation (Figure 2C).

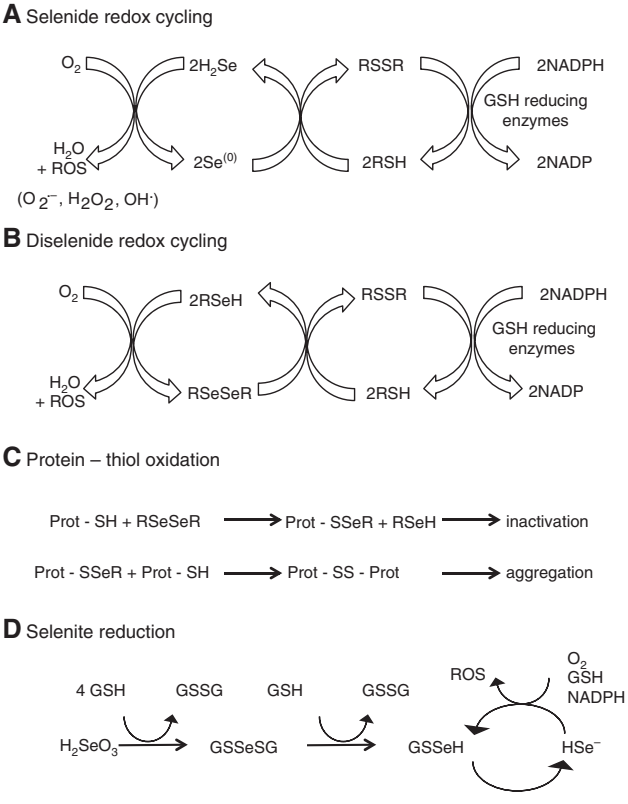

Figure 2: Redox reactions of selenium compounds.

Selenite/selenide toxicity

A large body of evidence, built up in course of the last decades, indicates that the toxicity of selenite is mainly caused by DNA damage (19, 28). In animal and yeast cells, selenite has been shown to induce ROS-dependent DNA strand breaks and/or base oxidation that lead to cell death by apoptosis or necrosis (30, 79–84). It is likely that reduction of selenite into hydrogen selenide *in vivo* (Figure 2D) (85–87), followed by redox cycling of selenide in the presence of oxygen and thiols (Figure 2A), accounts for selenite-induced DNA damage. Indeed, *in vitro* studies (68, 82) showed that selenide induced DNA single-strand breaks caused by hydroxyl or hydroxyl-like radicals produced upon oxidation of selenide by oxygen. In yeast, studies of individual deletion mutants (88–91) and more recently, two independent genome-wide analyses (68, 92) confirmed the importance of DNA repair systems, especially the homologous recombination pathway, in resistance to selenite/selenide exposure.

In addition, selenite exposure promotes redox imbalance and oxidative stress. Accumulation of superoxides and hydrogen peroxide was observed in various eukaryotic cell lines (80, 93–98), associated to a decrease of the GSH/GSSG ratio and to an increase of protein oxidation and lipid peroxidation (94, 99–101). Transcriptome analyses, in *S. cervisiae* (102) and *Caenorhabditis elegans* (103), revealed that selenite treatment up-regulated genes involved in the oxidative stress response. Several analyses in yeast showed that mutants of GSH or Grx metabolisms were more sensitive to selenite than the wild-type (68, 90, 92, 104, 105). These results indicate that maintaining a proper cellular redox homeostasis is crucial for selenite resistance.

Selenocyst(e)ine toxicity

One major difference between SeCys and cysteine is a significantly lower pK_a value of the selenol function relative to that of the corresponding thiol (5.2 for SeCys vs. 8.3 for cysteine) (106, 107). As a consequence, at physiological pH, reduced SeCys exists as a selenolate ion (RSe^-) whereas cysteine is mostly protonated. This property, as

well as the higher nucleophilicity of the selenolate, contributes to the greater reactivity of SeCys over cysteine in redox reactions (108–110). Thus, for example, a SeCys to cysteine substitution reduces 300 times the catalytic efficiency of the *Escherichia coli* selenoenzyme formate dehydrogenase (111). However, as discussed above, free selenolates can easily react with oxygen and thiols, potentially resulting in oxidative stress and disruption of cellular functions (112). The duality between the beneficial properties of catalytic SeCys vs. the harmful effects of free SeCys provides a rationale for the selection of a SeCys insertion machinery which does not require high concentrations of free SeCys in the cell.

Oxidation of SeCys to the diselenide form is fast at neutral pH [<10 min vs. >10 h for cysteine, in identical conditions (113)]. For this reason, most toxicity studies were performed starting from SeCystine, which is easily reduced intracellularly into SeCys, either by GSH (114), or by the Trx and Grx systems (69). SeCystine has been shown to be toxic to animal cells with IC_{50} ranging from a few μM to a few hundreds of μM (115, 116). Higher sensitivity of cancer cell lines over normal cells suggests a cancer-cell specificity making SeCystine a promising therapeutic agent (115). In various human cells, SeCystine treatment was reported to trigger (i) an increase in intracellular ROS, (ii) an accumulation of DNA strand breaks, (iii) an activation of the prosurvival Pi3K/AKT and MAPK/ERK pathways and (iv) an activation of the p53 pathway which, in turn, induces mitochondrial dysfunction and apoptosis (95, 115, 117, 118). Protection against DNA damage and apoptosis was afforded by various antioxidants and radical scavengers, suggesting that the pro-oxidant properties of SeCys mediate cytotoxicity (119). These results are in agreement with the generally accepted hypothesis that the cytotoxic effects of selenium compounds derive from their ability or that of their metabolites to produce an accumulation of reactive oxygen species. Redox cycling of the diselenide (SeCystine) or of mixed selenylsulfides (CysSeSR) with intracellular thiols and oxygen is a potential source of ROS.

Nevertheless, the ROS-dependent toxicity of SeCys has recently been challenged by the work of Wallenberg et al. (70) who reported that treatment of lung-derived H-157 cancer cells with cytotoxic concentrations of SeCystine did not induce ROS production. The same authors showed that SeCystine triggered endoplasmic reticulum (ER) stress, the unfolded protein response, an increase in protein ubiquitination and extensive cytoplasmic vacuolarization in HeLa cells, in the absence of DNA damage (120). In higher plants also, evidence was recently provided that SeCys treatment impairs protein homeostasis without elevation of superoxide levels (121, 122). It was also reported that a mutation of *Arabidopsis thaliana* BiP2 protein, an ER chaperone that binds to misfolded proteins, prevented germination when plants were grown on SeCys, suggesting errors in ER protein folding or quality control (121).

One mechanism that may explain SeCys-induced proteotoxic stress is non-specific incorporation of SeCys into proteins, which is expected to cause structural and functional alterations. Such a hypothesis was proposed more than 30 years ago from studies in higher plants (123). It was based on the observation that, although Se-tolerant *Astragalus* species accumulated considerably more selenium than did sensitive plants, Se concentration in the protein fractions from the Se-accumulators was reduced nearly 10-fold compared to that from the non-tolerant *Astragalus* species (124). Since then, misincorporation of SeCys in proteins was demonstrated in the plant *Vigna radiata* (mung bean) (125), in *S. cerevisiae* (126, 127) and in *E. coli* cells (128). Another mechanism that may contribute to protein damage is reaction of protein thiols with oxidized selenols such as SeCystine. As pointed out above, this reaction will lead to the formation of selenylsulfide or disulfide bonds, which may in turn cause structural changes leading to protein aggregation or to functional inactivation.

To summarize, SeCys toxicity seems to result from both ROS-dependent and ROS-independent mechanisms. The latter may be due to misincorporation of SeCys in proteins and/or protein-thiol oxidation. The contribution to toxicity of each of these mechanisms remains to be assessed more precisely and may depend on the cell type under study.

Selenomethionine toxicity

A great variability of results were reported on the SeMet dose necessary to elicit toxicity in cultured cells, with IC_{50} values ranging from a few μM to tenths of mM, depending on the cell type, culture conditions and type of assay (129, 130). Such a disparity may be partly explained by the strong inverse dependency of SeMet toxicity on the methionine concentration in the growth medium of yeast or mammalian cell cultures (113, 131). This dependency indicates that SeMet and methionine share common uptake and/or metabolic pathways. Therefore, it is important to take into account the SeMet/Met ratio rather than the selenium concentration only, when comparing the toxic effects of SeMet in various conditions.

Insights into the toxic mode of action of SeMet are scarce. Although misincorporation of SeMet in proteins might in principle generate toxicity, it has been shown in human cells that SeMet can support cell growth in the absence of methionine (131). In yeast mutants, the substitution of more than 90% of protein methioninyl residues by SeMet does not elicit significant toxicity (132–134). Moreover, a *S. cerevisiae* mutant unable to metabolize SeMet to its primary metabolic product, SeAM, displays low SeMet sensitivity (135). Therefore, SeMet toxic effects must be mediated by one or several of its metabolic products (see Figure 1) rather than by itself.

Comparison of the effects of SeMet and selenite on the growth of wild-type and mutant yeast cells indicated the lesser importance of DNA damage in SeMet vs. selenite toxicity (91). Likewise, SeMet treatment did not result in DNA damage in human lymphocytes, even at cytotoxic concentrations (136). Studies investigating the effects of toxic concentrations of SeMet and selenite in human carcinoma cell lines revealed apoptotic responses specific for each compound (137, 138). These results indicate that the mechanistic bases of SeMet and selenite toxicities are substantially different. Therefore, formation of selenide, which plays a central role in the toxic effects of selenite, is unlikely to be responsible for the toxicity of SeMet.

In 2004, Spallholz and colleagues showed that, *in vitro*, SeMet produced superoxide ions in the presence of bacterial methioninase (139). This result led to the hypothesis that SeMet toxic effects could be mediated by its direct cleavage to MeSe, followed by redox cycling of the latter resulting in the accumulation of ROS. However, no homologue of bacterial methioninase was found in animal genomes (53). Although a methioninase activity was reported in trout hepatocytes (140) and in mouse liver cell extracts (141), the mouse liver enzyme was later purified and found to be cystathionine γ-lyase, the enzyme converting cystathionine into cysteine (142, 143). Its high K_M value for SeMet (15 mM), suggests that the amount of MeSe generated from SeMet by this enzyme is low *in vivo* (142). Thus, the occurrence in animal cells of a γ-lyase enzyme able to efficiently cleave SeMet is unlikely. In agreement with this conclusion, speciation studies in rat liver or in human leukemia cells exposed to SeMet failed to detect MeSe or its oxidized form DMDSe suggesting that metabolization of SeMet to MeSe was insignificant in these models (144, 145). Therefore, MeSe may only be a minor determinant of SeMet-induced toxicity in animal cells.

To gain insights into the metabolic product(s) underlying SeMet toxicity, we compared the sensitivity to SeMet of several *S. cerevisiae* mutants compromised in individual pathways of sulfur metabolism (113). This analysis allowed us to exclude effects resulting from an accumulation of SeAM, SeAH or any compound in the methionine salvage pathway. Instead, it indicated that toxicity arised from metabolization of SeMet into selenols, SeHCys, SeCys and possibly γ-Glu-SeCys and GSeH. Another study showed that deletion of *CYS3*, the gene encoding cystathionine γ-lyase required for synthesis of SeCys, drastically reduced toxicity (134). Overall, these results implied that SeCys formation plays a central role in SeMet-induced growth inhibition, which suggests that SeMet mode of action should be similar to that of SeCys. In this context, it is interesting to note that, in a recent study by Kupsco and Schlenk (146), exposure of fish (Japanese medaka) embryos to SeMet combined with hypersalinity induced a 100-fold increase in transcripts of BiP, the major ER chaperone (146), suggesting an accumulation of misfolded proteins in the ER reminiscent of the SeCystine-induced ER stress observed by Wallenberg et al. (120) in HeLa cells.

Recently, a genome-wide screen of the *S. cerevisiae* deletion collection revealed that tolerance against SeMet mainly involves mechanisms related to the folding or removal of damaged proteins (127). In particular, genes related to ubiquitin-mediated protein degradation, either via the proteasome complex or via the multivesicular body sorting pathway were over-represented among deletion mutants sensitive to SeMet. In accordance with the idea that proteins are the main targets of SeMet-induced effects, an accumulation of aggregated proteins was observed in wild-type yeast cells exposed to SeMet. Deletion of *CYS3*, which prevents the formation of SeCys from SeMet, completely abolished protein aggregation. All these recent results suggest the involvement of a SeCys-induced proteotoxic stress as a major determinant of SeMet toxicity in yeast.

As discussed in this review concerning SeCys effects, oxidative stress may also contribute to SeMet toxicity. Exposure to SeMet was shown to increase the production of intracellular ROS in fish embryos (147) and hepatocytes (148). Suzuki et al. (138) reported that SeMet induced ROS generation and apoptosis in lung cancer A549 cells (138). Superoxide production was observed in SeMet-treated yeast cells and deletion of *SOD1*, the gene coding for superoxide dismutase, was shown to increase SeMet toxicity (113). In addition, metabolomic studies in yeast demonstrated that SeMet addition induced a redox imbalance (149, 150). How exposure to SeMet generates oxidative stress remains to be established. It could be the result of redox-cycling of selenols produced *in vivo* with

oxygen and intracellular thiols. In addition, superoxides produced in these reactions can catalyze the formation of unnatural selenylsulfide or diselenide bridges, which may, in turn, promote protein misfolding and aggregation.

Conclusion and future perspectives

Induction of oxidative stress has long been held to account for selenium toxicity. ROS-induced DNA damage mediated by selenide produced *in vivo* is now a fairly well established mechanism accounting for selenite cytotoxicity. Because cancer cells generally exhibit an increased vulnerability to ROS-producing compounds, there is an increasing interest in designing anticancer agents promoting oxidative stress. Among redox active drugs, selenite is a promising candidate as a cancer therapeutic agent (17, 151). The next step consists in evaluating its toxicity in cancer patients and the therapeutic potential of redox-active selenium compounds.

Recent studies suggest that the mode of action of selenoamino acids could be different from that of inorganic selenium compounds. In particular, studies in *S. cerevisiae* indicate that SeMet toxicity results from its conversion into SeCys by cellular metabolism. SeCys has been shown to mediate a proteotoxic stress rather than DNA damage, which may play a role in selenium toxicity that was underestimated until now. Several questions remain unanswered. Firstly, further investigations are needed to determine whether SeCys-induced growth inhibition mostly arises from unspecific incorporation of SeCys resulting in protein misfolding or from protein thiol-oxidation leading to aggregation and/or inactivation of folded proteins. The relative importance of these mechanisms in the cell is likely to depend on the rate of protein synthesis. Another question that needs answering is whether SeCys causes functional inactivation of a few proteins with essential functions or induces a general disruption in protein homeostasis. A promising avenue of research to address these issues will be to use a global proteomic approach in yeast to identify proteins that aggregate under SeMet stress.

The involvement of SeCys in the toxicity of SeMet emerged from studies in the yeast system. It is likely that SeCys also participates in SeMet toxicity in other cellular systems, in particular human cells, but this remains to be confirmed. In animals, the expression of the transsulfuration enzymes, cystathionine β-synthase and cystathionine γ-lyase, is tissue-specific (152). For example, the synthesis of cysteine from methionine is at least two orders of magnitude lower in the brain than in the liver and kidney (153), suggesting that if metabolization to SeCys contributes significantly to SeMet toxicity in animals, this mechanism should be strongly tissue-dependent. Additional studies on SeMet metabolism and toxicity in different animal and human cell lines are necessary to expand our understanding of the consequences of SeMet exposure on protein aggregation in higher eukaryotes. Another area of research that may be worth investigating is the potential of selenoamino acids as anticancer agents. Indeed, tumor cells have a high demand for amino acids and in particular for methionine, to sustain their high proliferation rates (154). In addition, tumorigenesis is often associated with an increased dependency on protein homeostasis networks (155). This makes the use of SeMet, alone or in association with agents that interfere with protein homeostasis, an interesting strategy to explore for cancer treatments.

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