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Regulation of the cadmium stress response through SCF-like ubiquitin ligases: comparison between *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and mammalian cells

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Abstract

Saccharomyces cerevisiae has developed several mechanisms to cope with exposure to cadmium. In particular, the sulfur compound glutathione plays a pivotal role in cadmium detoxification, and exposure to cadmium leads to a wide reorganization of *S. cerevisiae* transcriptome and proteome, resulting in a significant increase in glutathione synthesis. Met4, the transcriptional activator of the sulfur metabolism enzymes, is a critical actor in this reorganization. Recent work has uncovered a part of the mechanism of cadmium-induced Met4 regulation, and showed that it occurs trough the SCF ubiquitin ligase complex SCF^{Met30}. We discuss this regulation in *S. cerevisiae* and compare it with the regulation of two other transcriptional activators involved in cadmium detoxification: the *Schizosaccharomyces pombe* Zip1, regulated by SCF^{Pof1}, and the mammalian Nrf2, regulated by the SCF-like ubiquitin ligase Cul3:Rbx1:Keap1.

Keywords: Cadmium; Sulfur metabolism; SCF complex; Glutathione; Yeast; Met30; Pof1; Keap1

1. Effects of cadmium in the cell

1.1. Cadmium causes oxidative stress, lipid peroxidation and DNA damage

Oxidative stress is generally thought to originate from toxic levels of reactive oxygen species (ROS), mainly hydroxyl radicals ($OH^{\bullet}$), which are considered to be the most toxic ROS because they can attack and damage all macromolecules in the cell, and lead to protein oxidation, lipid peroxidation and DNA damage (for review, see [1,2]). Cadmium (Cd^{2+}), as other heavy metals, causes oxidative stress, lipid peroxidation, and mutagenesis; but the molecular mechanisms leading to these cellular effects remain elusive (for review, see [3]). Consistently, *Saccharomyces cerevisiae* genes encoding proteins involved in oxidative stress defense (superoxide dismutase, thioredoxin, glutathione and thioredoxin reductases) and Yap1, the basic leucine-zipper (bZIP) transcriptional activator required for efficient expression of these genes, are each necessary for cadmium tolerance [4–6].

Yeast cells exposed to cadmium show an elevated level of lipid peroxidation [7]. Three enzymes present in vitro phospholipid hydroperoxidase activity and are supposed to be able to remove lipid peroxides in *S. cerevisiae*: Gpx1, Gpx2 and Gpx3 [8]. Among them, Gpx3 has been shown to play a major role in cadmium resistance through its phospholipid hydroperoxidase activity [9], thus suggesting that an important toxic effect of cadmium is membrane lipid peroxidation.

Cadmium has strong mutagenic effects; it induces a high rate of recombination events [4], base substitutions and frame-shift mutations [10], even at low concentrations. Cadmium seems to cause hypermutability by inhibiting the mutation avoidance system rather than by direct DNA damage. Indeed, it strongly inhibits the mismatch repair system, thereby

Abbreviations: bZIP, basic leucine-zipper; GSH, glutathione; GSSH, disulfide glutathione; ROS, reactive oxygen species; *S. cerevisiae*, *Saccharomyces cerevisiae*; *S. pombe*, *Schizosaccharomyces pombe*.

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increasing the fixation of mutations resulting from endogenous processes [10].

1.2. Hypothetical molecular mechanisms for cadmium toxicity

Cadmium is a redox-inactive metal and cannot undergo simple oxidation reactions. The molecular mechanism(s) by which cadmium leads to ROS production and oxidative stress is (are) still largely unknown and is (are) probably indirect.

The mechanism usually proposed for cadmium toxicity is its binding to cellular proteins, resulting in the inhibition of some essential enzymes. As cadmium has a high affinity for thiol groups, it is thought to bind accessible cysteine residues in proteins [11]. Different studies [12,13] have confirmed this hypothesis, but also uncovered the importance of the carboxyl groups of aspartate and glutamate residues in peptides able to bind cadmium. In vitro analyses have shown that human thiol transferases (glutathione reductase, thioredoxin reductase, thioredoxin) are inhibited by cadmium, probably through direct binding of the two essential cysteine residues present in the thiol transferase active sites [14]. As these enzymes are involved in oxidative stress defense, their inhibition would lead to increased oxidative stress in the cell. Cadmium may also displace zinc and calcium ions from metalloproteins [11, 15,16], thus leading to inhibition of essential enzymes. It is not known how cadmium poisons the mismatch repair system, which components are targeted and whether this inhibition is the result of the replacement of an unidentified zinc site in an essential subunit of the MMR complex [17].

An alternative hypothesis for cadmium toxicity is the depletion of cytosolic glutathione (GSH) pools. The cadmium detoxification pathway, which involves chelation to glutathione and subsequent transport of $Cd(GS)_2$ complexes into the vacuole (see next paragraph), may contribute to deplete free glutathione pools from the cytosol. Consequently, it would reduce the activity of glutathione-dependent enzymes, such as glutathione peroxidases, glutathione S-transferases and glutaredoxins, which are involved in oxidative stress defense and other essential functions in the cell. Among these enzymes, the activity of the glutaredoxin Grx5 is particularly important for the cell, since it is required for the activity of mitochondrial iron/sulfur enzymes [18]. The inhibition of Grx5 would increase free iron levels in the cell, and thus, Fenton-type reactions and ROS production.

2. Glutathione and cadmium detoxification in *S. cerevisiae*

2.1. Glutathione, its different functions in the cell

Glutathione is the most abundant cellular thiol, with concentrations in the millimolar range in most living cells. It consists in the tripeptide γ-glu-cys-gly, in which the peptide bond between the glutamate and cysteine residues involves the γ-carboxyl group of glutamate. The cysteine residue is responsible for glutathione redox properties, particularly its nucleophilic property and its ability to react with other sulfydryl groups, thus producing disulfide bonds. Its abundance and redox properties, combined to its high GSH:GSSG ratio (see below), allows it to act as a redox buffer, keeping reducing redox balance in the cytosol. Note that glutathione is also present in the endoplasmic reticulum, with a lower GSH:GSSG ratio, but its function there remains unclear [19]. In standard conditions, approximately half of the total glutathione content is stored in the vacuole [20], where it can be catabolized and recycled to amino-acids through the action of γ-glutamyl-transpeptidases and a dipeptidase [21].

As indicated above, one of the major functions of cellular glutathione is to provide reducing equivalent source to two main classes of anti-oxidant enzymes, the glutathione peroxidases and glutaredoxin enzymes (for review, see [2,22]). Under oxidizing conditions (e.g. H_2O_2 challenge), part of the cellular glutathione is oxidized in disulfide glutathione (GSSG) or in disulfide protein-bonds. This protein glutathionylation probably protects the redox sensitive sulfydryl groups of some essential enzymes, such as Tdh3, during oxidative stress [23]. Oxidized glutathione GSSG is reduced to GSH by the glutathione reductase enzyme Glr1, and protein-bound glutathione is thought to be removed by some glutaredoxins.

Glutathione is also used for conjugation to electrophilic and lipophilic molecules that would be toxic for the cell, particularly xenobiotics. These reactions are supposed to be catalyzed by glutathione S-transferases (Gtt1 and Gtt2). The conjugates are transported into the vacuole by the vacuolar transporter Ycf1 [24] (see next paragraph). Glutathione also plays an essential role in nitric oxide, formaldehyde and methylglyoxal detoxification (for review, see [25]). Finally, glutathione serves as a sulfur and nitrogen reserve for the cell, in case of sulfur or nitrogen starvation [25].

Though dispensable in bacteria [26], glutathione is essential in *S. cerevisiae*; but its essential function(s) has (ve) not been identified yet [27]. Glutathione is probably vital for all eukaryotes, especially mammals, in which it plays a similar role as in *S. cerevisiae* (for review, see [28]). In particular, glutathione, which is present in all tissues, is very abundant and important in the liver, for detoxification of electrophiles and in defense against oxidative stress [28]. Consistent with its important role in antioxidant defense, cellular glutathione availability is a key factor of apoptosis, aging and several diseases (e.g. cancers, AIDS, cardiovascular diseases, Alzheimer's and Parkinson's diseases).

2.2. Cadmium detoxification

Depending on the cell type, there are two major mechanisms of cadmium detoxification. Mainly, in prokaryotes, cadmium is directly exported outside the cell [29], whereas in eukaryotes, the metal is inactivated and sequestrated by chelation to sulfydryl rich compounds, such as glutathione, phytochelatin or metallothioneins, depending on the organism (for review, see [30,31]).