— **Review** —

Role of the Ubiquitin-proteasome System in Methylmercury Toxicity in *Saccharomyces cerevisiae*

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(Received December 17, 2010)

Methylmercury (MeHg) is an important environmental pollutant that causes severe disorders of the central nervous system, but the mechanism underlying its toxicity and the corresponding biological defense mechanisms remain largely unknown. *Saccharomyces cerevisiae* (*S. cererisiae*) yeast cells were used to elucidate the defense mechanisms against MeHg toxicity and to search for novel genes involved in MeHg resistance. *S. cerevisiae* is a eukaryotic organism that possesses many gene products that are functionally similar to those of mammals such as humans. We have previously reported that Cdc34 and Rad23 confer MeHg resistance to yeast cells. Interestingly, the both proteins are related to ubiquitin-proteasome system (UP system) that is involved in the intracellular degradation of proteins. In our detailed experiments, we found that the UP system might play an important role in lending protection against MeHg toxicity. This review summarizes the results of our studies on the role of the UP system as a defense mechanism against MeHg toxicity in yeast cells.

Key words —— methylmercury, toxicity, ubiquitin, proteolysis, yeast

INTRODUCTION

Methylmercury (MeHg) is a well-known environmental pollutant that causes serious disorders of the central nervous system and gives rise to various other symptoms. Recent epidemiological studies have indicated that ingestion of MeHg through fish during pregnancy can result in neurological defects in the offspring. However, the mechanism underlying MeHg toxicity is not fully understood.

We used *Saccharomyces cerevisiae* (*S. cererisiae*) yeast cells to elucidate the defense mechanisms against MeHg toxicity, and search for novel genes involved in MeHg resistance because *S. cerevisiae* is a eukaryotic organism having many gene products that are functionally similar to those of mammals such as humans. We previously reported that Cdc34[1)] and Rad23[2)] confer MeHg resistance on yeast cells. Cdc34 is a ubiquitin-conjugating enzyme that is involved in the ubiquitin-proteasome system (UP system), and Rad23 is another factor related to the UP system. The UP system initiates protein degradation by the covalent attachment of ubiquitin to substrate proteins to yield ubiquitin-protein conjugates. The formation of a thiolester bond between ubiquitin and the ubiquitin-activating enzyme leads to ubiquitin activation, and the activated ubiquitin is then transferred to one of the many distinct ubiquitin-conjugating enzymes by transthiolation. The ubiquitin-conjugating enzymes catalyze the ubiquitination of substrate proteins either directly or in conjunction with a distinct ubiquitin ligase that is composed of multiple proteins. The ubiquitination of a substrate protein is followed by degradation of that protein through the proteasome (Fig. 1).[3)] This review mainly summarizes the results of our research that explores the role of the UP system in the mechanism of MeHg toxicity in yeast cells.

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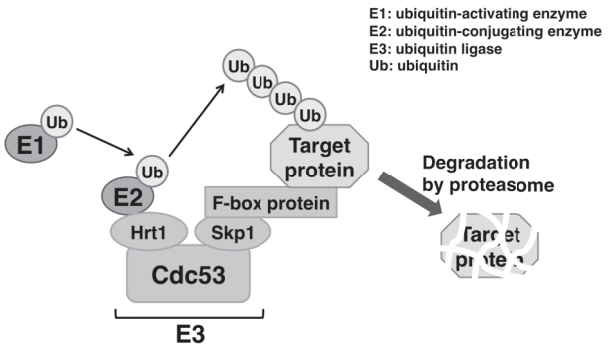

Fig. 1. Skp1/Cdc53/F-box Protein (SCF) Complex-mediated Proteasomal Degradation

REDUCTION IN MeHg TOXICITY BY UP SYSTEM-MEDIATED ACCELERATION OF PROTEOLYSIS

To identify the functional domain of Cdc34 that is essential for acquisition of resistance to MeHg, we constructed several Cdc34 mutants[4,5] that had no ubiquitin-conjugating activity and examined their MeHg sensitivity, thereafter. Our results showed that the MeHg sensitivity of yeast cells that overexpressed Cdc34 mutants did not significantly differ from that of the control yeast cells. In addition, the overexpression of wild-type Cdc34 led to an increase in the levels of total ubiquitinated proteins. However, this phenomenon was not recognized in yeast cells that expressed the Cdc34 mutants.[6] These results suggest that the function of Cdc34 as a ubiquitin-conjugating enzyme is essential for resistance to MeHg.

The Skp1/Cdc53/F-box protein (SCF) complex is known as ubiquitin ligase that together with Cdc34 is involved in the ubiquitination of proteins (Fig. 1).[7] Overexpression of individual subunits of SCF (particularly Cdc53, Hrt1, and Skp1) did not significantly affect the MeHg sensitivity of yeast cells or the levels of total ubiquitinated proteins. In contrast, overexpression of Uba1, a ubiquitin-activating enzyme, lent the cells limited resistance to MeHg, without any significant change in the levels of total ubiquitinated proteins. Increased levels of total ubiquitinated proteins were observed only in yeast cells that overexpressed Cdc34.

Ubiquitin-conjugating enzyme consists of the gene family, and 13 different ubiquitin-conjugating enzymes, including Cdc34, have been identified,[8] and these are involved in lending MeHg resistance to yeast cells. Interestingly, we found that the yeast cells that overexpressed several ubiquitin-conjugating enzymes apart from Cdc34 also showed some degree of resistance to MeHg. Further, these cells had increased levels of total ubiquitinated proteins, when compared with the control cells. The difference in the degree of resistance is thought to depend on the substrate specificity of each ubiquitin-conjugating enzyme.[6] Thus, in the UP system that requires Cdc34 as a ubiquitin-conjugating enzyme, the ubiquitin-conjugating enzyme seems to be rate limiting. The basal cellular concentration of Cdc34 might be lower than that of the other components of the UP system.

The conjugation of ubiquitin to target proteins serves as a signal that triggers the degradation of these proteins in the proteasome. Treatment with MG132, a proteasome inhibitor, almost completely eliminated the protective effect that Cdc34 overexpression had against MeHg toxicity. The proteasome-defective cells were hypersensitive to MeHg when compared with the wild-type cells.[6] These results suggested that proteasome activity is essential for the Cdc34-mediated resistance to MeHg. Therefore, it seems likely that certain proteins that have not yet been identified, but exist in cells, increase MeHg toxicity. However, the toxicity might be reduced by the enhanced degradation of these proteins when Cdc34 is overexpressed in the UP system.

The UP system is strongly conserved from yeast to human cells. Human cells overexpressing Cdc34 exhibited significant resistance to MeHg when compared with the control cells.[6] This result indicates that the UP system involving Cdc34 plays an important role in conferring protection against MeHg toxicity on both yeast and human cells. Therefore, identifying the protein involved in enhancing MeHg toxicity should help in elucidating the mechanism of underlying MeHg-induced damage to the central nervous system in humans.

IDENTIFICATION OF THE F-BOX PROTEIN INVOLVED IN REDUCING MeHg TOXICITY

SCF complex is a component of the UP system and consists of Skp1, the scaffold protein Cdc53, the RING-finger protein Hrt1, and a member of the family of F-box proteins. Among the factors that make up this SCF complex in yeast cells, 17 different F-box proteins are known to bind directly to

the substrate proteins that are then degraded by the UP system.[9] F-box proteins have their individual substrate-specificities that play important roles in the selection of proteins that are degraded by the UP system. When overexpression of the F-box protein in yeast cells, ubiquitinated protein and combination ratio with the SCF complex increase, it is thought that proteasomal degradation following ubiquitination of the target protein is promoted. In an attempt to identify the F-box protein involved in the protection of yeast cells against MeHg toxicity, we generated 17 yeast strains that overexpressed each of the 17 different F-box proteins. We observed that yeast cells that overexpressed Hrt3 or Ylr224w were strongly resistant to MeHg, when compared with the control cells.[10] Recently, we also found that Ymr258c, a new F-box protein, is involved in reduction of MeHg toxicity.[11] Furthermore, yeast cells that overexpressed wild-type Hrt3, Ylr224w, or Ymr258c were resistant to MeHg, while those that overexpressed proteins with deleted F-box domains were not. The MeHg resistance to the former group of yeast cells disappeared in the presence of the proteasome inhibitor MG132.[10, 11] These results suggest that the formation of the SCF complex and the proteasome-mediated degradation of ubiquitinated proteins might be necessary for the yeast cells to exhibit resistance to MeHg on overexpression of Hrt3, Ylr224w, and Ymr258c.

MeHg toxicity is believed to reduce with the overexpression of Cdc34 or F-box protein and the proteasomal degradation of the ubiquitinated protein, which is recognized by other F-box proteins (Hrt3 or Ylr224w). We recently succeeded in identifying the protein that specifically binds to the F-box protein and is involved in the reinforcement of MeHg toxicity. In the future, detailed studies on the role of the protein, which serves as a determination factor for MeHg toxicity, will help elucidate the mechanism of MeHg toxicity and the corresponding biological defense mechanism.

REDUCTION IN MeHg TOXICITY BY UP SYSTEM-MEDIATED SUPPRESSION OF PROTEOLYSIS

We found that Rad23, a UP system-related factor, is involved in the reduction of MeHg toxicity.[2] Rad23 has 2 domains: a ubiquitin-associated (UBA) domain that bind to the ubiquitin chain of ubiquitinated proteins, and a ubiquitin-like (UbL) domain that binds to the proteasome.[12] It has been reported that Rad23 has 2 contradictory functions. First, Rad23 inhibits the elongation of the ubiquitin chain by binding to the ubiquitin moiety of ubiquitinated proteins. Because the proteasome recognizes ubiquitinated proteins as substrates when more than a certain number of ubiquitin molecules have been attached, it has been postulated that proteins can elude proteasomal degradation when elongation of the ubiquitin chains is inhibited.[13] Second, Rad23 transports ubiquitinated proteins to the proteasome. Rad23 binds to the proteasome and enhances the degradation of ubiquitinated proteins.[14, 15] Thus, Rad23 seems to regulate the degradation of ubiquitinated proteins through its contradictory functions.

To understand the relationship between the MeHg toxicity and the 2 contradictory functions of Rad23, we investigated MeHg sensitivity of yeast cells with overexpressed truncated variants of Rad23 that had defective UbL or UBA domains. We found that yeast cells that overexpressed Rad23 with a defective UbL domain were more resistant to MeHg than were those that overexpressed wild-type Rad23. In contrast, yeast cells that overexpressed Rad23 with a defect in UBA domain were resistant only to very low levels of MeHg.[2] Our findings suggest that the UbL domain in Rad23 might be involved in the enhancement of MeHg toxicity, whereas the UBA domain might be involved in the conferring resistance to MeHg toxicity. The fact that yeast cells overexpressing wild-type Rad23 were resistant to MeHg indicates that the functions mediated by the UBA domain might be dominant over those mediated by the UbL domain with respect to the acquisition of MeHg resistance when Rad23 is overexpressed. We also found a marked increase in the levels of total ubiquitinated proteins in the yeast cells that overexpressed the wild-type Rad23, and an even greater increase in this expression in the yeast cells that overexpressed Rad23 with defect in the UbL domain. In contrast, we detected a marked reduction in the levels of total ubiquitinated proteins in the yeast cells that overexpressed Rad23 with a defect in the UBA domain.[2] These results clearly show that the UbL domain of Rad23 plays a role in reducing the cellular level of ubiquitinated proteins, whereas the UBA domain play a role in increasing the levels of these proteins. Thus, the proteasome-mediated degradation of ubiquitinated proteins is enhanced by the UbL-mediated transport of ubiquitinated proteins to the proteasome, whereas the degradation of ubiquiti-

nated proteins is suppressed when the elongation of the ubiquitin chains of ubiquitinated proteins is inhibited by the UBA domain of Rad23.

MeHg toxicity is probably reduced by UBA domain-mediated suppression of the degradation of ubiquitinated proteins, while it is increased by UbL domain-mediated enhancement of the degradation of ubiquitinated proteins. In yeast cells that overexpressed Rad23, Rad23 activity that suppresses the degradation of ubiquitinated proteins might be dominant over the activity that enhances the degradation of these proteins. This possibly explains how the Rad23-overexpressing yeast cells acquire resistance to MeHg. Therefore, we propose that certain proteins in yeast cells are involved in the reduction of MeHg toxicity and are degraded by the UP system, and that Rad23 might play a role in enhancing the protective actions of these proteins against MeHg toxicity by suppressing their degradation.

RELATIONSHIP BETWEEN CDC34 AND RAD23 IN MeHg TOXICITY

Unlike Cdc34, Rad23 reduces MeHg toxicity by suppressing the degradation of proteins that might reduce MeHg toxicity. Nevertheless, we could not rule out the possibility that both Cdc34 and Rad23 recognize the same proteins, which are indirectly involved in MeHg toxicity, as substrates, because Cdc34 is involved in protein ubiquitination, and Rad23 binds to the ubiquitin chain of ubiquitinated proteins. However, when we overexpressed Cdc34 in normal and Rad23-defective yeast cells, resistance to MeHg was enhanced to almost the same extent in both yeast cell lines. Thus, it is possible that the binding of Rad23 to ubiquitinated proteins might be regulated by a mechanism that involves the recognition of substrate proteins, and that the functions of Rad23 might not affect the protein-degradation system in which Cdc34 is involved as a ubiquitin-conjugating enzyme. Multiple ubiquitinated proteins that reduce or enhance MeHg toxicity might be present in cells. The UP system and related proteins might determine the extent of MeHg toxicity by regulating the cellular concentrations of the various proteins (Fig. 2).

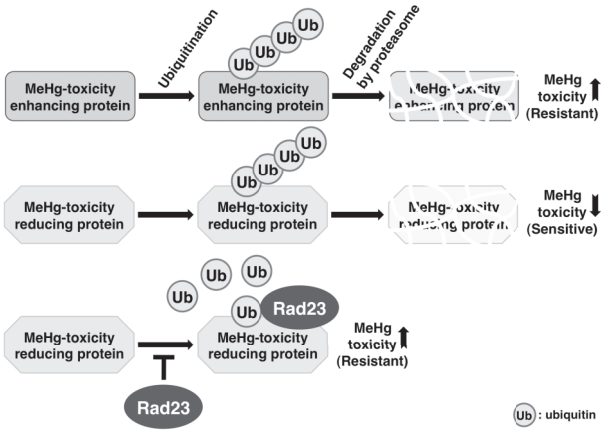

Fig. 2. Role of the UP System in MeHg Toxicity in Yeast Cells

CONCLUSIONS

Recently, many studies showed that the UP system is involved in a number of neurological disorders, and accumulating evidence indicates that defects in UP systems disrupt cellular homeostasis and induce degeneration, especially in the brain. However, attention should also be paid to the relationship between the UP system and MeHg toxicity, because the MeHg mainly causes central nerve disorder. Our findings suggest the possibility that the UP system plays an important role as a determination factor of sensitivity to MeHg. These findings will help in elucidating the role of the UP system as a mechanism in reducing MeHg toxicity. Although substrate proteins recognized by Cdc34 or Rad23 have not been identified, they may include proteins associated with MeHg toxicity. Identification of these proteins may be important for elucidating the largely unknown mechanism of MeHg toxicity.

Acknowledgements I thank Professor Akira Naganuma for his help and advice and all the graduate school students for who assisted me on this research. This work was supported partly by a Grant-in-Aid for Scientific Research (S) and partly by Grant-in-Aid for young scientists (B) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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