【S-5】

A ubiquitin-proteasome system as a determination factor involved in methylmercury toxicity

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The methylmercury (MeHg) is a toxic environmental pollutant, causing serious neurological and developmental effects in humans. Recent epidemiological studies have indicated that ingestion of MeHg in fish during pregnancy can result in neuroethological effects in the offspring. However, the mechanism underlying the MeHg-toxicity is not fully understood.

To elucidate the mechanisms of toxicity of MeHg and of defense against MeHg, we searched for factors that determine the sensitivity of yeast cells to MeHg, and found that overexpression of Cdc34, a ubiquitin-conjugating enzyme (E2) that is a component of the ubiquitin-proteasome (UP) system, induces a resistance to MeHg toxicity in both yeast and human cells. The UP system is involved in the intracellular degradation of proteins. When Cdc34 is overexpressed in cells, ubiquitination reactions are activated and the degradation of certain proteins by the UP system is enhanced. Therefore, it seems likely that certain as-yet-unidentified proteins that increase MeHg toxicity might exist in cells and that toxicity might be reduced by the enhanced degradation of such proteins, mediated by the UP system, when Cdc34 is overexpressed.

SCF ubiquitin-ligase is a component of UP system and consists of Skp1, the scaffold protein Cdc53, the RING-finger protein Hrt1, and one member of the family of F-box proteins. The F-box proteins directly bind to the substrates and are the determinants of substrate specificity of SCF. Therefore, we searched for the F-box protein that confers resistance to MeHg, and found that overexpression of Hrt3 or Yil224w induced resistance to MeHg toxicity in yeast cells. Since the protein(s) that enhance toxicity of MeHg might plausibly be induced in substrates of both F-box proteins, we next searched for substrate proteins that are recognized by Hrt3 or Ylr224w using two-hybrid screen. We found that Did3 or Grs1 interacts with Hrt3; and Eno2 interacts with Yir224w. The yeast cells that overexpressed each those proteins showed hypersensitivity to MeHg, respectively, indicating that those proteins enhance the MeHg toxicity. Both Dld3 and Eno2 are proteins involved in the synthesis of pyruvate, and

overexpression of both proteins might induce increase in interacellular levels of pyruvate. Deletion of Yil006w that transports pyruvate into the mitochondria induced a resistance to MeHg. These results suggest that the promotion of the pyruvate inflow into the mitochondria might enhance MeHg toxicity. This study providesimportant key for the elucidation of the molecular mechanism of MeHg toxicity.

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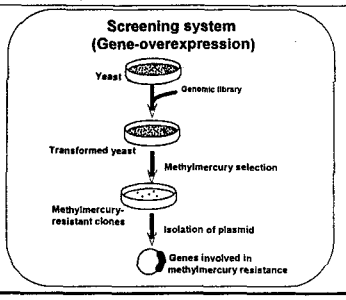
Model organism : Yeast (*Saccaromyces cerevisiae*)
Powerful genetic approaches can be used to elucidate fundamental but complex eukaryotic processes



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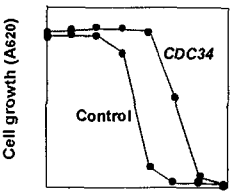

Effects of overexpression of Cdc34 on sensitivity of yeast to *methylmercury (MeHg)*

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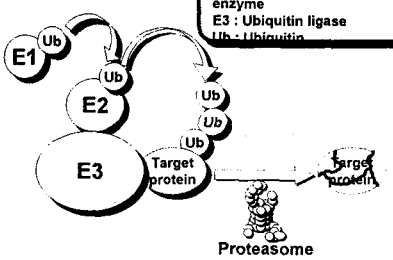
E1 : Ubiquitin-activating enzyme
E2 : Ubiquitin-conjugating enzyme
E3 : Ubiquitin ligase
Ub : Ubiquitin



Ubiquitin-proteasome system

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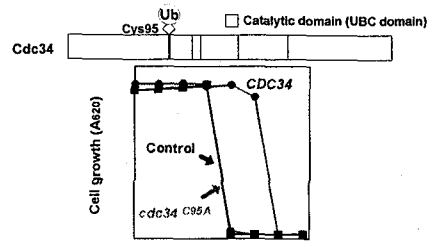
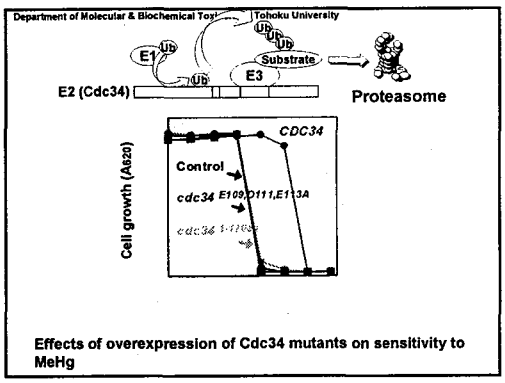
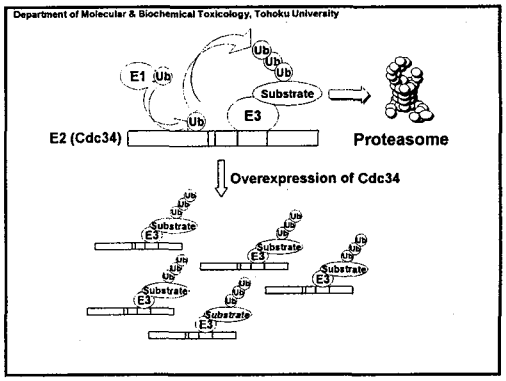
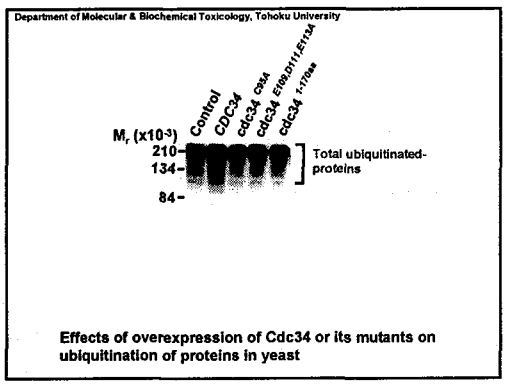


Effects of overexpression of Cdc34 mutant (point mutation of ubiquitin-binding site) on sensitivity to MeHg

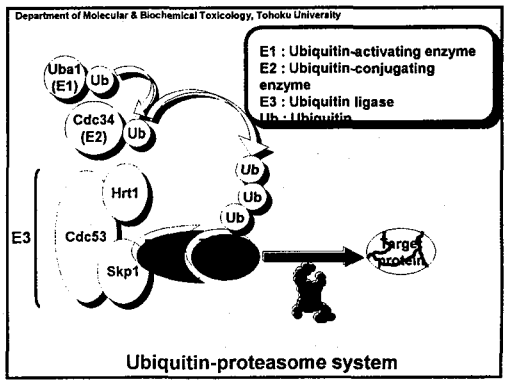


Effects of overexpression of Cdc34 mutants on sensitivity to MeHg

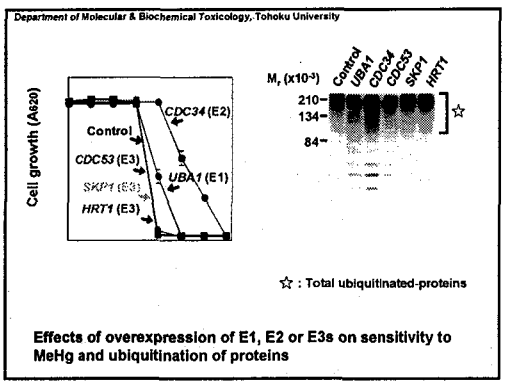




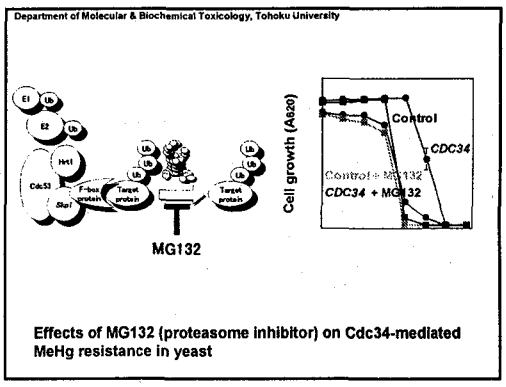
Effects of overexpression of Cdc34 or its mutants on ubiquitination of proteins in yeast



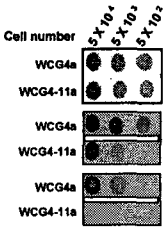
Ubiquitin-proteasome system



Effects of overexpression of E1, E2 or E3s on sensitivity to MeHg and ubiquitination of proteins



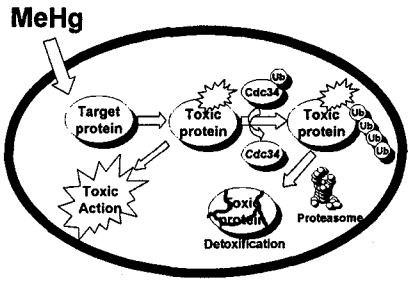
Effects of MG132 (proteasome inhibitor) on Cdc34-mediated MeHg resistance in yeast



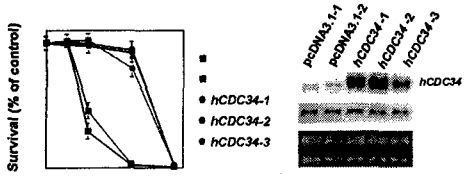
†WCG4a : Wild-type cells

†WCG4-11a : Proteasome-defective cells
Missense mutant of *PRE1* (Proteasome subunit)

Effects of proteasome activity on MeHg-sensitivity in yeast

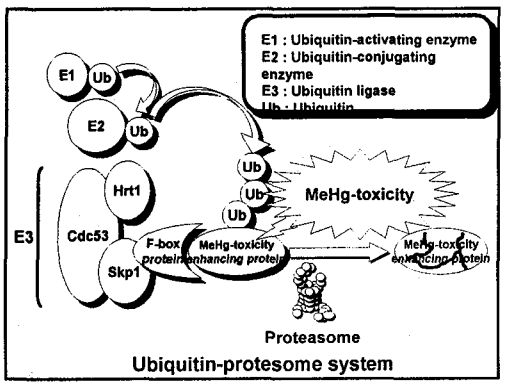


The model for Cdc34-mediated resistance to MeHg

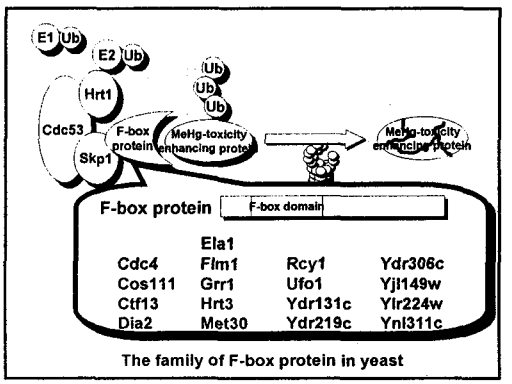


Effects of overexpression of human Cdc34 on sensitivity to MeHg in HEK293 cells

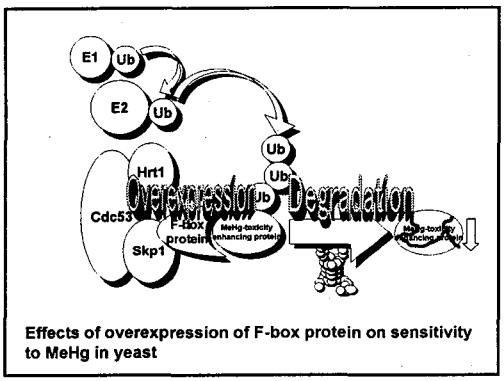
Screening of
MeHg-toxicity enhancing protein



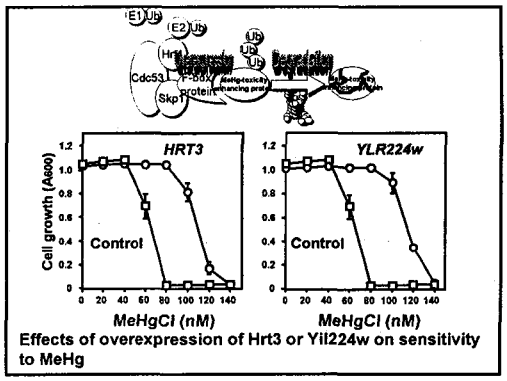
Ubiquitin-protesome system



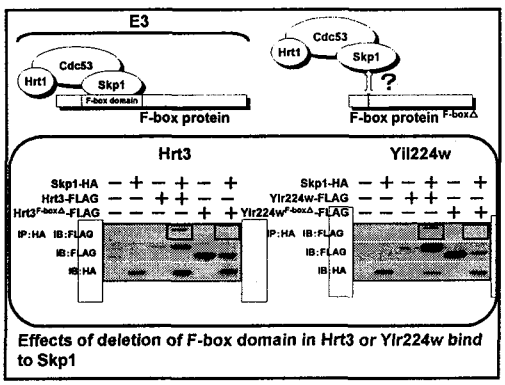
The family of F-box protein in yeast



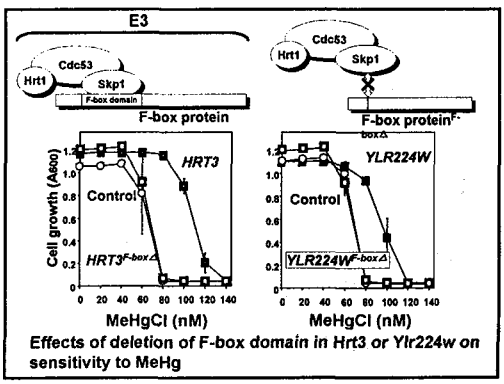
Effects of overexpression of F-box protein on sensitivity to MeHg in yeast



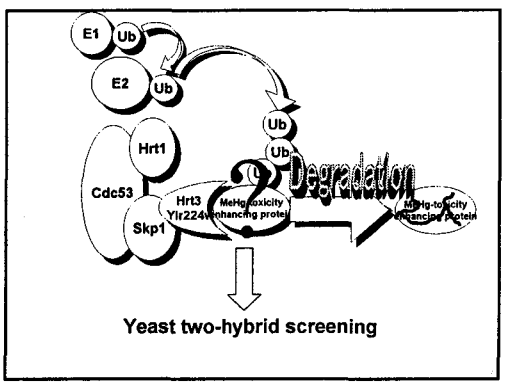
Effects of overexpression of Hrt3 or Yil224w on sensitivity to MeHg

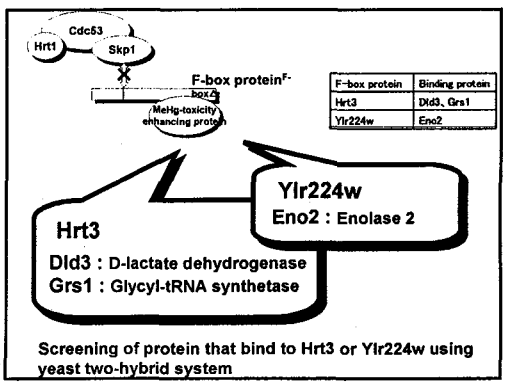


Effects of deletion of F-box domain in Hrt3 or Ylr224w bind to Skp1

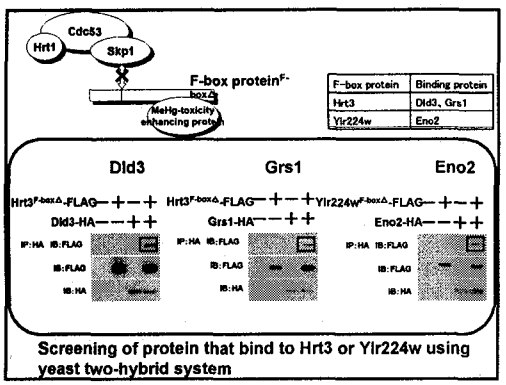


Effects of deletion of F-box domain in Hrt3 or Ylr224w on sensitivity to MeHg

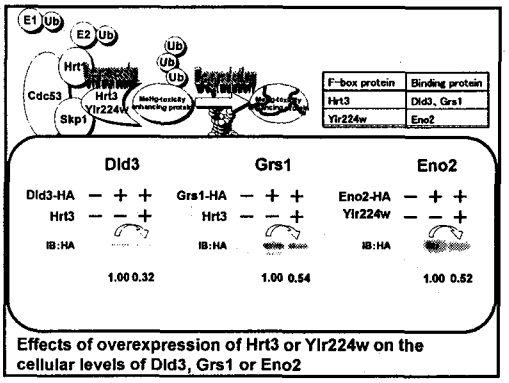




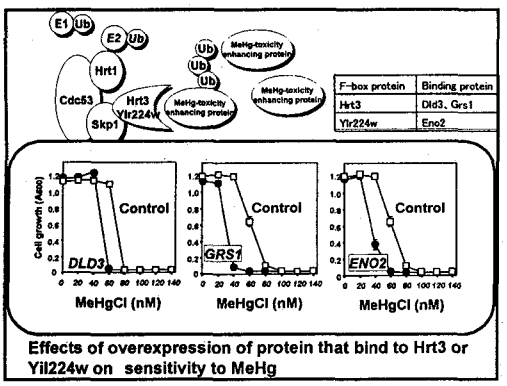
Screening of protein that bind to Hrt3 or Ylr224w using yeast two-hybrid system



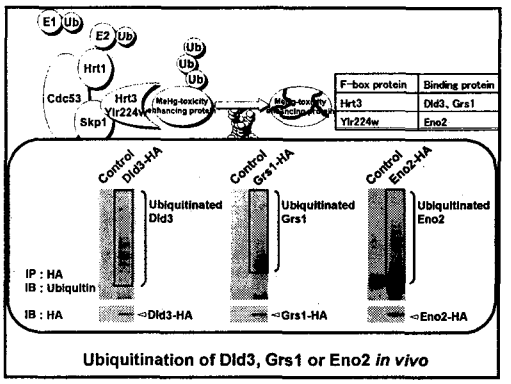
Screening of protein that bind to Hrt3 or Ylr224w using yeast two-hybrid system



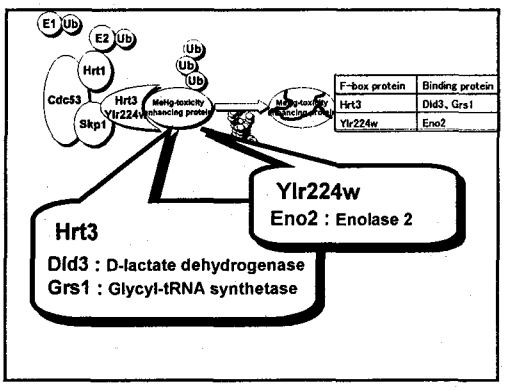
Effects of overexpression of Hrt3 or Ylr224w on the cellular levels of Dld3, Grs1 or Eno2

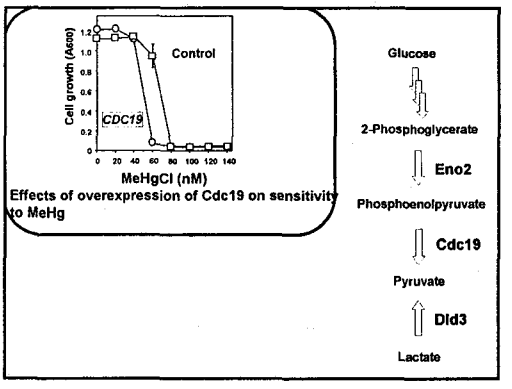


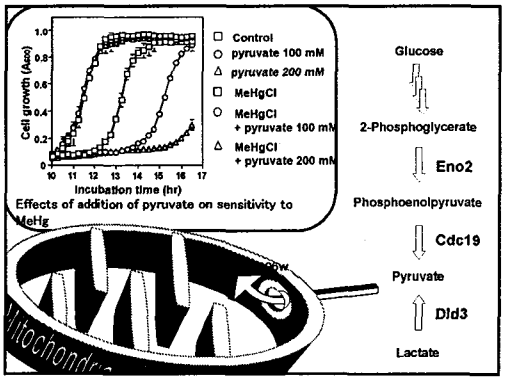
Effects of overexpression of protein that bind to Hrt3 or Yil224w on sensitivity to MeHg

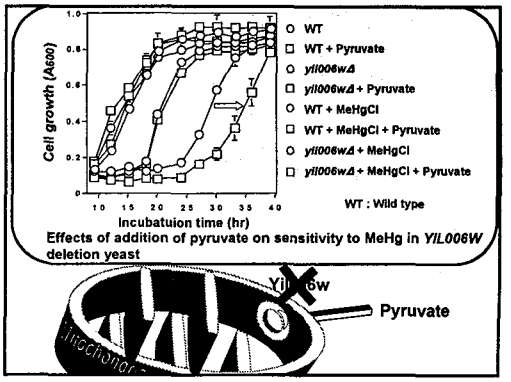


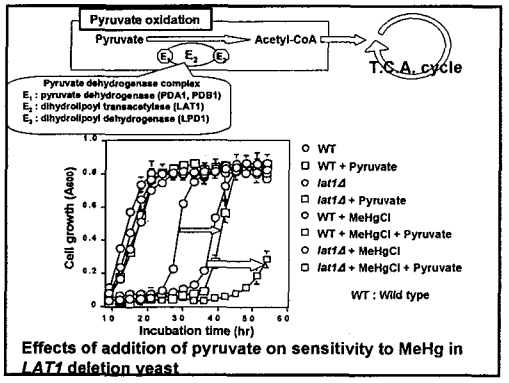
Ubiquitination of Dld3, Grs1 or Eno2 *in vivo*

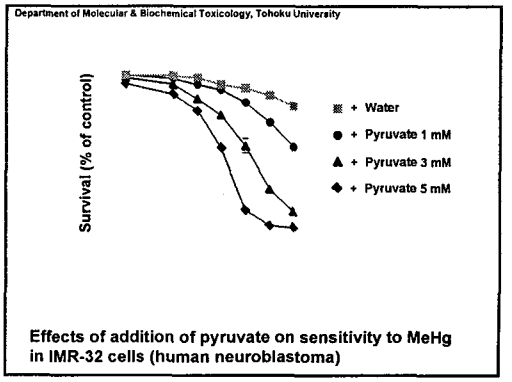


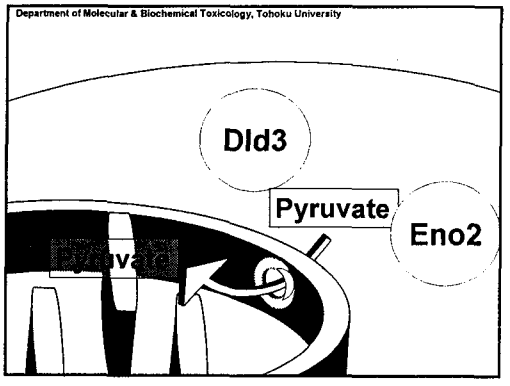


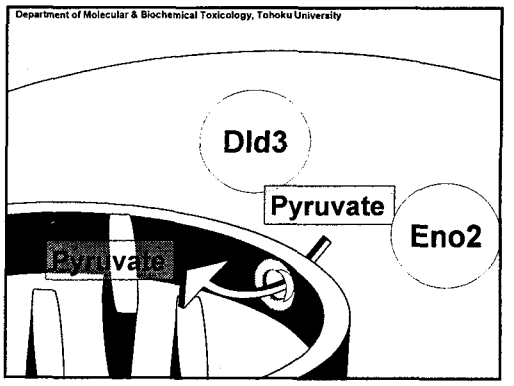

Cell growth (Aεοο)
Incubation time (hr)
Control
pyruvate 100 mM
pyruvate 200 mM
MeHgCl
MeHgCl + pyruvate 100 mM
MeHgCl + pyruvate 200 mM
Effects of addition of pyruvate on sensitivity to MeHg
Glucose
2-Phosphoglycerate
Eno2
Phosphoenolpyruvate
Cdc19
Pyruvate
Dld3
Lactate
Mitochondria

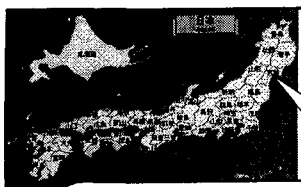

Cell growth (Aεοο)
Incubatuion time (hr)
WT
WT + Pyruvate
yil006wΔ
yil006wΔ + Pyruvate
WT + MeHgCl
WT + MeHgCl + Pyruvate
yil006wΔ + MeHgCl
yil006wΔ + MeHgCl + Pyruvate
WT : Wild type
Effects of addition of pyruvate on sensitivity to MeHg in YIL006W deletion yeast
Yil 6w
Pyruvate
Mitochondria


Pyruvate oxidation
Pyruvate
Acetyl-CoA
T.C.A. cycle
Pyruvate dehydrogenase complex
E1 : pyruvate dehydrogenase (PDA1, PDB1)
E2 : dihydrolipoyl transacetylase (LAT1)
E3 : dihydrolipoyl dehydrogenase (LPD1)
Cell growth (Aεοο)
Incubation time (hr)
WT
WT + Pyruvate
lat1Δ
lat1Δ + Pyruvate
WT + MeHgCl
WT + MeHgCl + Pyruvate
lat1Δ + MeHgCl
lat1Δ + MeHgCl + Pyruvate
WT : Wild type
Effects of addition of pyruvate on sensitivity to MeHg in LAT1 deletion yeast


Department of Molecular & Biochemical Toxicology, Tohoku University
Survival (% of control)
+ Water
+ Pyruvate 1 mM
+ Pyruvate 3 mM
+ Pyruvate 5 mM
Effects of addition of pyruvate on sensitivity to MeHg in IMR-32 cells (human neuroblastoma)


Department of Molecular & Biochemical Toxicology, Tohoku University
Dld3
Pyruvate
Eno2
Pyruvate
Mitochondria


Department of Molecular & Biochemical Toxicology, Tohoku University
Dld3
Pyruvate
Eno2
Pyruvate
Mitochondria



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