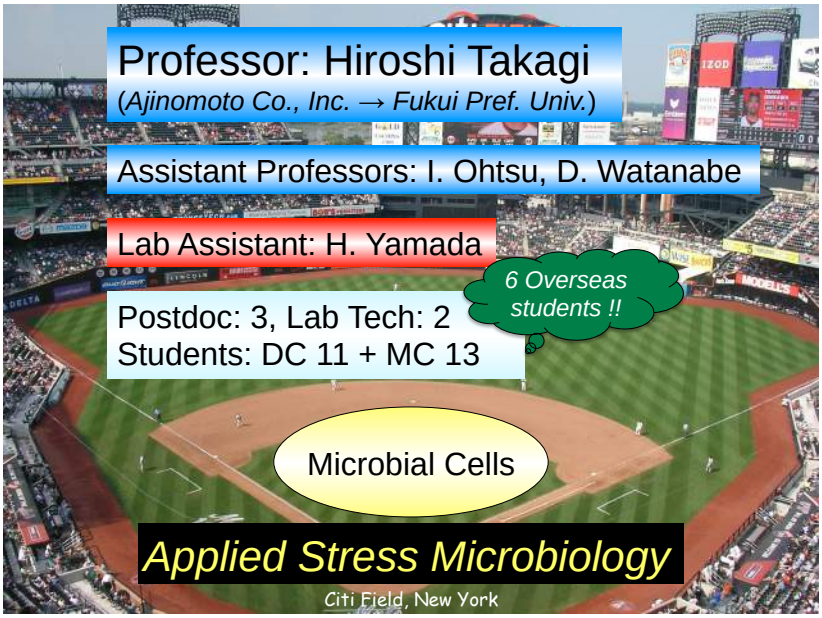
2015

Laboratory of Applied Stress Microbiology

Professor Hiroshi Takagi, Ph.D.

Originality !! *Serendipity !!*

The Takagi Laboratory



Microbial bioscience

Screening (mutants, isolates) *Genomic information*

Analysis and improvement of microbial functions

Gene engineering *Protein engineering*
Metabolic engineering *Cell engineering*

- Construction of useful microorganisms
- Production of valuable substances
- Development of promising technology

Food *Environment*
Life *Energy*

Application in biotechnology

Applied Stress Microbiology

TAKAGI LAB Since 2005

The 8th ANNIVERSARY

Stress response and adaptation mechanisms in yeast

①Proline ②*N*-Acetyltransferase Mpr1 ③Arginine/NO ④Ubiquitin system

＜Keywords＞ oxidative stress, ROS, proline, transporter, mitochondria, arginine, NO, NO synthase, *S*-nitrosylation, redox regulation, ubiquitination, permease, phospholylation, transcription factor (Msn2, Pog1) ion stress, signal transduction *etc*.

L-cysteine metabolism and its role in Escherichia coli

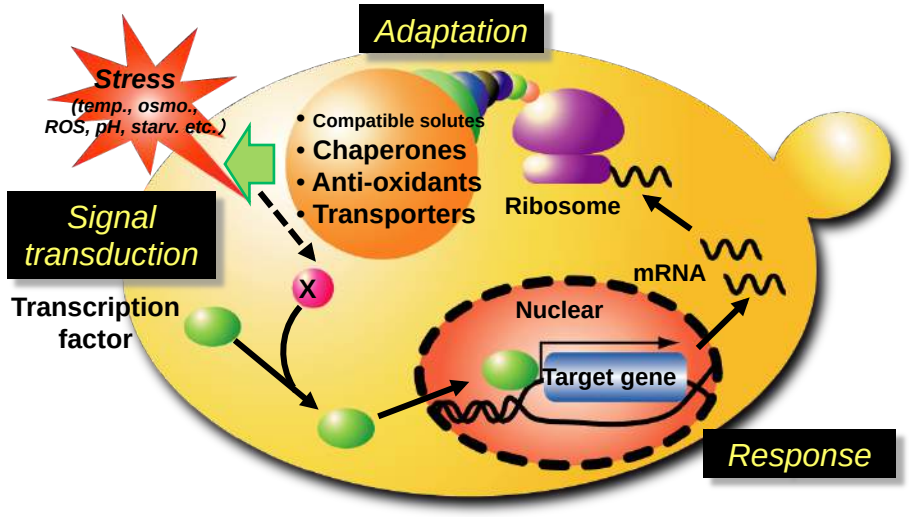
＜Keywords＞ cysteine, transporter, redox regulation, thiosulfate pathway *etc*.

CO_2 fixation system in super oligotroph Rhodococcus

＜Keywords＞ super oligotroph, CO_2 fixation, methanol metabolism, HCHO *etc*.

The budding yeast *Saccharomyces cerevisiae*

Cellular response and adaptation to environmental stresses



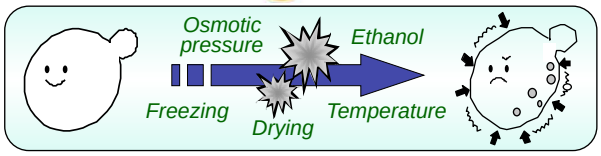
Industrial yeast strains

H. Takagi

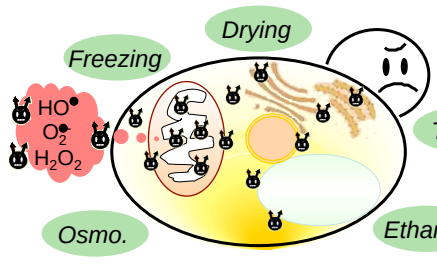
D. Watanabe

Breads | Alcoholic beverages | Bioethanol

Multiple severe stresses



ROS generation



·Growth inhibition
·Cell death

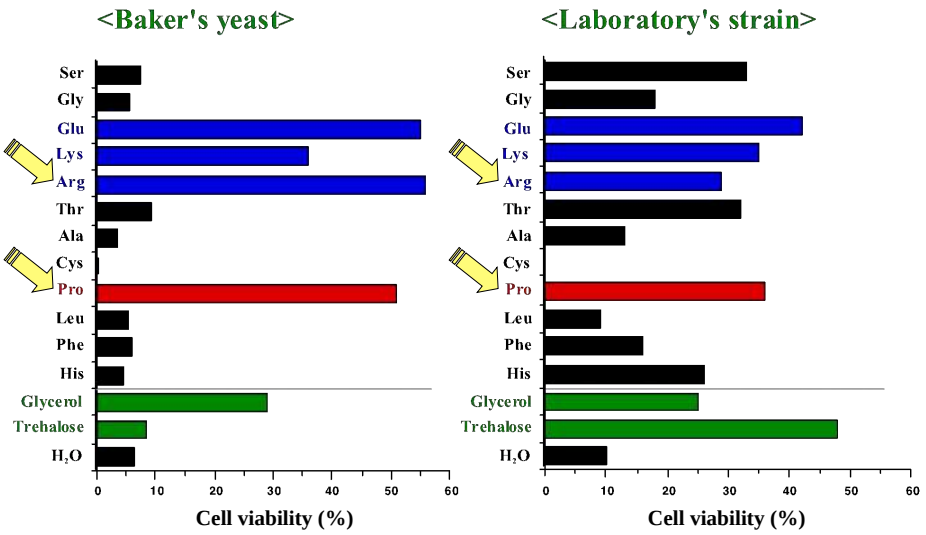
Fermentation ability is limited.

Stress tolerance is the key for yeast cells.

Proline has a cryoprotective activity.

(Takagi *et al.*, *Appl. Microbiol. Biotechnol.*, **47**, 405, 1997)

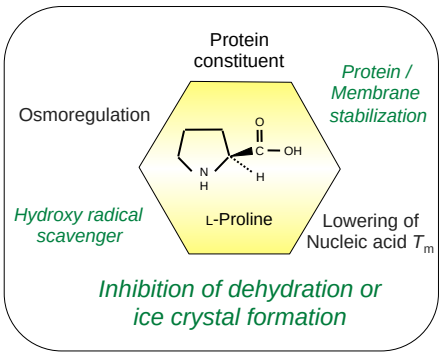
Effect of amino acids on yeast cells exposed to freezing



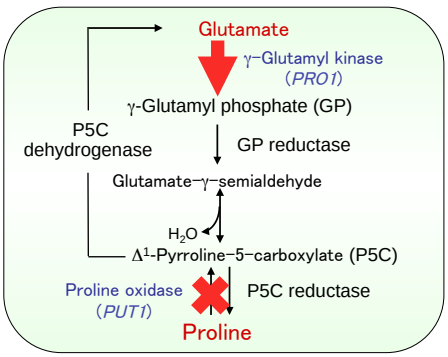
What is proline ?

In response to osmotic stresses, many bacterial and plant cells accumulate proline.
Yeast cells induce glycerol or trehalose synthesis, but do NOT increase the proline level.

Physiological functions



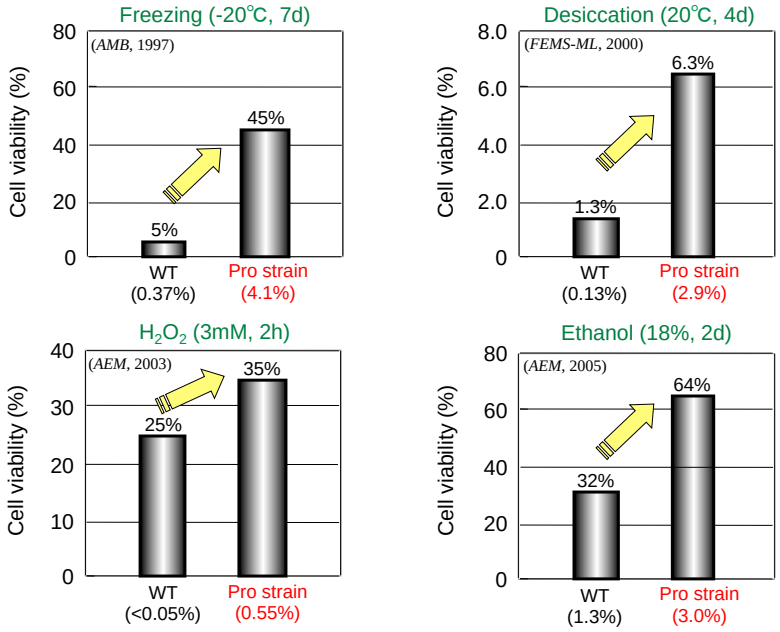
Metabolism in *S. cerevisiae*



Yeast cells that accumulate proline

- Weakened degradation → *PUT1* disruption
- Enhanced synthesis → proline analogue (AZC)-resistant mutant (*PRO1* mutation)

Pro-accumulating yeast cells are tolerant to various stresses.



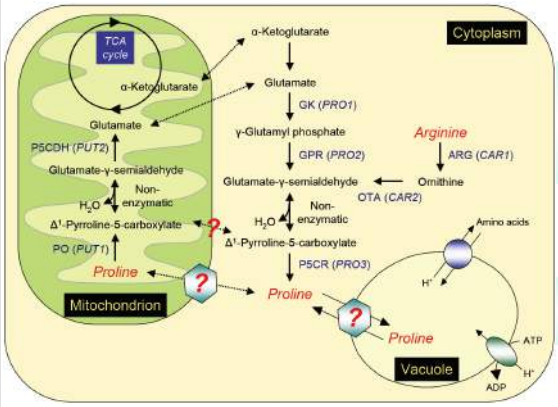
① Proline

Appl. Microbiol. Biotechnol., **47**, 405, 1997; **79**, 273, 2008, **81**, 211, 2008; *FEMS Microbiol. Lett.*, **184**, 103, 2000; *Appl. Environ. Microbiol.*, **69**, 212, 2003; **69**, 6527, 2003; **71**, 8656, 2005; **73**, 4011, 2007; **74**, 5845, 2008; *J. Biosci. Bioeng.*, **94**, 2002; **100**, 538, 2005; **103**, 277, 2007; **116**, 576, 2013; *Biosci. Biotech. Biochem.*, **73**, 2131, 2009; **76**, 454, 2012; *Int. J. Food Microbiol.*, **152**, 40, 2012.

< So far >

★ Proline protects yeast cells from various stresses as a ROS scavenger *!!*

★ The appropriate cellular level and localization of proline are important for the stress-protective effect *!!*



< Current projects >

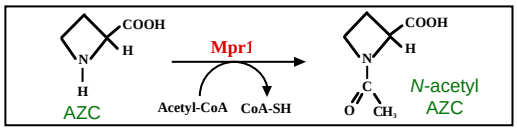
- Physiological functions (ROS)
- Transport to mitochondria/vacuole
- Functional analysis of GK and PO

Breeding of novel stress-tolerant yeast strains with Pro accumulation

What is Mpr1 ?

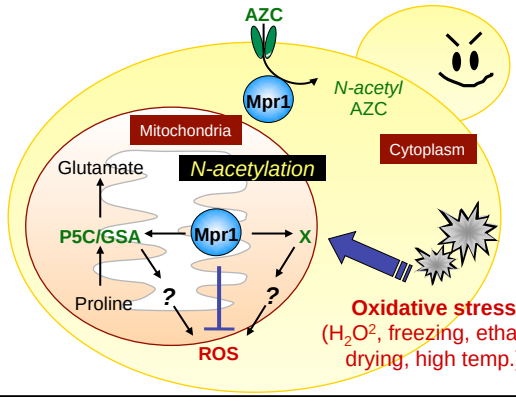
The yeast *Saccharomyces cerevisiae* Σ1278b
(sig**M**a 1278b genes for **P**roline-analogue **R**esistance)

★ *MPR1* encodes a novel *N*-acetyltransferase that detoxifies azetidine-2-carboxylate.



J. Bacteriol., 2000
J. Biol. Chem., 2001
Yeast, 2002
J. Biochem., 2003
Biosci. Biotech. Biochem., 2008
FEMS Yeast Res., 2008 *etc.*

★ Mpr1 protects yeast cells from oxidative stress by controlling ROS levels.



Proc. Natl. Acad. Sci. USA, 2004
J. Biochem., 2005
Appl. Microbiol. Biotechnol., 2007
Biotechnol. Bioeng., 2009
Int. J. Food Microbiol., 2010 *etc.*

Mpr1 is a novel antioxidant enzyme.

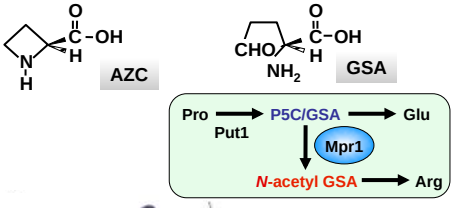
② Mpr1

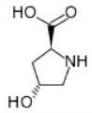
J. Bacteriol., **182**, 4249, 2000; *J. Biol. Chem.*, **276**, 41998, 2001; *Yeast*, **19**, 1437, 2002; **26**, 587, 2009; *J. Biochem.*, **133**, 67, 2003; **138**, 391, 2005; *Proc. Natl. Acad. Sci. USA*, **101**, 12616, 2004; **110**, 11821, 2013; *Appl. Microbiol. Biotechnol.*, **75**, 1343, 2007; **97**, 247, 2013; *FEMS Yeast Res.*, **8**, 607, 2008; *Biotechnol. Bioeng.*, **103**, 341, 2009; *Int. J. Food Microbiol.*, **138**, 181, 2010; *J. Biosci. Bioeng.*, **114**, 160, 2012.

< So far >

★ Mpr1 catalyzes *N*-acetylation of AZC, GSA and *cis*-4-hydroxy-L-proline *!!*

★ Mpr1 is involved in antioxidation by GSA *N*-acetylation and stress-induced arginine synthesis *!!*





Mpr1

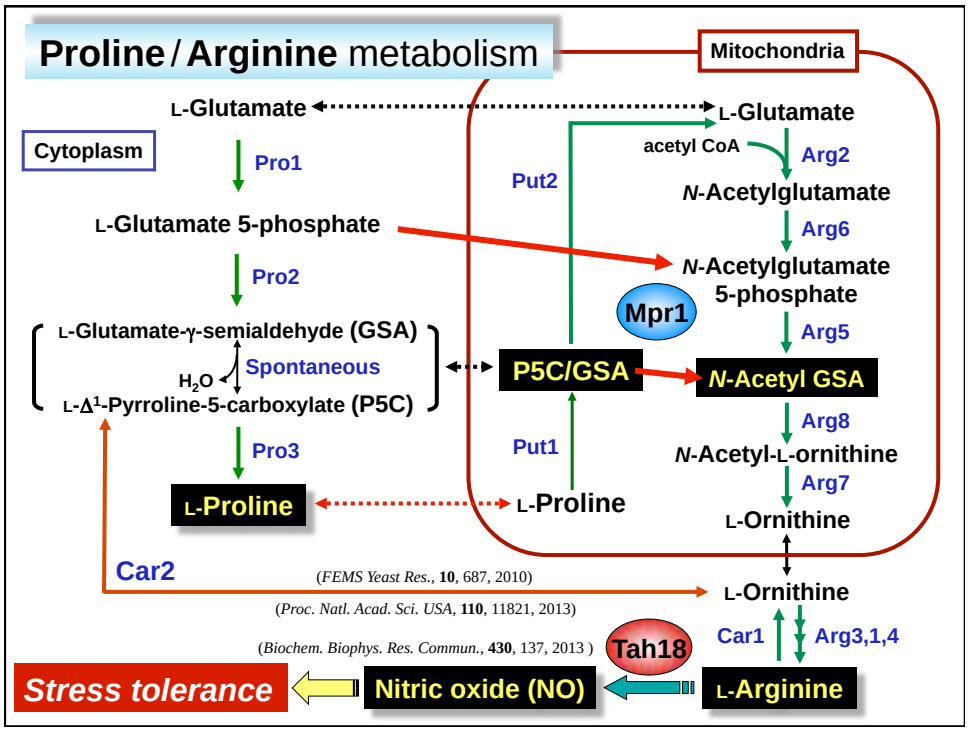
***N*-acetyl CHOP**

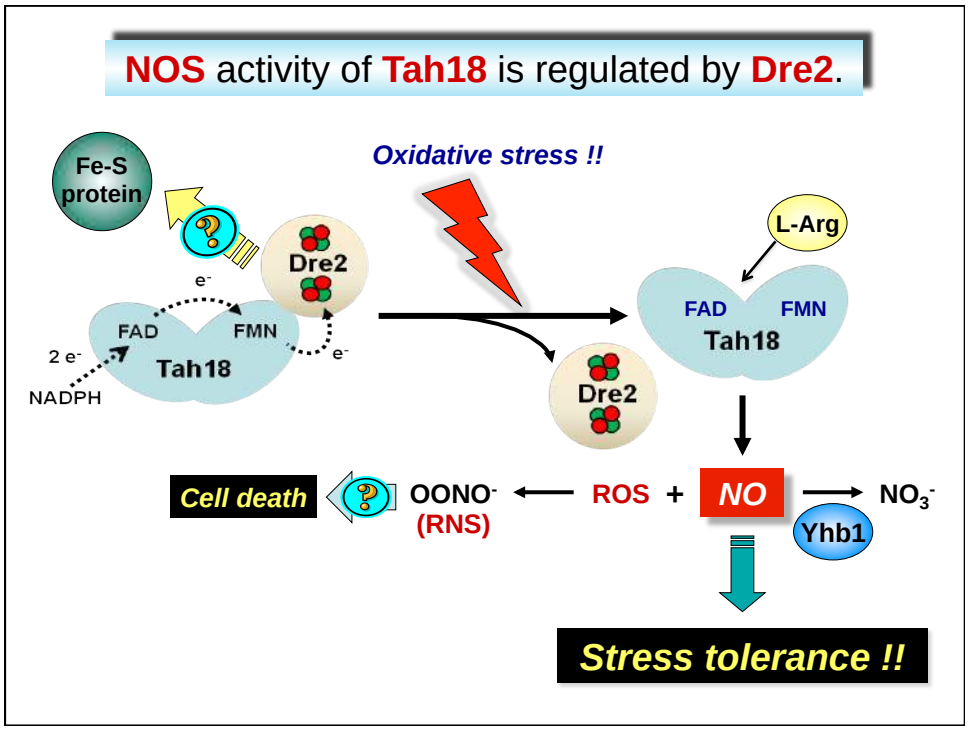
A novel anticancer drug

< Current projects >

- Determination of 3D structure
- Catalytic reaction mechanism
- Production of *N*-acetyl CHOP

Mpr1 engineering for industrial application


Proline / Arginine metabolism
Mitochondria
Cytoplasm
L-Glutamate
L-Glutamate
Pro1
acetyl CoA
Arg2
Put2
N-Acetylglutamate
L-Glutamate 5-phosphate
Arg6
N-Acetylglutamate 5-phosphate
Pro2
Mpr1
L-Glutamate-γ-semialdehyde (GSA)
Arg5
H2O
Spontaneous
P5C/GSA
N-Acetyl GSA
L-Δ1-Pyrroline-5-carboxylate (P5C)
Arg8
Put1
N-Acetyl-L-ornithine
Pro3
Arg7
L-Proline
L-Proline
L-Ornithine
Car2
(FEMS Yeast Res., 10, 687, 2010)
L-Ornithine
(Proc. Natl. Acad. Sci. USA, 110, 11821, 2013)
Car1
Arg3,1,4
(Biochem. Biophys. Res. Commun., 430, 137, 2013)
Tah18
Stress tolerance
Nitric oxide (NO)
L-Arginine


NOS activity of Tah18 is regulated by Dre2.
Oxidative stress !!
Fe-S protein
Dre2
e-
FAD
FMN
2 e-
Tah18
NADPH
e-
L-Arg
FAD
FMN
Tah18
Dre2
Cell death
OONO-
(RNS)
ROS
+
NO
NO3-
Yhb1
Stress tolerance !!

③ Nitric oxide

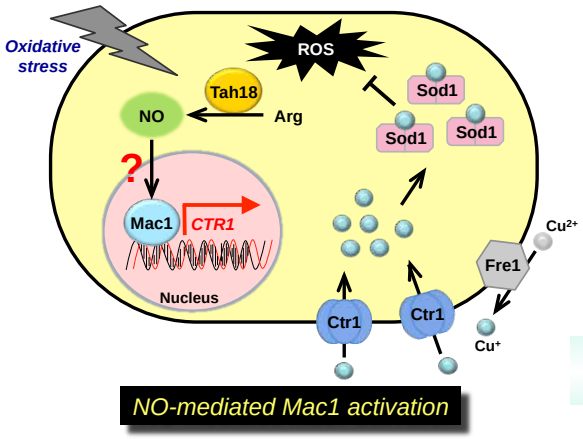
A novel antioxidative mechanism in yeast

FEMS Yeast Res., **10**, 687, 2010; *Microb. Cell Fact.*, **11**:40 doi: 10.1186/1475-2859-11-40, 2012: *Proc. Natl. Acad. Sci. USA*, **110**, 11821, 2013; *Biochem. Biophys. Res. Commun.*, **430**, 137, 2013; *PLoS One*, **9**, e113788, 2014.

★ Put1 and Mpr1 are required for stress-induced Arg synthesis *!!*

★ Arg-dependent NO synthesis confers stress tolerance on yeast cells *!!*

★ The Tah18 protein is first identified as the yeast NO synthase *!!*



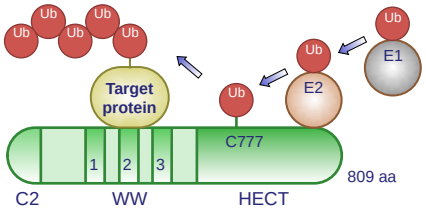
NO-mediated Mac1 activation

< Current projects >

- Characterization of Tah18
- Downstream pathway of NO
- Physiological role of NO

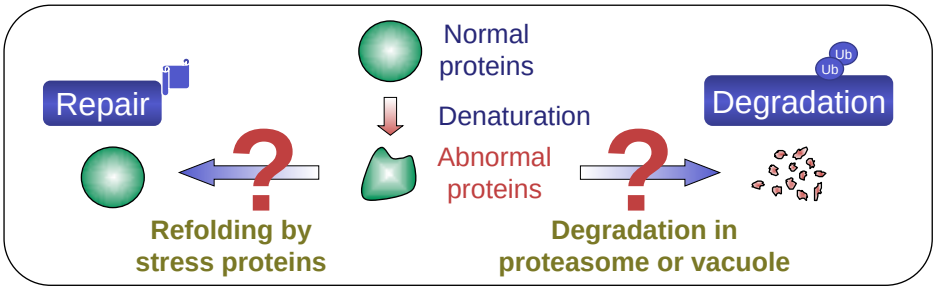
Breeding of novel stress-tolerant yeast strains with NO production

Rsp5 is an essential E3 ubiquitin ligase.



Rsp5 participates in many events through ubiquitination of target proteins;

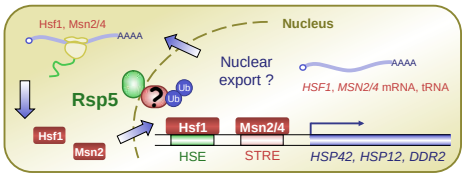
endocytosis of plasma membrane permeases, mitochondrial inheritance, degradation of the large subunit of RNA pol. II, biosynthesis of unsaturated fatty acids, actin cytoskelton organization, sporulation, ER-associated degradation etc.

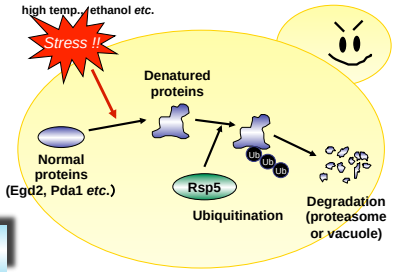


Rsp5 may be involved in repair / degradation of abnormal proteins.

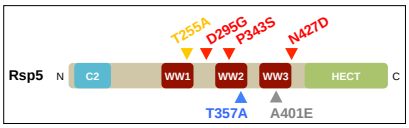
Rsp5

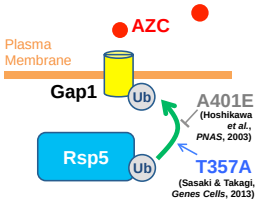
Involved in repair / degradation of abnormal proteins

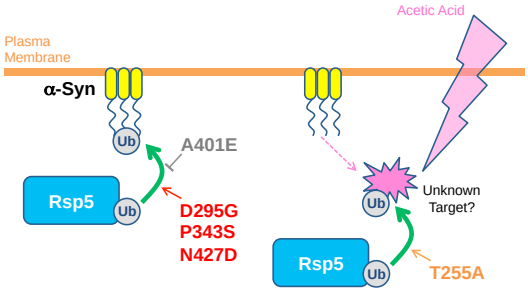




Required for degradation of Gap1







④ Ubiquitin system

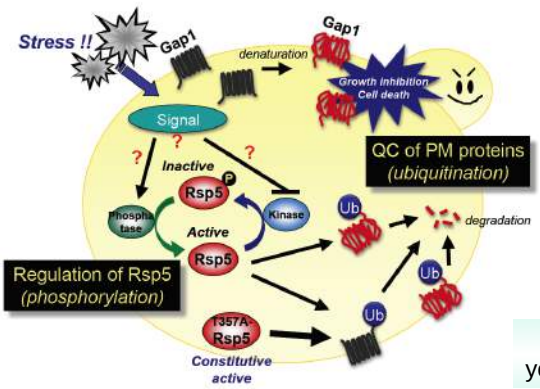
Proc. Natl. Acad. Sci. USA, **100**, 11505, 2003; *FEBS Lett.*, **580**, 3433, 2006; *Biosci. Biotech. Biochem.*, **70**, 2762, 2006; **73**, 2268, 2009; *FEMS Microbiol. Lett.*, **277**, 70, 2007; *Genes Cells*, **13**, 105, 2008; *FEMS Yeast Res.*, **9**, 73, 2009; **14**, 567, 2014; *FEBS J.*, **276**, 5287, 2009 ; *J. Brew. Distill.*, **3**, 1, 2012, *Genes Cells*, **18**, 459, 2013; *Eukaryot. Cell*, **13**, 1191, 2014; *J. Biochem.*, **157**, 251, 2015; *Biochem. Biophys. Res. Commun.*, in press. *etc.*

< So far >

★ Rsp5 is involved in quality control of plasma membrane proteins *!!*

★ Rsp5 activity is regulated by phosphorylation of a conserved Thr357 *!!*

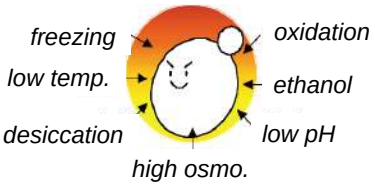
< Current projects >



- Recognition and degradation of abnormal proteins by Rsp5
- Functional improvement of Ub-system (Rsp5)
- Regulation of the Rsp5 activity via phosphorylation

Breeding of novel stress-tolerant yeast strains with improved Ub-system

Contribution to biotechnology



Stress-tolerant industrial yeasts

＜Expansion of **yeast**-related industry＞

・Improvement of fermentation ability

Efficient production of alcoholic beverages and breads

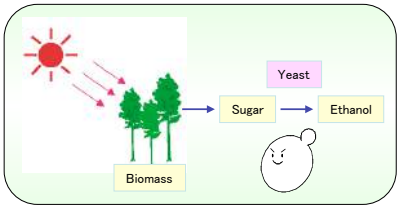


＜Creation of **yeast**-based new industry＞

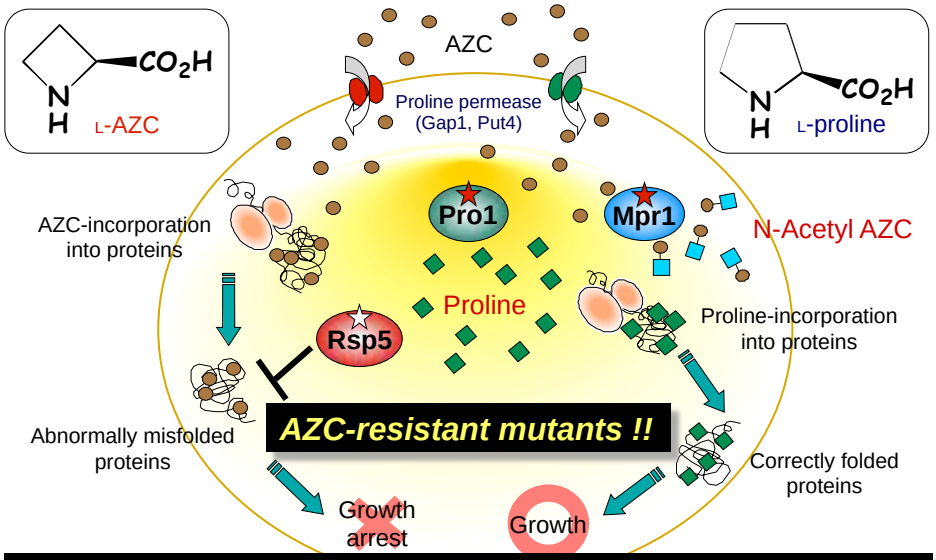
・Production of bioethanol

High ethanol productivity and tolerance





Construction of commercial industrial yeasts



Pro1/Mpr1/Rsp5 variants confer stress tolerance to yeast cells.

Cysteine: metabolic regulation and overprodution

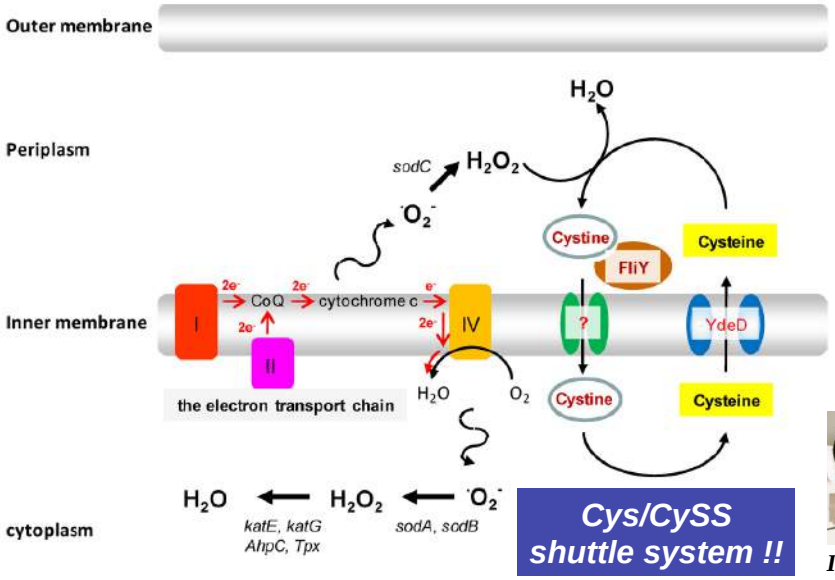
Appl. Environ. Microbiol., **64**, 1607, 1998;
FEBS Lett., **452**, 323, 1999;
FEMS Microbiol. Lett., **179**, 453, 1999;
J. Biochem., **136**, 629, 2004;
FEMS Microbiol. Lett., **255**, 156, 2006;
Protein Eng. Des. Sel., **19**, 163, 2006;
Microb. Cell Fact., **11**:62 doi: 10.1186/1475-2859-11-62, 2012 *etc.*

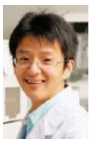
Appl. Environ. Microbiol., **72**, 4735, 2006;
Appl. Microbiol. Biotechnol., **81**, 903, 2009 *etc.*

FEMS Microbiol. Lett., **217**, 103, 2002;
Appl. Microbiol. Biotechnol., **62**, 239, 2003;
Appl. Environ. Microbiol., **71**, 4149, 2005 *etc.*

Cysteine is important for the H_2O_2 resistance in *E. coli*.

(*J. Biol. Chem.*, **285**, 17479, 2010; *PLoS One*, **10**, e0120619, 2015)





I. Ohtsu

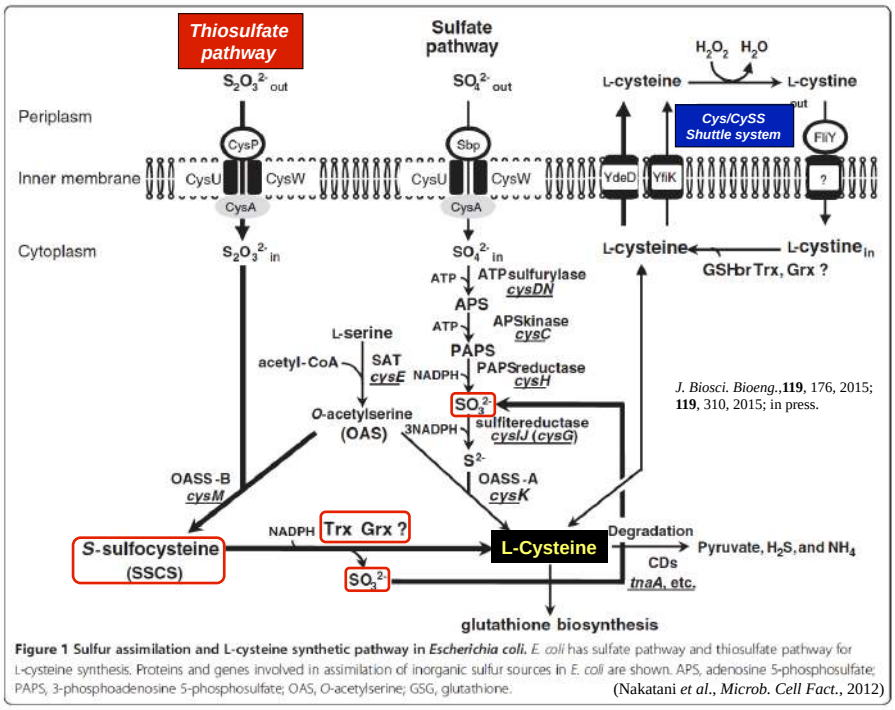


Figure 1 Sulfur assimilation and L-cysteine synthetic pathway in *Escherichia coli*. *E. coli* has sulfate pathway and thiosulfate pathway for L-cysteine synthesis. Proteins and genes involved in assimilation of inorganic sulfur sources in *E. coli* are shown. APS, adenosine 5-phosphosulfate; PAPS, 3-phosphoadenosine 5-phosphosulfate; OAS, *O*-acetylserine; GSG, glutathione.

J. Biosci. Bioeng.,**119**, 176, 2015; **119**, 310, 2015; in press.

(Nakatani *et al.*, *Microb. Cell Fact.*, 2012)