BioMetals 2005 18:467–474
DOI 10.1007/s10534-005-4663-0

Genome-wide screening of aluminum tolerance in *Saccharomyces cerevisiae*

Masayuki Kakimoto[1], Atsushi Kobayashi[1], Ryouichi Fukuda[2], Yasuke Ono[2], Akinori Ohta[2] & Etsuro Yoshimura[1,*]
[1]*Department of Applied Biological Chemistry and;* [2] *Department of Biotechnology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan; *Author for correspondence (E-mail: ayoshim@mail.ecc.u-tokyo.ac.jp)*

Received 7 August 2004; accepted 29 March 2005

Key words: Al-tolerant genes, PKC1-MAPK cascade, protein mannosylation, retrograde transport, *Saccharomyces cerevisiae*

Abstract

Genome-wide screening has identified 37 Al-tolerance genes in *Saccharomyces cerevisiae*. These genes can be roughly categorised into three groups on the basis of function, i.e., genes related to vesicle transport processes, signal transduction pathways, and protein mannosylation. The largest group is composed of genes related to vesicle transport processes; severe Al sensitivity was found in yeast strains lacking these genes. The retrograde transport of endosome-derived vesicles back to the Golgi apparatus is an important factor in determining the Al tolerance of the vesicle transport system. The PKC1-MAPK cascade signalling pathway is important in the Al tolerance of signal transduction. The lack of the gene implicated in this process leads to weakened cell wall architecture, rendering the yeast Al-sensitive. Alternatively, Al might attack the cell wall and/or plasma membrane, and, as signalling is prevented in cells devoid of the genes related to signalling processes, the cells may be unable to alleviate the damage. The genes for protein mannosylation are also associated with Al tolerance, demonstrating the importance of cell wall architecture. These genes are involved in cell integrity processes.

Introduction

The most abundant metal in the Earth's crust is Al (8.2% of the crust; Cox 1995). The metal is present mostly in the forms of oxides and silicates that are sparingly soluble under neutral pH conditions. Increased acidity, which may occur naturally or anthropogenically, promotes the release of Al in the form of metal ions. These ions are toxic to a variety of living organisms, including animals, higher plants, and microbes. Although a wealth of information is available regarding the physiological and biochemical changes caused by Al ions, the mechanisms whereby the ions exert these toxic effects are still poorly understood.

Several genes of *Saccharomyces cerevisiae* involved in Al metabolism have been identified. The deletion of the *SLT2* or *BCK1* (*SLK1*) gene makes yeast Al-sensitive (Schott & Gardner 1997). Slt2p and Bck1p proteins encode MAP kinase and MAP kinase kinase, respectively (Levin & Errede 1995), indicating the involvement of MAP kinase signalling pathways in Al tolerance. The expression of the *HSP150* gene is induced by Al stress and also plays a role in Al tolerance, as disruption of the gene renders the yeast Al-sensitive (Ezaki *et al.* 1998). The vacuolar H^+-ATPase (V-ATPase) is known to function in Al tolerance; deletion mutant strains (*vma1*, *vma2*) demonstrate a striking decrease in growth in the presence of Al (Hamilton *et al.* 2001). In contrast, the over-expression of two yeast genes, *ALR1* and *ALR2*, confers increased tolerance to Al (MacDiarmid & Gardner 1998). These findings lead to the hypothesis that yeast should possess cellular mechanisms that act as constitutive modules to ameliorate Al toxicity. To further under-

standing of Al tolerance in yeast, a genome-wide analysis has been undertaken using a series of single gene deletion mutants of *S. cerevisiae*.

Materials and methods

The total set (4826 strains) of a deletion series of the budding yeast *Saccharomyces cerevisiae* BY4741 (EUROSCARF) was employed throughout this study. The background of this strain is: *Mat*α; *his*3Δ1; *leu*2Δ0; *met*15Δ0; *ura*3Δ0. Low pH, low phosphate (LPP) medium (Schott & Gardner 1997) was solidified with 1% agarose. Yeast cells were suspended in LPP medium, and the suspension was spotted onto solidified medium with and without Al. Cells were grown for 5 d at 30 °C, and the growth rates on medium with and without Al were compared. An $AlCl_3$ concentration of 1 mM was employed in the first screening. Strains that showed no appreciable growth in the Al-containing medium were further evaluated in the second screening in which the media contained 0.2, 0.4, or 0.6 mM $AlCl_3$.

Patterns of uptake and release of Al by the strains were examined for the cells grown in YPD medium (100 ml) for 18 h. After the cells were washed in LPP medium and collected, they were inoculated in LPP medium (100 ml) containing 0.1 mM Al. Strains were cultured for 4 h, collected by centrifugation, and then washed in LPP medium without Al. After the cells were re-grown in this medium, they were collected by centrifugation at every 0.5 h, digested with HNO_3, and analyzed for Al by inductively coupled plasma emission spectrometry (SPS 1200 VR, Seiko Instruments, Tokyo). The Al concentrations in cells were expressed as in a $\mu g/OD_{600}$ unit.

Results

Approximately 200 strains of the deletion series showed no appreciable growth on 1 mM Al medium. Of these, one strain (*vps15*) did not grow on 0.2 mM Al, nine did not grow on 0.4 mM Al, and 27 did not grow on 0.6 mM Al medium. Database analysis of the disrupted genes in the selected strains enabled the categorization of these genes into three classes with respect to cellular functions (http://www.yeastgenome.org/). The group comprising genes related to vesicle transport was the largest with 14 genes (Table 1), followed by the group comprising genes related to signal transduction (Table 2), and finally the group comprising genes associated with protein mannosylation (Table 3). The remaining genes had miscellaneous cellular functions in transport and lipid biosynthesis (Table 4).

The concentrations of Al in cells cultured for 4 h in liquid LPP medium containing 0.1 mM Al are shown in Tables 1–4. The cell density (an optical density at 600 nm; OD_{600}) of each strain at harvest was also tabulated in these tables. Two strains, *cdc40* and *thp1*, did not grow in LPP medium without Al. The Al content of the other sensitive strains was almost equal to or higher than that of wild-type yeast. This was especially true for the strains with defective glycosylation genes (*ANP1*, *CAX4*, *MNN10*, and *MNN11*), in which the Al content was more than twice that of wild-type yeast. Strains with defects in genes involved in vesicle transport and signal transduction pathways also had increased Al levels.

When cells cultured in Al-containing medium were transferred to LPP medium without Al, the slowest Al releasing rate was seen in *pmr1* mutant among wild-type and four arbitrarily selected mutants (*mnn10*, *pmr1*, *rgp1* and *ypt6*) strains (data not shown). As shown in Fig.1, the cellular concentrations of Al in wild type and *pmr1* strains decreased at a rate approximated by biphasic first-order kinetics. Thus, it is likely that two distinct Al pools are present in yeast cells. As rate constants for the slow release process are closely related to cell growth rates, this process could be accounted for by a dilution effect caused by increased yeast biomass. In contrast, the fast process represents more rapid Al decay and indicates excretion of Al from the cells. As the fast process ends up within 1 h, rate constants of the fast decay were calculated based on the change in cellular Al concentrations up to 0.5 h. As shown in Tables 1–4, almost all Al-sensitive strains have a rate constant for fast Al release kinetics similar to that of the wild-type strain (except for mutants with defects in *ERV29* or *VPS51*, which belong to the group of genes involved in vesicle transport), where the rate constant was calculated by slope of the fast decay times −2.303. These mutants secrete Al at 30% of the rate observed in wild-type yeast.

Discussion

Genome-wide screening of *S. cerevisiae* identified 37 Al-tolerance genes. Clustering the genes with respect to their cellular activity revealed that the largest group was composed of genes involved in vesicle transport processes. Severe Al sensitivity was found in a strain lacking the genes belonging to this subset. One strain (*vps15*) was Al sensitive at Al concentrations of 0.2 mM, and seven strains (*rgp1*, *ric1*, *sso2*, *vps45*, *vps52*, *vps61*, and *ypt6*) were sensitive to 0.4 mM Al. Two strains (*bud19* and *ccr4*), categorized as signal transduction pathway genes, also demonstrated Al sensitivity at 0.4 mM Al. The other strains were sensitive at concentrations of 0.6 mM Al. By comparison, the wild-type strain without any defect grows in the presence of 1 mM Al.

Vesicular transport pathways

It appears that genes associated with secretory/endocytic pathway are implicated in Al tolerance (Fig. 2). Vps52p, Vps53p, and Vps54p form the VFT complex and have a tethering role (Whyte & Munro 2002). Vps51p is required for the association of the VFT complex with Tlg1p, a SNARE protein that is localized in endosome-derived

Table 1. Al-tolerance genes implicated in vesicle transport processes.

Gene	Screened Al conc. (mM)	Al content[a] (μg/OD_{600})	Cell density (OD_{600})	Rate constant[b] (h^{-1})	Molecular function	Biological process
–[c]	–	4.90	4.19	1.27		
COG6	0.6	5.79	8.34	1.04	Unknown	Intra-Golgi transport
COG8	0.6	6.11	9.94	1.31	Unknown	Intra-Golgi transport
ERV29	0.6	6.77	3.84	0.36	Unknown	ER to Golgi transport
GOS1	0.6	7.13	5.70	0.92	v-SNARE activity	Intra-Golgi transport; vesicle fusion
RGP1	0.4	6.55	7.20	0.99	Guanyl-nucleotide exchange factor activity	Intracellular protein transport
RIC1	0.4	5.13	7.74	1.45	Guanyl-nucleotide exchange factor activity	Intracellular protein transport
SSO2	0.4	6.82	3.75	1.63	t-SNARE	Golgi to plasma membrane transport; vesicle fusion
TLG2	0.6	7.42	10.00	1.27	t-SNARE activity, v-SNARE activity	Vesicle fusion
VPS15	0.2	13.79	2.45	1.31	Protein serine/threonine kinase activity	Protein amino acid phosphorylation; protein-Golgi retention; protein-vacuolar targeting; vacuolar transport
VPS45	0.4	9.72	1.84	1.14	Chaperone activity	Golgi to vacuole transport; osmoregulation; protein complex assembly; vacuole organization and biogenesis
VPS51	0.6	6.91	10.0	0.38	Protein binding	Apical bud growth; protein-vacuolar targeting; retrograde transport, endosome to Golgi; vesicle organization and biogenesis
VPS52	0.4	8.83	9.80	0.79	Protein binding	Golgi to vacuole transport; actin filament-based process; retrograde transport, endosome to Golgi
VPS61	0.4	6.05	8.60	0.83	Unknown	Protein-vacuolar targeting
YPT6	0.4	9.17	7.56	1.14	GTPase activity	Intracellular protein transport; retrograde transport, endosome to Golgi

[a] Al content of the cells was normalized with OD_{600nm}.
[b]The rate constants of the fast decay were calculated based on the change in the cellular Al concentration up to 0.5 h.
[c]Wild-type strain.

Table 2. Al-tolerance genes implicated in signal transduction pathways.

Gene	Screened Al conc. (mM)	Al content[a] (μg/OD_{600})	Cell density (OD_{600})	Rate constant[b] (h^{-1})	Molecular function	Biological process
BCK1	0.6	9.32	7.41	0.98	MAP kinase kinase activity	Establishment of cell polarity (sensu *Saccharomyces*); protein amino acid phosphorylation; protein kinase cascade; response to nutrients
BDF1	0.6	10.31	3.40	0.67	Transcription regulator activity	Chromatin remodelling; sporulation (sensu *Saccharomyces*)
BUD19	0.4	7.54	4.53	1.21	Unknown	Bud site selection
CCR4	0.4	7.68	3.94	1.43	3′-5′ exoribonuclease activity	mRNA catabolism, deadenylation-dependent; poly(A) tail shortening; regulation of transcription from Pol II promoter
CDC40	0.6	–[c]	–[c]	–[c]	Nucleic acid binding activity	DNA replication; S phase of mitotic cell cycle; mitotic spindle assembly (sensu Fungi); nuclear mRNA splicing, via spliceosome
PTK2	0.6	8.45	4.50	1.62	Protein kinase activity	G1/S transition of mitotic cell cycle; cell ion homeostasis; polyamine transport
SIT4	0.6	9.06	4.85	1.65	Protein serine/threonine phosphatase activity	G1/S transition of mitotic cell cycle; actin cytoskeleton organization and biogenesis; cell wall organization and biogenesis; protein amino acid phosphorylation; protein kinase cascade
SLT2	0.6	21.32	3.45	0.93	MAP kinase kinase activity	Cell wall organization and biogenesis; protein amino acid phosphorylation; signal transduction

[a]Al content of cells was normalized with $OD_{600\ nm}$.
[b]The rate constants of the fast decay were calculated based on the change in the cellular Al concentration up to 0.5 h.
[c]No growth was observed in LPP medium without Al.

vesicles (Siniossoglou & Pelham 2001; Siniossoglou & Pelham 2002). The GTPase Ypt6p is associated with the Golgi apparatus and is activated by a complex formed by Ric1p and Rgp1p via nucleotide exchange (Siniossoglou *et al.* 2000). Activated Ypt6p bridges the VFT complex with the Golgi apparatus (Siniossoglou & Pelham 2001). These gene products are implicated in retrograde transport from endosome-derived vesicles to late Golgi membranes. With the exception of *VPS53* and *VPS54*, these genes are all involved in basal Al tolerance (Table 1). The involvement of Tlg1p in basal Al tolerance has been unclear, because *TLG1* is essential for cellular existence. These findings strongly demonstrate the involvement of retrograde transport in basal Al tolerance.

TLG2 and *VPS45* were found to be Al-tolerance genes (Table 1). Similar to strains devoid of the genes involved in retrograde transport from endosome-derived vesicles to Golgi membrane, the strain lacking *TLG2* exhibits defects in retrieval of TGN-resident proteins (Abeliovich *et al.* 1998). Also, in the absence of Vps45p, synthesized Tlg1p is degraded by the proteosome (Bryant & James 2001). *VPS15*, which is needed for the retention of late Golgi membrane proteins (Northwehr *et al.* 1996), is also required for Al tolerance (Table 1). In addition, strains could not grow in medium containing 0.6 mM Al when either *COG6* or *COG8* was deleted (Table 1). Less severe Al sensitivity was observed in the *cog5* strain, which showed lethality at 1 mM Al (data not shown). An aberrant distribution of v-SNARE Snc1p was observed in the strain devoid of *COG5*, *COG6*, or *COG8*, suggesting that these genes are also important in recycling vesicles from endosomes to the Golgi (Whyte & Munro 2001). These findings are also in accordance with the importance to Al tolerance of retrograde transport back to the Golgi.

The strains lacking *ERV29* and *VPS51* are likely to be defective with regard to the Al exudation process (Table 1). Al efflux was severely depressed in these strains, with the rate constants reduced to 28% (for *erv29*) and 29% (for *vps51*) of the wild-type rate. In contrast, in both these strains, Al content of the cells marginally increased

Table 3. Al-tolerance genes implicated in trans-mannosylations.

Gene	Screened Al conc. (mM)	Al content[a] (μg/OD_{600})	Cell density (OD_{600})	Rate constant[b] (h^{-1})	Molecular function	Biological process
ANP1	0.6	12.82	4.49	1.41	Mannosyltransferase activity	N-linked glycosylation
CAX4	0.6	12.08	0.69	1.27	Pyrophosphatase activity	N-linked glycosylation; lipid biosynthesis
MNN10	0.6	12.74	8.49	1.53	Alpha-1,6-mannosyltransferase activity	N-glycan processing; actin filament organization; cell wall mannoprotein biosynthesis; mannan metabolism
MNN11	0.6	10.78	6.96	0.81	Alpha-1,6-mannosyltransferase activity	Protein glycosylation

[a]Al content of cells was normalized with $OD_{600\ nm}$.
[b]The rate constants of the fast decay were calculated based on the change in the cellular Al concentration up to 0.5 h.

Table 4. Al-tolerance genes implicated in miscellaneous cellular functions.

Gene	Screened Al conc. (mM)	Al content[a] (μg/OD_{600})	Cell density (OD_{600})	Rate constant[b] (h^{-1})	Molecular function	Biological process
AGP2	0.6	6.03	4.25	1.73	Hydrogen, amino acid symporter activity	Amino acid transport; carnitine transport; fatty acid metabolism; response to osmotic stress
COX16	0.6	10.62	5.00	1.25	Unknown	Aerobic respiration; protein complex assembly
FES1	0.6	8.04	4.05	1.62	Adenyl-nucleotide exchange factor activity	Protein biosynthesis
HUR1	0.6	6.94	5.48	0.83	Unknown	DNA replication
MCH5	0.6	6.74	6.00	1.04	NOT a monocarboxylic acid transport activity	Transport
OPI3	0.6	7.41	4.20	0.88	Phosphatidyl-N-methylethanolamine N-methyl transferase activity	Phosphatidylcholine biosynthesis
PMR1	0.6	3.47	2.72	0.66	Calcium-transporting ATPase activity	Calcium ion transport; manganese ion transport; secretory pathway
RPE1	0.6	8.59	4.95	1.50	Ribulose-phosphate 3-epimerase activity	Pentose-phosphate shunt
SAC1	0.6	11.16	3.80	1.32	Inositol/phosphatidylinositol phosphatase activity	Dephosphorylation; phosphoinositide metabolism
THP1	0.6	–[c]	–[c]	–[c]	Protein binding	DNA recombination; RNA elongation from Pol II promoter; RNA-nucleus export; bud site selection
VMA21	0.6	8.46	6.45	0.5	Unknown	Protein complex assembly

[a] Al content of cells was normalized with OD_{600nm}.
[b]The rate constants of the fast decay were calculated based on the change in the cellular Al concentration up to 0.5 h.
[c] No growth was observed in LPP medium without Al.

(Table 1). *ERV29* encodes a member of the COPII coat complex which is though to participate in anterograde transport from the ER to the Golgi (Otte *et al*. 2001). Vps51p has a crucial role in bridging the VFT complex and the endosome-derived vesicle. Therefore, both Erv29p and Vps51p

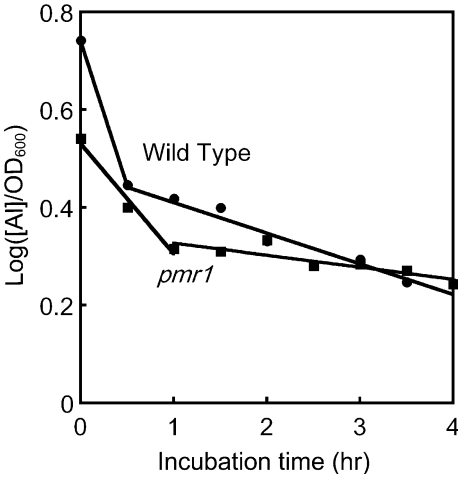

Figure 1. Biphasic decay of intracellular Al concentration. Cells cultured for 4 h in LPP medium containing 0.1 mM Al were washed and cultured in LPP medium without Al. The initial OD_{600} values for wild-type and *pmr1* strains were 4.2 and 2.7, respectively. The Al concentrations in cells were expressed as $\mu g/OD_{600}$.

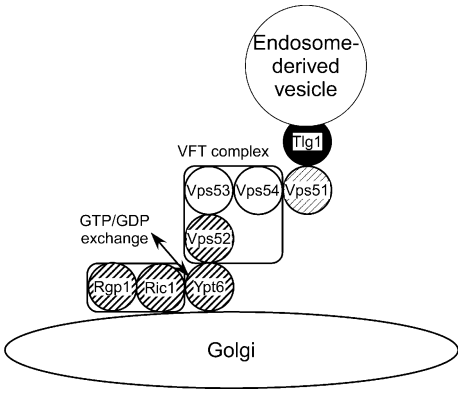

Figure 2. Scheme for retrograde transport of endosome-derived vesicles to the Golgi. Products of essential genes are represented by closed circles. Genes involved in Al tolerance at 0.4 mM Al are shown by hatched circles with bold lines, and those at 0.6 mM Al are indicated by hatched circles with fine lines.

might be implicated in the Al-releasing module, although the molecular mechanisms for Al release from yeast cell are unclear.

Signal transduction

In a previous report (Schott & Gardner 1997), it was shown that the deletion of *SLT2* or *BCK1*(*SLK1*) rendered yeast Al sensitive. These genes were also identified in our screens (Table 2). Additionally, in the first screen using 1 mM Al, *SLG1*, *SWI4*, and *SWI6*, were found to have roles in Al tolerance (data not shown). Swi4p and Swi6p form the SBF complex: Swi4p binds to DNA and Swi6p is phosphorylated by activated MAP kinase Slt2p (Fig. 3). Taking into account the fact that *RHO1* GTPase is essential, it is apparent that the signal transduction pathway (Fig. 3) plays an important role in Al tolerance.

Breakdowns in signalling cause weakened cell walls. The deletion of *MPK1* renders the yeast susceptible to zymolyase digestion (de la Torre-Ruiz *et al.* 2002). The deletion of *BCK1* results in temperature-dependent cell lysis defects (Lee & Levin 1992). Mutant strains lacking either the SBF component Swi4p or Swi6p are hypersensitive to calcofluor white and SDS (Igual *et al.* 1996). A weakened cell wall could account for the increased Al sensitivity in a way similar to that observed in mutants devoid of genes related to protein mannosylation (Table 3). Defective PKC1-MAPK cell integrity pathway may fail to activate genes related to the maintenance of cell wall integrity, resulting in Al sensitivity.

CCR4 was identified as an Al-tolerance gene (Table 2). Ccr4p was found to occur along with Paf1p, Cdc73p, and Hpr1p in a complex containing RNA polymerase II (Fig. 3) (Chang *et al.* 1999). Because it is more likely that this complex occurs downstream of the PKC1-MAPK pathway (Chang *et al.* 1999), Ccr4p would also contribute to the maintenance of cell wall integrity via a mechanism similar to that performed by the SBF complex.

The *sit4* mutant demonstrated Al sensitivity (Table 2). *SIT4* codes for a protein phosphatase and negatively regulates the PKC1-MAPK signalling pathway; thus, the deletion mutant *sit4* has enhanced signalling activity. This would lead to a rigid cell wall structure, since this strain displays enhanced tolerance to treatment with zymolyase (de la Torre-Ruiz *et al.* 2002). On the other hand, the *sit4* strain also displays another phenotype in which the G_1 state is elongated. It is possible that *SIT4* functions in cell cycle regulation. Pkc1p is implicated in many cellular process including actin cytoskeleton organization, ribosomal gene transcription, and G_1 arrest. The absence of Sit4p would provoke Pkc1 activation, resulting in a delay from G_1 to S (de la Torre-Ruiz *et al.* 2002). Several genes involved in cell

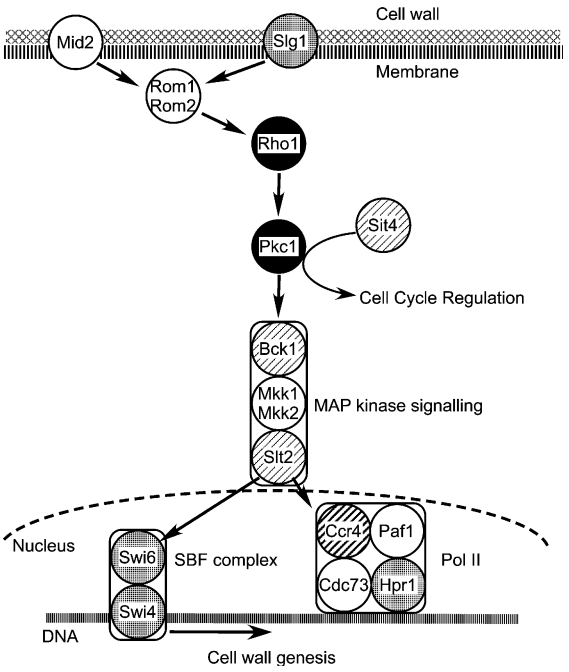

Figure 3. Scheme for cell integrity signalling pathway. Products of essential genes are represented by closed circles. Genes involved in Al tolerance at 0.4 mM Al are represented by hatched circles with bold lines, and those at 0.6 mM Al are indicated by hatched circles with fine lines. Genes identified in the first screening (1 mM Al) are represented by circles with dots.

cycle regulation-*BUD19*, *CDC40*, and *PTK2*–appear to have roles in Al tolerance (Table 2). Although the mechanisms are unclear, there could be a partial link between Al tolerance and cell cycle regulation.

N-linked mannosyl transferase

Mutant strains lacking *ANP1*, *MNN10*, or *MNN11* were unable to grow in the presence of 0.6 mM Al (Table 3). *N*-linked mannosylation is initiated by M-pol I (containing Mnn9p and Van1p), and the subsequent elongation is performed by M-pol II (containing Anp1p, Hoc1p, Mnn9p, Mnn10p and Mnn11p; Jungmann *et al.* 1999). Thus the deletion of the proteins constituting the M-pol II complex rendered the yeast Al-sensitive, and increased intracellular Al levels were evident (Table 3). Mannans may bind Al ions, preventing them from progressing closer to the yeast membrane. This may account for the higher levels of Al found in yeast strains devoid of the constituents of the M-pol II complex.

Other mechanisms

Three genes have so far been identified as being involved in Al tolerance: *HSP150* (Ezaki *et al.* 1998), which encodes a heat shock protein, and *TFP1* (*VMA1*) and *VMA2* (Hamilton *et al.* 2001) which encode subunits of the V-ATPase. In our screen, however, these genes demonstrated no clear Al-tolerance effect. These genes might function in Al tolerance at lower levels than those of the genes identified in our screen. Differences in culture conditions used for testing Al tolerance may also contribute to the discrepancy. Al tolerance was examined for *HSP150* in supplemented minimal medium (Ezaki *et al.* 1998) and for *VMA1* and *VMA2* in liquid LPP medium (Hamilton *et al.* 2001). This is in contrast to our study in which solidified LPP medium was used. *VMA21* was identified as an Al-tolerance gene in connection with V-ATPase (Table 4). Vma21p is an ER-resident accessory protein and is required for the assembly and transport of V-ATPase. The lack of any of these accessory proteins resulted in destabilization of V-ATPase (Herrmann *et al.* 1999). This supports the suggestion that V-ATPase activity is implicated in Al tolerance.

Enhanced Al tolerance was found for yeast strains over-expressing *ALR1* or *ALR2*, the yeast uptake system for Mg (MacDiarmid & Gardner 1998). These genes were not identified in our study, perhaps owing to redundancy of the genes. The cation pump protein Pmr1p also seemed to have a role in Al tolerance (Table 4). This protein is located in the Golgi and mediates ATP-dependent transport of Ca^{2+} and Mn^{2+} ions (Dürr *et al.* 1998). The *pmr1* strain has a defect in protein glycosylation that results from decreased Mn^{2+} concentration in the Golgi (Dürr *et al.* 1998). This could contribute to the Al sensitivity of this strain.

Conclusion

Genome-wide screening of the Al-tolerance genes in *S. cerevisiae* has disclosed some of the tolerance mechanisms in yeast. Three cellular processes – vesicle transport, signal transduction pathways, and protein mannosylation-appear to play vital roles in Al tolerance. In particular, retrograde transport of endosome-derived vesicles to the Golgi apparatus is important in determining the Al

tolerance of the vesicle transport system. The PKC1-MAPK cascade signalling pathway is the critical Al-tolerance factor in the signal transduction pathway. These cellular processes probably do not function independently, but may be acting cooperatively. In particular, the signal transduction pathway could be closely related to the protein mannosylation pathway in terms of Al tolerance.

Acknowledgements

The authors are indebted to Professor Koji Yoda for helpful advice and discussions of detailed experimental procedures and to Professor Masahiko Saigusa for helpful discussions. This work was supported by a Grant-in-Aid for Scientific Research (No. Z3070009) from the Ministry of Education, Science, Culture, and Sports of Japan.

References

Abeliovich H, Grote E, Novick P, Ferro-Novick S. 1998 Tlg2p, a yeast syntaxin homolog that resides on the Golgi and endocytic structure. *J Biol Chem* **273**, 11719–11727.

Brady D, Starke AD, Duncan JR. 1994 Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Saccharomyces cerevisiae*. *Biotech Bioengineer* **44**, 297–302.

Bryant NJ, James DE. 2001 Vps45p stabilizes the syntaxin homologue Tlg2p and positively regulates SNARE complex formation. *EMBO J* **20**, 3380–3388.

Chang M, French-Cornay D, Fan H-Y, Klein H, Denis CL, Jaehning JA. 1999 A complex RNA polymerase II, Paf1p, Cdc73p, Hpr1p, and Ccr4p plays a role in protein kinase C signalling. *Mol Cell Biol* **19**, 1056–1067.

Cox PA. 1995 The Elements on Earth. Oxford University Press, Oxford.

Dürr G, Strayle J, Plemper R, Elbs S, Klee SK, Catty P, Wolf DH, Rudolph HK. 1998 The medial-Golgi ion pump Pmr1 supplies the yeast secretory pathway with Ca^{2+} and Mn^{2+} required for glycosylation, sorting, and endoplasmic reticulum-associated protein degradation. *Mol Biol Cell* **9**, 1149–1162.

Ezaki B, Gardner RC, Ezaki Y, Kondo H, Matsumoto H. 1998 Protective roles of two aluminum (Al)-induced genes, HSP150 and SED1 of *Saccharomyces cerevisiae*, in Al and oxidative stress. *FEMS Microbiol Lett* **159**, 99–105.

Hamilton CA, Good AG, Taylor GJ. 2001 Vacuolar H^+-ATPase, but not mitochondrial F_1F_0-ATPase, is required for aluminum resistance in *Saccharomyces cerevisiae*. *FEMS Microbiol Lett* **205**, 231–236.

Herrmann JM, Malkus P, Schekman R. 1999 Out of the ER-outfitters, escorts and guides. *Trend Cell Biol* **9**, 5–7.

Igual JC, Johnson AL, Jonston LH. 1996 Coordinated regulation of gene expression by the cell cycle transcription factor SWI4 and the protein kinase C MAP kinase pathway for yeast cell integrity. *EMBO J* **15**, 5001–5013.

Jungmann J, Rayner JC, Munro S. 1999 The *Saccharomyces cerevisiae* protein Mnn10p/Bed1p is a subunit of a Golgi mannosyltransferase complex. *J Biol Chem* **274**, 6579–6585.

Lee KS, Levin DE. 1992 Dominant mutations in a gene encoding a putative protein kinase (BCK1) bypass the requirement for a *Saccharomyces cerevisiae* protein kinase C homolog. *Mol Cell Biol* **12**, 172–182.

Levin DE, Errede B. 1995 The proliferation of MAP kinase signaling pathway in yeast. *Curr Opin Cell Biol* **7**, 197–202.

MacDiarmid CW, Gardner RC. 1998 Overexpression of the *Saccharomyces cerevisiae* magnesium transport system confers resistance to aluminum ion. *J Biol Chem* **273**, 1727–1732.

Northwehr SF, Bryant NJ, Stevens TH. 1996 The newly identified yeast GDR genes are required for retention of late-Golgi membrane protein. *Mol Cell Biol* **16**, 2700–2707.

Otte S, Belden WJ, Heidtman M, Liu J, Jensen ON, Barlowe C. 2001 Erv41p and Erv 46: new component of COPII vesicles involved in transport between the ER and Golgi complex. *J Cell Biol* **152**, 503–517.

Schott EJ, Gardner RC. 1997 Aluminum-sensitive mutants of *Saccharomyces cerevisiae*. *Mol Gen Genet* **254**, 63–72.

Siniossoglou S, Peak-Chew SY, Pelham HRB. 2000 Ric1p and Rgp1p form a complex that catalyses nucleotide exchange on Ypt6p. *EMBO J* **19**, 4885–4894.

Siniossoglou S, Pelham HRB. 2001 An effector of Ypt6p binds the SNARE Tlg1p and mediates selective fusion of vesicles with late Golgi membranes. *EMBO J* **20**, 5991–5998.

Siniossoglou S, Pelham HRB. 2002 Vps51p links the VFT complex to SNARE Tlg1p. *J Biol Chem* **277**, 48318–48324.

de la Torre-Ruiz MA, Torres J, Ariño J, Herrero E. 2002 Sit4 is required for proper modulation of the biological functions mediated by Pkc1 and cell integrity pathway in *Saccharomyces cerevisiae*. *J Biol Chem* **277**, 33468–33476.

Whyte JRC, Munro S. 2001 The Sec/34/35 Golgi transport complex is related to exocyst, defining a family of complexes involved in multiple steps of membrane traffic. *Dev Cell* **1**, 527–537.

Whyte JRC, Munro S. 2002 Vesicle tethering complexes in membrane traffic. *J Cell Sci* **115**, 2627–2637.