Screening for yeast mutants defective in recipient ability for transkingdom conjugation with *Eschelichia coli* revealed importance of vacuolar ATPase activity in the horizontal DNA transfer phenomenon

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Running title:
Yeast mutants defective in recipient ability for transkingdom conjugation with *E. coli*

Key words: Horizontal gene transfer, Gene transfer from bacteria to eukarya, Transkingdom sex, Broad host range plasmid

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Abstract

Proteobacterium *Escherichia coli* strains harboring wide-transfer-range conjugative plasmids are able to transfer these plasmids to several yeast species. Whole plasmid DNA is mobilizable in the transkingdom conjugation phenomenon. Owing to the availability of various conjugative plasmids in bacteria, the horizontal DNA transfer has potential to occur between various bacteria and eukaryotes. In order to know host factor genes involved in such conjugation, we systematically tested the conjugability of strains among a yeast library comprising single-gene-knockout mutants in this study. This genome-wide screen identified 26 host chromosomal genes whose absence reduced the efficiency of the transkingdom conjugation. Among the 26 genes, 20 are responsible for vacuolar ATPase activity, while 5 genes (*SHP1*, *CSG2*, *CCR4*, *NOT5*, and *HOF1*) seem to play a role in maintaining the cell surface. Lack of *ZUO1* gene, which encodes a component of the ribosome-associated cytoplasmic molecular chaperone, also strongly affected transkingdom conjugation.

Key words: Horizontal gene transfer / Conjugation / Transfomation / *Saccharomyce cerevisiae* / Interaction between bacteria and eukarya

Introduction

Bacteria are promiscuous in exchanging genetic materials even among different kingdom. The Gram negative bacterium *Escherichia coli* harboring a wide transfer range type conjugative plasmid can transfer mobile plasmid DNAs even to bacteria of a different kingdom group (Bushman, 2002) —i.e. Gram positive bacteria (Gormley and Davies, 1991). Recent genomic studies have revealed a number of remnants of horizontal DNA transfer between bacteria and eukaryotes. It is well known that pathogenic *Agrobacterium* species can genetically transform plants by putting a portion of their pathogenic plasmid pTi and pRi into plant nuclei by the virulence gene action in nature (Zupan et al., 2000; Suzuki et al., 2009). In addition, experimentally reproducible examples of DNA transfer from bacteria to eukaryotes are increasing (Heinemann and Sprague, 1989; Waters, 2001; Schröder et al, 2011; Fernández-González, 2011). Gram-negative bacteria harboring wide-host-range conjugative plasmids can transfer these plasmids to yeasts (Heinemann and Sprague, 1989; Sikorski et al., 1990; Nishikawa et al., 1990 and 1992; Hayman and Bolen, 1993; Inomata et al., 1994; Bates et al., 1998). Whole plasmid DNA is mobilizable in the *t*rans*k*ingdom *c*onjugation (TKC) phenomenon. Various conjugative plasmids are available in bacteria (Thomas,

1989). TKC is interesting from an evolutional view point and increase of its efficiency is expected from a biotechnogical view point. Combination of a derivative of RK2 as ahelper with incQ-type mobilizable plasmids allowed us to demonstrate the rare integration of mobilized DNA into yeast chromosomes (Nishikawa et al., 1992).

The conjugation plasmids are essential in TKC (Heinemann and Sprague, 1989). Cell density and choice of suitable yeast gene and plasmids affect the *E. coli*-yeast conjugation (Heinemann and Sprague, 1989; Bates et al., 1998). Mechanisms for yeast cells to recognize donor bacterial cells and accept plasmid DNAs are not yet reported. Mutant strains defective in TKC if available would help to study the molecular mechanism of TKC. In this study, we screened yeast mutants with decreased efficiency as the recipients of TKC.

Materials and methods

Donor and recipient strains

Bacterial and yeast strains used in this study are listed in **Table 1**. Yeast strains BY4742 and the complete set of *MATα* knockout mutants (the *Saccharomyces* Genome Deletion Project consortium) derived from BY4742 were purchased from Invitrogen (CA, USA). Yeast strains BY4741, BY4743 and several knockout mutants were kindly provided by Drs. T. Miyakawa and M. Mizunuma (Hiroshima University), J. Shima and S. Andoh (National Food Research Institute, Tsukuba, Japan).

E. coli -yeast mating conditions

Quantitative *E. coli*-yeast mating experiment was carried out essentially as described by Nishikawa et al (1990) with some modification: one hr co-cultivation on YPD agar {2% peptone (Daigo-Eiyo, Tokyo), 1% yeast extract (Difco), 2% glucose and 2% agar} in place of 12 hr co-cultivation.

For mutant screening using the yeast deletion strains, the following co-cultivation

and selection was carried out. Yeast strains were streaked on YPD agar using toothpicks and incubated overnight at 28^{o}C for preculture. Five μl of cell suspension ($3x10^{9}$ cells/ml) of the donor *E. coli* strain HB101(pRH210, pAY205) was spotted on TN agar {TrisHCl (pH 7.5)}. A small quantity of yeast cells was taken from the overnight YPD agar culture, and then mixed with the donor *E. coli* cell suspension on the TN agar using a plastic inoculation loop. The liquid portion of the mixture was absorbed in the agar in several minutes. The mixture was kept overnight at 28^{o}C, and then transferred using a plastic inoculation loop onto SC-ura agar (0.67% yeast nitrogen base w/o amino acid (Difco), 2% glucose, 2% agar supplemented with lysine, leucine and histidine), which is selective for the resulting yeast transconjugant cells.

When recipient yeast strains were prototrophic for uracil (Ura^{+}) and/or had an additional mutation that causes auxotrophy for uracil, the donor strain was replaced with HB101(pRH220, pMZ1) or HB101(pRH220, pMZ2) and leucine in the selective agar was omitted in place of uracil.

Plasmids and plasmid construction

The plasmids used in this study are listed in **Table 1.** Transkingdom mobilization

plasmids pMZ1 and pMZ2 were constructed as follows. Plasmid pHRP309 (Parales and Harwood, 1993) was cleaved with *Sma*I and *Hin*dIII. A 9-kbp fragment of the digest was ligated with YEp351 cleaved with the same pair of enzymes, resulting in a 14.6-kbp plasmid pMZ1 (**Table 1**). The 9-kbp fragment was ligated with YEp351 cleaved with *Aat*II and *Hin*dIII, resulting in a 12.4-kbp plasmid pMZ2 (**Table 1**). Yeast chromosomal genes were amplified by PCR by KOD-plus DNA polymerase (Toyobo, Japan) using primers designed based on the genomic sequences, 0.5-kbp upstream and 0.5-kbp downstream from the target ORF. Recognition sequences for restriction enzymes were added artificially to 5' part of the primer sequences, where necessary. Chromosomal genes were cloned in either one of pRS313, YEp351 and YEp352. pUC19-STV1 is a pUC19 harboring 4.5-kbp *Xba*I-*Kpn*I fragment containing the *STV1* gene. Two *Ban*III fragments (1.4 kbp in total) inside the *STV1* gene were removed and replaced with *HIS3* gene released from pUC19-HIS3 by digestion with *Sma*I and *Hin*cII, resulting in pUC19-stv1::HIS3. pTF63 and pCWH36 were kindly provided by Dr. J. Kaplan (University of Utah).

Miscellaneous genetic and biochemical methods

Growth and or drug sensitivity was tested as follows. A series of serially diluted yeast cell suspension were spotted onto solid media, and then incubated at 28^{o}C for several days to check cell growth.

Yeast Li transformation method followed Gietz and Woods (2002). Techniques for extraction and purification of genomic DNA and plasmid DNA, and construction of recombinant plasmid DNA were essentially as described by Maniatis et al. (1982).

Results

In order to identify the chromosomal genes important for TKC, we screened the series of yeast mutants, each of which has a G418-resistance gene tag in place of respective chromosomal ORF in the yeast nuclear genome.

Identification of the 26 yeast chromosomal genes affecting transkingdom conjugation

The TKC condition described in “Materials and methods” enabled the wild-type

parental strain BY4742 to exhibit confluent growth of transconjugant yeast cells on selective agar (SC-ura) after co-cultivation on TN agar with the donor *E. coli* HB101(pRH210, pAY205). We screened the strains in the complete set of *MAT*α knockout strains for mutants defective in the ability to form such transconjugant cells. We detected yeast mutant strains exhibiting no transconjugant growth or growth of an apparently smaller number of transconjugant colonies on the selective SC-ura agar after the co-cultivation. The strains were tested further twice by the same method. To evaluate mutants that had an additional auxotrophic mutation, the required additional nutrient for each of the mutants was supplemented to the selective agar at the repetitive steps. In further screening steps and TKC test was carried out again using the selective agar, the co-cultivation condition was modified because there was a possibility that the overnight co-cultivation on TN agar induced serious damage, owing to starvation, cell lysis and something like those, in some mutants. We therefore replaced the TN agar with the rich medium YPD agar, which was used for preculture, and shortened the length of co-cultivation with the donor *E. coli* down to 1 hour. In addition, not only the number of transconjugant cells but also the number of viable yeast cells was counted after co-cultivation to normalize the transconjugant frequency by dividing the transconjugant cell number by the number of viable cells. We called the resultant normalized value

" TKC efficiency." As shown in **Table 2**, a total of 27 strains exhibited less than about 15% of the wild-type efficiency and most strains exhibited only several or less percent of the wild-type efficiency. **Table 2** also shows the function of each gene that is disrupted in each mutant strains. In total, a deletion at each of 26 genes was found to affect TKC efficiency well.

We confirmed the linkage between each mutation and the defect in the transkingdom conjugability (**Table 2) as** the following. Mutant strains in *MATa* background also exhibited TKC deficiency in the TKC and introduction of a plasmid containing the corresponding wild type gene recovered the TKC activity.

vATPase mutants

Of the 27 mutants, 21 had deletions of genes responsible for vATPase activity. Among the 20 genes involved, 13 were structural genes for the subunits of the enzyme (Graham et al., 2003; Davis-Kaplan et al., 2004). The strain YCL007C (*cwh36Δ*) had a deletion at the overlapping gene *VMA9*, which encodes the smallest subunit of vATPase. In all, genes for every subunit proteins except one were contained in these 13 genes, whereas the subunit "a" gene did not appear in the list. The "a" subunit in the V_0 sector of

vATPase is encoded by two structural genes—namely, *VPH1* and *STV1* (Kawasaki-Nishi et al., 2001). As shown in **Fig. 1**, the *stv1Δ* mutation (strain 149B8) had a subtle effect, while the *vph1Δ* mutation (strain 144H12) indicated a little effect. Therefore, we prepared a double-mutant strain by disrupting *STV1* gene in the *vph1Δ* strain. As shown in **Table 3(a)**, the double mutant (*vph1Δ* and *stv1::HIS3*) exhibited very low efficiency, comparable to those of the other subunit mutants (**Table 2**). Therefore, it is clear that every subunit of vATPase is prerequisite for TKC.

Each mutant in three genes (*VPH2*/*VMA12*, *VMA21* and *VMA22*) responsible for the assembly of the vATPase subunits in ER (Graham et al., 2003) showed low TKC efficiency (**Table 2**).

TKC defect was also observed in mutants in each of two genes *VPS34* and *FAB1* for biosynthesis of phosphatidylinositol- 3,5-biphosphate, a gene (*VPS15*) for recruiting Vps34p to Golgi, and a gene (*PEP3*) for traffic between endosomes and vacuoles (Slessareva et al., 2006; Srivastava et al., 2000; Stack et al., 1995), as shown in **Table 2**.

It stands to reason that the vATPase and related mutants were collected in the first screening with TN agar for co-cultivation condition, because the vATPase mutants are sensitive to an alkaline environment and starvation (Nelson and Nelson, 1990; Sambade et al., 2005). Thereby, the co-cultivation condition was replaced with YPD agar

(approximately pH 6) for 1 hour to observe TKC efficiency at the final screening step in this study. Since the viable cell number was included to consider the efficiency, it is apparent that mutant cells kept viability but were unable to establish transconjugant colonies.

In *E. coli*-yeast TKC, higher efficiency is observable when *LEU2* gene is employed in place of *URA3* as a selection marker (Heinemann and Sprague, 1991). We applied a *LEU2* mobilizable plasmid pMz1 in place of pAY205. Simultaneously, in the experiment, we replaced YPD with the synthetic agar containing leucine to count total output yeast cells. The replacement minimizes difference with the selective agar. As a result, TKC efficiency was improved in the wild type strain and in most of the mutants. The resulting % wild type ratio of the mutants was as low as that obtained using *URA3* (**Table 2**). Growth of the mutants on YPD agar was compared with that on the synthetic agar (**Fig. 1**). Colony-forming-units was almost the same, although growth rate on the synthetic agar was slower than that on YPD.

Genes other than those for vacuolar function

Besides the mutants defective in vATPase activity mentioned above, 6 mutants showed

much reduced TKC efficiency (**Table 2**). This result suggests some participation of six genes (*CSG2*, *SHP1*, *HOF1*, *CCR4*, *NOT5* and *ZUO1*) in TKC.

CSG2 is required for cell wall integrity. This gene is involved in the mannosylation of inositol phosphorylceramid (Stock et al., 2000). Mutations in the phosphoprotein phosphatase 1 gene *SHP1* (Zhang et al., 1995) result in pleiotrophic changes, including a slender morphology of cell shape according to the *Saccharomyces* genome database (http://www.yeastgenome.org/). Our own analysis of the *shp1Δ* mutant revealed a hypersensitivity to calcofluor white (CFW) and apparently increased binding of CFW to the cell surface (data not shown). The hypersensitivity to CFW and enhanced binding of CFW suggest increased chitin content in the cell wall. *HOF1* is important for formation of the contractile ring and the bud neck (Kamei et al., 1998). Mutations in *CCR4* and *NOT5* induce a pleiotrophic effect including abnormal cell wall according to the *Saccharomyces* genome database. Products of the two genes form a complex called the Ccr4-Not complex (Chen et al., 2001).

Although relation of the characteristics of the mutants with TKC remains unclear, the mutations in the six genes mentioned above seem commonly to cause defects in the composition and shape of the cell surface, which is the initial site of interaction with the donor bacteria.

The product of the *ZUO1* gene is a component of a molecular chaperone associated with cytoplasmic ribosomes. Zuo1p associates with Ssz1p as well as with either Ssb1p or Ssb2p (Gautschi et al., 2002). Therefore we examined the transkingdom conjugability of the *ssz1Δ* mutant (131G3) and the *ssb1Δ* mutant (136G11). As shown in **Table 3(b)**, the *ssz1Δ* mutant showed low efficiency, as did the *zuo1Δ* mutant. On the other hand, the *ssb1Δ* mutant was normal, probably owing to the presence of the near identical protein encoded by *SSB2*. The difference in conjugability between these mutants was consistent with that of sensitivity to CFW (**Fig. 2B**). It is supposed that bacterial proteins such as TraI mobilized into yeast cells along with the plasmid DNA, and then yeast proteins start to associate and some attack the protein-DNA complex. Facilitated recovery from such a damage by the shuffle and enhancement of the complex formation between the mobilized materials and yeast proteins are a possible explanations for the involvement of the chaperone activity in the conjugation.

Relation with transformation-related mutations

In the yeast *Saccharomyces cerevisiae*, transformation using purified plasmid DNA is commonly used in laboratories. Kawai et al. (2004) have identified mutations that

cause defect in the transformation of intact yeast cells (i.e., *pde2Δ, spf1Δ,* and *pmr1Δ*). The mechanism of protoplast transformation is explained as endocytotic DNA uptake. Neither the mutants found by Kawai et al. (2004) nor the endocytosis gene mutants were included among the low-TKC mutants, which we found in this study. Our examination of TKC in endocytosis mutants (**Fig. 3**) indicated no or trivial effect on TKC efficiency (data not shown). These results indicate that TKC is not the same as transformation with naked DNA molecules released from lysed donor bacterial cells but rather a form of direct transport by the type IV secretion system from the donor bacterium to yeast cells.

Discussion

In E. coli, a large number of mutants deficient in F-factor conjugation were isolated and analysed. Despite the efforts, host-specific factor genes are limited (Frost, 2002). Altered lipopolysaccharide mutants (*waa*) and OmpA protein mutants impair the conjugation in liquid (Manoil and Rosenbush, 1982). However, conjugation on filter membrane surface is not seriously affected by the same mutations.

This paper revealed that vATPase is very important for TKC. It still remains

unknown how vATPase participates in TKC mechanism. The vATPase mutants are sensitive to various stresses. It is possible that the attenuated cellular environment in all perturbs the interaction between donor and recipient cells. Kane (2007) and Tarsio et al (2011) indicated decrease of extracellular and cytoplasmic pH in vATPase mutants. The lower pH might damage the TSS structure on bacteria surface before the donor cells attach to the mutant yeast, and or affect a putative bond between the donor T4SS structure and recipient yeast membrane. Another explanation is that mobilized plasmid DNA molecules are degraded in the mutant yeast cytoplasm by nucleases induced by the elevated stresses in the mutants.

Recently, Silby et al. (2007) expressed a fusion protein between Gfp and TraI proteins. The latter is the pilot protein of the IncP conjugation system. They observed the localization of the fusion protein in yeast nuclei. The datum supports an idea of facilitated transport of plasmid DNA from yeast cytoplasm to nuclei with the aid of the pilot protein, as in the *Agrobacterium* virulence system for T-DNA transfer to plant nuclei. Suppose highly sensitive detection for TraI protein is available, we can distinguish the above two possibilities.

Most and possibly all yeast and fungal species possess the genes identified in this study. The difference in the efficiency of conjugation between strains and species

remains unknown. Putative factors that mask some receptors on yeast cells might determine this difference. The latter genes may be identified by screening mutants of an enhanced efficiency type. So far, extensive studies for various cell functions have been done in *E. coli* and separately in the yeast as model microbes. However, there has been little information how the two microbes interact mutually when they encounter. In natural environments such as in soil, eukaryotic and prokaryotic cells are localized together. In order to extend this line of study, we should know how bacterial cells and yeast cells affect mutually. In any way, this study is a good model for physiological and genetic interaction between bacterial and eukaryotic microorganisms and for analysis of transkingdom gene transfer.

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Leferences

Bates S, Cashmore AM, Wilkins BM. IncP plasmids are unusually effective in mediating conjugation of *Escherichia coli* and *Saccharomyces cerevisiae*: Involvement of the Tra2 mating system. J Bacteriol 1998; 180: 6538-6543.

Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P, Boeke JD**.** Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 1998; 14: 115-132.

Bushman F. *Lateral DNA transfer : mechanisms and consequences*. Cold Spring Harbor Laboratory Press, NY; 2002.

Chen J, Rappsilber J, Chiang YC, Russell P, Mann M, Denis CL. Purification and characterization of the 1.0 MDa CCR4-NOT complex identifies two novel components of the complex. J Mol Biol 2001; 314: 683-694.

Davis-Kaplan SR, Ward DM, Shiflett SL, Kaplan J. Genome-wide analysis of iron-dependent growth reveals a novel yeast gene required for vacuolar acidification. J Biol Chem 2004; 279: 4322-4329.

Fernández-González E, de Paz HD, Alperi A, Agúndez L, Faustmann M, Sangari FJ,

Dehio C, Llosa M. Transfer of R388 derivatives by a pathogenesis-associated type IV secretion system into both bacteria and human cells. J Bacteriol. 2011; in press.

Frost, SL. Conjugation, bacterial, in *Encyclopedia of Microbiology, second edition*, edited by J. Lederberg. Academic Press; 2000. p. 847-862.

Gautschi M, Mun A, Ross S, Rospert S. A functional chaperone triad on the yeast ribosome. Proc Natl Acad Sci USA 2002; 99: 4209-4214.

Gietz RD, Woods RA. Transformation of yeast by Li Acetate/single-stranded carrier DNA/polyethylene glycol method. Methods Enzymol 2002; 350: 87-96.

Gormley EP, Davies J. Transfer of Plasmid RSF1010 by Conjugation from *Escherichia coli* to *Streptomyces lividans* and *Mycobacterium smegmatis*. J Bacteriol 1991; 173: 6705-6708.

Graham LA, Flannery AR, Stevens TH. Structure and assembly of the yeast V-ATPase. J Bioenerg Biomembr 2003; 35: 301-312.

Hayman GT, Bolen PL. Movement of shuttle plasmids from *Escherichia coli* into yeasts other than *Saccharomyces cerevisiae* using trans-kingdom conjugation. Plasmid 1993; 30: 251-257.

Heinemann JA, Sprague GFJ**.** Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. Nature 1989; 340: 205-209.

Inomata K, Nishikawa M, Yoshida K**.** The yeast *Saccharomyces kluyveri* as a recipient eukaryote in transkingdom conjugation: behavior of transmitted plasmids in transconjugants. J Bacteriol 1994; 176: 4770-4773.

Kamei T, Tanaka K, Hihara T, Umikawa M, Imamura H, et al. Interaction of Bnr1p with a novel Src homology 3 domain-containing Hof1p. Implication in cytokinesis in *Saccharomyces cerevisiae*. J Biol Chem 1998; 273: 28341-28345.

Kane PM. The long physiological reach of the yeast vacuolar H^+-ATPase. J Bioenerg Biomembr 2007; 39:415-421.

Kawai S, Pham TA, Nguyen HT, Nankai H, Utsumi T et al. Molecular insights on DNA delivery into *Saccharomyces cerevisiae*. Biochem Biophys Res Commun 2004; 317: 100-107.

Kawasaki-Nishi S, Nishi T, Forgac M. Yeast V-ATPase complexes containing different isoforms of the 100-kDa a-subunit differ in coupling efficiency and in vivo dissociation. J Biol Chem 2001; 276:17941-17948.

Maniatis T, Fritsch EF, Sambrook J**.** *Molecular cloning. A laboratory manual.* Cold Spring Harbor Laboratory Press, NY; 1982.

Manoil C, Rosenbusch JP. Conjugation-deficient mutants of Escherichia coli distinguish classes of functions of the outer membrane OmpA protein. Mol Gen Genet 1982;

187:148-156.

Nelson H, Nelson N. Disruption of genes encoding subunits of yeast vacuolar H(+)-ATPase causes conditional lethality. Proc Natl Acad Sci USA 1990; 87: 3503-3507.

Nishikawa M, Suzuki K, Yoshida K. Structural and functional stability of IncP plasmids during stepwise transmission by trans-kingdom mating: promiscuous conjugation of *Escherichia coli* and *Saccharomyces cerevisiae*. Jpn J Genet 1990; 65: 323-334.

Nishikawa M, Suzuki K, Yoshida K. DNA integration into recipient yeast chromosomes by trans-kingdom conjugation between *Escherichia coli* and *Saccharomyces cerevisiae*. Curr Genet 1992; 21: 101-108.

Parales RE, Harwood CS. Construction and use of a new broad-host-range *lacZ* transcriptional fusion vector, pHRP309, for Gram$^-$ bacteria. Gene 1993; 133: 23-30.

Sambade M, Alba M, Smardon AM, West RW, Kane PM. A genomic screen for yeast vacuolar membrane ATPase mutants. Genetics 2005; 170: 1539-1551.

Schröder G, Schuelein R, Quebatte M, Dehio C. Conjugative DNA transfer into human cells by the VirB/VirD4 type IV secretion system of the bacterial pathogen Bartonella henselae. Proc Natl Acad Sci U S A. 2011;108:14643-14648.

Sikorski RS, Michaud W, Levin HL, Boeke JD, Hieter P. Trans-kingdom promiscuity.

Nature 1990; 345: 581-582.

Silby MW, Ferguson GC, Billington C, Heinemann JA**.** Localization of the plasmid-encoded proteins TraI and MobA in eukaryotic cells. Plasmid 2007; 57: 118-130.

Slessareva JE, Routt SM, Temple B, Bankaitis VA, Dohlman HG. Activation of the phosphatidylinositol 3-kinase Vps34 by a G protein alpha subunit at the endosome. Cell 2006; 126: 191-203.

Srivastava A, Woolford CA, Jones EW. Pep3p/Pep5p complex: a putative docking factor at multiple steps of vesicular transport to the vacuole of *Saccharomyces cerevisiae*. Genetics 2000; 156: 105-122.

Stack JH, DeWald DB, Takegawa K, Emr SD**.** Vesicle-mediated protein transport: regulatory interactions between the Vps15 protein kinase and the Vps34 PtdIns 3-kinase essential for protein sorting to the vacuole in yeast. J Cell Biol 1995; 129: 321-334.

Stock SD, Hama H, Radding JA, Young DA, Takemoto JY. Syringomycin E inhibition of *Saccharomyces cerevisiae*: requirement for biosynthesis of sphingolipids with very-long-chain fatty acids and mannose- and phosphoinositol-containing head groups. Antimicrob Agents Chemother 2000; 44: 1174-80.

Suzuki K, Tanaka K, Yamamoto S, Kiyokawa K, Moriguchi K, Yoshida K. Ti and Ri plasmids, in *Microbial Megaplasmids,* edited by E. Schwartz. Springer Verlag, Heidelberg; 2009. p. 133-147.

Tarsio M, Zheng H, Smardon AM, Martínez-Muñoz GA, Kane PM. Consequences of loss of Vph1 protein-containing vacuolar ATPases (V-ATPases) for overall cellular pH homeostasis. J Biol Chem 2011; 286:28089-96.

Thomas C. Introduction. In: edited by Thomas C. *Promiscuous plasmids of Gram-negative bacteria*, Academic Press, London; 1989. p. vii – viii.

Zhang S, Guha S, Volkert FC. The *Saccharomyces SHP1* gene, which encodes a regulator of phosphoprotein phosphatase 1 with differential effects on glycogen metabolism, meiotic differentiation, and mitotic cell cycle progression. Mol Cell Biol 1995; 15: 2037-50.

Zupan J, Muth TR, Draper O, Zambryski P. The transfer of DNA from *Agrobacterium tumefaciens* into plants: a feast of fundamental insights. Plant J 2000; 23: 11-28.

Figure legends

Fig. 1. Vegetative growth of low TKC mutant yeast strains. A series of 5 μl of serially diluted yeast cell suspension was spotted onto YPD agar and synthetic agar, and then incubated for three days.

Fig. 2. Sensitivity to CFW of the molecular chaperone mutants. A series of 10 μl of serially diluted yeast cell suspension was spotted onto YPD agar and YPD agar with 0.001% CFW, and then incubated for three days.

Table

Table 1. List of strains and plasmids used in this study.

Strains	Genotype or relevant character	Source
E. coli		
HB101	*F-, recA13, proA2*	Boyer and Rolland-Dusoix (1969)
HB101(pAY205, pRH210)	donor strain	M. Nishikawa et al. (1992) Ref. 21
HB101(pMZ1, pRH220)	donor strain	This study
HB101(pMZ2, pRH220)	donor strain	This study
S. cerevisiae		
BY4742	*MATα his3Δ1*, *leu2Δ0*, *lys2Δ0*, *ura3Δ0*	Brachmann et al (1998) Ref. 2
Mutants derived from BY4742		the yeast genome deletion project*
BY4741	*MATa his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*	Brachmann et al (1998) Ref. 2
Mutants derived from BY4741		the yeast genome deletion project*

Plasmid	Relevant characters	Source or reference
pAY205	*ARS1, TRP1, URA3, oriV-Q, oriT-Q, mob-Q,* Km^R	M. Nishikawa et al. (1992) Ref. 21
pRH210	*oriV-C, oriT-P, mob-P, tra-P,* Ap^R	M. Nishikawa et al. (1992) Ref. 21
pRH220	*oriV-C, oriT-P, mob-P, tra-P,* Cm^R	M. Nishikawa et al. (1992) Ref. 21
pHRP309	*oriV-Q, oriT-Q, mob-Q,* Gm^R	Parales and Harwood (1993) Ref. 22
pMZ1	*2μori, LEU2, oriV-Q, oriT-Q, mob-Q,* Ap^R, Gm^R	This study
pMZ2	*2μori, LEU2, oriV-Q, oriT-Q, mob-Q,* Gm^R	This study
pUC19	*oriV-ColE1* Ap^R	Yanish et al (1985)
pUC19-HIS3	*HIS3* in pUC19	This lab
pUC19-STV1	*STV1* in pUC19	This study
pUC19-stv1::HIS3	*stv1::HIS3* in pUC19	This study
pRS313	*URA3 cen6 arsH4* Ap^R	
YEp351	*LEU2, 2μori,* Ap^R	Hill et al (1986)
YEp352	*URA3, 2μori,* Ap^R	Hill et al (1986)
pTF63	*URA3, 2μori,* Ap^R	Davis-Kaplan et al (2004) Ref. 6
pVMA4	*VMA4* in pRS316	This study
pVMA6	*VMA6* in pRS316	This study
pSHP1	*SHP1* in YEp352	This study
pCSG2	*CSG2* in YEp351	This study
pCWH36	*VMA9* in pTF63	Davis-Kaplan et al (2004) Ref. 6
pZUO1	*ZUO1* in YEp352	This study
pYKL118W(VPH2)	*YKL118W*(*VPH2*) in YEp351	This study
pHOF1	*HOF1* in YEp352	This study
pNOT5	*NOT5* in YEp351	This study

*) http://www-sequence.stanford.edu/group/yeast_deletion_project/

Table 2. List of mutants exhibiting low transkingdom conjugation efficiency

Strain	Disrupted gene	Gene function	TKC efficiency (Ura+) %WTRatio +/- σ	TKC efficiency(Leu+) %WTRatio +/- σ	TKC phenotype in *MATa* strain*	Plasmid rescue**
BY4742 (WT)			(100)	(100)		
139C6	*TFP1*/*VMA1*	vATPase subunit A in V1 sector	1.7 +/- 2.9	1.8 +/- 2.4	Low	nt
146A1	*VMA2*	vATPase subunit B in V1 sector	0.7 +/- 0.8	0.0 +/- 0.0	nt	nt
112A1	*CUP5*/*VMA3*	vATPase subunit c in V0 sector	0.1 +/- 0.2	2.2 +/- 3.9	nt	nt
107A2	*VMA4*	vATPase subunit E in V1 sector	1.5 +/- 2.5	1.9 +/- 2.3	nt	Yes
117A5	*VMA5*	vATPase subunit C in V1 sector	0.3 +/- 0.6	0.9 +/- 0.8	nt	nt
135D3	*VMA6*	vATPase subunit d in V0 sector	0.4 +/- 0.7	2.4 +/- 3.1	nt	Yes
123B10	*VMA7*	vATPase subunit F in V1 sector	0.4 +/- 0.7	0.0 +/- 0.0	nt	nt
112B10	*VMA8*	vATPase subunit D in V1 sector	0.4 +/- 0.7	1.6 +/- 1.0	nt	nt
115B7	*VMA9*, *CWH36*	vATPase subunit e in V0 sector	1.6 +/- 2.8	1.9 +/- 2.0	Low	Yes
130C1	*VMA10*	vATPase subunit G in V1 sector	0.4 +/- 0.6	1.9 +/- 1.0	nt	nt
108D8	*TFP3*/*VMA11*	vATPase subunit c' in V0 sector	1.6 +/- 2.8	0.0 +/- 0.0	nt	nt
117C8	*VPH2*/*VMA12*	vATPase assembly factor	0.3 +/- 0.5	0.6 +/- 1.1	nt	nt
133B12	*VMA13*	vATPase subunit H in V1 sector	1.6 +/- 1.9	6.1 +/- 6.2	nt	nt
134G5	*PPA1*/*VMA16*	vATPase subunit c'' in V0 sector	0.7 +/- 1.2	2.0 +/- 1.7	nt	nt
117C7	*YKL118W*, *VPH2*	vATPase assembly factor	0 +/- 0	0 +/- 0	Low	Yes
117C8	*VPH2/VMA12*	vATPase assembly factor	0.9 +/- 1.5	0.3 +/- 0.5	Low	Yes
118C7	*VMA21*	vATPase assembly factor	0.4 +/- 0.9	3.8 +/- 5.4	nt	nt
114C2	*VMA22*	vATPase assembly factor	0.9 +/- 1.2	0.6 +/- 0.3	nt	nt
171C10	*VPS15*	protein kinase, recruits Vps34 to Golgi	10.6 +/- 13.6	4.3 +/- 3.7	nt	nt
171A2	*VPS34*	phosphatidylinositol 3-kinase catalytic subunit	18.2 +/- 8.2	6.1 +/- 10.5	nt	nt
145B9	*FAB1*	phosphatidylinositol-3-phosphate 5-kinase	20.8 +/- 15.5	11.2 +/- 3.2	nt	nt
170H10	*PEP3*	vacuolar membrane protein, promotes endosomal vesicles to fuse to vacuole	12.4 +/- 4.2	6.1 +/- 5.7	nt	nt
101C10	*CCR4*	Component of CCR-NOT transcriptional comlex	0.4 +/- 0.7	4.7 +/- 8.1	Low	nt
126A3	*SHP1*	UBX domain-containing protein, regulates Glcp phosphatase	9.4 +/- 13.2	5.4 +/- 3.1	Low	Yes
140C4	*CSG2*	ER membrane protein required for mannosylation of inositolphosphorylce ramide	9.9 +/- 7.1	17.2 +/- 13.8	Low	Yes

Table 2. (Continued)

140C4	*CSG2*	ER membrane protein required for mannosylation of	9.9 +/-	7.1	17.2 +/-	13.8	Low	Yes
130B7	*ZUO1*	cytoplasmic ribosome-associated	6.2 +/-	7.0	1.1 +/-	0.2	Low	Yes
103G6	*HOF1*	bud neck-localized protein, regulates	6.3 +/-	5.7	21.2 +/-	12.5	Low	Yes

*) TKC phenotype was observed in the corresponding mutant in the *MATa* background. Low, low efficiency; nt, not tested.

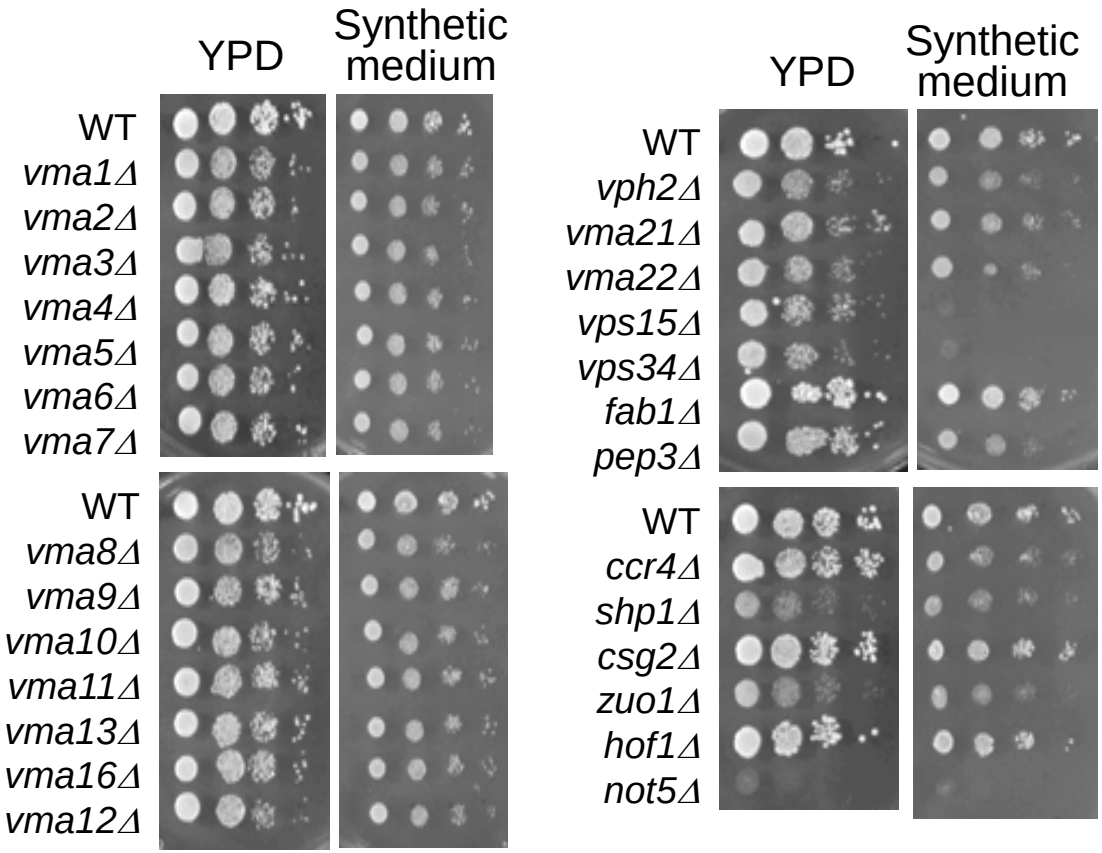
**) TKC phenotype was found in the mutant strain transformed with a plasmid containing the corresponding wild

Table

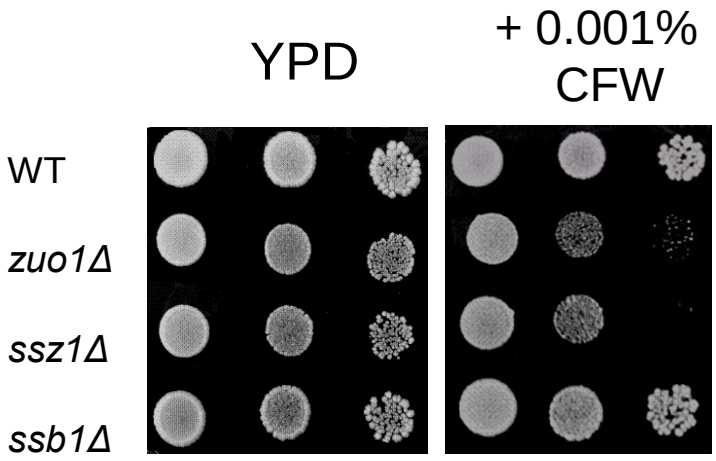
Table 3. Transkingdom conjugation efficiency of vATPase "a" subunit gene mutants, chaperone gene mutants and endocytosis mutants.

Strain	Disrupted gene	TKC efficiency (Ura+) %WTRatio +/- σ	TKC efficiency(Leu+) %WTRatio +/- σ
WT		(100)	(100)
(a) vATPase "a" subunit gene mutants			
149B8	*STV1*	90 +/- 34	103 +/- 41
144H12	*VPH1*	60 +/- 25	47 +/- 41
stv1 vph1	*STV1 VPH1*	0.0 +/- 0.0	3.5 +/- 3.7
(b) chaperone gene mutants			
130B7	*ZUO1*	3.0 +/- 3.0	6.1 +/- 8.1
131G6	*SSZ1*	6.0 +/- 8.0	8.8 +/- 3.4
136G11	*SSB1*	79 +/- 4.0	96 +/- 69
(c) endocytosis mutants			
141F9	*END3*	187 +/- 36	136 +/- 70
139A10	*ENT1*	115 +/- 42	116 +/- 2.2
116C7	*ENT2*	114 +/- 43	84 +/- 19
142G12	*TPT53*	117 +/- 35	128 +/- 77
142H8	*INP52*	152 +/- 60	142 +/- 57

Figure



New Figure 1



New Figure 2