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Relation	

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

4

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7

8

9 **Running title:**

10 Yeast mutants defective in recipient ability for transkingdom conjugation with *E. coli*

11

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

14

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1 **Abstract**

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

15

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

# 1 **Introduction**

2

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

1 1989). TKC is interesting from an evolutionary view point and increase of its efficiency  
2 is expected from a biotechnological view point. Combination of a derivative of RK2 as  
3 a helper with incQ-type mobilizable plasmids allowed us to demonstrate the rare  
4 integration of mobilized DNA into yeast chromosomes (Nishikawa et al., 1992).

5 The conjugation plasmids are essential in TKC (Heinemann and Sprague, 1989). Cell  
6 density and choice of suitable yeast gene and plasmids affect the *E. coli*-yeast  
7 conjugation (Heinemann and Sprague, 1989; Bates et al., 1998). Mechanisms for yeast  
8 cells to recognize donor bacterial cells and accept plasmid DNAs are not yet reported.

9 Mutant strains defective in TKC if available would help to study the molecular  
10 mechanism of TKC. In this study, we screened yeast mutants with decreased efficiency  
11 as the recipients of TKC.

12

1 **Materials and methods**

2

3 **Donor and recipient strains**

4

5 Bacterial and yeast strains used in this study are listed in **Table 1**. Yeast strains BY4742  
6 and the complete set of *MAT $\alpha$*  knockout mutants (the *Saccharomyces* Genome Deletion  
7 Project consortium) derived from BY4742 were purchased from Invitrogen (CA, USA).

8 Yeast strains BY4741, BY4743 and several knockout mutants were kindly provided by  
9 Drs. T. Miyakawa and M. Mizunuma (Hiroshima University), J. Shima and S. Andoh  
10 (National Food Research Institute, Tsukuba, Japan).

11

12 ***E. coli* -yeast mating conditions**

13

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

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

1 and selection was carried out. Yeast strains were streaked on YPD agar using toothpicks  
2 and incubated overnight at 28°C for preculture. Five µl of cell suspension ( $3 \times 10^9$   
3 cells/ml) of the donor *E. coli* strain HB101(pRH210, pAY205) was spotted on TN agar  
4 {TrisHCl (pH 7.5)}. A small quantity of yeast cells was taken from the overnight YPD  
5 agar culture, and then mixed with the donor *E. coli* cell suspension on the TN agar using  
6 a plastic inoculation loop. The liquid portion of the mixture was absorbed in the agar in  
7 several minutes. The mixture was kept overnight at 28°C, and then transferred using a  
8 plastic inoculation loop onto SC-ura agar (0.67% yeast nitrogen base w/o amino acid  
9 (Difco), 2% glucose, 2% agar supplemented with lysine, leucine and histidine), which is  
10 selective for the resulting yeast transconjugant cells.

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

15

## 16 **Plasmids and plasmid construction**

17

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

1 plasmids pMZ1 and pMZ2 were constructed as follows. Plasmid pHRP309 (Parales and  
2 Harwood, 1993) was cleaved with *SmaI* and *HindIII*. A 9-kbp fragment of the digest  
3 was ligated with YEp351 cleaved with the same pair of enzymes, resulting in a 14.6-kbp  
4 plasmid pMZ1 (**Table 1**). The 9-kbp fragment was ligated with YEp351 cleaved with  
5 *AatII* and *HindIII*, resulting in a 12.4-kbp plasmid pMZ2 (**Table 1**). Yeast chromosomal  
6 genes were amplified by PCR by KOD-plus DNA polymerase (Toyobo, Japan) using  
7 primers designed based on the genomic sequences, 0.5-kbp upstream and 0.5-kbp  
8 downstream from the target ORF. Recognition sequences for restriction enzymes were  
9 added artificially to 5' part of the primer sequences, where necessary. Chromosomal  
10 genes were cloned in either one of pRS313, YEp351 and YEp352. pUC19-STV1 is a  
11 pUC19 harboring 4.5-kbp *XbaI-KpnI* fragment containing the *STV1* gene. Two *BanIII*  
12 fragments (1.4 kbp in total) inside the *STV1* gene were removed and replaced with *HIS3*  
13 gene released from pUC19-HIS3 by digestion with *SmaI* and *HincII*, resulting in  
14 pUC19-stv1::HIS3. pTF63 and pCWH36 were kindly provided by Dr. J. Kaplan  
15 (University of Utah).

16

## 17 **Miscellaneous genetic and biochemical methods**

18



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

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

7

8

## 9 **Results**

10

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

14

### 15 **Identification of the 26 yeast chromosomal genes affecting transkingdom**

#### 16 **conjugation**

17

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

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

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

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

10

#### 11 **vATPase mutants**

12

13 Of the 27 mutants, 21 had deletions of genes responsible for vATPase activity. Among  
14 the 20 genes involved, 13 were structural genes for the subunits of the enzyme (Graham  
15 et al., 2003; Davis-Kaplan et al., 2004). The strain YCL007C (*cwh36Δ*) had a deletion at  
16 the overlapping gene *VMA9*, which encodes the smallest subunit of vATPase. In all,  
17 genes for every subunit proteins except one were contained in these 13 genes, whereas  
18 the subunit “a” gene did not appear in the list. The “a” subunit in the V<sub>0</sub> sector of

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

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

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

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

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

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

15

## 16 **Genes other than those for vacuolar function**

17

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

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

3 *CSG2* is required for cell wall integrity. This gene is involved in the mannosylation  
4 of inositol phosphorylceramid (Stock et al., 2000). Mutations in the phosphoprotein  
5 phosphatase 1 gene *SHP1* (Zhang et al., 1995) result in pleiotrophic changes, including  
6 a slender morphology of cell shape according to the *Saccharomyces* genome database  
7 (<http://www.yeastgenome.org/>). Our own analysis of the *shp1* $\Delta$  mutant revealed a  
8 hypersensitivity to calcofluor white (CFW) and apparently increased binding of CFW to  
9 the cell surface (data not shown). The hypersensitivity to CFW and enhanced binding of  
10 CFW suggest increased chitin content in the cell wall. *HOF1* is important for formation  
11 of the contractile ring and the bud neck (Kamei et al., 1998). Mutations in *CCR4* and  
12 *NOT5* induce a pleiotrophic effect including abnormal cell wall according to the  
13 *Saccharomyces* genome database. Products of the two genes form a complex called the  
14 Ccr4-Not complex (Chen et al., 2001).

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

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

14

#### 15   **Relation with transformation-related mutations**

16

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

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

10

11

## 12 **Discussion**

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

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



1 unknown how vATPase participates in TKC mechanism. The vATPase mutants are  
2 sensitive to various stresses. It is possible that the attenuated cellular environment in all  
3 perturbs the interaction between donor and recipient cells. Kane (2007) and Tarsio et al  
4 (2011) indicated decrease of extracellular and cytoplasmic pH in vATPase mutants. The  
5 lower pH might damage the TSS structure on bacteria surface before the donor cells  
6 attach to the mutant yeast, and or affect a putative bond between the donor T4SS  
7 structure and recipient yeast membrane. Another explanation is that mobilized plasmid  
8 DNA molecules are degraded in the mutant yeast cytoplasm by nucleases induced by  
9 the elevated stresses in the mutants.

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

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

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

11

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9

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15

1 **Figure legends**

2

3 **Fig. 1.** Vegetative growth of low TKC mutant yeast strains. A series of 5  $\mu$ l of  
4 serially diluted yeast cell suspension was spotted onto YPD agar and synthetic agar, and  
5 then incubated for three days.

6 **Fig. 2.** Sensitivity to CFW of the molecular chaperone mutants. A series of 10  $\mu$ l  
7 of serially diluted yeast cell suspension was spotted onto YPD agar and YPD agar with  
8 0.001% CFW, and then incubated for three days.

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

Strains	Genotype or relevant character	Source
<i>E. coli</i>		
HB101	<i>F<sup>+</sup>, recA13, proA2</i>	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
<i>S. cerevisiae</i>		
BY4742	<i>MATa his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0</i>	Brachmann et al (1998) Ref. 2
Mutants derived from BY4742		the yeast genome deletion project*
BY4741	<i>MATa his3Δ1, leu2Δ0, met15Δ0, ura3Δ0</i>	Brachmann et al (1998) Ref. 2
Mutants derived from BY4741		the yeast genome deletion project*
Plasmid	Relevant characters	Source or reference
pAY205	<i>ARS1, TRP1, URA3, oriV-Q, oriT-Q, mob-Q, Km<sup>R</sup></i>	M. Nishikawa et al. (1992) Ref. 21
pRH210	<i>oriV-C, oriT-P, mob-P, tra-P, Ap<sup>R</sup></i>	M. Nishikawa et al. (1992) Ref. 21
pRH220	<i>oriV-C, oriT-P, mob-P, tra-P, Cm<sup>R</sup></i>	M. Nishikawa et al. (1992) Ref. 21
pHRP309	<i>oriV-Q, oriT-Q, mob-Q, Gm<sup>R</sup></i>	Parales and Harwood (1993) Ref. 22
pMZ1	<i>2μori, LEU2, oriV-Q, oriT-Q, mob-Q, Ap<sup>R</sup>, Gm<sup>R</sup></i>	This study
pMZ2	<i>2μori, LEU2, oriV-Q, oriT-Q, mob-Q, Gm<sup>R</sup></i>	This study
pUC19	<i>oriV-ColE1 Ap<sup>R</sup></i>	Yanish et al (1985)
pUC19-HIS3	<i>HIS3</i> in pUC19	This lab
pUC19-STV1	<i>STV1</i> in pUC19	This study
pUC19-stv1::HIS3	<i>stv1::HIS3</i> in pUC19	This study
pRS313	<i>URA3 cen6 arsH4 Ap<sup>R</sup></i>	
YEp351	<i>LEU2, 2μori, Ap<sup>R</sup></i>	Hill et al (1986)
YEp352	<i>URA3, 2μori, Ap<sup>R</sup></i>	Hill et al (1986)
pTF63	<i>URA3, 2μori, Ap<sup>R</sup></i>	Davis-Kaplan et al (2004) Ref. 6
pVMA4	<i>VMA4</i> in pRS316	This study
pVMA6	<i>VMA6</i> in pRS316	This study
pSHP1	<i>SHP1</i> in YEp352	This study
pCSG2	<i>CSG2</i> in YEp351	This study
pCWH36	<i>VMA9</i> in pTF63	Davis-Kaplan et al (2004) Ref. 6
pZUO1	<i>ZUO1</i> in YEp352	This study
pYKL118W(VPH2)	<i>YKL118W(VPH2)</i> in YEp351	This study
pHOF1	<i>HOF1</i> in YEp352	This study
pNOT5	<i>NOT5</i> in YEp351	This study

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

**Table 2.** (Continued)

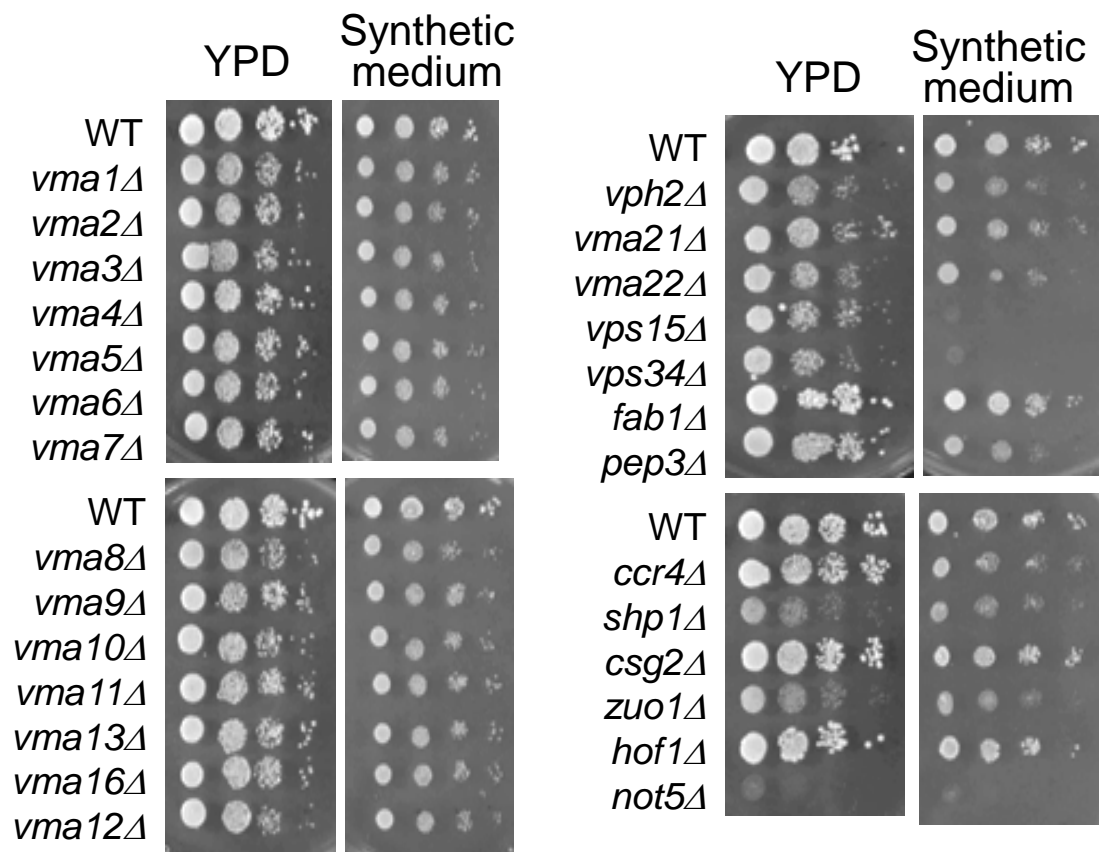
140C4	<i>CSG2</i>	ER membrane protein required for mannosylation of cytoplasmic ribosome-associated bud neck-localized protein, regulates	9.9 +/-	7.1	17.2 +/-	13.8	Low	Yes
130B7	<i>ZUO1</i>		6.2 +/-	7.0	1.1 +/-	0.2	Low	Yes
103G6	<i>HOF1</i>		6.3 +/-	5.7	21.2 +/-	12.5	Low	Yes

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

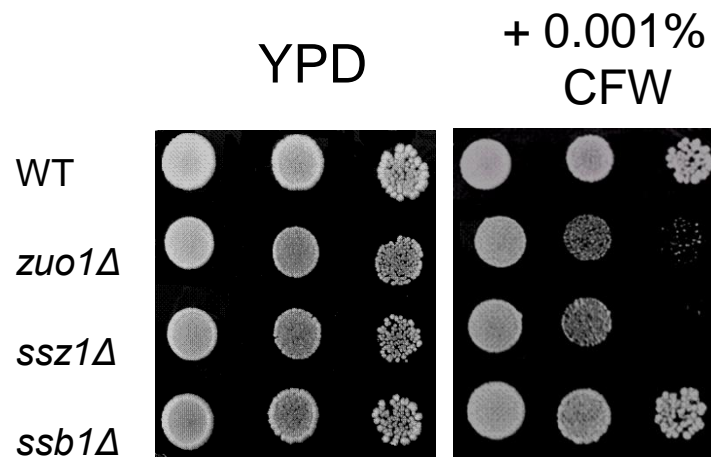
\*\*\*) TKC phenotype was found in the mutant strain transformed with a plasmid containing the corresponding wild

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

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



**New Figure 1**



**New Figure 2**