RESEARCH ARTICLE

Single-gene deletions that restore mating competence to diploid yeast

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Abstract

Using the *Saccharomyces cerevisiae MAT***a**/*MAT*α ORF deletion collection, homozygous deletion strains were identified that undergo mating with *MAT***a** or *MAT*α haploids. Seven homozygous deletions were identified that confer enhanced mating. Three of these, lacking *CTF8*, *CTF18*, and *DCC1*, mate at a low frequency with either *MAT***a** or *MAT*α haploids. The products of these genes form a complex involved in sister chromatid cohesion. Each of these strains also exhibits increased chromosome loss rates, and mating likely occurs due to loss of one copy of chromosome III, which bears the *MAT* locus. Three other homozygous diploid deletion strains, *ylr193c*Δ/*ylr193c*Δ, *yor305w*Δ/*yor305w*Δ, and *ypr170c*Δ/*ypr170c*Δ, mate at very low frequencies with haploids of either or both mating types. However, an *ist3*Δ/*ist3*Δ strain mates only with *MAT***a** haploids. It is shown that *IST3*, previously linked to splicing, is required for efficient processing of the *MAT***a***1* message, particularly the first intron. As a result, the *ist3*Δ/*ist3*Δ strain expresses unbalanced ratios of Matα to Mat**a** proteins and therefore mates with *MAT***a** haploids. Accordingly, mating in this diploid can be repressed by introduction of a *MAT***a***1* cDNA. In summary, this study underscores and elaborates upon predicted pathways by which mutations restore mating function to yeast diploids and identifies new mutants warranting further study.

Introduction

Mating in *Saccharomyces cerevisiae* is one of the most-studied signaling processes at the molecular level (Herskowitz, 1989; Cook & Tyers, 2004). Yeast haploid strains can exist as one of two mating types, **a** or α, which can mate with each other to form **a**/α diploids (Herskowitz, 1995; Wittenberg & La Valle, 2003). The mating process involves secretion of peptide mating pheromones by each haploid, which interact with cell-surface receptors expressed by haploids of the opposite mating type. The interaction of a mating pheromone with its receptor leads to activation of a MAP kinase cascade, ultimately resulting in enhanced activation of the transcription factor Ste12 and induction of the mating response. Subsequently, stimulated **a** and α haploids generate projections toward each other, fuse, and become **a**/α diploids. Diploid yeast are no longer able to mate and do not produce or respond to mating pheromones, but can sporulate to create two **a** and two α haploid spores.

Haploid identity is determined by the genes present at the *MAT* locus. Haploid **a** cells have *MAT***a***1* and *MAT***a***2* present at the *MAT* locus, whereas α cells have *MAT*α*1* and *MAT*α*2*. Genes at the *MAT* locus encode homeobox-containing transcription factors that act in combination with each other or with additional transcription factors to regulate sets of target genes and dictate cell identity (Johnson, 1995). In α cells, Matα1 acts in a complex with Mcm1 to activate α-specific genes, while α2 acts with Mcm1 to repress **a**-specific genes. In contrast, **a** cells represent a default state for mating, in which **a**-specific genes are activated by an Mcm1-Ste12 complex and α-specific genes are not expressed because *MAT*α*1* is not present. The expression of another set of haploid-specific genes in both mating types is regulated by Mcm1.

In diploid yeast, Mat**a**1 and Matα2 proteins form a heterodimer that represses transcription of both haploid-specific

genes and *MAT*α*1*, resulting in no expression of α-specific genes. Excess Matα2 also interacts with Mcm1 to repress **a**-specific genes. Mutations at the *MAT* locus result in changes in mating behavior. For instance, deletion of *MAT*α in an **a**/α diploid (or gene conversion to *MAT***a**) results in cells with diploid DNA content but **a** mating behavior. Similarly, **a**/α diploids lacking functional Mat**a**1 mate as α.

Among the few genes that have been reported to be required uniquely for proper mating behavior in diploids are *DIG1/RST1* and *DIG2/RST2* (Gelli, 2002). Homozygous deletion of both *DIG1/RST1* and *DIG2/RST2* causes **a**/α diploid cells to express **a**-specific genes and consequently mate as **a** cells. Dig1/Rst1 and Dig2/Rst2 interact with and act as repressors of Ste12 (Cook *et al*., 1996; Pi *et al*., 1997; Tedford *et al*., 1997). Activation of Ste12 during the mating response relies in part on phosphorylation and inactivation of Dig1/Rst1 and Dig2/Rst2 by the MAPK Fus3 (Cook *et al*., 1996; Tedford *et al*., 1997). Of note, Ste12 activity is also required in nonmating cells for basal levels of cell-type specific transcription (Fields & Herskowitz, 1985; Fields *et al*., 1988). Hypomorphic mutations in the essential U5 snRNP component, *AAR2*, also lead to diploid mating due to inefficient splicing of the *MAT***a***1* message, which contains two introns (Nakazawa *et al*., 1991). Apart from the aforementioned mutations and those directly at the *MAT* locus, no other mutations have been reported to permit mating specifically in diploids to the authors' knowledge.

Here, the results are reported of a screen of the homozygous deletion set for diploid yeast strains that mate with **a**, α, or both **a** and α haploids. Mating-competent homozygous deletion strains were verified by recreating a homozygous deletion from the corresponding haploid mutants contained in the **a** and α haploid deletion sets and retesting for mating. Because many of these deletions also affect mating in haploids, they were excluded from the retest. Also many sterile strains are missing entirely from the deletion set. It was decided to focus on deletions that affect mating specifically in diploids. Among the deletion strains identified directly in this screen are four (*ctf8*Δ/*ctf8*Δ, *ctf18*Δ/*ctf18*Δ, *ylr193c*Δ/*ylr193c*Δ, and *yor305w*Δ/*yor305w*Δ) that mate at a low frequency with either **a** or α strains likely due to an enhanced rate of genome instability, and one deletion (*ypr170c*Δ/*ypr170c*Δ) that mates as an **a**. Unexpectedly, one deletion was also identified (*ist3*Δ/*ist3*Δ) that mates as α due to inefficient splicing of one of the two introns of the *MAT***a***1* transcript.

Materials and methods

Yeast strains and growth conditions

All strains, except controls and mating testers (described below), were derived from the yeast ORF homozygous diploid or haploid gene deletion sets (Open Biosystems) (Brachmann *et al*., 1998; Winzeler *et al*., 1999). Control strains were BY4741 (*MAT***a** *his3 leu2 ura3 met15*), BY4742 (*MAT*α *his3 leu2 ura3 lys2*), and BY4743 (*MAT***a**/*MAT*α *his3/his3 leu2/leu2 ura3/ura3 LYS2/lys2 met15/MET15*). Mating tester strains were used for diploid and/or triploid selection and obtained from S. Fields (University of Washington): AM227a (*MAT***a** *lys1 cry1*) and AM227α (*MAT*α *lys1*). For rescue of the mating phenotype in *ist3*Δ/*ist3*Δ cells, the mating tester used was TSY011 (*MAT***a** *leu2 trp1 ura3*) and the triploid selection medium was synthetic minimal+Ura medium in order to force retention of the *LEU2*-bearing plasmids containing the *MAT***a** gene throughout the experiment. Standard growth conditions were used for all assays (Sherman, 1991). The mating testers used provide full auxotrophic complementation to the strains being tested, and in all cases the phrase 'selective minimal medium' refers to synthetic medium with no amino acids added.

For general mating assays, strains were grown overnight in YPD, and then samples of both the mutant and tester strains were added together to fresh YPD and allowed to grow over a second night. Cultures were then plated to selective minimal medium to assay for diploid or triploid growth.

High-throughput mating assay of the diploid deletion collection

The entire diploid homozygous deletion set (fifty-seven 96-well plates) was inoculated into fresh YPD medium in 96-well plates (~1 μL per well) using a high-density Biomek FX replica pinning robot (Beckman Coulter). Concurrently, the mating testers AM227a (*MAT***a** *cry1 lys1*) and AM227α (*MAT*α *lys1*) were inoculated into fresh YPD. Cells were cultured overnight at 30 °C. Mating testers were first inoculated from the overnight cultures to one set each of 96-well plates by transferring ~1 μL to each well containing 100 μL YPD. Mating assays were then initiated by transferring ~1 μL per well from overnight cultures of the deletion set into individual wells of 96-well plates containing either AM227a or AM227α. These cultures were grown overnight at 30 °C before pinning ~1 μL to plates of minimal medium selective for triploids formed by mating. Plates were incubated at 30 °C for several days and inspected for colony growth. A mating was considered to be positive if four or more colonies grew.

Quantitative mating assays

A modification of the standard quantitative mating assay (Sprague, 1991) was used to test simultaneously multiple colonies of remade diploids from various strains. To minimize effects due to the suspected chromosome loss phenotype of some of the strains, homozygous deletion diploids

were remade from the haploid deletion strains by selection on Minimal+His+Leu+Ura each time the assay was performed. For the quantitative assay, several 96-well plates were prepared with 50 μL YPD agar in each well. Samples of both the tester and the mutant diploid strain were added to each well as appropriate, with fivefold more of the tester strain. The plates were spun down to pellet the cells, the supernatant was discarded, and the plates were incubated at 30 °C for 5 h. The cells were resuspended and serial dilutions were plated to various selection media to differentiate the resulting triploids from both the diploids and the tester strains. The selection plates were incubated at 30 °C for several days and then the colonies were counted.

Shmoo analysis

Single cells that formed shmoos in response to α-factor were isolated and allowed to form colonies. DNA from the colonies was then isolated (Hoffman & Winston, 1987) and assayed via PCR for the presence or the absence of both the *MAT***a***1* and the *MAT*α*1* locus. (See Fig. 4 and Supplementary Table S1 for primer locations and sequences, respectively.)

RNA preparation for PCR analysis and cloning

RNA was isolated from BY4743 and *ist3*Δ/*ist3*Δ using the hot acid phenol method (http://derisilab.ucsf.edu/data/microarray/protocols.html) up to the point where the chloroform is added and the sample is spun down. Approximately 100 μg of RNA was removed from the aqueous phase and purified on Qiagen RNeasy columns as specified by the manufacturer.

*MAT***a***1* expression

For the rescue experiment, the spliced, partially spliced, and unspliced versions of the *MAT***a***1* gene were expressed using plasmid p415 (Mumberg *et al.*, 1995).

Splicing analysis

For reverse transcription, the RNA prep was treated with DNase and then amplified using oligo(dT) and RNA reverse transcriptase (Promega). PCR of DNA isolated by reverse transcriptase from BY4743 and the *ist3*Δ/*ist*Δ strain was performed using Biolase Taq DNA polymerase (Bioline). (See Fig. 5 for primer locations and Supplementary Table S1 for sequences, respectively.)

Quantitative PCR (QPCR) analysis

Cell lysates were prepared using a high-salt buffer and detergents as described previously (MacKay *et al.*, 2004), and RNA was purified using Qiagen RNeasy minicolumns. Approximately 2 μg total RNA was converted to cDNA with Invitrogen SS III reverse transcriptase and an oligo$(dT)_{25}$ primer with a G/C/A anchor. Specific cDNAs were quantitated with an iCycler (Bio-Rad) and SYBRGreen detection of products, using primers designed to detect specific mRNAs or splicing precursors. All reactions were performed in triplicate and the average quantities determined were normalized to *CDC28* transcript levels before performing calculations.

Results

Identifying deletions conferring diploid mating potential

To assay the diploid homozygous deletion set for mating ability, the entire set of *c.* 4800 single-gene deletion strains was screened as described in 'Materials and methods' and illustrated in Fig. 1a. Deletion strains were scored positive for mating if four or more colonies grew on triploid selection media (Fig. 1b). Frequently, one to three colonies were observed, likely reflective of the high background mating potential of the BY4743 strain relative to other diploids tested (data not shown). One-hundred potential diploid maters were selected for further analysis (see Supplementary Table S2).

In spite of the effectiveness of the high-throughput approach that was used to generate the deletion set strains, some deletion strains in the diploid set are known to have other genetic anomalies. For instance, aneuploidy has been discovered in a small but significant percentage of these strains (Hughes *et al.*, 2000; Deutschbauer *et al.*, 2005), and the putative diploid may in fact be a haploid that would then be mating competent. These anomalies could lead to incorrect attribution of the diploid mating phenotype to the known gene deletion in that strain. Therefore, the diploid-mating phenotype was verified in each of the hundred candidate deletion strains, recreating a homozygous diploid deletion strain by crossing the corresponding haploids in the **a** and α ORF deletion collections. Each of these was then tested for the ability to mate with the same mating testers used in the initial screen (Fig. 2a). In 11 cases, a diploid could not be constructed for testing, either because one or both of the haploid strains was sterile or they displayed genetic anomalies (e.g. the wrong mating type or an inappropriate set of auxotrophic markers) or because the gene deletion in question was required for assembly of an amino acid necessary for diploid or triploid selection.

Of the 89 newly created diploids that were tested for mating, only six displayed reproducible mating capability (Fig. 2b, Table 1). This low rate of retesting can be explained by one of three possibilities. First, a substantial number of the diploid strains in the deletion set may not in fact be

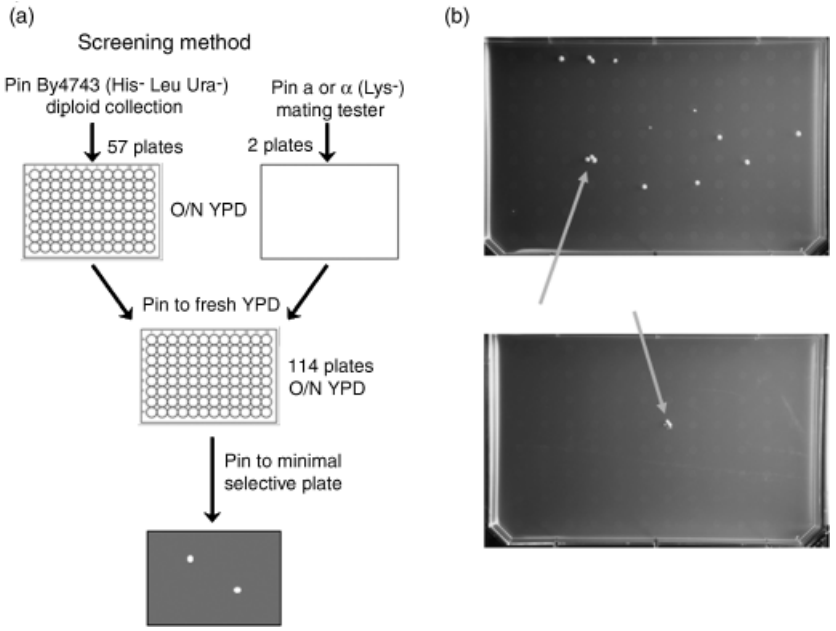

Fig. 1. A screen for gene deletions permitting diploid mating (a) Schematic showing the diploid mating screen performed as described in 'Materials and methods.' (b) Sample plates showing positive and negative maters. These plates are the diploid homozygous deletion collection plate #66 mated with a *MAT***a** haploid (top) or *MAT*α haploid (bottom). Well E4 (*ylr193c*Δ/*ylr193c*Δ) was scored positive for mating with the *MAT***a** tester (top, arrow), and well D7 (*swi6*Δ/*swi6*Δ) was scored positive for mating with the *MAT*α tester (bottom, arrow). All other wells were scored as non-mating diploids.

diploids. Second, genetic anomalies may have arisen in the strain from the homozygous diploid ORF deletion collection that are not present in the corresponding strains from the haploid ORF deletion collections and hence not present when a diploid is created by mating the haploid deletion strains. Third, the original screen may have been performed at low stringency, leading to the identification of a high rate of false positives. In some cases, deletions identified as maters may initially have an elevated rate of mating but were not identified in the second screen due to stochastic effects. This also raises the possibility that the screen had a high rate of false negatives, especially among chromosome segregation mutants, due to the stochastic nature of these events. Therefore, the list of deletion strains identified in the initial screen has been included for reference (Supplementary Table S2).

Characterization of diploid maters

Six diploid deletion strains demonstrated elevated mating ability in both the initial screen and the follow-up analyses. Included among these is *ctf8*Δ/*ctf8*Δ, which exhibited elevated mating with both **a** and α testers (Fig. 2b, Table 1). Interestingly, the *ctf18*Δ/*ctf18*Δ strain appeared to mate only as an **a** strain in the initial screen, but demonstrated mating capacity as both **a** and α in follow-up analysis. Possible reasons for this discrepancy are discussed below. In addition to *ctf8*Δ/*ctf8*Δ and *ctf18*Δ/*ctf18*Δ, two other diploid deletions (*ylr193c*Δ/*ylr193c*Δ and *yor305w*Δ/*yor305w*Δ) were identified that mate with both **a** and α testers: one that mates as an **a** (*ypr170c*Δ/*ypr170c*Δ) and the other that mates as α (*ist3*Δ/*ist3*Δ).

Quantitative mating assays were then performed on selected diploid deletion strains to determine the extent to which these strains can mate relative to haploid controls. These assays were performed on solid media as opposed to the liquid YPD used in the initial screen to improve mating efficiency. For a diploid strain lacking *CTF8* or *DCC1*, mating efficiency with either **a** or α testers was highly variable (Fig. 3), although consistently elevated above the background seen with the diploid control, BY4743. Similar results were seen with *ctf18*Δ/*ctf18*Δ diploids (not shown). This variability is likely due to stochastic loss of the *MAT* locus. Quantitative mating assays for *ist3*Δ/*ist3*Δ and *yor305w*Δ/*yor305w*Δ diploids were also performed. Each of these diploid deletions mated at levels at least fivefold higher than the BY4743 wild-type (WT) diploid (Fig. 3) but still at dramatically reduced levels compared with haploid WT controls. It should also be noted that the mating rates in both *ist3*Δ/*ist3*Δ and *yor305w*Δ/*yor305w*Δ diploids may be lower in the quantitative mating assay due to the slow growth of both of these strains (Steinmetz *et al.*, 2002; Deutschbauer *et al.*, 2005). Although the *yor305w*Δ/*yor305w*Δ strain mated solely as an **a** in this assay, α mating has been observed in this strain as well (Fig. 2b). The results obtained with the *ist3*Δ/*ist3*Δ strain were more consistent from experiment to experiment, suggesting that stochastic loss of genes at the *MAT* locus was not a prerequisite for diploid mating in this case. The fact that none of the single gene mutations isolated results in mating competence similar to that of WT haploid strains is likely indicative of either the multiple levels of regulation in place to inhibit diploid mating or the low number of cells that lose a single copy of chromosome III.

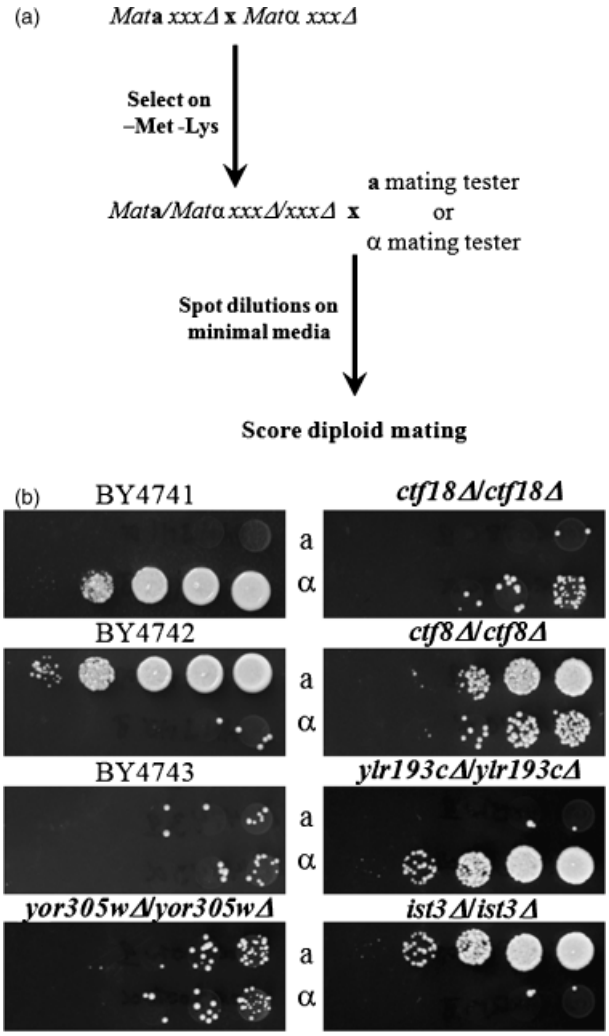

Fig. 2. Verification of mating in diploid deletion strains. (a) Diagram of the method used to retest genes identified in the initial screen. Diploids were made from the haploid deletion collections and then crossed with the mating testers and assayed for triploid growth as described in 'Materials and methods.' (b) Tenfold serial dilutions (from right to left) of diploid deletions mixed with excess mating testers were spotted onto selective media.

Mating and chromosome instability

Strains lacking *CTF8* and *CTF18* are known to exhibit decreased fidelity of chromosome transmission (Spencer *et al.*, 1990). Therefore, it was considered highly likely that rare cells in diploids lacking these genes mate due to loss of the *MAT* locus. However, it was surprising that only these two genes should be identified in the screen, because a large number of other genes are also required for chromosome stability. Moreover, Ctf18 and Ctf8 exist in a protein complex with Dcc1 (Mayer *et al.*, 2001), making a diploid strain lacking this protein a candidate for elevated mating. Because diploid deletion strains with elevated mating could have been missed in the initial screen, the *dcc1*Δ/*dcc1*Δ strain was examined for increased mating (Fig. 3, Table 1). The diploid deletion strain was remade from the haploid deletion collections and it also showed enhanced mating with **a** and α testers, consistent with findings that Ctf8, Ctf18, and Dcc1 act in a complex to maintain chromosome transmission fidelity.

Although only **a**-specific mating was observed for the *ypr170c*Δ/*ypr170c*Δ strain, it is suspected that it also has a weak chromosome mis-segregation phenotype. Mis-segregation is indicated from the characterization of several isolates from this diploid that formed shmoos in the presence of α-factor. PCR analysis of these colonies demonstrated the loss of the MATα locus (Fig. 4). Ylr193c and Yor305w may also assist in proper chromosome segregation because they were each found to sometimes mate with both **a** and α testers, and at variable levels depending upon the particular isolate tested (data not shown). To the author' knowledge, they have not been reported previously to have such an effect. The mating levels in these deletions were consistently lower than in strains lacking the chromosome transmission the fidelity components described above, suggesting that their role in fidelity of chromosome transmission, if any, may be less important than that of Ctf8, Ctf18, and Dcc1. Owing to the low, stochastic nature of chromosome loss in the *ylr193c*Δ/*ylr193c*Δ and *yor305w*Δ/*yor305w*Δ strains, shmoos could not be isolated to confirm loss of the *MAT* locus.

Table 1. Diploid maters identified in genome-wide screen*

ORF	Gene	Function	Mates as
YHR191C	*CTF8*	Member of an alternative RFC complex required for sister chromatid cohesion	**a** or α
YMR078C	*CTF18*	Member of an alternative RFC complex required for sister chromatid cohesion	**a** or α
YLR193C	*UPS1*	Regulates alternative processing and sorting of mitochondrial GTPase Mgm1p	**a** or α
YOR305W		Uncharacterized ORF	**a** or α
YPR170C		Uncharacterized ORF	**a**
YIR005W	*IST3*	Splicing factor	α
YCL016C†	*DCC1*	Member of an alternative RFC complex required for sister chromatid cohesion	**a** or α

*Strains that were identified in the initial screen but did not demonstrate enhanced mating potential in the second screen are listed in Supplementary Table S2.

†*YCL016C* was not identified in the initial screen but demonstrated mating ability as **a** or α upon retest.

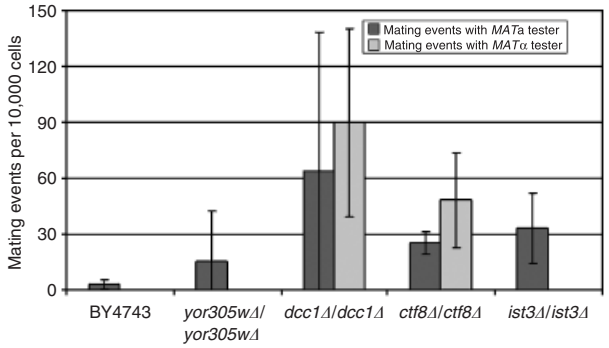

Fig. 3. Quantitation of mating proficiency in selected diploid deletion strains. Quantitative mating analysis was performed for the four diploid deletion strains shown (*yor305wΔ/yor305wΔ*, *dcc1Δ/dcc1Δ*, *ctf8Δ/ctf8Δ*, and *ist3Δ/ist3Δ*) as well as *ctf18Δ/ctf18Δ* (data not shown).

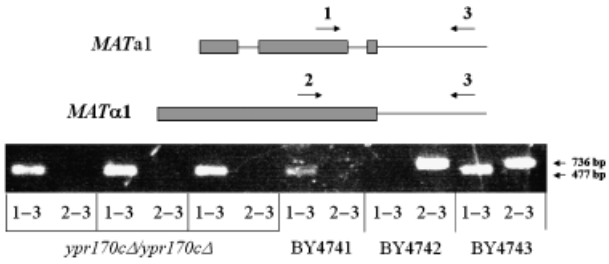

Fig. 4. Loss of *MAT*α locus in the *ypr170cΔ/ypr170cΔ* strain. PCR analysis of colonies formed from three individual diploid cells that formed shmoos in the presence of α-factor. The primers used are listed in Supplementary Table S1.

The initial screen may not have been sensitive enough to identify other genes that are known to affect chromosome loss, such as *CHL1* (Haber, 1974; Liras *et al*., 1978; Gerring *et al*., 1990). In the case of the *chl1Δ/chl1Δ* mutant, it was found that it formed only three colonies when mated with the *MAT***a** tester strain, and no colonies when crossed with the *MAT*α tester. As stated, mating was considered positive if four or more colonies grew.

Splicing of *MAT***a***1* by Ist3

IST3 was originally identified in a screen for mutants with *I*ncreased *S*alt *T*olerance, but encodes the U2 snRNP protein, Snu17 (Entian *et al*., 1999; Gottschalk *et al*., 2001; Wang *et al*., 2005). Unlike many eukaryotes, only a small percentage of yeast genes contain introns. It was speculated that the α-mating behavior of the diploid *ist3Δ/ist3Δ* strain might be attributable to inefficient splicing of the *MAT***a***1* RNA, which contains two introns. In such diploids, there would be insufficient Mat**a***1* activity to repress transcription of α-specific and haploid-specific genes and to promote transcription of diploid genes.

To examine this possibility, RNA isolated from the *ist3Δ/ist3Δ* strain and the isogenic WT diploid was analyzed by reverse transcription-coupled PCR with primers designed to compare spliced and unspliced *MAT***a***1* message (Fig. 5a). Using sets of primers that flank either one or both introns, a *MAT***a***1* splicing defect was detected in a diploid strain lacking *IST3*. Thus, it is proposed that mating in this strain arises from inefficient splicing of this RNA. Interestingly, recessive mutations in *IST1* have been reported to cause increased salt tolerance. Because *IST1* also has an intron, it was reasoned that it might be inefficiently spliced in the *ist3Δ/ist3Δ* strain, which was verified by PCR analysis (Fig. 5b). Splicing is an essential function in yeast, and yet the *ist3Δ* strain was only marginally slow growing (Steinmetz *et al*., 2002). Because some splicing of both *MAT***a***1* and *IST1*

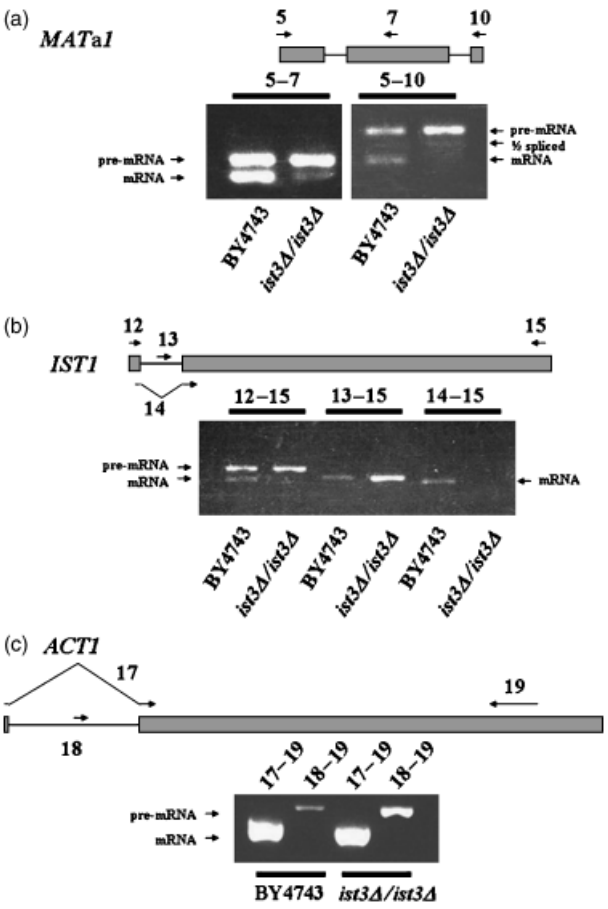

Fig. 5. Reverse transcriptase-PCR assay for splicing defects in the *ist3Δ/ist3Δ* strain. RNA was prepared from either BY4743 or the *ist3Δ/ist3Δ* strain. After reverse transcription, PCR analysis was performed to compare levels of spliced to unspliced message for the following genes: (a) *MAT***a***1*, (b) *IST1*, and (c) *ACT1*. (a) '1/2 spliced' indicates that at this position, one of the two *MAT***a***1* introns has been removed. There is a faint but visible band, but the similar sizes of introns 1 and 2 (52 and 54 bp, respectively) prohibit concluding, which has been removed. (b) The bands for the product 13–15 are pre-mRNA bands that nearly co-migrate with the 12–15 and 14–15 mRNA bands. The primers used are listed in Supplementary Table S1.

was apparent in the *ist3*Δ/*ist3*Δ diploid, one possibility is that Ist3 is a general splicing factor but not totally required for splicing. Alternatively, Ist3 may be specifically required for processing of a subset of yeast introns. To test this possibility, *ACT1* (actin) splicing was examined in the *ist3*Δ strain (Fig. 5c); *ACT1* splicing is reduced only to a small extent in the *ist3*Δ/*ist3*Δ strain. Thus, it is suspected that Ist3 plays a general role in splicing but may influence the splicing of some RNAs to a greater extent than others. This hypothesis is consistent with findings from a reported genome-wide splicing survey (Clark *et al.*, 2002).

The above findings indicate strongly that the diploid *ist3*Δ/*ist3*Δ strain mates as an α with increased frequency due to inappropriate *MAT***a***1* splicing. However, the authors wanted to rule out the formal possibility that splicing defects arise in this strain due to mutation(s) directly in *MAT***a***1*. To test this possibility, the *MAT***a** *ist3*Δ strain was mated to either a *MAT*α *IST3* strain or a *MAT*α *ist3*Δ strain and the resultant **a**/α diploids were examined for mating ability (Fig. 6). The *ist3*Δ/*ist3*Δ diploid exhibited high mating ability with the *MAT***a** tester, but the *IST3*/*ist3*Δ diploid did not. The *IST3*/*ist3*Δ strain would be expected to mate if the *ist3*Δ *MAT***a** parent strain contained an inactivating mutation at the *MAT* locus. In addition, the possibility that the *ist3*Δ/*ist3*Δ diploid also had a chromosome loss phenotype was tested. PCR analysis of colonies derived from diploids that formed shmoos in the presence of α-factor showed that the *MAT*α locus was still present in these cells (not shown). Together, these findings indicate that elevated mating in *ist3*Δ/*ist3*Δ diploids results from loss of Ist3 splicing function and not due to inherent defects at the *MAT***a** locus or to chromosome loss.

To quantify the nature of the splicing defect, QPCR analysis was performed on cDNA generated from both the WT diploid and mutant *ist*Δ/*ist3*Δ strain (Table 2). A slight increase was found in the amount of unspliced message at *MAT***a***1* Intron2 (2.43-fold increase) in the *ist*Δ/*ist3*Δ diploid, but a nearly 10-fold increase in unspliced transcript at *MAT***a***1* Intron1, compared with the WT control. The total *MAT***a***1* transcript levels were the same in the two strains. Splicing efficiency is also reduced, although to a lesser extent, in both the *IST1* and *ACT1* messages, where only a sixfold and approximately threefold increase in unspliced message were observed, respectively. Comparing the spliced message of *IST1* in the mutant and WT by amplifying a product with a primer at the splice junction, a 50% decrease in the spliced message is seen. These findings suggest that the importance of Ist3 in splicing is likely to be at least partially dependent on the RNA being spliced, consistent with the report that Ist3 has a role in Mer1-dependent splicing (Spingola *et al.*, 2004).

It was next attempted to repress the mating phenotype in this strain by constructing plasmids containing unspliced, partially spliced (missing intron 2), or totally spliced *MAT***a***1* and transforming them into the *ist3*Δ/*ist3*Δ mutant, the WT *MAT*α haploid (BY4742), WT *MAT***a** haploid (BY4741), and WT **a**/α diploid (BY4743) controls. These strains were then mated with either the *MAT***a** or *MAT*α mating tester and plated to selective media. As expected, the mating phenotype is suppressed in both the *ist3*Δ/*ist3*Δ mutant and the

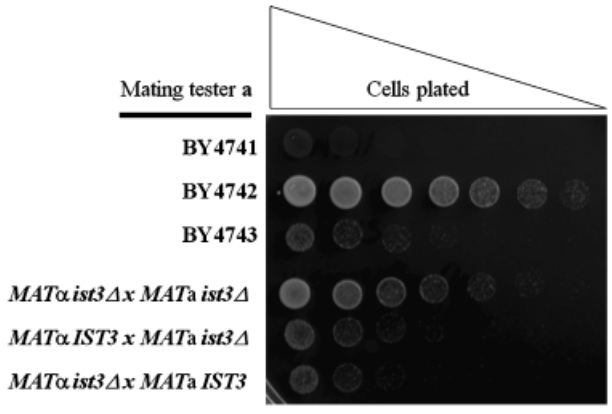

Fig. 6. Increased mating in the *ist3*Δ/*ist3*Δ strain is not due to defects in the *MAT* locus. *ist3*Δ/*ist3*Δ, *IST3*/*ist3*Δ, and *ist3*Δ/*IST3* **a**/α diploids were created from the haploid deletion collections and mating assays were performed on these strains. Threefold serial dilutions (left to right) of the indicated strain mixed with excess *MAT***a** mating tester were spotted onto selective plates.

Table 2. Transcript levels of intron-containing RNAs in *ist3*Δ/*ist3*Δ (Ist3^{-}) compared with BY4743 (WT)

Primers*	Gene	Region	Size (bp)	Strain	Relative amount§
38–39	*MAT***a***1*†	Exon1-Intron1	120	WT	1
				Ist3^{-}	9.56
36–55	*MAT***a***1*†	Exon2-Intron2	149	WT	1
				Ist3^{-}	2.43
40–41	*MAT***a***1*†	Exon2-Exon2	116	WT	1
				Ist3^{-}	1.02
42–43	*IST1*	Exon1-Exon2‡	158	WT	1
				Ist3^{-}	0.53
44–45	*IST1*	Exon1-Intron1	112	WT	1
				Ist3^{-}	5.72
46–47	*IST1*	Exon2-Exon2	125	WT	1
				Ist3^{-}	0.73
48–49	*ACT1*	Intron1-Exon2	176	WT	1
				Ist3^{-}	3.03
50–51	*ACT1*	Exon2-Exon2	136	WT	1
				Ist3^{-}	1.06

*The sequences of the primers used can be found in Supplementary Table S1.

†We were unable to design appropriate QPCR primers for *MAT***a***1* that crossed the splice junctions.

‡The reverse primer in this case crosses the splice junction, allowing amplification only of message that has been spliced.

§All of the numbers were normalized to the level of *CDC28* cDNA before comparison.

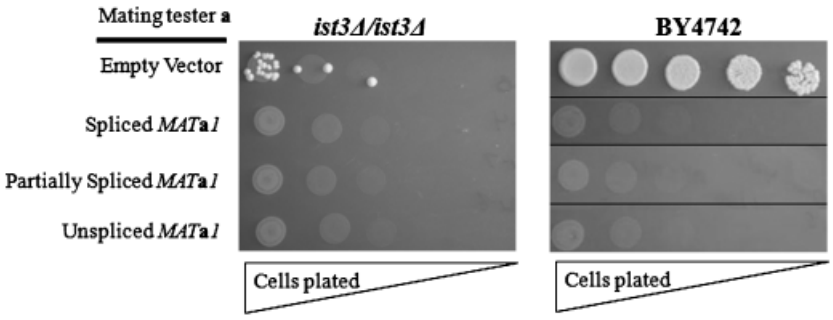

Fig. 7. Addition of *MAT***a***1* message represses the mating phenotype in the *ist3*Δ/*ist3*Δ strain. Plasmids containing unspliced, partially spliced (missing intron 2), or totally spliced *MAT***a***1* were transformed into the *ist3*Δ/*ist3*Δ mutant and the WT *MAT*α haploid, and mating assays were performed on these strains. Fivefold serial dilutions (left to right) of the indicated strain mixed with excess *MAT***a** mating tester were spotted onto selective plates. For the right panel, all images were taken from the same plate but were realigned for presentation.

Table 3. *MAT***a***1* transcript levels in *ist3*Δ/*ist3*Δ (Ist3^{-}) and BY4743 (WT) diploid transformants

Primers*	Region	Strain	Relative amount†
40–41	Exon2-Exon2	Ist3^{-}+vector	1.09
		WT+vector	1
40–41	Exon2-Exon2	Ist3^{-}+unspliced	1.67
		WT+unspliced	1
40–41	Exon2-Exon2	WT+vector	1
		WT+unspliced	18.25
40–41	Exon2-Exon2	Ist3^{-}+vector	1
		Ist3^{-}+unspliced	28.24
38–39	Exon1-Intron1	Ist3^{-}+vector	5.04
		WT+vector	1
38–39	Exon1-Intron1	Ist3^{-}+unspliced	15.50
		WT+unspliced	1
36–55	Exon2-Intron2	Ist3^{-}+vector	2.27
		WT+vector	1
36–55	Exon2-Intron2	Ist3^{-}+unspliced	2.63
		WT+unspliced	1

*The sequences of the primers used can be found in Supplementary Table S1.

†All of the numbers were normalized to the level of *CDC28* cDNA.

WT *MAT*α haploid strains (Fig. 7). Although the *ist3*Δ/*ist3*Δ mutant is thought to have a splicing defect, it is not a complete lack of splicing. The high levels of total transcript from the partially spliced and unspliced *MAT***a***1* plasmids (Table 3) will yield sufficient completely spliced message to allow suppression of the mating phenotype equivalent to what is seen from the spliced plasmid. In the WT diploid strain, transformation with any of the three versions of *MAT***a***1* leads to a decrease in low-level background mating with the **a** tester strain, while there is no apparent effect in the WT *MAT***a** strain (data not shown).

Transcription of *MAT***a***1* is equivalent in the WT and *ist3*Δ/*ist3*Δ mutant for either the chromosomal gene or the plasmid-borne copy, although transformants with the plasmid bearing the unspliced *MAT***a***1* gene showed an increase in total *MAT***a***1* message of *c.* 18- and 28-fold, respectively, when compared with vector controls (Table 3). In the *ist3*/*ist3* mutant+vector control, message from the chromosomal *MAT***a***1* gene unspliced at intron 1 was increased fivefold relative to the WT+vector strain, while the increase was over 15-fold in transformants with the unspliced *MAT***a***1* gene. The increase in message not spliced at intron 2 was only 2.27 with the vector control and 2.63 with the unspliced *MAT***a***1* added.

Although splicing is an essential function in yeast, there are a number of non-essential factors that assist splicing. However, only the *ist3*Δ/*ist3*Δ strain was identified as having increased diploid mating. *AAR2*, which has been previously shown to affect the splicing efficiency of *MAT***a***1* in yeast (Nakazawa *et al.*, 1991), is not part of the homozygous diploid deletion collection and therefore would not have been found in the screen. However, other yeast splicing factors were examined directly to determine whether they allowed diploid mating and were missed in the initial screen. Deletion strains were selected based on genome-wide microarray data that showed a similar effect to Ist3 (Snu17) on splicing of *MAT***a***1* message (Clark *et al.*, 2002). Of the seven strains identified, one (*prp4*Δ/*prp4*Δ) is inviable and another (*msl1*Δ/*msl1*Δ) appeared to be diploid in the haploid sets (failed to mate, contained auxotrophic markers of the diploid). Of the five remaining diploid deletion strains remade from the haploid deletion sets and tested together with *ist3*Δ/*ist3*Δ (*brr1*Δ/*brr1*Δ, *ecm2*Δ/*ecm2*Δ, *mud2*Δ/*mud2*Δ, *prp18*Δ/*prp18*Δ, and *snu66*Δ/*snu66*Δ), only the *ist3*Δ/*ist3*Δ strain showed enhanced mating (Fig. 8). From these findings, it is proposed that Ist3 has a particularly important role in splicing the *MAT***a***1* message.

Discussion

A screen has been performed using the yeast ORF deletion collection to identify diploid strains that have increased mating capacity, which may be used to identify homozygous diploid mutants with a chromosome missegregation or a rearrangement phenotype (Kouprina *et al.*, 1988). Three genes were identified that are involved in sister chromatid cohesion (*CTF8*, *CTF18*, and *DCC1*); deletion of any one of these enhances chromosome loss and therefore increased mating in diploids. Nineteen genes are listed as being

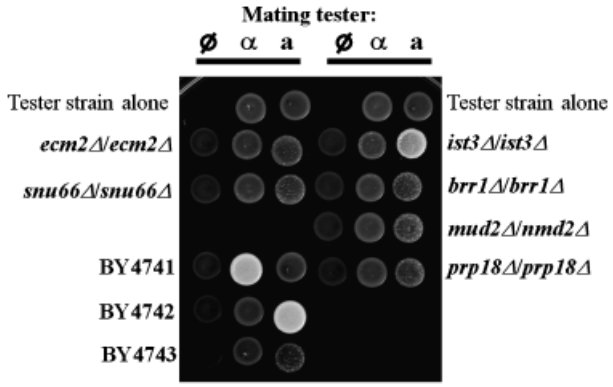

Fig. 8. Deletion of other splicing factors does not result in increased diploid mating. Mating assay with homozygous deletions of five known, nonessential splicing genes that were not identified in the initial genome-wide screen compared with the *ist3*Δ/*ist3*Δ strain. Indicated diploid deletion strains were incubated with excess mating testers and spotted onto media selecting for mating events. The Ø indicates that there is no mating tester in that column.

important for sister chromatid cohesion in yeast, according to the Saccharomyces Genome Database (http://www.yeastgenome.org/). Of these, *bim1*Δ/*bim1*Δ was originally identified as having increased mating (see Supplementary Table S2); however, the remade diploid deletion did not exhibit elevated mating. Given that mating is variable in these deletions due to stochastic chromosome loss events, it is speculated in retrospect that this strain does indeed exhibit elevated mating and was discarded because elevated loss of chromosome III was not evident in the retest due to the stochasticity of the phenotype. Of the other 15 genes, nine were not present in the diploid deletion set, either because they encode essential proteins or because elevated chromosome loss caused them to be discarded during quality control while the deletion sets were being constructed. Therefore, it is concluded that proteins involved in sister chromatid exchange are particularly important for proper segregation of chromosome III and for diploid sterility.

UPS1 (*YLR193C*) and two genes of unknown function (*YOR305W* and *YPR170C*) were identified in the screen. *YPR170C* is listed as a dubious ORF, because it is not conserved in closely related *Saccharomyces* species. It may encode a protein or, alternatively, deletion of *YPR170C* may affect the expression of genes adjacent on chromosome XVI, something that occurs at a significant level due to the close apposition of yeast genes. In genome-wide localization screens, Yor305w is reported to localize to the mitochondria, whereas Ylr193c has been localized both to the nucleus and the mitochondria (Kumar *et al.*, 2002; Huh *et al.*, 2003). Ylr193c (Ups1) has recently been reported to be required for processing and sorting of the mitochondrial GTPase Mgm1p (Sesaki *et al.*, 2006). Ups1p, a conserved intermembrane space protein, regulates mitochondrial shape and alternative topogenesis of Mgm1p, and is thus required for normal mitochondrial morphology. The human ortholog of Mgm1, OPA1, has been implicated in autosomal dominant optic atrophy (Olichon *et al.*, 2006). It is possible that deletion of *YLR193C* may have some effect on the respiratory pathway, leading to an increase in oxidative DNA damage and increased chromosome loss rates. It is noted, however, that no genes known to increase oxidative damage in the cell were identified in the screen; thus, it is possible that there is an unknown function of this protein unrelated to the reported mitochondrial role.

The initial screen may not have been sensitive enough to identify other genes that are known to affect chromosome segregation. In order to optimize the screen to identify such mutants, it may be helpful to grow the cultures over several nights by diluting aliquots from the previous culture into fresh media to allow defects to accumulate. Longer growth might also allow mutants to show positive mating during the testing of freshly made diploids. Strains with homozygous deletions at *CSM1*, *CSM2*, *HUR1*, and *NPL6* failed to retest, although each has been implicated in some aspect of chromosome maintenance either during chromosome segregation (*CSM1*, *CSM2*) (Huang *et al.*, 2003; Smith *et al.*, 2004; Wysocka *et al.*, 2004) or maintenance of telomeres (*HUR1*, *NPL6*) (Askree *et al.*, 2004; Smith *et al.*, 2004). Additionally, deletion strains for three genes that have been implicated in pre-mRNA processing (*MUD1*, *LSM12*, and *BRR1*) (Horsthemke *et al.*, 1992; Liao *et al.*, 1993; Noble & Guthrie, 1996), one that has been shown to affect mating (*ARD1*) (Whiteway & Szostak, 1985), and one that apparently affects mRNA translation (*SGN1*) (Winstall *et al.*, 2000), all mated in the initial screen but failed during later testing.

In summary, seven homozygous deletion strains have been identified that show elevated diploid mating ability. At least three of these deletions (and perhaps as many as six) show enhanced mating due to elevated loss of chromosome III. The other homozygous deletion strain (*ist3*Δ/*ist3*Δ) shows elevated α mating due to diminished splicing of the *MAT***a***1* message. Further work will be needed to better understand the importance and enhanced specificity of Ist3 in splicing this particular RNA. All of these deletion strains can be explained based on known activities that indirectly promote diploid sterility. The fact that no other deletion strains were identified with enhanced mating suggests that sterility in diploids is a robust phenotype depending largely on the expression of *MAT***a**-and *MAT*α-encoded proteins.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Primers used for analysis as indicated.

Table S2. Mating results from original screen.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1567-1364.2007.00322.x (This link will take you to the article abstract).

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