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Cell Wall Functions Required for Mating in Saccharomyces cerevisiae

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TABLE OF CONTENTS

Abstract

In order for two haploid yeast cells to undergo normal fusion during mating, adhesive contacts are formed by a general class of fungal proteins termed “adhesins”. Within this family there is a subgroup of cell wall proteins termed “agglutinins” which are known to mediate aggregation, thereby increasing mating efficiency in liquid medium (Aga1p, Aga2p, Agα1p). In the absence of sufficient mating adhesive contacts we observed that cells undergo defective morphogenesis, apparently continuing to grow at their tips after fusing at the expense of laterally expanding their conjugation bridge. Based on these qualitative observations, I utilized differential interference microscopy to capture micrographs of mating cells of different mutants that lacked one or more of the yeast adhesin/agglutinin proteins Aga1p, Aga2p, Fig2p, and Agα1. From the micrographs collected, I determined the relative dimensions of the mating cells and analyzed them using various statistical methods. The study elucidated the necessity of these proteins for proper morphogenesis. In a related project I sought to identify cell wall genes whose inactivation led to deficiencies in mating when the mating partner lacked specific adhesins. Cell wall residency of the adhesins and the remodeling that the cell wall undergoes during cell-cell fusion are important and incompletely understood processes whose components might affect such crosses. I performed a large scale genomic screen of strains known to be defective in some aspect of cell wall organization. A collection of 170 cell wall mutant strains, each containing a replacement of a single yeast gene with a dominant drug resistance marker, was tested for mating deficiency in crosses to wild type and adhesion deficient partner strains. From this screen seven candidate gene mutations were identified that reproducibly satisfied the screen and hold promise for further study.

Preface

Completing an honors thesis serves as a concrete culmination to the last four years as an undergraduate at Syracuse University. Now I look back on my experience with skewed hindsight as I try to articulate how this process has been for me. Perhaps a more accurate assessment of this experience and my education as a whole would be to look at the changes that have occurred in me as a result. Nevertheless I would like to share some of the more tangible insights derived from this experience. I began my studies with interests in nearly every subject including, but not limited to, Biology, Philosophy, Literature and Psychology. I had few inclinations concerning what I would concentrate my attention and career on for the ensuing years, and after four years I can't say that my interests have diminished or that the view of my future is substantially clearer. I can say however that I have gained a tremendously deeper understanding of many subjects, primarily biology. I have learned some of what it takes to be a scientist, from day to day. The majority of my research was completed in the summer of 2004. For much of this time I found myself locked up in the darkest room in the lab peering through a pair of lenses, looking at thousands of yeast cells and taking hundreds of micrographs. My description may paint a negative picture of this process, but I found it quite interesting, tiresome and even monotonous at times, but I truly enjoyed moments when I realized how compelling the microscopic world of biology can be. I was quite pleased that this became a substantial part of my project

and when I finally got the data it was that much sweeter; I felt fulfilled and it was time well spent.

Although the subject of this preface is my research in biology department, I feel the need to digress about the education I received in general because the whole process has contributed to this work, not only the in the lab, but also the classroom. What I mean by this is that scientists benefit from a broad and diverse education. Outside influences inspire me in and outside the classroom by enhancing creativity and enforcing independent thought. If everyone were to receive the same education then where will the new thoughts come? This being said, the laboratory provided me with a tremendous experience which I look back on as one of the most beneficial parts of my college education. It offered a break from the mundane classroom atmosphere and *ad nauseam* memorization that biological study entails. As I have progressed in my education I also realize that the laboratory and the scientific process are not solely applicable to scientific pursuits, or perhaps more clearly science is applicable to all pursuits. I can't remember how many times I have used principles of science in other classes, and in everyday life, whether in analyzing the mind of Don Quixote in Spa 444, the ethics of Kant in Phi 191, or the psychology of Freud in Psy 393. Therefore I will always be a proponent of the student with a diverse education, diverse environment, and a diverse mind who in the end can achieve anything they desire.

Advice to Future Honors Students

There are a few things that I wish I had realized before I arrived at Syracuse. One of them is to do independent research and do it early. This provided me with the opportunity to really develop in a productive setting and to make the important personal connections with professors and graduate students which add immeasurably to the education experience. If you do choose to get involved in a project, ask lots of questions and do not be afraid to ask the dreaded "stupid" question. Also, as a first research endeavor it will be frustrating, but make mistakes and be resilient because that is natural.

Aside from research don't hesitate to travel abroad. Science majors are typically either discouraged from going abroad or the curriculum makes it substantially more difficult to graduate on time. With that in mind go anyway, think of it as an experiment on yourself, you will be immersed in a completely new culture without the same comforts or luxuries that one is used to, of course it is not for everyone but those who are interested in other cultures, and enjoy traveling independently should consider. Other wisdom of course would be to start the thesis early and keep it in mind from the beginning of the experience, but I leave it for last because I know that reading this will probably not change anyone's decisions and naturally I am not the only one providing this insight.

Acknowledgements

I would like to thank Dr. Erdman for his support in the lab. It was surprising when I found out that he had grown up in the same region as I, essentially because it is not the typical place that churns out academicians. Nevertheless this was just one area of common ground. I always appreciated the many entertaining stories he told, and where there was one, there were usually many. He also has been a tremendous mentor, providing insight in biology, career choices, or just dining in Syracuse (good food is never trivial matter). Once Scott mentioned that the only thing that bothered him in the lab was silence, and I always liked that philosophy. I can easily say that, directly or indirectly, he taught me the most of any teacher I have had. I would also like to thank Guohong Huang. During my time in the lab she has patiently guided me in my projects, giving me advice, teaching me the proper method, and helping me analyze data.

I also thank Dr. Belote and Dr. Wolf not only for the past three semesters in the honors seminar, but as teachers. I have taken classes with each of them and I feel they are two of the finest teachers from whom I have learned a tremendous amount.

Finally I thank the Biology Department as a whole for the facilities and the opportunities that they have provided. I wish to acknowledge the Ruth Meyer Research Award for the financial support of my research.

Introduction

The process of cell adhesion provides a mechanism for the interactions necessary for persistence and propagation or invasion into new environments by symbiotic and pathogenic fungi. There is a family of proteins which have previously been shown to be integral in the formation of these adhesive contacts for the saprophytic fungi *Saccharomyces cerevisiae* they are termed "adhesins" (Erdman, et al., 1998). The expression of these proteins is regulated by various environment cues and stages of the life cycle. The mating pathway is one such stage which is especially relevant. *S. cerevisiae* cells have the ability of both asexual division and sexual mating. Thus, haploid cells have one of two different mating types: MAT**a** and MAT***α***. Cells of different mating types can fuse to form a diploid (Fig1). For mating to occur properly when cells are suspended in a liquid environment, the process of agglutination is essential. This involves the adherence of one cell type to the partner from the opposite mating type which serves to stabilize the complex during subsequent cell-cell fusion.

Agglutination is regulated by the agglutinin genes AGA1 and AGA2 in MAT**a** cells and AGα1 (SAG1) in MAT**α** cells. Aga1p is a 703 amino acids long, secretory pathway processed and GPI anchored protein, bound to the 69 amino acid polypeptide, Aga2p protein (Huang, et al., 2003). Aga1p and Aga2p complex with one another on MATa cells in order to bind Agα1p (Sag1p), a GPI anchored cell surface binding

protein. Therefore binding interactions are heterotypic in nature since the proteins involved are distinct for each cell type. The adhesin Fig2p is of interest as well and causes increased agglutination when absent in both MATa and MATα cells. This could be because of increased access to the anchorage components when absent or there may be an indirect effect involving trans-membrane signaling (Zhang, et al., 2002).

Fig.1 Yeast Life Cycle

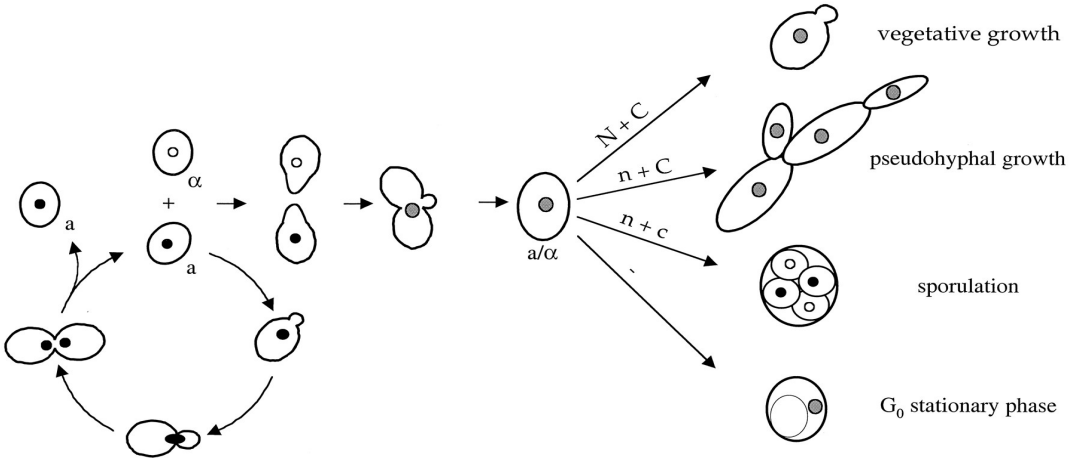


Fig. 1 Diploid yeast cells adopt alternative fates depending on the availability of nutrients. N + C, abundant nitrogen and fermentable carbon source; n + C, limiting nitrogen and abundant fermentable carbon source; n + c, limiting nitrogen and non-fermentable carbon source; no nutrients. (Lengeler, et al., 2000)

Evidence suggests that the loss of any of the agglutinins causes a deficiency in mating partner adherence in liquid mating, before cell fusion. While in liquid medium cells must be able to stably bind their partner long enough to carry out fusion. In general these characteristics of the agglutinin system make it an excellent one for understanding the nature of microbial cell adhesion processes.

Furthermore, because of genetic conservation among other yeast species, the yeast model is extremely useful for the understanding and control of fungal pathogenesis. Of specific relevance is the adhesin protein Hwp1p from the pathogenic fungal species *Candida albicans* that is homologous to Aga1p as it contains several active conserved domains, which are implicated in the adhesive contacts necessary for fungal host association (Huang, et al. in preparation).

The adhesins, as a larger class of proteins, have an established role in stabilizing the mating complex through adhesive contacts. From observations made of adhesin mutants, adhesins have been purported to have an additional role in ensuring normal morphogenesis during the mating pathway (Erdman et al., 1998; Zhang et al. 2002). In an attempt to extend these studies, we aimed to quantify the effects that adhesin deletions have on zygote morphology.

Another goal of my research was to carry out a screen of strains mutated in genes implicated in cell wall activities as highlighted by Pagé, et al., (2003) and Lussier, et al., (1997). We hypothesized that some of these genes may function in the mating

pathway and also may interact directly or indirectly with one or more of the cell wall localized adhesin proteins. This screen was greatly facilitated by the already sequenced and annotated *S. cerevisiae* genome. This technology has enabled a "program of precise gene disruption" to create a collection of mutant strains deficient in each gene (Lussier et al., 1997). The cell wall is of considerable interest for its dynamic nature, one prime example being the remodeling that occurs during fusion events. The cell wall is composed of highly mannosylated proteins (mannan) and three kinds of polysaccharide chains: 1,3-β-glucan, 1,6-β-glucan component, and chitin. Glucans make up about half of the dry weight of the cell wall, while chitin accounts for only 1 to 2%. Evidence has suggested that the different components of the yeast cell wall are linked covalently, in a complex considered a "flexible building block" (Rodriguez-Pena, et al., 2000). Rodriguez-Pena et al. have also observed that major constituents are linked covalently: mannoproteins are GPI anchored to 1,6-β-glucan which is similarly bound to 1,3-β-glucan chains. They also suggest that chitin could bind either 1,3- or 1,6-β-glucan. In addition, the cell wall dynamically interacts with the plasma membrane, secretory organelles, the cytoskeleton and cytoplasmic components in order to monitor cell integrity during growth and morphogenesis. The cell surface of a yeast cell undergoes considerable change in shape and composition as a result of the life cycle; importantly for this study, during mating projection formation and cell fusion in haploid cell conjugation (Lussier, et al., 1997). One change of particular interest occurs by the activation of the RHO1 signaling pathway due to

polarized growth arising during mating due to pheromone signaling in local areas of the cell cortex. The Rho1 protein then activates the cell wall biogenesis enzyme encoded by FKS1 and protein kinase C (PKC1) which in turn activates the effector mitogen-activated protein kinase (MAPK) that regulates a variety of cellular responses. The RHO1 pathway regulates transcription factors and chromatin-binding proteins that modulate the transcription of many genes involved in cell wall biosynthesis (Valdivia and Schekman, 2003). Other changes lead to a thinner glucan layer in the region of the mating projection, altered chitin and glucan compositions, an increase in β 1,3 synthesis, as well as chitin synthesis. The adhesins function during mating and interact closely with cell wall components during these fusion events. The adhesin proteins Aga1p, Fig2p are anchored by GPI groups, which have been shown to be covalently linked to β 1,6 glucan (Lu, et al., 1994). This linkage to the cell wall probably functions to aid in exposing the adhesins on the cell wall surface, because the average cell wall is 100-200nm thick the adhesins must span this distance for proper binding (Lipke, et al., 1995), (Huang, et al., 2003).

Materials and Methods

2.1 Strains

Strain	Genotype	Source/Reference[a]
YSE430	*MAT***a** *ura3□ leu2□ his3□ TRP1 met15□ LYS2*	
YSE431	*MAT□ ura3□ leu2□ his3□ TRP1 MET15 lys2□*	
YSE913	*MAT***a** *aga1::URA3 leu2□ his3□ TRP1 met15□ LYS2*	
YSE914	*MAT□ aga1::URA3 leu2□ his3□ TRP1 MET15 lys2□*	
YSE548	*MAT***a** *fig2::KanR ura3□ leu2□ his3□ TRP1 met15□ LYS2*	
YSE549	*MAT□ fig2::KanR ura3□ leu2□ his3□ TRP1 MET15 lys2□*	
YSE1046	*MAT***a** *aga1::URA3 fig2::KanR leu2□ his3□ TRP1 met15□ LYS2*	this study
YSE1047	*MAT**a** aga2::KanR fig2::KanR ura3□ leu2□ his3□ TRP1 met15□ LYS2*	this study
YSE1048	*MAT***a** *aga1::URA3 aga2::KanR leu2□ his3□ TRP1 met15□ LYS2*	this study
YSE1049	*MAT□ aga1::URA3 sag1::KanR leu2□ his3□ TRP1 MET15 lys2□*	this study
YSE1050	*MAT□ sag1::KanR fig2::KanR ura3□ leu2□ his3□ TRP1 MET15 lys2□*	this study

YSE1051	*MAT□ aga1::URA3 fig2::KanR leu2□ his3□ TRP1 MET15 lys2□*	this study
YSE1090	*MAT***a** *aga1::URA3 aga2::KanR fig2::KanR leu2□ his3□ TRP1 met15□ LYS2*	this study
YSE1091	*MAT□ aga1::URA3 sag1::KanR fig2::KanR leu2□ his3□ TRP1 MET15 lys2□*	this study

[a]All strains are derived from the Yeast Genome Deletion collection unless otherwise indicated.

2.2 Tetrad Dissection

Cultures of strains to be sporulated were grown overnight on GNA presporulation mediums. 1.5 ml of cells were pelleted in a sterile eppendorf tube, then resuspended in 0.5-1.9 ml sterile water and re-pelleted. Pellets were resuspended in 0.5ml 2% KAc and then a small aliquot of cells was transferred to a fresh culture tube containing 2.5ml of 2% KAc. The tube was placed on the roller drum at room temperature for 3 days. The cells were pelleted in a microcentrifuge tube resuspended in 1.0 ml sterile water, and pelleted again. The asci were resuspended in 0.5 ml of sterile water and stored at 4 degrees. To dissect tetrads, the asci were resuspended well, 50 microliters was removed and 25 microliters and 25 microliters of sterile distilled water was added to a new microcentrifuge tube and then 5 microliters of 2mg/ml Zymolase 100T was added. The asci were allowed to incubate with tube upright in the 30C incubator 8-10min. Then 5-7 microliters were removed from the bottom of the tube and spread on a narrow stripe down the center of YPD plate. Then cells were dissected using a Nikon Eclipse 6400 microscope equipped with a micromanipulator and incubated at room temperature or 30C until colonies were evident.

2.3 Double Mutant Construction

Beginning with strains that were single mutants of fig2Δ, aga1Δ and sag1Δ as MATα and MAT**a,** strains were crossed to form diploids containing both mutations in heterozygous state. Sporulation of these diploids yielded the desired double mutants

among the meiotic progeny. Diploid strains were grown on GNA-presporulation media to promote sporulation. Sporulated cells that had mated were collected by centrifugation and plated onto YPD plates for tetrad dissection. Tetrads dissection was performed in order to find the spore that contains the double deletion. Double mutants were selected by markers and then presence of the inferred deletions confirmed by PCR. Mutants constructed in this fashion included: aga2fig2Δ, aga1sag1Δ, aga2fig2Δ, fig2sag1Δ, aga1aga2Δ, fig2sag1Δ, aga1aga2Δ, and aga1sag1Δ.

2.4 Liquid Mating Assays

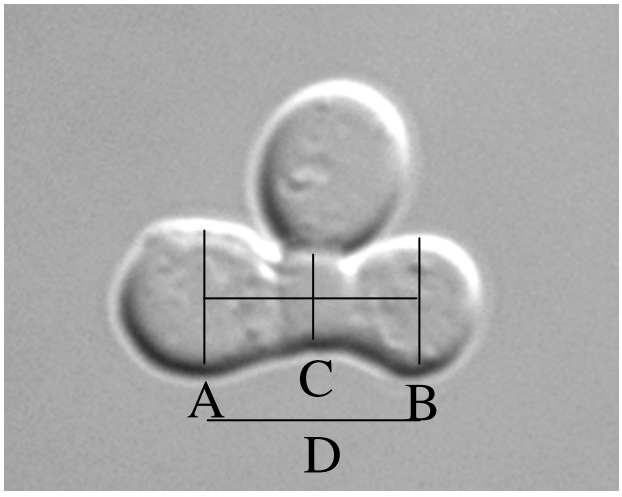
Cells to be mated were grown separately overnight in liquid YPAD media, on a roller drum. The following day they were adjusted in culture density to OD_{600}=0.1, and grown until early log phase (OD_{600}=0.5). Cells were mixed in 18mm culture tubes, and one equivalent of fresh YPAD liquid media was added (1ml MATa, 1ml MATα, 1ml YPAD). The mixture was vortexed gently to mix, and vacuum filtered. Mating cells were filtered and placed under sterile conditions onto solid YPAD agarose media and mated at room temperature.

2.5 Photo-microscopy

Two measurements were utilized in analysis of cell fusion and morphogenesis of crosses. Measurements of inter-parental width were first quantified by analyzing widths of parental cells relative to the length between parent to parent midpoints. In addition, measurements of conjugation bridge morphogenesis were quantified by

analyzing the widths of parental cells relative to the width of the conjugation bridge joining them in zygotes (Zhang et al. 2002). In order to assess the trait among different strains and mating conditions, 50 zygotes were photographed for each sample cross with a Nikon TE-300 microscope fitted with an 100x objective and differential interference contrast (DIC) optics. Images were captured with a Roper/Princeton Instruments MicroMax 5-Mhz charge-couple device (CCD) camera. A ruler tool in Photoshop was used to determine the four relevant measurements for each zygote. These data were then tabulated and compared by spreadsheet analysis (Excel).

Fig. 2 Method of Measurement



A model of the method of measurement which was then used to quantify the morphology of zygotes for all strains analyzed. Inter-parental Distance Measurement: W=D/(A+B)/2. Conjugation Bridge Height Measurement H=C/(A+B)/2

2.6 Statistical Analysis

For analysis of statistical significance T-tests were performed utilizing the following website. In addition to this ANOVA, tests were performed by fellow researchers in order to confirm significance.

http://www.graphpad.com/quickcalcs/ttest1.cfm?Format=SD

2.7 Strains Used for Genomic Screen

For our purposes strains from Lussier and Pagé were selected (Tables 1-4) and organized into a database using Microsoft Access. Strains were obtained from a collection of five thousand MAT**a** strains in Dr. Erdman's laboratory kept frozen and organized in 96 well plates by specific addresses. The selected strains were patched onto YPAD plates for further experimentation.

Table 1 Genes Screened

Gene	ORF	Chr.	Function/Homology/Domain
DEP1/FUN54	YAL013W	I	Transcriptional modulator In phospholipid biosynthesis
FUN30	YAL019W	I	Chromosome stability
ATS1	YAL020C	I	Regulatory interactions between microtubules and the cell cycle
CCR4/FUN27/NUT21	YAL021C	I	Component of the CCR4-NOT transcriptional complex
PMT2	YAL023C	I	Protein O-mannosyltransferase
DRS2, FUN38, SWA3	YAL026C	I	Integral membrane Ca(2+)-ATPase
FUN12, Yif2	YAL035W	I	GTPase
ERV46	YAL042W	I	Protein localized to COPII-coated vesicles
CNE1	YAL058W	I	Endoplasmic reticulum protein quality control
ECM1	YAL059w	I	Protein of unknown function
GDH3, FUN51	YAL062w	I	NADP(+)-dependent glutamate dehydrogenase
BUD14	YAR014C	I	Bud-site selection and bud pattern
KIN3, FUN52, NPK1	YAR018C	I	Nonessential protein kinase with unknown cellular role
ORF, MERGED SWH1	YAR044W	I	Protein possibly involved in ergosterol synthesis
MMM1,YME6	YLL006W	XII	Mitochondrial outer membrane protein
ECM31	YBR176w	II	Ketopantoate hydroxymethyltransferase
RPS6B,LPG18	YBR181C	II	Protein component of the small (40S) ribosomal subunit
BEM1, SRO1	YBR200W	II	Cell polarity and morphogenesis
ROT2, GLS2	YBR229C	II	Glucosidase II catalytic subunit
ECM18	YDR125c	IV	Unknown
SWF1	YDR126W	IV	Spore Wall Formation
SAC6, ABP67	YDR129C	IV	Fimbrin, actin-bundling protein
VPS61	YDR136C	IV	Dubious open reading frame, unlikely to encode a protein
VPS7	YDR349C	IV	Putative GPI-anchored aspartic protease
VID21, EAF1	YDR359C	IV	Component of the NuA4 histone acetyltransferase complex
CDC40, PRP17	YDR364C	IV	Pre-mRNA splicing factor
VPS74	YDR372C	IV	Protein-vacuolar targeting
BCS1	YDR375C	IV	ATP-dependent chaperone in mitochondrial inner membrane
SAC7	YDR389W	IV	GTPase activating protein (GAP) for Rho1p
DIT2, CYP56	YDR402c	IV	Sporulation-specific enzyme required for spore wall maturation
ERD1, LDB2	YDR414c	IV	Potential role in the retention of endoplasmic reticulum proteins
NPL3, MTR13	YDR432W	IV	RNA-binding protein
VAC8	YEL013W	V	Cytoplasm-to-vacuole targeting (Cvt) pathway

Table 2 Genes Screened Cont'd

Gene	ORF	Chr.	Function/Homology/Domain
ECM10, SSC3	YEL030w	V	Heat shock protein of the Hsp70 family
RAD23	YEL037c	V	Role in DNA repair
GDA1	YEL042W	V	Guanosine diphosphatase located in the Golgi
GLY1	YEL046C	V	Threonine aldolase; involved in glycine biosynthesis
MAK10	YEL053C	V	Non-catalytic subunit of N-terminal acetyltransferase
GPA2	YER020W	V	Nucleotide binding alpha subunit of the heterotrimeric G protein
MOT2	YER068W	V	Component of the CCR4-NOT transcription regulatory complex
RPS24A	YER074W	V	Protein component of the small (40S) ribosomal subunit
NSR1, SHE5	YGR159C	VII	Nucleolar protein that binds nuclear localization sequences
KRE11, TRS65	YGR166W	VII	Protein involved in biosynthesis of cell wall beta-glucans
FYV8	YGR196C	VII	Protein of unknown function
ECM29	YHL030w	VIII	Major component of the proteasome
ECM34	YHL043w	VIII	Non-essential protein of unknown function
DIA4	YHR011W	VIII	Probable mitochondrial seryl-tRNA synthetase
SBE22	YHR103W	VIII	Transport of cell wall components
ECM14	YHR132C	VIII	Putative metalloprotease
RPS4B	YHR203C	VIII	Protein component of the small (40S) ribosomal subunit
ACE2	YLR131C	XII	Activation of G1-specific genes
ECM15	YBL001c	II	Non-essential protein of unknown function
CDC50	YCR094W	III	Endosomal protein that regulates cell polarity
SAP155	YFR040W	Vi	Protein that forms a complex with the Sit4p protein phosphatase
Unknown	YFR043C	VI	Unknown
URA7	YBL039c	II	CTP synthase
ECM13	YBL043w	II	Unknown
PIN4, MDT1	YBL051C	II	Protein involved in G2/M phase progression
ORF Unknown	YGL007W	VII	Unknown
ERG4	YGL012W	VII	C-24(28) sterol reductase
CWH41	YGL027c	VII	Processing alpha glucosidase I
ERV14	YGL054C	XIV	Protein localized to COPII-coated vesicles
Unknown	YNL213C	XIV	Unknown
ALG9	YNL219c	XIV	Catalyzes the transfer of mannose
ATG4	YNL223W	XIV	Cysteine protease required for autophagy
URE2	YNL229c	XIV	Nitrogen catabolite repression regulator
SAC1, RSD1	YKL212W	XI	Lipid phosphoinositide phosphatase of the ER and Golgi

Table 3 Genes Screen Cont'd

Gene	ORF	Chr.	Function/Homology/Domain
VPS1, GRD1	YKR001C	XI	Vacuolar protein sorting
FOX2, POX2	YKR009c	XI	Multifunctional enzyme of the peroxisome
VPS51, WH16	YKR020W	XI	Forms a tetramer with VPS52, VPS53, and VPS54
UTH1	YKR042W	XI	Yeast longevity; involved in cell growth
YCK2	YNL154c	XIV	membrane-bound casein kinase I homolog
RPS17A	YML024W	XIII	Ribosomal protein 51 (rp51) of the small (40s) subunit
GSF2, ECM6	YML048w	XIII	ER localized integral membrane protein
PEX12, PAS11	YMR026C	XIII	RING-finger peroxisomal membrane peroxin
IPK1, GSL1	YDR315C	IV	Inositol 1,3,4,5,6-pentakisphosphate 2-kinase
SWA2, AUX1	YDR320C	IV	auxilin-like protein
KRE27	YIL027C	IX	Protein of unknown function
BUD27	YFL023W	XI	Bud-site selection, nutrient signaling
Unknown	YGR263C	VII	Unknown
YTA7	YGR270W	VII	Unknown function
SCW4	YGR279C	VII	Cell wall protein with similarity to glucanases
SYG1	YIL047C	IX	Lethality suppressor of G-alpha protein deficiency
RPS24B	YIL069C	IX	Protein component of the small (40S) ribosomal subunit
FYV10;GID9	YIL097W	IX	Protein of unknown function
ECM12	YHR021w-a	XIII	Unknown
ECM4	YKR076w	XI	Non-essential protein of unknown function; similar to Ygr154cp
ECM40, ARG7	YMR062c	XIII	Mitochondrial ornithine acetyltransferase
RPL13B	YMR142C	XIII	Protein component of the large (60S) ribosomal subunit
GLC8	YMR311C	XIII	Glycogen metabolism and chromosome segregation
ELP6, HAP3	YMR312W	XIII	HAP subcomplex of Elongator
MSN1, FUP1	YOL116w	XV	Regulation of invertase and glucoamylase expression
ASC1	YMR116C	XIII	WD repeat protein (G-beta like protein) involved in translation
STO1, CBC1	YMR125W	XIII	Large subunit of the nuclear mRNA protein complex
COX11, LPI13	YPL132w	XVI	Copper delivery
APL4	YPR029C	XVI	Gamma-adaptin
LAS21	YJL062W	X	Synthesis of the glycosylphosphatidylinositol (GPI) core structure

Table 4 Genes Screened Cont'd

Gene	ORF	Chr.	Function/Homology/Domain
SPE3	YPR069c	XVI	Spermidine synthase; biosynthesis
SRB2	YHR041C	VIII	RNA polymerase II holoenzyme/mediator subunit
FYV4	YHR059W	VIII	Protein of unknown function
EAF6	YJR082C	X	Esa1p-associated factor, subunit of the NuA4 acetyltransferase
BUD4	YJR092w	X	Bud-site selection and axial budding pattern
ECM17, MET5	YJR137c	X	Sulfite reductase beta subunit; involved in amino acid biosynthesis
SIR1	YKR101W	XI	Transcription repression at the silent mating-type loci
Unknown	YLL007C	XII	Unknown
ACS1	YAL054c	I	Acetyl-coA synthetase isoform
FYV5	YCL058C	III	Protein of unknown function; ion homeostasis
ECM30	YLR436C	XII	Non-essential protein of unknown function
CNA1, CMP1	YLR443w	XII	calmodulin binding protein homologous to mammalian calcineurin
FYV1	YDR024W	IV	Dubious open reading frame, unlikely to encode a protein
RPS11A	YDR025W	IV	Non-essential protein of unknown function
SOD1	YJR104C	X	Cu, Zn superoxide dismutase
ECM27	YJR106w	X	Non-essential protein of unknown function
ILM1	YJR118C	X	Protein of unknown function
ATP2	YJR121W	X	Evolutionarily conserved enzyme complex
MGM101	YJR144W	X	Protein involved in mitochondrial genome maintenance
ERV41	YML067C	XIII	Protein localized to COPII-coated vesicles
MRPL27	YBR282W	II	Mitochondrial ribosomal protein of the large subunit
Unknown	YBR284W	II	Unkown
IMG2	YCR071C	III	Mitochondrial ribosomal protein of the small subunit
VPS54	YDR027C	IV	Component of the GARP (Golgi-associated retrograde protein)
RPS16A	YMR143W	XIII	Protein component of the small (40S) ribosomal subunit
ECM5	YMR176w	XIII	Non-essential protein of unknown function
ERG2	YMR202W	XIII	C-8 sterol isomerase
DFG5	YMR238W	XIII	Mannosidase; GPI anchor
RGT2	YDL138w	IV	Plasma membrane glucose receptor
BUD30	YDL151C	IV	Unknown
PPH2	YDL188c	IV	Catalytic subunit of phosphatase 2A
UTR1	YJR049C	X	NAD kinase
ECM21	YBL101c	II	Unknown
COG6	YNL041C	XIV	Component of Golgi complex

Table 5 Genes Screen Cont'd

Gene	ORF	Chr.	Function/Homology/Domain
VPS27	YNR006W	XIV	Hydrophilic protein; putative zinc finger esential for function
ECM39	YNR030w	XIV	Alpha-1,6-mannosyltransferase localized to the ER
SSK2	YNR031C	XIV	Suppressor of Sensor Kinase (SLN1); MAP Kinase
ECM2	YBR065c	II	Pre-mRNA splicing factor
ECM8	YBR076w	II	Non-essential protein of unknown function
MTQ1	YNL063W	XIV	Methyltransferase
YDJ1	YNL064C	XIV	Yeast DNA homolog (nuclear envelope protein)
FYV6	YNL133C	XIV	Unknown
SNF3	YDL194w	IV	Glucose transport
ECM37	YIL146c	IX	Non-essential protein of unknown function
RRD1	YIL153W	IX	Regulates G1 phase progression, the G2/M phase transition
IMP2'	YIL154c	IX	Ion homeostasis; DNA repair
MKS1	YNL076W	XIV	Ras-CAMP and lysine biosynthetic pathways
HMO1	YDR174W	IV	Genome maintenance; rDNA-binding
YUR1	YJL139C	X	Mannosyltransferase of the KTR1 family; N-glycosylation
HAL5	YJL165c	X	Putative protein kinase
CWP2	YKL096w-a	XI	Major constituent of the cell wall containing GPI-anchor
CTK1	YKL139W	XI	Catalytic (alpha) subunit of C-terminal domain kinase I (CTDK-I)
SAP190	YKR028W	XI	Complexs with the Sit4p protein phosphatase
IMG1	YCR046C	III	Respiration and maintenance of the mitochondrial genome
TUP1	YCR084C	III	Transcription repression; glucose repression mediator
DCG1	YIR030C	IX	Unknown
ARC18	YLR370C	XII	Motility and integrity of cortical actin patches
NAM2, MSL1	YLR382C	XII	Mitochondrial leucyl-tRNA synthetase; mitochondrial splicing
IWR1	YDL115C	IV	Unknown
PBS2	YJL128C	X	MAP kinase; osmosensing signal-transduction pathway
VAN1	YML115c	XIII	Mannosyltransferase with a role in protein N-glycosylation
POP2, CAF1	YNR052C	XIV	RNase of the DEDD superfamily, subunit of the Ccr4-Not complex
TOS1	YBR162C	II	Unknown
SEC66	YBR171W	II	Non-essential subunit of Sec63 complex; ER import
PTC1	YDL006W	IV	Type 2C protein phosphatase (PP2C)
RPS16B	YDL083C	IV	Protein component of the small (40S) ribosomal subunit
PMT1	YDL095W	IV	Protein O-mannosyltransferase, protein residue transfer
ECM11	YDR446w	IV	Non-essential protein of unknown function
ADA2, SWI8	YDR448W	IV	Transcription coactivator

2.8 YPAD Media Preparation

800 ml of H20 was added to 10G yeast extract, 20 g bacto-peptone, 20g dextrose, 30 ml 10MM adenine then stirred. The mixture was poured into a graduated cylinder and brought up to 1 liter. The mixture was stirred for 15 minutes. Then aliquoted into two 2-liter flasks (500ml of ypad each) and 10 g of agar was added to each flask. Last, the liquid was sterilized by autoclaving for 25 minutes on liquid cycle.

2.9 Synthetic Complete (SC) Media Preparation

800 ml of distilled water was added to 1.5g yeast nitrogen base without amino acids, 5g ammonium sulfate, 2 g yeast stock powder and 20g dextrose. The mixture was poured into a graduated cylinder and brought up to 1 liter. The mixture was stirred for 15 minutes, then aliquoted into two 2 liter flasks (500ml of ypad each) and 10g of agar was added to each flask. Last, the liquid was sterilized by autoclaving for 25 minutes on liquid cycle.

2.10 –Met-Lys Media Preparation

800 ml of H20 was added to 1.5g yeast nitrogen base without amino acids, 5g ammonium sulfate, 2 g –Met-Lys yeast stock powder and 20g dextrose. The mixture was poured into a graduated cylinder and brought up to 1 liter. The mixture was stirred for 15 minutes, then aliquot into two 2 liter flasks (500ml of YPAD each) and

10g of agar was added to each flask. Last, the liquid was sterilized by autoclaving for 25 minutes on liquid cycle.

2.11 Lawn Preparation

500 ml of YPAD liquid medium was pipetted into a sterile centrifuge tube. A matchstick size amount of cells was picked up on a sterile toothpick and put in the YPAD. Then 120 microliters of solution was spread onto each of 3 plates and grown overnight at room temperature.

2.12 Solid Mating

Candidate strains were patched on complete YPAD media or SC media. MAT**a** patches were grown overnight at room temperature. Mat**α** lawns were made as described above. MAT**a** patches of selected strains were taken and pressed against velvet on a replica plating block, then the lawns of MAT**α** partners, similarly, were pressed against velvet, and finally these cells were pressed onto –Met-Lys plates. The MAT**α** partners utilized in this study were sag1fig2Δ, aga1fig2Δ, and aga1sag1Δ and WT was added for second round screens.

Results & Discussion

3.1 Quantification of Zygote Morphology

To test for defects in aspects of zygote morphogenesis that could occur in zygotes produced by parents lacking one or more of the adhesins we created mutants strains with these genes deleted (Materials and Methods 2.3). The mutant strains were crossed (Materials and Methods 2.4) and then prepared on a slide for observation by microscopy. Photographs were taken (Materials and Methods 2.5) and for each strain, means were generated from the measurements taken. We observed that the following mutants for width: Aga1p-Fig2p, Fig2-Aga1p, Fig2-Fig2p, and the following mutants for height: Aga1-Fig2p, Fig2-Aga1p, Fig2-Fig2p; were significantly altered in morphogenesis relative to WT. In addition, the zygotes formed by mutants lacking the adhesins (Fig2-Fig2p) were also significantly altered with respect to conjugation bridge height relative to other mutants.

From these data, statistical tests of the means were performed (ANOVA, T-test) which showed that the absence of specific adhesins clearly alters the morphogenesis of the mating cells. Relative to wild type, adhesin mutants generally exhibited elongated conjugation bridges, and the distances between the parental cells were increased in addition to their possessing narrower conjugation bridges. The data also suggest that some adhesins have a more significant effect on the morphology. In other research (Huang et al., in preparation) genetic interactions have pointed to contacts between Aga1p and Fig2p, and Fig2p and Fig2p in the opposite mating types. When Fig2-

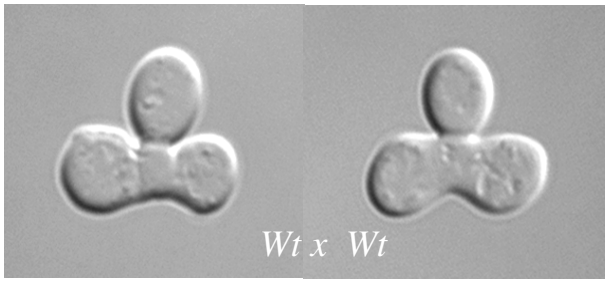
Fig2p is only contact among the adhesins present, a more severe narrowing of the conjugation bridge is observed. In this cross several agglutinin proteins are absent which suggests that these proteins may cooperate to play a role in zygote morphogenesis control, with respect to the conjugation bridge. Among the agglutinins it is not evident which have a greater function in zygote morphogenesis and this may be complicated by a question of the importance of the anchorage subunit versus the binding subunit for theAga1p-Aga2p adhesin complex. Our data suggest that Aga1p is likely to play a dual role in mating cell adhesion, both as a support for Aga2p and as a receptor or binding partner for Fig2p. It has been noted that Aga1p in MATα cells can interact only with Fig2p in MATa cells, therefore suggesting a specific role in adhesive contacts. The data we collected for Aga1p-Aga2--Sag1p mediated matings demonstrated the lowest of zygote interparental widths, suggesting that this may be the dominant adhesive interaction among the several adhesive interactions occurring during mating, however without further sampling this study cannot substantiate the claim that Aga1p is therefore more important in zygote morphogenesis.

Now that a correlation has been established between adhesin presence and normal morphogenesis, the question arises, how do adhesins affect morphogenesis? The reasons are suggested to involve changes in the polarization of growth that occurs in mating cells. In response to mating pheromone a mating projection forms as an extension of the cell and grows towards the mating partner to initiate fusion following contact. At this point cells grow apically and pheromone signaling is concentrated in

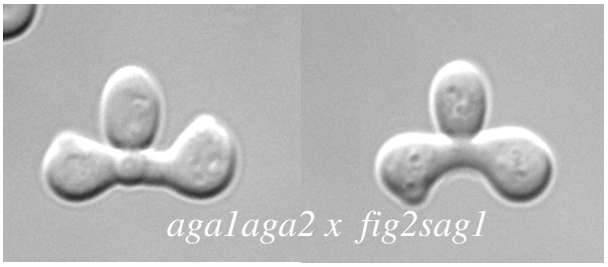
the region of the projection. In the normal mating pathway adhesins are present at the projection tip and stabilize the complex during fusion, but there is a point whereby the cells reduce the apical growth and begin to grow isotropically which gives the normal conjugation bridge its thick appearance following cell-cell fusion. We speculate that in adhesin mutant cells the conjugation bridge is probably significantly less stable and this alters morphogenesis in the following manner: The remaining contacts present in the different situations we studied might cause the cells to delay cell fusion slightly and continue to grow apically for a longer duration, instead of switching to isotropic growth, an explanation which would account for both elongation and narrowing of the conjugation bridge in the zygotes formed from matings of many of the different adhesin mutants. While a model in which such effects are mediated solely through alterations in adhesion interactions is an attractive explanation, it may over simplify the situation to some extent since previous studies also motivate consideration of effects of adhesins on growth factor signaling (Zhang et al. 2002). In addition to the adhesins which are present at the mating projection, there are growth factors which function in the fusion of mating cells, one of these is Rho1/Pkc1 cell integrity signaling. It has been shown that Fig2p causes increased Rho1/Pkc1 signaling therefore causing delays in fusion which leads to a longer time spent in projection morphogenesis and delayed lateral expansion (isotropic) of the conjugation bridge (Zhang et al. 2002). This does not seem to be the case, however, with Aga1p.

Therefore in the future additional studies might be concentrated on the connection between signaling and adhesins in order to get a clearer explanation of all factors that may influence the morphogenesis of zygotes. Further experiments will also be aimed at understanding the biochemistry underlying the interactions of the adhesin proteins containing conserved repeats shared by Aga1p and Fig2p.

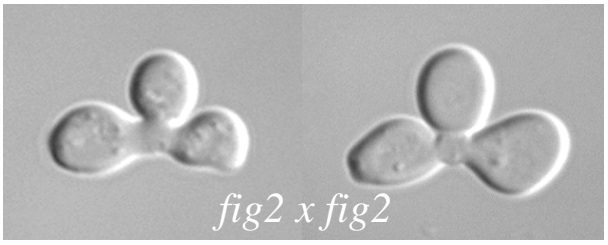
Figure 3. Qualitative View of Adhesin Morphology



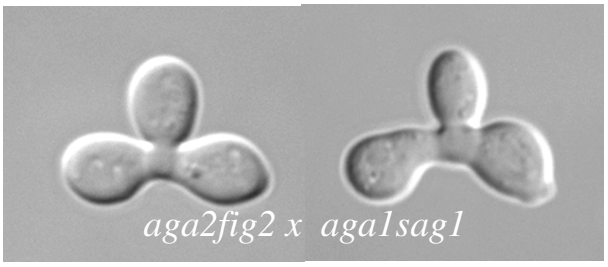
Aga2p - Sag1p
Fig2p - Aga1p
Aga1p - Fig2p
Fig2p - Fig2p
Wt x Wt



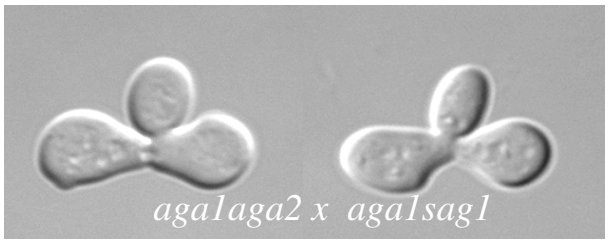
Aga2p - Sag1p
Fig2p - Aga1p
Aga1p - Fig2p
Fig2p - Fig2p
aga1aga2 x fig2sag1



Aga2p - Sag1p
Fig2p - Aga1p
Aga1p - Fig2p
Fig2p - Fig2p
fig2 x fig2



Aga2p - Sag1p
Fig2p - Aga1p
Aga1p - Fig2p
Fig2p - Fig2p
aga2fig2 x aga1sag1



Aga2p - Sag1p
Fig2p - Aga1p
Aga1p - Fig2p
Fig2p - Fig2p
aga1aga2 x aga1sag1

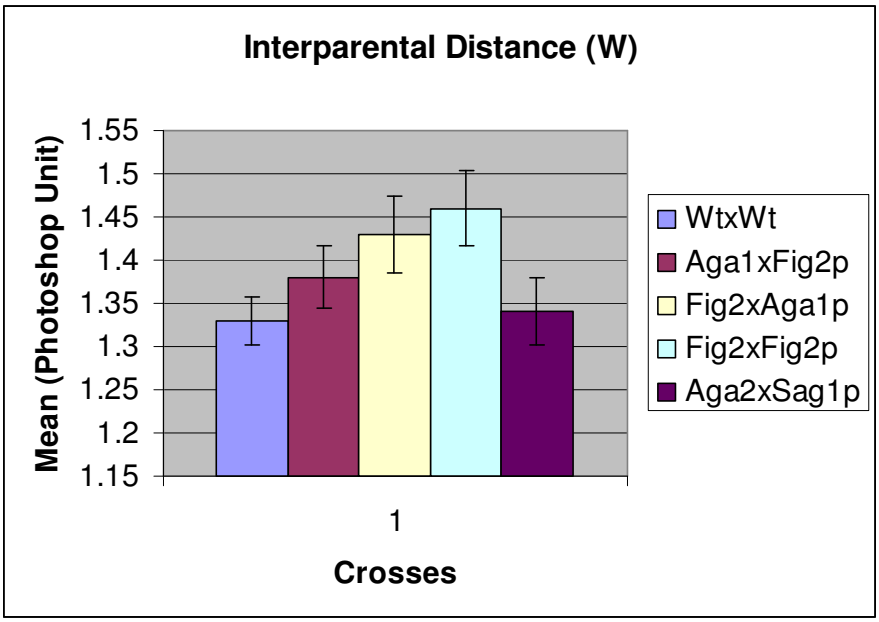
Crosses quantified and the respective adhesin genes present (black) and absent (grey) are displayed. Photos provide a representation of the morphogenesis of zygotes for the particular mutant crosses.

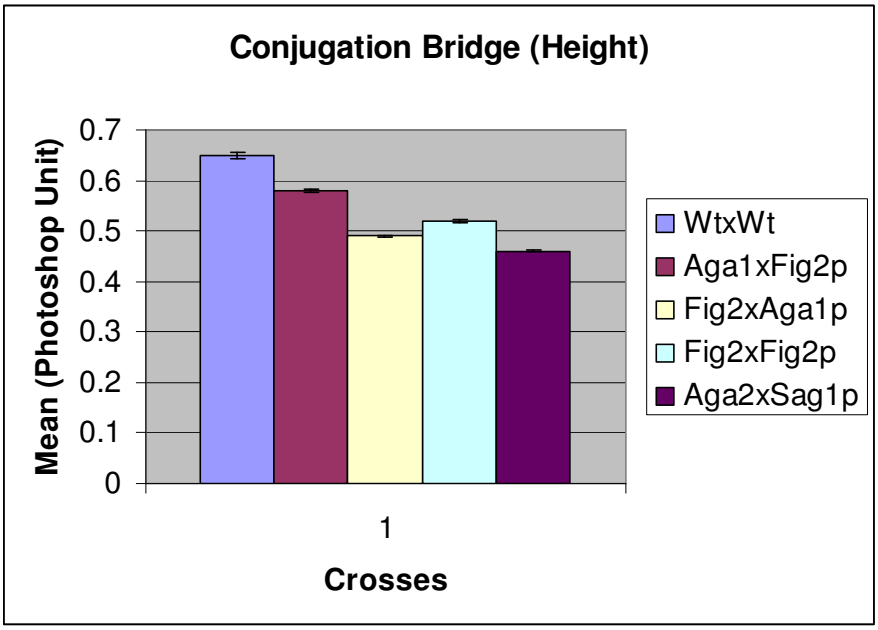
Table 6 Means of Zygote Dimensions

Cross	Width	Height
WtxWt	1.33 ± 0.016	0.65 ± 0.014
Aga1xFig2p	1.38 ± 0.019 S	0.49 ± 0.010 S
Fig2xAga1p	1.43 ± 0.022 S	0.52 ± 0.009 S
Fig2xFig2p	1.46 ± 0.021 S	0.46 ± 0.013 SS
Aga2xSag1p	1.34 ± 0.022	0.50 ± 0.008 S

Displays the data from the photo-microscopy and measures utilizing the ruler tool in Photoshop. S signifies statistical significance relative to Wt, SS signifies statistical significance with Wt and mutants with a single S.

Figure 4. Width and Height Measurement Data





Width graph shows the means found for the measurements of inter-parental distance. Height graph shows the means found for measures of conjugation bridge. The crosses used are listed on the left.

3.2 Genomic Screening - Mating Deficient Strains

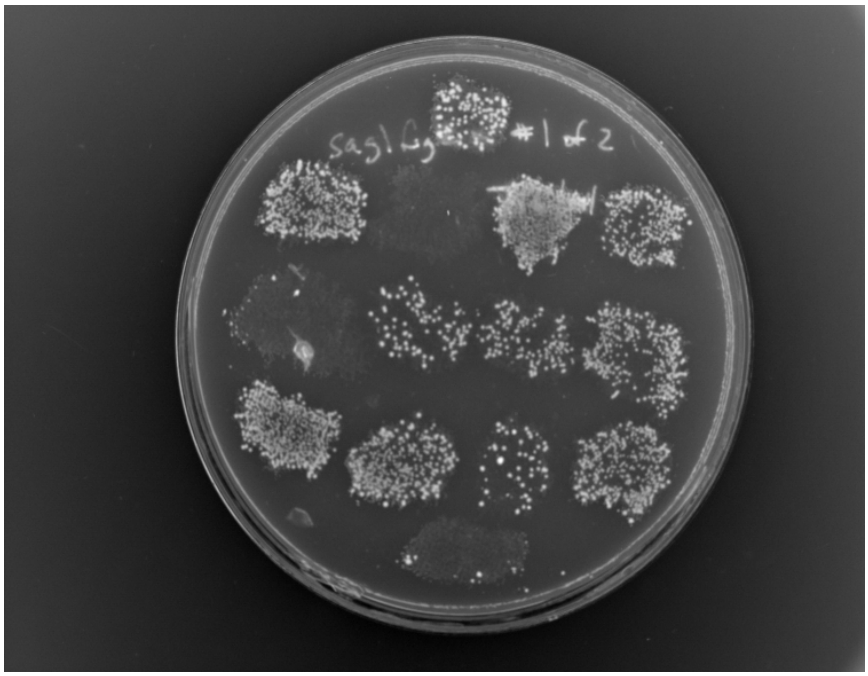
To perform the genomic mating screen on 170 genes involved in cell wall activities a series of mating assays were carried out on solid medium. Lawns of MAT**α** strains were made as described in (Materials and Methods 2.11). These were mated to the patched MAT**a** strains which each contained a deletion of a different gene implicated in cell wall function. Mated cells were then grown at 30°C for ~48hrs and scored according to the following simple rating system. The strains that received a "poor" rating showed a significant mating deficiency in mating on solid medium; either none or very few resulting diploid colonies were present. Strains receiving "fair" rating showed fewer diploid colonies than wildtype. Strains that received a "good" rating showed no difference relative to wildtype. Strains markedly deficient (receiving fair or poor rating) were grouped for second trial mating. For this trial a wild type MAT**α** partner was included in order to compare relative to the adhesin mutants. The method was then repeated and the strains that did not exhibit wild type rating were concluded to be deficient in mating. Seven strains were found to be deficient in mating after the second trial (Table 7). There was no overall observable difference in the mating quality of these strains when they were mated with wild type or with the adhesin deletions; except when partnered with MAT**α** aga1sag1Δ for which all strains produced fewer diploid cells.

The majority of the strains containing mutations in cell wall functioning genes that were examined for mating defects were found to be normal for mating under the various conditions we employed in our screen. To some degree this is

a testament to the ability for yeast cells to function normally despite individual defects in the function of many different types of genes, which may perhaps be explained by redundancy across the genome for some gene functions. Viewed another way, while the cell wall is dynamically regulated throughout the process of mating, surprisingly few genes specific to cell wall function are essential to the mating process. Moreover, there seem to be no functional relationships among the seven genes identified in our screen. Nevertheless, three of the seven strains that were found to be deficient in mating individually warrant comment as they have been identified to alter a major component of the cell wall, or they have interesting genetic interactions. The uncharacterized ORF YGL007W is known to have a synthetic interaction with SWF1 a gene implicated in spore wall formation. Further research could be aimed at fully characterizing this small gene and determine its role in the mating pathway, and the nature of its interaction with SWF1, if any. Two other genes ERG4, implicated in ergosterol synthesis and GLY1 implicated in threonine aldolase activity, relating to glycine synthesis, have the characteristic, that when deleted cause an increase in β 1,3 or β 1,6 glucan composition of the cell wall. This screen functions as a starting point for further investigation into cell wall activities during mating. For example, though the genes highlighted did not exhibit different mating effects when crossed with adhesin mutants, the fact that two are implicated in β 1,6 glucan, and β 1,3 glucan composition alteration does, putatively, link them to the GPI anchored adhesins, and therefore merits further study to understand

interactions if any do exist. Furthermore, differences may not have been evident merely because the growth deficiency was so severe.

Fig. 5 Sample of Mating Assay Rating



Top of plate is positive control, bottom negative control; all other patches are meant as samples of varying mating success.

Table 7. Strains Deficient in Mating in Two Separate Trials

Gene	ORF	T 1 Rate	T 2 Rate	Function
ERG4	YGL012W	Poor	Poor	Ergosterol biosynthesis
GLY1	YEL046C	Poor	Fair	Theonine aldolase activity
ORF Dub	YGL007W	Poor	Poor/Fair	Unknown (SWF Synthetic Interaction)
URE2	YNL229C	Fair	Poor	Nitrogen regulation
IMP2'	YIL154C	Poor	Poor	Transcriptional activator; ion homeostasis; DNA damage
YPS7	YEL046C	Fair	Poor	GPI-anchored aspartic protease
MOT2	YER068W	Poor	Fair	CCR4-Not txn regulation

Notes

Gly1–deletion causes elevation of β 1,6 glucan from 45% to 65% and β 1,3 glucan from 25% to 45%

Erg4-deletion causes elevation of beta 1,6 from 45% to 65%

Trial rating for strains found to be deficient in mating after two trials and their respective functions as provided by the Yeast Genome Database

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