Mechanisms that ensure monogamous mating in *Saccharomyces cerevisiae*

Corrina G. Robertson, Manuella R. Clark-Cotton, and Daniel J. Lew*
Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710

ABSTRACT **Haploid cells of the budding yeast *Saccharomyces cerevisiae* communicate using secreted pheromones and mate to form diploid zygotes. Mating is monogamous, resulting in the fusion of precisely one cell of each mating type. Monogamous mating in crowded conditions, where cells have access to more than one potential partner, raises the question of how multiple-mating outcomes are prevented. Here we identify mutants capable of mating with multiple partners, revealing the mechanisms that ensure monogamous mating. Before fusion, cells develop polarity foci oriented toward potential partners. Competition between these polarity foci within each cell leads to disassembly of all but one focus, thus favoring a single fusion event. Fusion promotes the formation of heterodimeric complexes between subunits that are uniquely expressed in each mating type. One complex shuts off haploid-specific gene expression, and the other shuts off the ability to respond to pheromone. Zygotes able to form either complex remain monogamous, but zygotes lacking both can re-mate.**

Monitoring Editor
Patrick Brennwald
University of North Carolina, Chapel Hill

Received: Dec 3, 2020
Revised: Feb 1, 2021
Accepted: Feb 9, 2021

INTRODUCTION

Sexual reproduction entails cycles of ploidy reduction through meiosis and ploidy restoration through fertilization. A key requirement is that fertilization occurs through the fusion of exactly two haploid gametes, and mechanisms have been uncovered that actively block polyspermy in animal fertilization to ensure this outcome (Wong and Wessel, 2006). The most common mechanisms center around the specialized extracellular matrix (ECM) of the egg cell. Before fertilization, the ECM attracts and activates sperm to promote sperm–egg fusion. After fusion, release of egg cell cortical granules leads to ECM remodeling so that the ECM now imposes both physical and chemical barriers to any subsequent sperm seeking entry. Because ECM remodeling takes time, some animals also exhibit a faster but transient block to polyspermy, based on ion fluxes that alter the fertilized egg's membrane potential (Wong and Wessel, 2006). Although much less well understood, plants (Tekleyohans *et al.*, 2017) and fungi (Vjestica *et al.*, 2018) have also evolved mechanisms to block fusion between more than two gametes. Here, we address the mechanisms that ensure monogamous mating in the budding yeast *Saccharomyces cerevisiae*.

In contrast to animal cells, yeast gametes are not specialized egg or sperm, but rather haploid cells of different mating type that can either proliferate or mate. The cells are surrounded by rigid cell walls, so in order to mate, two haploids must degrade the intervening cell walls (Ydenberg and Rose, 2008). This is a dangerous exercise, as the cells are under turgor pressure, and wall degradation that is not precisely targeted to points of cell–cell contact can lead to lysis. Cell wall degradation is mediated by polarized secretion of wall polysaccharide hydrolases. The location of secretion is governed by polarity factors, including the highly conserved Rho-family GTPase, Cdc42, which is a master regulator of polarity in many eukaryotes (Park and Bi, 2007; Chiou *et al.*, 2017). Imaging of mating cells containing polarity probes revealed that before fusion, the mating partners concentrate polarity factors at the points of cell–cell contact (Bendezu and Martin, 2013; Hegemann *et al.*, 2015; Henderson *et al.*, 2019; Wang *et al.*, 2019). Partners that have "committed" to each other in this way take about 20 min to remove the intervening walls and fuse (Henderson *et al.*, 2019). Because mating often takes place in crowded conditions where a cell may be surrounded by several mating partners (McClure *et al.*, 2018), it is not clear what prevents cells from committing to and fusing with more than one partner.

A recent study of mating of the fission yeast *Schizosaccharomyces pombe* demonstrated that re-mating after an initial fusion can be blocked by a gene expression cascade initiated by a

This article was published online ahead of print in MBoC in Press (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E20-12-0757) on February 17, 2021.

*Address correspondence to: Daniel J. Lew (Daniel.lew@duke.edu).

Abbreviations used: CSM-Dex, complete synthetic media with dextrose; ECM, extracellular matrix; PCR, polymerase chain reaction; SEM, standard error of the mean; WT, wild-type.

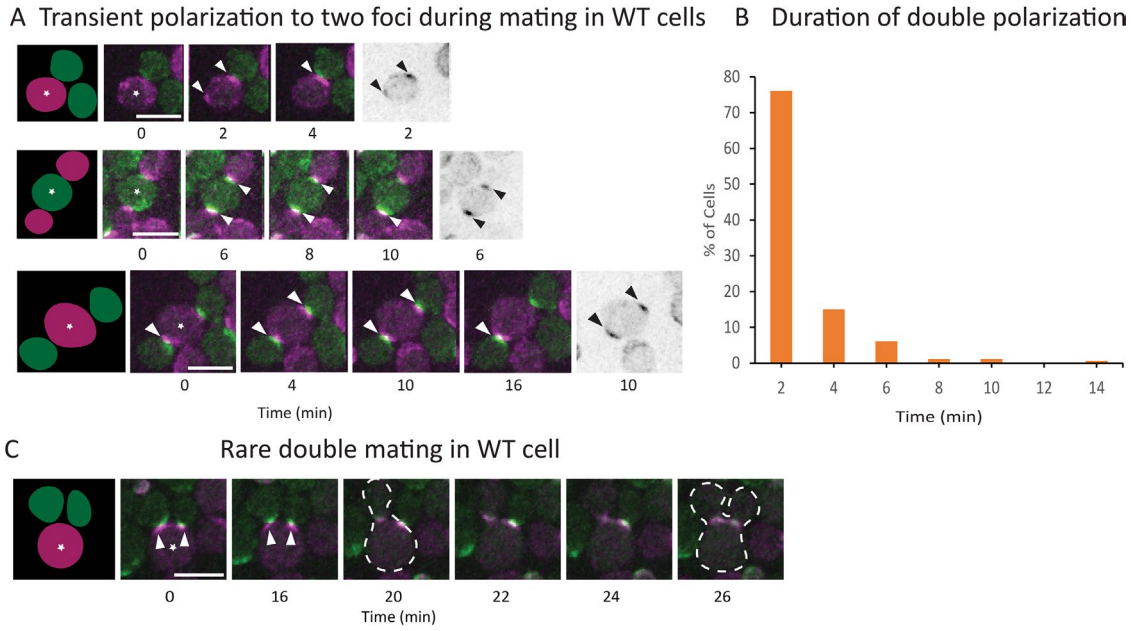

FIGURE 1: WT cells can transiently polarize towards two partners. (A) Examples of double polarization. Mating mixes were imaged where one parent expressed Bem1-GFP (green) and the other parent expressed Bem1-tdTomato (red). For one set, MATa cells expressed Bem1-GFP and MATα cells expressed Bem1-tdTomato (DLY9069 × DLY12944). For the other set, MATa cells expressed Bem1-tdTomato and MATα cells expressed Bem1-GFP (DLY12943 × DLY9070). Comparable results were obtained in both cases. Cartoon on the left indicates double-polarizing cell (asterisk). Merged color panels are maximum projection images from the indicated time points. Arrowheads indicate polarity foci. Inverted one-color image (right) illustrates two polarity foci at the indicated time. Scale bar, 5 μm. (B) Duration of double polarization. Instances (180) of double polarization were scored as described in *Materials and Methods*. (C) A rare instance of double fusion observed between WT cells where the cell involved in double fusion is denoted by an asterisk. Display as in A. Dashed outlines indicate fusion events.

heterodimeric transcription factor (Vjestica *et al.*, 2018). The constituent subunits of the transcription factor are expressed in haploids of different mating type, so the heterodimer does not form until the cells fuse. Similarly, budding yeast cells of different mating type express different transcription factors including a1 (in cells of mating type **a**) and α2 (in cells of mating type α), which can dimerize to form the transcription factor a1/α2 following fusion (Herskowitz, 1988). a1/α2 represses expression of all haploid-specific genes, including those that encode pheromones and pheromone receptors. Thus, it could be that budding yeast, like fission yeast, block re-fusion by switching off communication through this transcriptional pathway. However, the timescale for shutting down haploid transcription in zygotes is not known, and it is unclear whether this process would be fast enough to block re-fusion.

A potentially faster block based on the same logic as a1/α2 involves a protein called Asg7. Asg7 is expressed only in cells of mating type **a**, but it can bind to the **a**-factor pheromone receptor Ste3, which is expressed only in cells of mating type α. When both are expressed in the same haploid cell, binding of Asg7 to Ste3 post-translationally inhibits pheromone signaling (Cross, 1990; Roth *et al.*, 2000; Rivers and Sprague, 2003). It remains unclear to what extent either a1/α2 dimerization or binding of Asg7/Ste3 contribute to preventing zygote re-mating.

Here we show that mating cells frequently develop polarity foci directed toward two partners, but that such situations are unstable due to competition between the polarity foci. Slowing such competition allows mating to two partners, with the two fusion events occurring within a few minutes of each other. Subsequently, we show that Asg7/Ste3 and a1/α2 dimerization provide redundant blocks to re-mating in zygotes.

RESULTS AND DISCUSSION

The mating process in budding yeast involves cell cyle arrest in G1 phase accompanied by an early search period in which adjacent cells exhibit weak and mobile polarity foci (Henderson *et al.*, 2019). This "indecisive phase" can last up to 2 h in lab conditions but then a mating pair of cells "commits," and each partner develops a strong and stable polarity focus oriented toward the other partner. During the indecisive phase, the cells can transiently form more than one polarity focus (Henderson *et al.*, 2019). Imaging cells that expressed fluorescent versions of the polarity marker Bem1, we found that approximately 40% of mating cells displayed instances of double polarization during the indecisive phase (Figure 1A). Double polarization was always transient and resolved before fusion to a partner (Figure 1B). In approximately 8% of these cases, double polarization events were oriented toward two potential partners, and these instances were, on average, longer-lived (mean ± SEM, 5.6 ± 1 min when both polarity foci faced opposite mating-type partners, 2.5 ± 0.1 min otherwise; $p < 0.01$). The final fusion site could be either of the original foci or a different one altogether. Thus, cells can polarize simultaneously in two directions. Such events are usually transient, but can to some extent be stabilized when both polarity foci are directed toward potential mating partners.

Recent work indicates that when partner cells' polarity foci align with each other, both are stabilized, leading to commitment (Henderson *et al.*, 2019). Why, then, would a cell fail to fully stabilize such foci when it has two interested partners? We did detect two instances (out of more than 3800 zygotes imaged) where a cell did mate with two partners (Figure 1C), consistent with previous detection of rare triparental mating (Rogers and Bussey, 1978). Fusion was detected by the mixing of the fluorescent probes from different

parents, using contrast enhancement to detect cytoplasmic fluorescence (Supplemental Figure S1). However, in the vast majority of cases one focus disappeared, and mating was monogamous. Polarity foci are highly dynamic: constituent proteins (including Cdc42 and Bem1) are recruited from the cytoplasm and reside in a focus for just a few seconds before returning to the cytoplasm (Wedlich-Soldner *et al.*, 2004; Slaughter *et al.*, 2009; Dyer *et al.*, 2013). In addition, polarity foci are formed and sustained by positive feedback, in which proteins in the focus recruit more proteins from the cytoplasm (Chiou *et al.*, 2017). As the cytoplasmic pool is shared, this means that proteins lost from one focus may be captured by another, leading to competition between foci (Goryachev and Pokhilko, 2008; Howell *et al.*, 2009, 2012; Wu *et al.*, 2015). Thus, one possible reason for the transient nature of double polarization is that competition between foci eliminates all but one focus.

To test whether competition reduces double polarization in mating cells, we exploited a yeast strain that has been shown to slow competition in budding cells (Wu *et al.*, 2015). By increasing the propensity of Cdc42 and Bem1 to associate with the plasma membrane (*Materials and Methods*), the residence time of the proteins at polarity foci is increased, which slows competition and can lead to the formation of two or more buds (Wu *et al.*, 2015). Bem1 in this strain is replaced with a membrane-tethered Bem1-CAAX variant, which became concentrated in polarity foci (Supplemental Figure S2A) that were functional for mating (Supplemental Figure S2B). We readily detected instances of double polarization (Figure 2A), whose duration was significantly extended in this strain (mean ± SEM, 13.5 ± 1 min in mutant cells, compared with 2.8 ± 0.1 min in wild-type (WT) cells; $p < 0.01$; Figure 2B). In more than half of the cases ($n = 42$ out of 74) the double polarization remained until fusion (Figure 2, C and D). These cells either mated with both partners (Figure 2C; $n = 28$), or lost the second polarity focus after mating with the first partner (Figure 2D; $n = 14$). Double mating was observed in 2.4% of matings between slow-competition and WT parents, as opposed to <0.05% in matings between WT strains. We conclude that rapid competition between polarity foci is an important mechanism to prevent double mating.

When the cell mated with two partners, the fusion events were not simultaneous, and were separated by 5.7 ± 0.6 min (mean ± SEM; Figure 2E). The finding that an initial fusion event could be followed by another fusion a few minutes later indicated that it takes several minutes to shut off the mating potential of the zygote. However, in some instances a polarity focus disappeared shortly after (4–6 min) the initial fusion with the first partner (Figure 2D), suggesting that loss of the focus might be due to the fusion. Fusion enables the formation of two heterodimeric complexes, a1/α2 and Asg7/Ste3, that can shut down pheromone signaling (see *Introduction*). To investigate the impact of these species, we set up mating mixes in which the partner of mating type **a** lacked either a1 or Asg7.

Deletion of the mating-type locus (*mat*Δ) creates a cell that lacks mating type–specific transcription factors. However, because expression of **a**-specific genes is the default program in *S. cerevisiae* (Herskowitz, 1988), *mat*Δ cells behave just like MAT**a** cells until they mate. After mating to a MATα cell, the matΔ/MATα diploid expresses only α-specific information, and can therefore mate again with a MAT**a** cell. However, we found that zygotes formed by matΔ × MATα mating did not re-mate: instead, they went on to form buds with a timing indistinguishable from that of MAT**a** × MATα zygotes (WT 35.3 ± 1 min; *mat*Δ/MATα 33 ± 0.8 min, mean ± SEM; Figure 3). We conclude that a1/α2 is not necessary to block zygote re-mating, and that there must be some other pathway that can block re-mating, perhaps involving Asg7/Ste3.

To ask whether Asg7/Ste3 was important for blocking re-mating, we set up MAT**a** *asg7*Δ x MATα mating mixes. As previously reported (Roth *et al.*, 2000), zygotes from these matings became morphologically abnormal, with bulges occurring in the bridge between the parent cell bodies, and delayed subsequent bud emergence (*asg7*Δ/MATα 74.1 ± 1.5 min vs. WT 35.3 ± 1 min, $p < 0.01$; Figure 4, A and B). During the delay, Bem1 remained polarized to a region in the center of the zygote (Figure 4A, denoted by orange arrowhead) but more diffusely than when the cells eventually prepared to bud. We interpret these findings to indicate that without Asg7, zygotes take longer to shut down pheromone signaling. Continued signaling leads to continued cell-cycle arrest in G1 (delaying bud formation) and continued polarization to the central part of the zygote, yielding aberrant morphologies. Nevertheless, these zygotes did proceed to bud and they did not re-mate within the 2.5-h movie.

We next eliminated both a1/α2 and Asg7/Ste3. In *mat*Δ *asg7*Δ × MATα mating mixes, we observed rampant re-mating (Figure 4C), with 25% of all zygotes re-mating during the 2.5-h movie. It is also worth noting that the other 75% of zygotes did not go on to bud, which suggests that the zygotes were still "sensing" pheromone (either from surrounding haploid cells or from the zygotes themselves) and might have re-mated if observed for longer. We conclude that a1/α2 and Asg7/Ste3 provide redundant blocks to re-mating.

In summary, our findings elucidate a combination of mechanisms that ensure monogamous mating in budding yeast. First, when a yeast cell polarizes toward more than one partner, competition between polarity foci ensures that only one focus persists for long enough (20 min) to degrade the intervening cell walls and allow fusion (Figure 5A). When competition is slowed by manipulation of the affinity of polarity factors for the plasma membrane, double fusions are observed. Double fusions were separated by 2–14 min, suggesting that it takes a few minutes to establish an effective postzygotic block to re-mating.

The zygote inherits proteins from both parents, allowing the association of **a**-specific and α-specific proteins to form Asg7/Ste3 and a1/α2 dimers (Figure 5B). Asg7/Ste3 shuts down pheromone signaling at the level of the G protein that is coupled to pheromone receptors (Roth *et al.*, 2000; Rivers and Sprague, 2003), so the zygotes exit the mating program and enter the cell cycle. In mutants lacking this pathway, pheromone signaling continues after fusion. But if signaling continues, then why don't these zygotes re-mate? Our data implicate a1/α2 in this process. The a1/α2 heterodimer shuts off transcription of haploid-specific genes (Figure 5B), including those encoding pheromones, pheromone receptors, and signaling proteins. While perdurance of receptors and signaling proteins may allow continued signaling for 50–108 min after fusion (as seen in zygotes lacking Asg7), we speculate that pheromone production is shut off more rapidly. In yeast, most mRNAs are unstable, with half-life <5 min (Chan *et al.*, 2018), and pheromone secretion also takes <5 min (Govindan *et al.*, 1995). Thus, the redundant blocks provided by Asg7/Ste3 and a1/α2 may reflect a fast block of signaling by Asg7/Ste3 and a fast block of pheromone production by a1/α2. On longer timescales, a1/α2 repression would also lead to the elimination of haploid-specific receptors and signaling proteins, generating diploids uninterested in mating.

MATERIALS AND METHODS

Yeast strains

Standard molecular and yeast genetic methods were used for strain construction and all new genome modifications were confirmed via

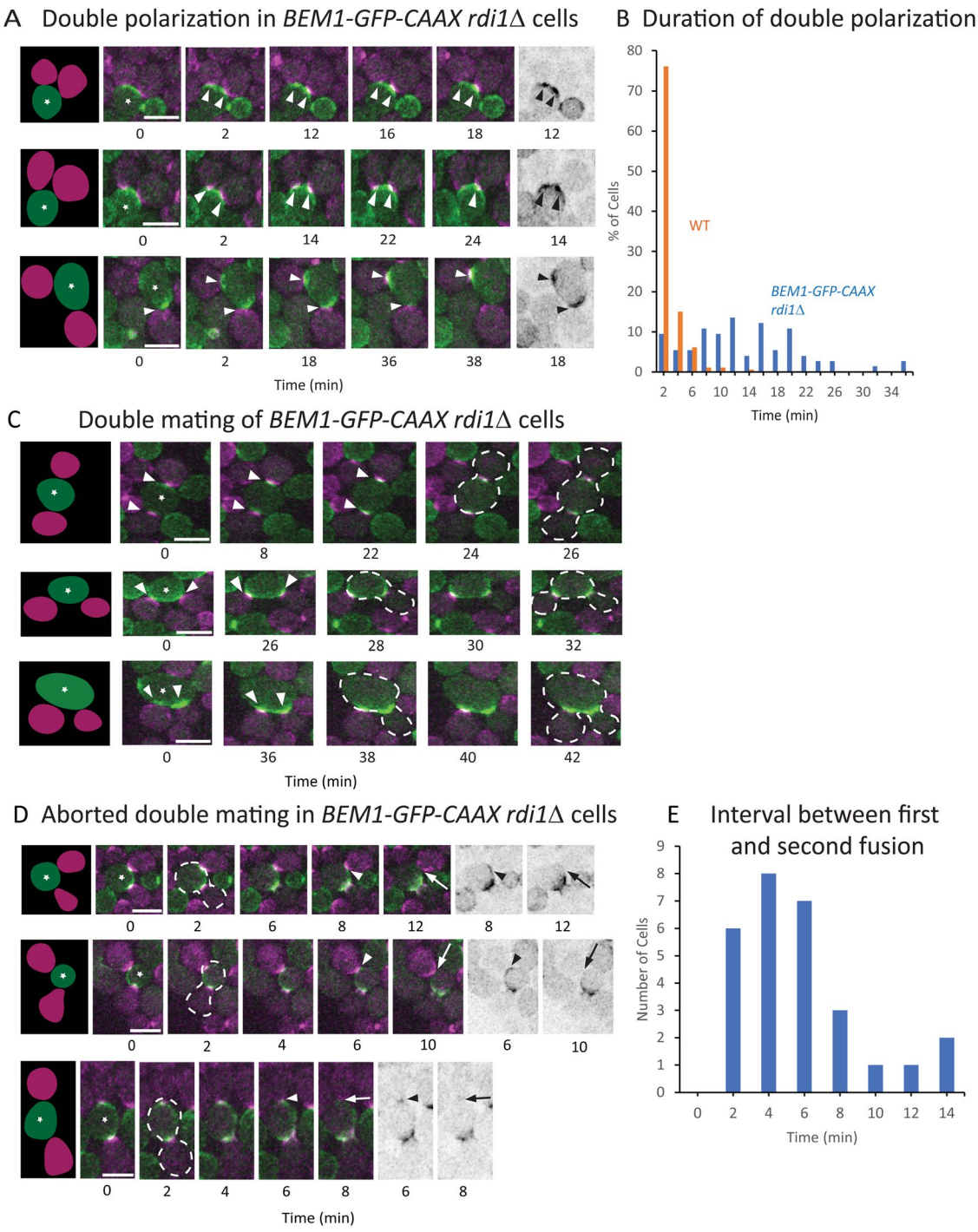

FIGURE 2: Slowing competition between polarity foci stabilizes double polarization and can lead to double fusion. (A) Examples of double polarization in slow-competition mutants. Double-polarizing cells are denoted by an asterisk. Scale bar, 5 μm. Arrowheads indicate polarity foci. (B) Extended duration of double polarization in mutant cells (blue). Instances (74) of double polarization were scored as described in *Materials and Methods* and compared with data from WT strains (orange, from Figure 1B). (C) Examples of double mating. Slow-competition mutants (DLY23555, DLY23556 or DLY23653, Bem1-GFP-CAAX, green) were mixed with WT partners (DLY12943 or DLY12944, Bem1-tdTomato, red) and imaged during mating. Cells that fuse with two partner cells are denoted by an asterisk. Dashed outline indicates zygote immediately after fusion. (D) Examples where first fusion was followed by loss of the second polarity focus. Cells that polarize toward two partners but eventually lose the second focus are denoted by an asterisk. Display as in Figure 1C. Arrow indicates loss of second focus after fusion (zygote indicated by dashed outline). (E) Interval between first and second fusion for cases of double mating. Instances (28) of double fusion were scored.

PCR. The yeast strains are listed in Table 1: all are in the YEF473 background (*his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52*; Bi and Pringle, 1996).

Bem1-GFP or Bem1-tdTomato were used to visualize the polarity foci and were previously shown to be functional: *BEM1-GFP:LEU2* (Kozubowski *et al.*, 2008), *BEM1-tdTomato:HIS3* (Howell *et al.*, 2012).

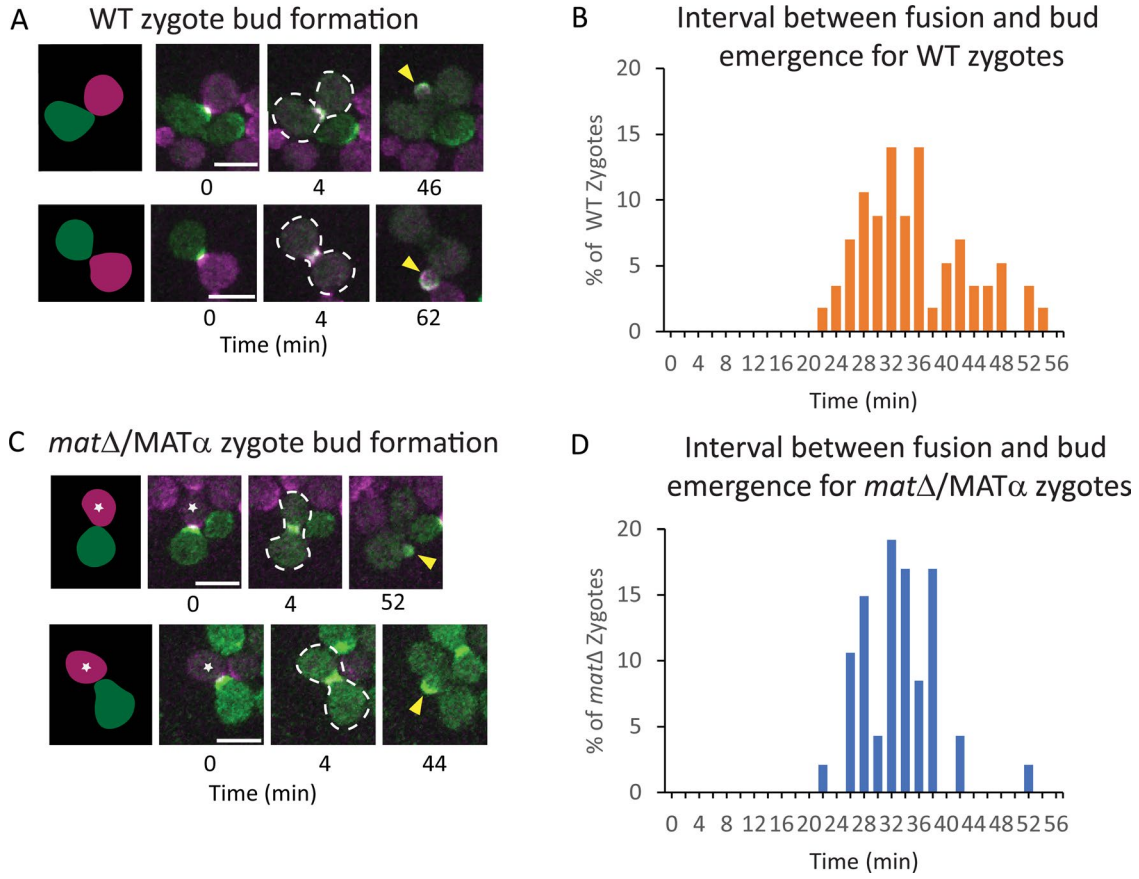

FIGURE 3: Deletion of the MAT locus does not affect zygote behavior after fusion. (A) Examples of WT (MATa/α) zygotes. Strains and probes as in Figure 1A. Cartoon on the left indicates cells that go on to fuse. Merged color panels are maximum projection images from the indicated time points. Dashed outlines indicate fusion events. Yellow arrowheads indicate budding by zygote. Scale bar, 5 μm. (B) Interval between fusion and zygote budding. Zygotes (57) were scored as described in *Materials and Methods*. (C) Examples of *mat*Δ/α zygotes. *mat*Δ (DLY23788, Bem1-tdTomato, red) and MATα (DLY9070, Bem1-GFP, green) cells were mixed and imaged during mating. *mat*Δ cells are denoted by an asterisk. Display as in A. (D) Interval between *mat*Δ/α fusion and zygote budding. Zygotes (47) were scored.

The genetic modifications that slow competition (*rdi1Δ BEM1-GFP-CAAX*) were described previously (Wu *et al.*, 2015). Deletion of the guanine nucleotide dissociation inhibitor *RDI1* slows the exchange of Cdc42 between the membrane and the cytoplasm (Slaughter *et al.*, 2009), and adding a prenylation motif (C-terminal CAAX box) to Bem1 similarly slows exchange of Bem1 (Wu *et al.*, 2015). Each manipulation slows competition between polarity foci and thereby increases the frequency of two-budded cells; the combination increases that frequency even further (Wu *et al.*, 2015).

The *asg7::NAT*^{R} allele was generated by one-step gene disruption (Longtine *et al.*, 1998) using a PCR fragment in which the NAT^R cassette was amplified using primers (rcc35 and rcc36) with homology to the sequences flanking the *ASG7* open reading frame.

The *mat::URA3* allele was generated by one-step gene disruption (Longtine *et al.*, 1998) using a PCR fragment in which the URA3 gene was amplified using primers (mj214 and mj215) that targeted homologous recombination at MATα, deleting most of the α1 and α2 coding regions but leaving the flanking genes *BUD5* and *TAF2* intact.

Microscopy and image analysis

For imaging, cells were grown overnight at 30°C in complete synthetic media (MP Biomedicals) with 2% dextrose (CSM-dex) to mid-log phase (~10^7/ml) and mounted on CSM-dex slabs with 2% agarose for imaging. Time-lapse videos were acquired at 30°C. Imaging was performed using an Andor XD Revolution Spinning Disk microscope with MetaMorph software (Universal Imaging) and an Andor Ixon3 897 512 electron-multiplying charge-coupled device camera. A 100×/1.4 UplanSApo oil-immersion objective was used. Images (z-stacks of 15 z-planes with 0.47-μm spacing) were captured at 2-min intervals using 250-ms exposure and 200 gain on the camera. Laser power was 10% for the 488 green-fluorescence channel and 8% for the 561 red-fluorescence channel. Images were denoised using the ImageJ Hybrid 3D Median Filter plug-in (2007). All images are maximum projections with the exception of Supplemental Figure S1A.

Scoring mating efficiency: Only cells that had an opportunity to mate were scored: those that had a G1 cell of opposite mating type (i.e., a potential partner) touching them early enough in the movie (>20 min before the end of the movie). Cells whose potential partners mated with another cell were excluded. Among this population (cells with the opportunity to mate during the movie), all cells were scored as mated or not mated and the percentage mated is reported as mating efficiency.

Fusion time was scored by the mixing of the fluorescent probes (Bem1-GFP and Bem1-tdTomato). To better detect cytoplasmic Bem1, inverted maximum projection images were visualized using

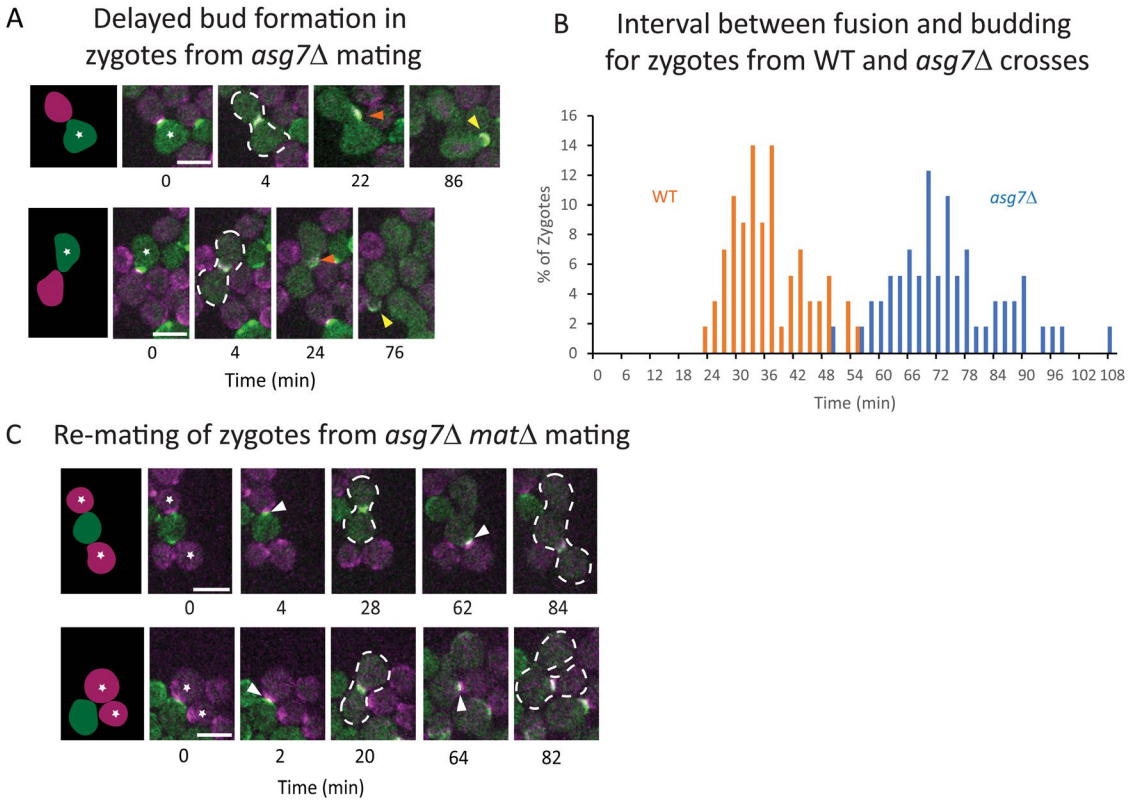

FIGURE 4: a1/α2 and Asg7/Ste3 provide redundant blocks to zygote re-mating. (A) Deletion of *ASG7* delays zygote budding. *asg7*Δ MATa (DLY23757, Bem1-GFP, green) and MATα (DLY12944, Bem1-tdTomato, red) cells were mixed and imaged during mating. An *asg7*Δ MATa cell is denoted with an asterisk. Dashed outlines indicate fusion events. Orange arrowheads indicate polarization at the center of the zygote. Yellow arrowheads indicate budding by zygote. Scale bar, 5 μm. (B) Interval between fusion and zygote budding. Zygotes (57) from the *asg7*Δ mating were scored (blue) and compared with the WT data from Figure 3B. (C) Combined deletion of *mat*Δ and *asg7*Δ allows frequent re-mating. *asg7*Δ *mat*Δ (DLY23796, Bem1-tdTomato, red) and MATα (DLY9070, Bem1-GFP, green) cells were mixed and imaged during mating. *asg7*Δ *mat*Δ cells are denoted by an asterisk. White arrowheads indicate polarity foci.

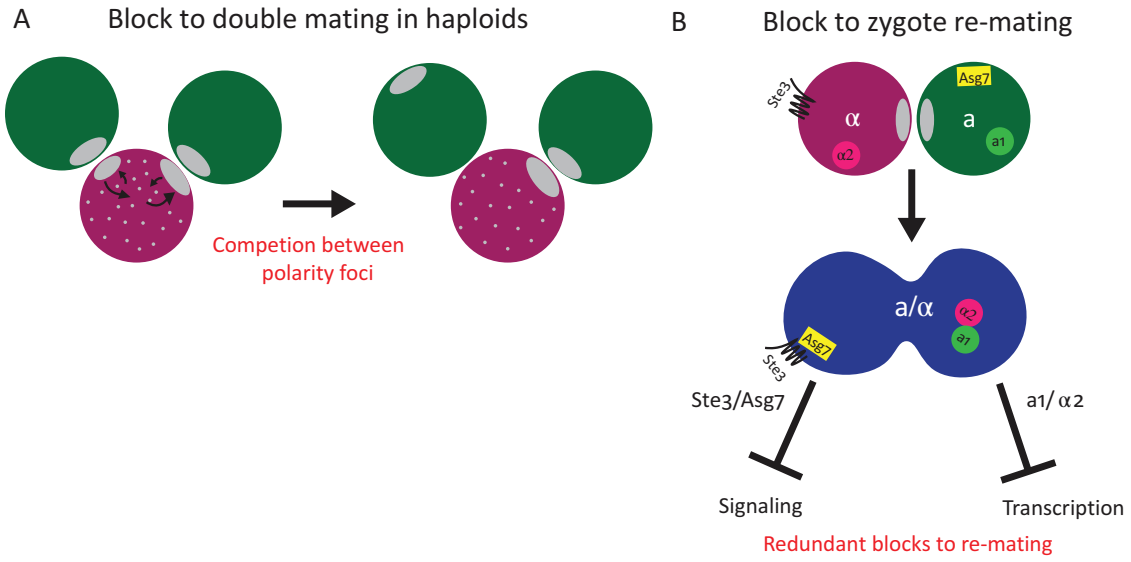

FIGURE 5: Mechanisms ensuring monogamy in yeast mating. (A) Competition between polarity foci for cytoplasmic polarity factors leads to a single focus. Thus, a cell with multiple partners can only orient stably towards one. This blocks simultaneous mating to more than one partner in haploids. (B) Zygote re-mating is blocked by formation of Ste3/Asg7 complexes that block pheromone signaling and the a1/α2 transcription factor that represses haploid-specific transcription of genes, including those making pheromones.

Strain	Relevant genotype	Source
DLY9069	MAT**a** *BEM1-GFP:LEU2*	Kozubowski *et al.*, 2008
DLY9070	MATα *BEM1-GFP-CAAX*	Kozubowski *et al.*, 2008
DLY12943	MAT**a** *BEM1-tdTomato:HIS3*	Howell *et al.*, 2012
DLY12944	MATα *BEM1-tdTomato:HIS3*	Howell *et al.*, 2012
DLY23555	MATα *BEM1-GFP-CAAX:LEU2 rdi1::TRP1*	This study
DLY23556	MATα *BEM1-GFP-CAAX:LEU2 rdi1::TRP1*	This study
DLY23653	MAT**a** *BEM1-GFP-CAAX:LEU2 rdi1::TRP1*	This study
DLY23757	MAT**a** *BEM1-GFP:LEU2 asg7::NAT*^{R}	This study
DLY23788	*matα::URA3 BEM1-tdTomato:HIS3*	This study
DLY23796	*matα::URA3 BEM1-tdTomato:HIS3 asg7::NAT*^{R}	This study

TABLE 1: Yeast strains.

contrast enhancement to observe the difference between background and cytoplasmic fluorescence (Supplemental Figure S1). Fusion time was scored as the time when cytoplasmic fluorescence of the partner cell's probe became visible.

Two-foci intermediate states in both WT and mutant cells were documented in inverted maximum projection images, and the 488 and 561 fluorescence channels were split to gain better visualization of the foci in individual cells. The interval of double polarization was scored from the time when a cell first exhibited two foci to the time when only one focus was detected. Thus, all durations are integer multiples of the 2-min interval between timepoints.

The interval from zygote formation to bud emergence from the zygote was scored from the time of fusion to the time a noticeable bud emerged.

Statistics: two-tailed *t* tests were used to test the null hypothesis that there was no difference in the mean durations of the time intervals compared in each case.

ACKNOWLEDGMENTS

We thank members of the Lew lab for stimulating discussions and comments on the manuscript. M.R.C.-C. was a Howard Hughes Medical Institute Gilliam Fellow and received a Graduate Diversity Enrichment Program Award from the Burroughs Wellcome Fund. This work was funded by National Institutes of Health/ National Institute of General Medical Sciences Grant no. R35GM-122488 to D.J.L.

REFERENCES

Bendezu FO, Martin SG (2013). Cdc42 explores the cell periphery for mate selection in fission yeast. Curr Biol 23, 42–47.

Bi E, Pringle JR (1996). ZDS1 and ZDS2, genes whose products may regulate Cdc42p in *Saccharomycescerevisiae*. Mol Cell Biol 16, 5264–5275.

Chan LY, Mugler CF, Heinrich S, Vallotton P, Weis K (2018). Non-invasive measurement of mRNA decay reveals translation initiation as the major determinant of mRNA stability. eLife 7, e32536.

Chiou JG, Balasubramanian MK, Lew DJ (2017). Cell polarity in yeast. Annu Rev Cell Dev Biol 33, 77–101.

Cross FR (1990). The DAF2-2 mutation, a dominant inhibitor of the STE4 step in the α-factor signaling pathway of *Saccharomycescerevisiae* MATα cells. Genetics 126, 301–308.

Dyer JM, Savage NS, Jin M, Zyla TR, Elston TC, Lew DJ (2013). Tracking shallow chemical gradients by actin-driven wandering of the polarization site. Curr Biol 23, 32–41.

Goryachev AB, Pokhilko AV (2008). Dynamics of Cdc42 network embodies a Turing-type mechanism of yeast cell polarity. FEBS Lett 582, 1437–1443.

Govindan B, Bowser R, Novick P (1995). The role of Myo2, a yeast class V myosin, in vesicular transport. J Cell Biol 128, 1055–1068.

Hegemann B, Unger M, Lee SS, Stoffel-Studer I, van den Heuvel J, Pelet S, Koeppl H, Peter M (2015). A cellular system for spatial signal decoding in chemical gradients. Dev Cell 35, 458–470.

Henderson NT, Pablo M, Ghose D, Clark-Cotton MR, Zyla TR, Nolen J, Elston TC, Lew DJ (2019). Ratiometric GPCR signaling enables directional sensing in yeast. PLoS Biol 17, e3000484.

Herskowitz I (1988). Life cycle of the budding yeast *Saccharomycescerevisiae*. Microbiol Rev 52, 536–553.

Howell AS, Jin M, Wu C-F, Zyla TR, Elston TC, Lew DJ (2012). Negative feedback enhances robustness in the yeast polarity establishment circuit. Cell 149, 322–333.

Howell AS, Savage NS, Johnson SA, Bose I, Wagner AW, Zyla TR, Nijhout HF, Reed MC, Goryachev AB, Lew DJ (2009). Singularity in polarization: rewiring yeast cells to make two buds. Cell 139, 731–743.

Kozubowski L, Saito K, Johnson JM, Howell AS, Zyla TR, Lew DJ (2008). Symmetry-breaking polarization driven by a Cdc42p GEF-PAK complex. Curr Biol 18, 1719–1726.

Longtine MS, McKenzie A III, DeMarini DJ, Shah NG, Wach A, Brachat A, Philippsen P, Pringle JR (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomycescerevisiae*. Yeast (Chichester, England) 14, 953–961.

McClure AW, Jacobs KC, Zyla TR, Lew DJ (2018). Mating in wild yeast: delayed interest in sex after spore germination. Mol Biol Cell 29, 3119–3127.

Park HO, Bi E (2007). Central roles of small GTPases in the development of cell polarity in yeast and beyond. Microbiol Mol Biol Rev 71, 48–96.

Rivers DM, Sprague GF Jr (2003). Autocrine activation of the pheromone response pathway in matα2$^-$ cells is attenuated by SST2- and ASG7-dependent mechanisms. Mol Genet Genomics 270, 225–233.

Rogers D, Bussey H (1978). Fidelity of conjugation in *Saccharomyces cerevisiae*. Mol Gen Genet 162, 173–182.

Roth AF, Nelson B, Boone C, Davis NG (2000). Asg7p-Ste3p inhibition of pheromone signaling: regulation of the zygotic transition to vegetative growth [In Process Citation]. Mol Cell Biol 20, 8815–8825.

Slaughter BD, Das A, Schwartz JW, Rubinstein B, Li R (2009). Dual modes of cdc42 recycling fine-tune polarized morphogenesis. Dev Cell 17, 823–835.

Tekleyohans DG, Mao Y, Kagi C, Stierhof YD, Gross-Hardt R (2017). Polyspermy barriers: a plant perspective. Curr Opin Plant Biol 35, 131–137.

Vjestica A, Merlini L, Nkosi PJ, Martin SG (2018). Gamete fusion triggers bipartite transcription factor assembly to block re-fertilization. Nature 560, 397–400.

Wang X, Tian W, Banh BT, Statler BM, Liang J, Stone DE (2019). Mating yeast cells use an intrinsic polarity site to assemble a pheromone-gradient tracking machine. J Cell Biol 218, 3730–3752.

Wedlich-Soldner R, Wai SC, Schmidt T, Li R (2004). Robust cell polarity is a dynamic state established by coupling transport and GTPase signaling. J Cell Biol 166, 889–900.

Wong JL, Wessel GM (2006). Defending the zygote: search for the ancestral animal block to polyspermy. Curr Top Dev Biol 72, 1–151.

Wu CF, Chiou JG, Minakova M, Woods B, Tsygankov D, Zyla TR, Savage NS, Elston TC, Lew DJ (2015). Role of competition between polarity sites in establishing a unique front. eLife 4, e11611.

Ydenberg CA, Rose MD (2008). Yeast mating: a model system for studying cell and nuclear fusion. Methods Mol Biol 475, 3–20.