Identification of Kel1p, a Kelch Domain-containing Protein Involved in Cell Fusion and Morphology in *Saccharomyces cerevisiae*

Jennifer Philips and Ira Herskowitz

Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143-0448

**Abstract.** We showed previously that protein kinase C, which is required to maintain cell integrity, negatively regulates cell fusion (Philips, J., and I. Herskowitz. 1997. *J. Cell Biol.* 138:961–974). To identify additional genes involved in cell fusion, we looked for genes whose overexpression relieved the defect caused by activated alleles of Pkc1p. This strategy led to the identification of a novel gene, *KEL1*, which encodes a protein composed of two domains, one containing six kelch repeats, a motif initially described in the *Drosophila* protein Kelch (Xue, F., and L. Cooley. 1993. *Cell.* 72:681–693), and another domain predicted to form coiled coils. Overexpression of *KEL1* also suppressed the defect in cell fusion of *spa2Δ* and *fps1Δ* mutants. *KEL2*, which corresponds to ORF YGR238c, encodes a protein highly similar to Kel1p. Its overexpression also suppressed the mating defect associated with activated Pkc1p. Mutants lacking *KEL1* exhibited a moderate defect in cell fusion that was exacerbated by activated alleles of Pkc1p or loss of *FUS1*, *FUS2*, or *FPS1*, but not by loss of *SPA2*. *kel1Δ* mutants form cells that are elongated and heterogeneous in shape, indicating that Kel1p is also required for proper morphology during vegetative growth. In contrast, *kel2Δ* mutants were not impaired in cell fusion or morphology. Both Kel1p and Kel2p localized to the site where cell fusion occurs during mating and to regions of polarized growth during vegetative growth. Coimmunoprecipitation and two-hybrid analyses indicated that Kel1p and Kel2p physically interact. We conclude that Kel1p has a role in cell morphogenesis and cell fusion and may antagonize the Pkc1p pathway.

Key words: Pkc1 • kelch • cell fusion • yeast mating • morphology

**Cell** fusion occurs during a variety of biological processes—for example, in sperm–egg fusion during fertilization, in myoblast fusion during myotube formation, and in microorganisms such as yeast during mating. In yeast, this process is initiated when haploid cells respond to the peptide pheromone secreted by cells of the opposite mating type (a cells responding to α-factor, and α cells to α-factor). The pheromones bind a seven-transmembrane receptor, stimulating a signal transduction cascade that results in cell cycle arrest, transcriptional induction of genes required for mating and cell fusion (Trueheart et al., 1987; McCaffrey et al., 1987; Elion et al., 1995), and a morphological response called shmoo formation (for reviews see Bardwell et al., 1994; Herskowitz, 1995). Cells polarize their actin cytoskeleton towards their mating partner by detecting a pheromone gradient (Jackson and Hartwell, 1990; Madden and Snyder, 1992; Segall, 1993), resulting in polarized protein deposition to the region of future cell contact (Trueheart et al., 1987; Elion et al., 1995).

Once the mating partners come into contact, a series of events, poorly understood at the molecular level, must be executed successfully to produce a diploid zygote. First, the cell wall must be removed in the region separating the two cells. Since inappropriate removal of cell wall material could result in lysis, correct spatial and temporal regulation of this event is critical for maintaining cell integrity. Hence, cell wall degradation does not occur until a cell comes into contact with its mating partner. The cell wall is then degraded beginning in the center of the region of contact and proceeding towards the edges (Osumi et al., 1974; Gammie et al., 1998). Cell wall degradation normally occurs quickly, and thus few cells are observed that have adhered but failed to fuse (Trueheart et al., 1987). Once cell wall material is removed, plasma membranes fuse, and...
then intracellular organelles such as nuclei and mitochondria fuse to produce an α/α diploid zygoate.

Our previous work demonstrated that Pkc1p and Fps1p regulate cell fusion (Philips and Herskowitz, 1997). Mutants defective in the FPS1 gene, predicted to encode a glycerol transporter (Luyten et al., 1995), accumulate intracellular glycerol and exhibit a defect in cell fusion (Luyten et al., 1995; Philips and Herskowitz, 1997). The fusion defect can be alleviated if intracellular glycerol concentration is restored to wild-type levels or if 1 M sorbitol is provided to osmotically stabilize the cells, suggesting that osmotic imbalance is the cause of the defect in cell fusion. Since the Pkc1p pathway responds to conditions that threaten cell integrity such as hypoosmotic shock (Davenport et al., 1995; Kamada et al., 1995), we proposed that Pkc1p inhibits cell fusion if cells are not osmotically balanced, a situation that makes cell fusion particularly dangerous (Philips and Herskowitz, 1997). Supporting the idea that Pkc1p negatively regulates cell fusion, cells expressing an activated allele of PKC1 (PKC1-R398P) exhibit a defect in cell fusion (Philips and Herskowitz, 1997). PKC1-R398P alters the pseudosubstrate binding site of Pkc1p, creating a dominant, activated allele (Nonaka et al., 1995). Pkc1p is proposed to function upstream of an α-factor production and mating (Adames et al., 1996; Brizzio et al., 1997). They were originally identified as the Mpk1p/Slt2p (Torres et al., 1991; Mazzoni et al., 1992), two proteins required for processes in addition to cell fusion and mating. This class includes Rvs161p, Fps1p, Spa2p, Pea2p, Bni1p, and Chs5p (Brizzio et al., 1998; Philips and Herskowitz, 1997; Dorer et al., 1997; Gammie et al., 1998; Santos et al., 1997). These proteins regulate morphogenesis and cell integrity, which may be modulated during mating to bring about cell fusion. Rvs161p is required to stabilize Fus2p during mating, a function that is distinct from its role in actin organization and endocytosis (Brizzio et al., 1998).

Here we describe a novel protein, Kel1p, which is a member of this final class of fusion proteins. We identified Kel1p because its overexpression suppressed the mating defect associated with an activated allele of Pkc1p. Overexpression of Kel1p also suppressed the mating defect of fps1Δ and spa2Δ mutants but not fus1Δ or fus2Δ mutants. Kel1p localizes to regions of polarized growth during mating and vegetative growth, and mutants lacking Kel1p exhibit defects in cell fusion and morphology.

Materials and Methods

Yeast Strains and Media

Yeast strains are described in Table I. Standard yeast growth conditions and genetic manipulations are described in Rose et al. (1990). Cells were grown at 30°C in YEPD medium unless otherwise noted. DNA manipulations were performed as in Sambrook et al. (1989).

Yeast Plasmids and Transformations

Plasmids are listed in Table I. pJP72 is a pRS306-derived plasmid containing the PKC1-R398P allele as described in Nonaka et al. (1995) (Philips and Herskowitz, 1997). This plasmid was used to integrate PKC1-R398P at its genomic locus, generating strain JP517. Control strain JP338 was constructed by integrating pRS306 at ura3. Yeast transformations were performed by the lithium acetate method (Ito et al., 1983). pJW192 codes for a RAS2-GEF fusion protein under control of the GDP1 promoter (kindly provided by J. Whistler, University of California, Berkeley; Philips and Herskowitz, 1997).

Cloning of KEL1 and KEL2

KEL1 was cloned by suppressing the mating defect of JP317. A plasmid (pPHS11) containing an ~6-kb insert was isolated from a 2µ library (Nasmyth; see Fig. 1 C). A HindIII–HindIII fragment from pPHS11 was subcloned into the HindIII site of YEp551 to generate pJP83. An SphI–SphI fragment from pHPS11 was subcloned into the SphI site of YEp531 to generate pJP81. A BamHI–BamHI fragment from pPHS11 was subcloned into the BamHI site of YEp531 to generate pJP84. pJP81, which contains YHR158c, was indistinguishable from pPHS11 in its ability to suppress the mating defect of JP317 (data not shown). To confirm that the ORF responsible for suppression was YHR158c, YHR158c was disrupted by digesting pJP81 with SpeI, treating with Klenow fragment and deoxynucleoside triphosphates, and ligating, to generate pJP82. pJP82 failed to suppress the mating defect exhibited by JP317. Additionally, a construct that contains approximately half of the YHR158c sequence (pJP84) partially suppressed the mating defect JP317, whereas a construct that removes the first 89 nucleotides of YHR158c (pJP83) failed to suppress the mating defect (Fig. 1 C).

Oligonucleotides OJP27 (5'-ACC TCT TGT AAC TAC TAC ATA CG) and OJP28 (5'-TCT TCT TCT GGA CAT TGG) were used to amplify KEL2 from yeast genomic DNA by PCR. The amplified product was cut with PstI and cloned into the PstI site of YEp551 to generate pJP92.

Strain Construction

The KEL1 gene was deleted from yeast strains using pJP94, a construct that replaces KEL1 with hisG/URA3-hisG (Aanli et al., 1987). This plas-
### Table I. Yeast Strains and Plasmids

| Plasmid Name | Description | Source |
|--------------|-------------|--------|
| pJP2         | *fus1Δ::TRP1* | Philips and Herskowitz (1997) |
| pJP5         | *fps1Δ::URA3* | Philips and Herskowitz (1997) |
| pJP67 (YCp50-DS1) | *YCp50-PKC1-R398P* | Nonaka et al. (1995) |
| pJP72        | *pRS06-PKC1-R398P* | Philips and Herskowitz (1997) |
| pJP81        | *YEp351-KEL1* | This study |
| pJP92        | *YEp351-KEL2* | This study |
| pJP94        | *kel1Δ::hisG-URA3-hisG* | This study |
| pJP113       | *kel2Δ::LEU2* | This study |
| pJP123       | *YEp351-KEL2* | This study |
| pJP126       | *YEp351-KEL2-GFP* | This study |
| pJP127       | *YEp351-KEL1* | This study |
| pJP131       | *YEp351-KEL2-PA* | This study |
| pJP138       | *kel2Δ::hisG-URA3-hisG* | This study |
| pJP139       | *YIp5-KEL1* | This study |
| pJP143       | *YIp5-KEL1* | This study |
| pJP158       | *pEG02-KEL2-DBD* | This study |
| pJP160       | *pRS426-GAL-KEL2* | This study |
| pJP167       | *pGI4-5-KEL1-AD* | This study |
| pJP168       | *pGI4-5-KEL2-AD* | This study |
| pJP202       | *YEp351-KEL1-PA* | This study |
| pJP207       | *YCplac111-KEL1* | This study |
| pJP209       | *YCplac111-KEL1-PA* | This study |
| pJSW192      | *RAS2-GFP* | J. Whistler |
| pKOFUS2      | *fus2Δ::URA3* | Philips and Herskowitz (1997) |
| pRFHM1       | *Bicoid-DBD* | Gyuris et al. (1993) |
| pSH18-34     | *LexAop-LacZ* | Gyuris et al. (1993) |
| pRS306       | *YCplac111* | This study |
| YEpl5        | *YEpl5* | This study |
| pJG4-5       | *pRS426* | This study |

* pRS306, pRS426, YEpl5, and YIp5 are described in Guthrie and Fink (1991). pEG202 and pGI4-5 are described in Gyuris et al. (1993). YCplac111 is described in Gietz and Sugino (1988). *Isogenic to IH2350. ** Isogenic derivatives in the EG123 strain background, whose full genotype is trp1::ura3 his4-34 leu2::can1. All other strains are isogenic derivatives of IH3160, whose full genotype is *MATa ade2-101 ura3-52 met1-1 his3-11 HMLa HMRa.*
mid was constructed by ligating the SphI–SphI fragment containing KEL1 into the SphI site of YEp351 to generate pJP126. To construct an HA-tagged version of Kel1p, an oligonucleotide (Kunkel et al., 1987) using oligonucleotides OJP43 (5′-GCG TAC GCA-3′) for the 5′-end and 3′-TAT TGT CTT TTA AGA TCT ATC GCT GTC AGC ATC-3′) for the 3′-end of both Kel1p and Kel2p. BglII sites were introduced just before the stop codon of KEL1 and KEL2 using the Minigel system (Bio-Rad Laboratories, Hercules, CA). The probes used were a 1.7-kb HindIII–BamHI fragment containing coding sequence for FUS1, a 1.7-kb HindIII–BamHI fragment containing coding sequence for YHR158c, and a 0.8-kb Hpal–SalII fragment for TCM1 (Schultz and Friesen, 1983).

Microscopy

Localization of Kel1p was analyzed in strains harboring pJP139 or pJP143 grown in SD-Ura. Localization of Kel2p was determined using strains harboring pJP126 or pJP123 grown in SD-Leu. Cells were grown to mid-log phase, sonicated, pelleted in a microcentrifuge, and resuspended in Fluormount G (Southern Biotechnology Associates, Inc.). Samples were viewed with a BX50 microscope at 100× (Olympus America, Inc., Melville, NY).

Morphology of wild-type (HI3196), kel1Δ (JP363), kel2Δ (JP371), and kel1Δ kel2Δ (JP385) strains was determined by growing cells to mid-log phase in synthetic complete medium. HI3196 harboringYep531 or pJP51 was grown to mid-log phase in SD-Leu. HI3196 harboring pRS426 or pJP160 was grown to mid-log phase in SGal-Ura. Cells were sonicated, pelleted in a microcentrifuge, and resuspended in 50% glycerol before viewing with an Axioskop microscope at 100× (Carl Zeiss, Inc., Thornwood, NY).

Northern Analysis

RNA preparations and sample analysis were performed as described previously (Cross and Tinkelenberg, 1991). The probes used were DNA restriction fragments that were gel-purified and labeled by random-prime labeling using a Prime-it kit (Stratagene, La Jolla, CA). The fragments used were a 1.7-kb PstI–HincII fragment containing coding sequence for FUS1, a 1.7-kb HindIII–BamHI fragment containing coding sequence for YHR158c, and a 0.8-kb Hpal–SalII fragment for TCM1 (Schultz and Friesen, 1983).

Coinmunoprecipitation Analysis

Yeast cells were prepared by growing cells overnight in SD-Ura Leu media. Cells were inoculated into YEPD and allowed to grow for approximately two generations to an OD600 of ~0.4. 100 ml cultures were harvested, resuspended in 250 μl ice-cold lysis buffer (50 mM Tris–HCl pH 8.0, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 50 mM NaF, 80 mM β-glycerophosphate, 1 mM Na3VO4, and a cocktail of protease inhibitors (Boehringer Mannheim Corp., Indianapolis, IN) and mixed with an equal volume of glass beads. After vortexing vigorously ten times with 10-s pulses, samples were centrifuged at 14 000 rpm for 15 min in a microfuge at 4°C. The supernatants were removed, and lysis buffer was added to a final volume of 0.5 ml. Samples were centrifuged again for 15 min at 4°C. 50 μl of supernatant was removed for detecting Kel1p-GFP in the extract (data not shown). Immunoprecipitations were performed on the remaining sample at 4°C for 2 h by incubating the remaining supernatant with 25 μl of 50% slurry of protein G-sepharose bound to anti-HA antibodies (12CA5) with rocking. Precipitates were washed four times with 0.5 ml cold lysis buffer, resuspended in 50 μl loading buffer, and boiled for 5 min before resolving by 7.5% SDS-PAGE. Immunoblot analysis was performed with anti-HA (HA11; Berkeley Antibody Co., Inc., Richmond, CA) or anti-GFP antibodies as described below.

Immunoblot Analysis

Extracts that were not used for coinmunoprecipitations were prepared as follows. Cells were grown to an OD600 of ~0.3. 15 ml of culture was pelleted in a microfuge and incubated on ice for 5 min. Pellets were resuspended in 150 μl ice-cold solution 1 (1.85 mM NaOH, 7.4% β-mercaptoethanol) and incubated on ice for 10 min. 150 μl ice-cold 50% TCA was added. Cells were incubated on ice for 10 min, followed by centrifugation for 2 min at 4°C. Pellets were washed with 1 ml ice-cold acetone. Samples were centrifuged for 2 min at 4°C. The pellet was resuspended in sample buffer, boiled for 5 min, and resolved by 7.5% SDS-PAGE. SDS-PAGE was followed by electroblotting onto nitrocellulose filters using the Minigel system (Bio-Rad Laboratories, Hercules, CA). Blots were incubated in TBST (TBS with 0.1% Triton X-100) with 10% nonfat dry milk for ~1 h. Blots were incubated with primary antibody (mAb HA11 diluted 1:10 000 [Berkeley Antibody Co., Inc.]; mAb anti-GFP [C63]; a gift from P. O’Farrell, University of California, San Francisco, CA) diluted 1:2) in TBST supplemented with 2% nonfat dry milk at 4°C overnight. Blots were washed three times for 5 min in TBST and incubated with peroxidase-linked secondary antibody (Bio-Rad Laboratories) diluted 1:2 000 in TBST with 2% nonfat dry milk for 1 h. Blots were washed twice for 5 min in TBS supplemented with 0.3% Triton X-100, followed by three washes in TBST. Blots were developed using an enhanced chemiluminescence detection kit (Amersham Corp., Arlington Heights, IL).
Two-hybrid plasmids pJP158 and pJP168 were constructed by introducing a BamHI site before the start codon of KEL2 by oligonucleotide-mediated mutagenesis (Kunkel et al., 1987) using oligonucleotide OJP50 (5'-GG CAG GCA ACC CGG ATC GTA GCT ATG GTA C). A BamHI-NcoI fragment containing KEL2 was cloned into pEG202 to create pJP158. A BamHI-NotI fragment containing KEL2 from pJP158 was ligated into the BamHI–NotI sites of pG4-5 to generate pJP168. pJP167 was generated by introducing a SalI site before the start codon of KEL1 by oligonucleotide-mediated mutagenesis (Kunkel et al., 1987) using oligonucleotide OJP49 (5'-GCT GAA TCC AGC CAT GTT TGG TCG ACT TTC TAG GTG C). A SalI–SalI fragment containing Kel1p-AD was induced with galactose for ~1 h. Data are expressed in Miller units (OD420 min/OD600 min). Cultures were grown overnight in S raffinose-His Ura Trp. Expression of Kel1p-AD was induced with galactose for ~1 h. Data are expressed in Miller units (OD420 min/OD600 min) as determined from at least three independent transformants, tested in duplicate.

**Analysis of mpk1Δ kel1Δ and mpk1Δ kel2Δ Strains**

An mpk1Δ strain (IH3077) was crossed with two independent kel1Δ strains (JP445A and JP445B) generated by gene replacement or two independent kel2Δ strains (JP446A and JP446B) generated by gene replacement. Segregants were allowed to germinate on YEPD containing 1 M sorbitol at 30°C. Cells were streaked for single colonies on either YE PD or YE PD + 1 M sorbitol and grown for 2 d at 34.5°C.

### Results

**Overexpression of KEL1 Suppresses Mutants Defective in Cell Fusion**

Cells expressing an activated allele of PKC1 (PKC1-R398P) exhibit impaired mating due to a defect in cell fusion (Philips and Herskowitz, 1997). Expression of this allele under control of its own promoter resulted in a mating defect that was easily detected when cells were mated to a fus1Δ fus2Δ mutant strain, which is also partially defective in cell fusion (Fig. 1 A). To identify additional genes important for cell fusion, we performed a high-copy suppressor screen for improved mating of cells expressing PKC1-R398P. Strain JP317 (PKC1-R398P) was transformed with a high-copy library, and ~4,500 transformants were screened by replica plating for mating with a fus1Δ fus2Δ mutant strain. Four plasmids containing distinct inserts were identified that restored mating when retransformed. One of them, designated pHHS11, is described here.

Sequencing revealed that pHHS11 contained two full-length ORFs, YHR158c and REC104, and part of YHR159w and YHR156c. Subcloning demonstrated that YHR158c was responsible for suppression (see Materials and Methods and Fig. 1 C). Fig. 1 A shows that a 2 μ plasmid containing YHR158c (pJP81) suppressed the mating defect of JP317. To ascertain whether overexpression of YHR158c suppressed the fusion defect of JP317, we examined the matings microscopically. We found that pJP81 reduced the number of prezygotes that accumulated in a strain expressing PKC1-R398P by >40% (Table II, lines 2 and 3). To determine whether pJP81 could suppress the mating defect of additional cell fusion mutants, we examined its effect on the mating ability of fus1Δ, fus2Δ, spa2Δ, and fps1Δ mutants. Similar to what we observed in the PKC1-R398P mutant, pJP81 partially restored mating and cell fusion to spa2Δ and fps1Δ mutants (Fig. 1 B and Table II). In contrast, there was no significant effect of pJP81 on fus1Δ and fus2Δ mutants (data not shown). We conclude that overexpression of YHR158c partially suppresses the mating defect exhibited by a subset of mutants defective in cell fusion.

YHR158c is predicted to encode an 1164-amino acid protein. Analysis of the sequence revealed six internal repeats in the amino-terminal half of the protein followed by a domain predicted to form coiled coils in the carboxy-terminal half of the protein (Fig. 2 A). The repeats of ~50...
Table II. The Effect of KEL1 Overexpression on Cell Fusion in spa2Δ, fps1Δ, and PKC1-R398P Mutants

| Genotype | Plasmid       | Prezygotes* |
|----------|---------------|-------------|
| WT       | vector        | 4.7 ± 1.1   |
| PKC1-R398P | vector      | 51 ± 0.5    |
| PKC1-R398P | KEL1        | 29 ± 7      |
| WT       | vector        | 2.3 ± 0.4   |
| spa2Δ    | KEL1         | 25 ± 4      |
| fps1Δ    | KEL1         | 67 ± 3      |

For lines 1–3, strains were: WT (JP338) and PKC1-R398P (JP317). Strains were transformed with YEp351 containing KEL1 (pJP1) or YEp351 alone as indicated. For lines 4–8, strains were: WT (H31963), spa2Δ (H3204), and fps1Δ (JP147). For lines 1–3, strains were grown overnight in SD-Leu. All strains were mated to a wild-type strain (H2350). At least 100 partnered cells were counted in each experiment to determine % prezygotes. Values are means of three experiments ± SD. *Percent prezygotes represents the number of prezygotes/zygotes + prezygotes.

Figure 2. Relationship of Kel1p, Kel2p, and Tea1p. (A) Domain structure of Kel1p, Kel2p, and Tea1p. Grey boxes indicate kelch repeats. Striped boxes indicate predicted coiled-coil domains. (B) Alignment of the kelch repeats in Kel1p, Kel2p, and Tea1p. Consensus residues are indicated in bold (residues present in at least three of the six repeats). Consensus sequence is listed underneath repeats in bold. *Indicates presence of amino acids excluded from the alignment. (C) Dendrogram indicating the relationship among Kel1p, Kel2p, Tea1p, and the protein predicted by the S. pombe sequencing project (Z98603). Dendrogram was generated using Pileup and Cluster analysis.

Segall, 1993). Many proteins required for cell fusion, including Fus1p, Fus2p, and Spa2p, localize to the region of future cell contact (Trueheart et al., 1987; Elion et al., 1995; Gehrung and Snyder, 1990). To determine whether Kel1p similarly localized, a fusion protein was constructed in which GFP was joined to the carboxy terminus of Kel1p (see Materials and Methods). Cells were treated with α-factor, and localization of Kel1p-GFP was examined by fluorescence microscopy. Kel1p-GFP expressed from 2μ or CEN-ARS, or integrating plasmids localized to the shmoo tip (Fig. 3 A, a and data not shown). Focusing through sections of cells indicated that Kel1p-GFP was localized to the periphery of the shmoo tip. When overexpressed, Kel1p-GFP suppressed the fusion defect associated with PKC1-R398P. In this case, Kel1p-GFP remained localized to the shmoo tip, but the green fluorescent signal was more intense and more broadly localized than when the fusion construct was integrated (data not shown).

Northern blot analysis indicated that the KEL1 transcript was present in a, α, and a/α diploid cells, consistent with it having a function during vegetative growth, and was not induced by pheromone (Fig. 4). Hence, we exam-
ined its localization during vegetative growth. As with pheromone treatment, Kel1p-GFP localized to regions of polarized growth in vegetative cells (Fig. 3, a and b). Kel1p-GFP could be seen as a single spot in unbudded cells. In cells with small- or medium-sized buds, Kel1p localized to the bud tip. In large-budded cells, Kel1p-GFP was often localized to the neck region separating the mother and daughter cell.

To determine if Kel2p could function similarly to Kel1p, we examined its subcellular localization. GFP was fused to the carboxy terminus of Kel2p. We could only detect Kel2p-GFP signal when expressed from a 2μm plasmid, but not when expressed from a CEN-ARS plasmid. Like Kel1p-GFP, Kel2p-GFP localized to the shmoo tip in pheromone-treated cells (Fig. 3, B, a). Also, like Kel1p, Kel2p-GFP localized to regions of polarized growth during vegetative growth. Kel2p-GFP was found as a single spot in unbudded cells. In cells with small- or medium-sized buds, Kel2p-GFP localized to the bud tip. In large-budded cells, Kel2p-GFP was detected at the bud tip or at the neck between mother and bud (Fig. 3, B, b). We con-

![Figure 3. Localization of Kel1p and Kel2p. (A) An integrating plasmid containing Kel1p-GFP (pJP139) was used to localize Kel1p in wild-type cells (IH3196) (a and b) and in kel2Δ (JP370) cells (c and d). No signal was observed in wild-type cells expressing an untagged control plasmid (pJP143) (e and f). Cells in a, c, and e were treated with 25 μg/ml α-factor for ~2 h. b, d, and f are composites from two different photographs. (B) YEp351 containing Kel2p-GFP (pJP126) was used to localize Kel2p in wild-type cells (a and b) and in kel1Δ cells (JP363; c and d). No signal was observed in wild-type cells expressing an untagged control plasmid (pJP123; e and f). Cells in a, c, and e were treated with 25 μg/ml α-factor for ~2 h. b, d, and f are composites from two different photographs. (C) Kel2p is present in kel1Δ strains. Wild-type (IH3196) and kel1Δ (JP363) strains harboring plasmids encoding Kel2p-GFP (pJP126) or untagged control plasmid (pJP123) were analyzed by Western blot as described in Materials and Methods. Monoclonal antibodies recognizing GFP (C163) were a generous gift from P. O’Farrell. Molecular weight standards are indicated on the left. (D) Kel1p is more highly expressed than Kel2p. JP317 was transformed with YEp351-derived plasmids containing Kel1p-HA (pJP202), untagged Kel1p (pJP127), Kel2p-HA (pJP131), or untagged Kel2p (pJP123). +, HA-tagged versions; −, untagged versions. Extracts were prepared as described in Materials and Methods. Lanes were equally loaded, and Western blot analysis was performed with monoclonal antibodies recognizing the HA tag (HA11; Berkeley Antibody Co., Inc.). Molecular weight standards are indicated on the left.

![Figure 4. Northern analysis of KEL1 and FUS1 mRNA. mRNA was prepared as described in Materials and Methods from a/α wild-type (JP54), α wild-type (IH3197), a wild-type (IH3196), a kel1Δ (JP358), and a fus1Δ (JP52) strains after cells were treated with α-factor for 20 min (+, treated; −, untreated). Northern blots were hybridized with a probe to KEL1 (top) or FUS1 (third panel). Blots were stripped and hybridized with a probe to TCM1 to serve as a loading control.](https://doi.org/10.1083/jcb.118.8.805)
The absence of Kel1p both with and without pheromone treatment depends upon each other. In localization, we determined whether their localization depended upon KEL1 because Kel1p and Kel2p exhibit a similar pattern of localization. We conclude that Kel1p is required to localize Kel2p to regions of polarized growth and that Kel2p is dispensable for Kel1p localization. Despite the considerable homology between Kel1p and Kel2p, Kel1p apparently interacts with a determinant at the site of polarized growth with which Kel2p fails to interact in the absence of Kel1p.

Kel1p and Kel2p Are Present in a Complex

Since localization of Kel2p was dependent upon Kel1p, we wondered whether the two proteins physically interact. To address this question, coimmunoprecipitation and two-hybrid analyses were performed. An epitope-tagged version of Kel2p (Kel2p-HA) was constructed in which a double hemagglutinin (HA)1 tag was fused to the carboxy terminus of Kel2p as described in Materials and Methods. Kel2p-HA was expressed in a strain that harbored a GFP-tagged version of Kel1p or an untagged version. In cells expressing Kel2p-HA, we were able to immunoprecipitate Kel2p with anti-HA antibodies (Fig. 5 A, bottom panel, lanes 1 and 2). If the cells coexpressed Kel1-GFP, Kel1p co-immunoprecipitated with Kel2p-HA as detected by immunoblot with anti-GFP antibodies (Fig. 5 A, top panel, lane 2). To determine whether Kel1p-GFP was specifically coimmunoprecipitated by Kel2p-HA, immuno-precipitations were performed from cells expressing the untagged version of Kel2p. We were unable to immunoprecipitate Kel1p-GFP with anti-HA antibodies in such extracts (Fig. 5 A, top panel, lane 3).

Similar experiments were performed to determine whether Kel1p can associate with itself. An HA-tagged version of Kel1p was expressed in a cell that also harbored an integrated copy of a GFP-tagged version of Kel1p. Kel1p-HA was immunoprecipitated using antibodies that recognize the HA epitope (HA11; Berkeley Antibody Co., Inc.). Equal amounts of Kel2p-HA were immunoprecipitated in lanes 1 and 2, whereas no band was detected in extracts prepared from strains expressing the untagged control (lane 3). Equal amounts of Kel1p-HA were immunoprecipitated in lanes 4 and 3, whereas no band was detected in extracts prepared from strains expressing the untagged control (lane 6). Cells coexpressed Kel1p-GFP (pJP139), indicated by +, or untagged Kel1p (pJP143), indicated by −. Monoclonal antibodies recognizing GFP were used to detect coimmunoprecipitated Kel1p-GFP in the upper blots. Arrowhead with an asterisk indicates full-length Kel1p-GFP. Other arrowheads indicate breakdown products of Kel1p-GFP that also coimmunoprecipitate. Molecular weight standards are indicated on the left of each gel. (B) Two-hybrid analysis of Kel1p and Kel2p. Cells expressed Kel2p or Bicoid fused to the lexA DNA-binding domain (DBD; pJP158 and pRFH1, respectively), and Kel1p fused to a transcriptional activation domain (AD; pJP167) or vector. Ability to activate transcription from a reporter construct was determined as described in Materials and Methods. Miller Units were determined as described in Materials and Methods. Values are means ± SD.

Kel2p fails to interact in the absence of Kel1p.

Localization of Kel2p Depends Upon KEL1

Because Kel1p and Kel2p exhibit a similar pattern of localization, we determined whether their localization depends upon each other. In kel1Δ strains, localization of Kel1p was indistinguishable from that in wild-type strains (Fig. 3 A, c and d). However, Kel2p failed to localize in the absence of Kel1p both with and without pheromone treat-
Figure 6. Kel1p and Kel2p are involved in mating and morphology. (A) kel1Δ mutants are defective in mating. kel1Δ strain JP363 was transformed with a CEN-ARS plasmid containing KEL1 (pJP207), a 2μ plasmid expressing Kel2p (pJP92), or vector (YCPplac111). The wild-type control (IH3196) was transformed with vector (YCPplac111). Strains were mated to an α fus1 fus2 strain (IH2351) as described in Materials and Methods. (B) Wild-type (a–d) and kel1Δ (JP363; e–h) mutants were mated on filters to wild-type strain IH2350 carrying the RAS2-GFP fusion plasmid (pJW192) as described in Materials and Methods. Nuclei were visualized by DAPI staining (d and h). RAS2-GFP was visualized by fluorescence microscopy (b and f). (C) kel1Δ and kel1Δ kel2Δ mutants exhibit altered morphology. Morphology of isogenic (a) wild-type (IH3196), (b) kel1Δ (JP363), (c) kel2Δ (JP371), and (d) RAS2-GFP expression plasmid (pJW191) transformed into wild-type strain. Prezygotes were scored as unfused, as evidenced by two distinct DAPI-staining structures in which the nuclei of mating partners remained separate. RAS2-GFP was visualized by fluorescence microscopy (a and d). (E) Morphological phenotype exhibited by cells overexpressing KEL1 or KEL2. Morphology exhibited by a wild-type strain (IH3196) transformed with (a) vector (YEp351), (b) 2μ KEL1 (pJP81), (c) vector (pRS426), or (d) pGAL KEL2 (pJP160) grown in SD-Ura (a and b) or S galactose-Ura (c and d).

kel1Δ mutants are defective in cell fusion. To examine further the defect in cell fusion, strains were transformed with a CEN-ARS plasmid containing KEL1 (pJP207), a 2μ plasmid expressing Kel2p (pJP92), or vector (YCPplac111). The wild-type control (IH3196) was transformed with vector (YCPplac111). Strains were mated to an α fus1 fus2 strain (IH2351) as described in Materials and Methods. (B) Wild-type (a–d) and kel1Δ (JP363; e–h) mutants were mated on filters to wild-type strain IH2350 carrying the RAS2-GFP fusion plasmid (pJW192) as described in Materials and Methods. Nuclei were visualized by DAPI staining (d and h). RAS2-GFP was visualized by fluorescence microscopy (b and f). (C) kel1Δ and kel1Δ kel2Δ mutants exhibit altered morphology. Morphology of isogenic (a) wild-type (IH3196), (b) kel1Δ (JP363), (c) kel2Δ (JP371), and (d) RAS2-GFP expression plasmid (pJW191) transformed into wild-type strain. Prezygotes were scored as unfused, as evidenced by two distinct DAPI-staining structures in which the nuclei of mating partners remained separate. RAS2-GFP was visualized by fluorescence microscopy (a and d). (E) Morphological phenotype exhibited by cells overexpressing KEL1 or KEL2. Morphology exhibited by a wild-type strain (IH3196) transformed with (a) vector (YEp351), (b) 2μ KEL1 (pJP81), (c) vector (pRS426), or (d) pGAL KEL2 (pJP160) grown in SD-Ura (a and b) or S galactose-Ura (c and d).

kel1Δ kel2Δ (JP385) strains. (D) Morphological phenotype exhibited by cells overexpressing KEL1 or KEL2. Morphology exhibited by a wild-type strain (IH3196) transformed with (a) vector (YEp351), (b) 2μ KEL1 (pJP81), (c) vector (pRS426), or (d) pGAL KEL2 (pJP160) grown in SD-Ura (a and b) or S galactose-Ura (c and d).

kel1Δ mutants are defective in cell fusion. To determine whether KEL1 has a role in cell fusion, we examined the phenotype of a strain in which KEL1 was deleted. In a diploid strain, one copy of the KEL1 open reading frame was replaced with the URA3 gene (see Materials and Methods). Examination of haploid segregants indicated that deletion of KEL1 had no effect on growth rate, budding pattern (in kel1Δ haploids and in a/a kel1Δ/ kel1Δ diploids), or sensitivity to high and low temperatures. kel1Δ mutants did exhibit a mating defect (Fig. 6 A). This defect was not due to inability to produce or respond to pheromone normally, as determined by halo assays and shmoo formation (Fig. 3 B, c and d not shown). Additionally, kel1Δ mutants signaled in response to pheromone as evidenced by normal transcriptional induction of FUS1 (Fig. 4). kel1Δ mutants were examined microscopically to ascertain if prezygotes accumulated when they were mated to a wild-type strain. Prezygotes were scored as structures in which the nuclei of mating partners remained unfused, as evidenced by two distinct DAPI-staining structures, and in which a septum was visible between adherent mating partners. kel1Δ mutants did exhibit a defect in cell fusion, displaying a four- to fivefold increase in the number of prezygotes when compared with wild-type cells (Table III, lines 1 and 2; Table IV, lines 1 and 2). The defect was not exacerbated by mating to kel1Δ cells (data not shown). Similarly, the mating defect of fps1Δ mutants is not exacerbated by mating to fps1Δ cells (Philips and Herskowitz, 1997). In contrast, a number of other mutants defective in cell fusion, such as fus1Δ and fus2Δ mutants, exhibit a severe defect when mated to a mutant partner (Trueheart et al., 1987).

To examine further the defect in cell fusion, strains were

kel1Δ mutants are defective in cell fusion. To determine whether KEL1 has a role in cell fusion, we examined the phenotype of a strain in which KEL1 was deleted. In a diploid strain, one copy of the KEL1 open reading frame was replaced with the URA3 gene (see Materials and Methods). Examination of haploid segregants indicated that deletion of KEL1 had no effect on growth rate, budding pattern (in kel1Δ haploids and in a/a kel1Δ/ kel1Δ diploids), or sensitivity to high and low temperatures. kel1Δ mutants did exhibit a mating defect (Fig. 6 A). This defect was not due to inability to produce or respond to pheromone normally, as determined by halo assays and shmoo formation (Fig. 3 B, c and d not shown). Additionally, kel1Δ mutants signaled in response to pheromone as evidenced by normal transcriptional induction of FUS1 (Fig. 4). kel1Δ mutants were examined microscopically to ascertain if prezygotes accumulated when they were mated to a wild-type strain. Prezygotes were scored as structures in which the nuclei of mating partners remained unfused, as evidenced by two distinct DAPI-staining structures, and in which a septum was visible between adherent mating partners. kel1Δ mutants did exhibit a defect in cell fusion, displaying a four- to fivefold increase in the number of prezygotes when compared with wild-type cells (Table III, lines 1 and 2; Table IV, lines 1 and 2). The defect was not exacerbated by mating to kel1Δ cells (data not shown). Similarly, the mating defect of fps1Δ mutants is not exacerbated by mating to fps1Δ cells (Philips and Herskowitz, 1997). In contrast, a number of other mutants defective in cell fusion, such as fus1Δ and fus2Δ mutants, exhibit a severe defect when mated to a mutant partner (Trueheart et al., 1987).

To examine further the defect in cell fusion, strains were
mated to a partner that produces RAS2-GFP fusion protein (kindly provided by J. Whistler, University of California, Berkeley) that localized green fluorescence around the cell periphery (Philips and Herskowitz, 1997; Dorer et al., 1997). In matings between two wild-type partners, the green fluorescent signal was seen throughout the entire zygote (Fig. 6 B, b). In contrast, in matings between kel1Δ mutants and a wild-type partner containing RAS2-GFP, prezygotes were found in which the green fluorescent signal remained restricted to one cell, indicating a failure in plasma membrane fusion and cytoplasmic mixing (Fig. 6 B, f). These structures could also be observed in wild-type matings, but were found at an elevated frequency in matings in which one partner was lacking KEL1. The increased frequency of prezygotes as determined by RAS2-GFP staining was similar to that quantitated by Nomarski optics and DAPI staining (data not shown).

To investigate further the role of KEL1 in cell fusion, we examined genetic interactions between kel1Δ mutants and other mutants defective in cell fusion. Because fus1Δ and fus2Δ mutants display only mild defects in cell fusion, whereas fus1Δ fus2Δ double mutants exhibit a more severe defect than either single mutant alone, it has been suggested that FUS1 and FUS2 function in parallel pathways (Trueheart et al., 1987; Brizio et al., 1998). To determine whether KEL1 functions in either of these pathways, we examined the fusion competence of kel1Δ fus1Δ and the kel1Δ fus2Δ double mutants. In matings between a kel1Δ fus1Δ double mutant and a wild-type mating partner, ~54% of the partnered cells were prezygotes, a defect significantly worse than that seen with kel1Δ or fus1Δ single mutants (9.4% and 22.4%, respectively; Table III).

Table III. The Effect of Deletion of KEL1 and KEL2 on Cell Fusion

| Genotype | Prezygotes* |
|----------|-------------|
|          | %           |
| WT       | 2.6 ± 0.7   |
| kel1Δ    | 9.4 ± 1.9   |
| kel2Δ    | 3.7 ± 0.8   |
| kel1Δ kel2Δ | 7.1 ± 1.4   |
| fus1Δ    | 22.4 ± 3.8  |
| fus1Δ kel1Δ | 54.0 ± 10.6 |
| fus1Δ kel2Δ | 31.9 ± 3.5  |
| fus2Δ    | 44 ± 7      |
| fus2Δ kel1Δ | 70 ± 3      |
| fus2Δ kel2Δ | 38 ± 2      |
| fps1Δ    | 62 ± 7      |
| fps1Δ kel1Δ | 93 ± 4      |
| fps1Δ kel2Δ | 66 ± 13     |
| fps1Δ kel1Δ kel2Δ | 99.3 ± 0.2 |
| spa2Δ    | 39 ± 12     |
| spa2Δ kel1Δ | 42 ± 10     |

Analysis of lines 1–7, 8–10, 11–14, and 15–16 were carried out separately, in each case with wild-type and kel1Δ controls that behaved similarly to lines 1 and 2, respectively. Strains were WT (BH3196), kel1Δ (JP363), kel2Δ (JP371), fus1Δ (JP52), fus2Δ (JP410), kel1Δ kel2Δ (JP385), fus2Δ (JP418), fus1Δ kel1Δ (JP417), fus2Δ kel2Δ (JP416), fps1Δ (JP147), fps1Δ kel1Δ (JP144), fps1Δ kel2Δ (JP415), fps1Δ kel1Δ kel2Δ (JP500), spa2Δ (IH3204), and spa2Δ kel1Δ (JP92). Cells were grown in YEPD and were mated to a wild-type z strain (BH2350). For lines 1–7, values are means of at least four experiments in which a total of at least 900 partnered cells were counted ± SD. For lines 8–14, values are the means of three experiments in which more than 100 partnered cells were counted per experiment. For lines 15 and 16, more than 200 partnered cells were counted in three experiments.

*Percent prezygotes represents the number of prezygotes/zygotes + prezygotes.

Loss of KEL1 exacerbated the defect in cell fusion of a fus1Δ and α fus1Δ cells, indicating that KEL1 is required in both cell types for efficient cell fusion (Table III and data not shown). Similar to the fus1Δ mutant, a fus2Δ single mutant exhibited 44% prezygotes, whereas the kel1Δ fus2Δ double mutant exhibited 70% prezygotes (Table III). These results demonstrate that loss of KEL1 exacerbates the defect in cell fusion of fus1Δ and fus2Δ mutants, suggesting that KEL1 is not in either the FUS1 or FUS2 pathways. In both fus1Δ and fus2Δ mutants, loss of KEL1 caused a 30% increase in the number of prezygotes. A 30% increase in the number of prezygotes was also seen in fps1Δ mutants, in which the number of prezygotes increased from 62% for fps1Δ mutants to 93% for fps1Δ kel1Δ double mutants (Table III). In contrast, loss of KEL1 did not significantly affect the ability of spa2Δ mutants to fuse. In this case, spa2Δ single mutants exhibited 39% prezygotes, whereas the spa2Δ kel1Δ double mutant exhibited 42% prezygotes, suggesting that Kel1p may function in the same pathway as Spa2p. We conclude that loss of KEL1 causes a mild defect in cell fusion, which is exacerbated by loss of FUS1, FUS2, or FPS1, but not by loss of SPA2.

Table IV. The Effect of PKC1-R398P on Cell Fusion in kel1Δ and kel2Δ Mutants

| Genotype | Prezygotes* |
|----------|-------------|
|          | %           |
| WT       | 5.5 ± 1.0   |
| kel1Δ    | 30.2 ± 2.4  |
| kel2Δ    | 8.7 ± 2.8   |
| fus1Δ    | 47.3 ± 3.7  |
| kel1Δ kel2Δ | 19.9 ± 2.0  |

Strains were: WT (BH3196), kel1Δ (JP363), kel2Δ (JP371), fus1Δ (JP52), and kel1Δ kel2Δ (JP385). Strains were transformed with either YCP50 containing PKC1-R398P (pJP67) or YCP50 alone as indicated. Strains were grown in SD-Ura, and were mated to a wild-type z strain (BH2350). More than 200 partnered cells were counted in each experiment to determine % prezygotes. Values are means of three experiments ± SD.

*Percent prezygotes represents the number of prezygotes/zygotes + prezygotes.

kel2Δ Mutants Do Not Exhibit a Defect in Cell Fusion

To determine whether kel2Δ was also required for cell fusion, we constructed a strain in which Kel2p was deleted, and analyzed its phenotype. One copy of the KEL2 open reading frame was replaced with the LEU2 gene (see Materials and Methods) in a diploid strain. Haploid segregants were examined for their ability to mate. Unlike kel1Δ mutants, kel2Δ mutants exhibited normal mating and cell fusion (Tables III and IV). In fus1Δ, fus2Δ, fps1Δ, and PKC1-R398P mutant strains, loss of Kel2p had little effect on cell fusion (Table III and IV). Since there is no detectable requirement for Kel2p in cell fusion in a wild-type strain, the primary function of Kel1p during fusion does not appear to be for localization of Kel2p.

Because kel1Δ mutants fail to localize Kel2p, one possible cause of the defect in cell fusion in such strains is mislocalization of Kel2p, as opposed to the absence of Kel1p or Kel2p at the shmoo tip. If such an explanation were correct, then deletion of KEL2 in the kel1Δ mutant should
suppress the defect in cell fusion. When grown in rich medium and mated to a wild-type partner, kel1Δ mutants accumulated 9.4 ± 1.9% prezygotes, whereas kel1Δ kel2Δ double mutants accumulated 7.1 ± 1.4% prezygotes compared with 2.6 ± 0.7% prezygotes for wild-type cells (Table III). When cells were grown in minimal medium, there was a higher background level of prezygotes. In this case, kel1Δ strains accumulated 30.2 ± 2.4% prezygotes, whereas kel1Δ kel2Δ double mutants accumulated 19.9 ± 2.0% prezygotes compared with 5.5 ± 1.0% for wild-type cells (Table IV, compare lines 2, 5, and 1, respectively).

We conclude that loss of KEL2 may modestly suppress the fusion defect of kel1Δ strains, but that mislocalized Kel2p does not completely account for the fusion defect of kel1Δ strains. Consistent with this interpretation, overexpression of KEL2 did not significantly alter the mating ability of kel1Δ mutants (Fig. 6 A). Taken together, these data indicate that Kel1p has a role in cell fusion that is not solely to localize Kel2p. In contrast, there is little requirement for Kel2p in cell fusion.

kel1Δ Mutants Have a Defect in Cell Morphology

In addition to the defect in cell fusion, kel1Δ mutants exhibited a defect in morphology, appearing slightly elongated and heterogeneous in shape when compared with wild-type control cells (Fig. 6 C, b). This morphological defect was observed in two different strain backgrounds (data not shown). kel2Δ mutants exhibited normal morphology (Fig. 6 C, c). Examination of the kel1Δ kel2Δ double mutant revealed the same elongated morphology as seen in the kel1Δ single mutant (Fig. 6 C, d), suggesting that, like the fusion defect, the altered morphology of kel1Δ mutants is not due to mislocalized Kel2p.

Although loss of Kel2p did not alter morphology, overexpressing Kel2p from the GAL1,10 promoter caused ~5% of cells to display a grossly aberrant morphology (Fig. 6 D, d). When Kel1p was overexpressed from a 2μ plasmid, an equivalent fraction of cells displayed a similar morphology (Fig. 6 D, b). We did not observe this morphology in cells containing vector when grown in galactose (Fig. 6 D, c), or when cells containing pGAL-KEL2 were grown in glucose (data not shown). These data show that although only Kel1p is required for proper morphology, overexpression of either Kel1p or Kel2p disrupts normal cellular morphology.

Because of the potential role of kelch repeats in mediating interaction with cytoskeletal components (see Discussion), we examined actin in kel1Δ strains by staining with rhodamin-phalloidin. We did not detect any obvious differences between wild-type and kel1Δ mutants. Microtubules were visualized in kel1Δ, kel2Δ, and kel1Δ kel2Δ mutants by indirect immunofluorescence both with and without pheromone treatment. Again, we did not detect any obvious differences (data not shown), but subtle defects cannot be excluded. Additionally, loss of Kel1p or Kel2p did not affect sensitivity to the microtubule-stabilizing drug benomyl, and kel1Δ mutants did not exhibit genetic interactions with tub1-1 mutants (Carminati, J., personal communication). Hence, it is currently unclear whether Kel1p interacts with the actin or microtubule cytoskeleton.

Relationship of Kel1p to the Pkc1p Pathway during Mating and Vegetative Growth

KEL1 was identified by its ability to suppress an activated allele of Pkc1p. In principle, Kel1p could be a target of Pkc1p, a negative regulator or Pkc1p, or could act in parallel to promote fusion. To examine the relationship between Kel1p and Pkc1p, we analyzed the phenotype of a kel1Δ mutant expressing PKC1-R398P. If Kel1p were the sole downstream target inhibited by Pkc1p to prevent cell fusion, then we would expect the PKC1-R398P kel1Δ double mutant to behave like the kel1Δ and PKC1-R398P single mutants. Examination of a kel1Δ PKC1-R398P kel1Δ double mutant revealed a defect in cell fusion that was worse than that seen in a kel1Δ mutant or in a wild-type cell expressing PKC1-R398P (Table IV). Matings between kel1Δ mutants and wild-type partners yielded ~30% prezygotes, whereas the kel1Δ PKC1-R398P strain exhibited 79% prezygotes, higher than that seen with wild-type strains carrying the PKC1-R398P allele (48%). These data suggest that Kel1p is not the sole target of Pkc1p. Kel1p could be one of several Pkc1p targets or could function upstream or parallel to Pkc1p to promote cell fusion.

To examine the relationship between Kel1p and Kel2p and the Pkc1p pathway during vegetative growth, we analyzed the phenotype of strains lacking MPK1 and KEL1 or KEL2. MPK1 encodes a MAP kinase that appears to function downstream of Pkc1p (Lee and Levin, 1992; Ka-
mada et al., 1995). mpk1Δ mutants grow slowly at 34.5°C and fail to grow at 37°C; the growth defect at high temperature is suppressed by 1 M sorbitol. To determine whether loss of KEL1 or KEL2 affects growth of an mpk1Δ mutant, kellΔ mpk1Δ and ke12Δ mpk1Δ double mutant strains were constructed (see Materials and Methods). All 18 kellΔ mpk1Δ and 13 ke12Δ mpk1Δ double mutants grew more poorly than mpk1Δ single mutants at 34.5°C (Fig. 7 A), and the growth defect was suppressed by 1 M sorbitol (Fig. 7 B). We conclude that loss of KEL1 or KEL2 exacerbates the growth defect of mpk1Δ mutants.

Discussion

Identification and Analysis of Kel1p and Kel2p

We identified a novel gene, KEL1, whose overexpression can partially relieve the defect in cell fusion caused by PKC1-R398P. We found that overexpression of Kel1p could partially reduce the defect in cell fusion exhibited by spa2Δ and fps1Δ mutants. Kel1p localizes to the region of the cell where fusion is initiated during mating and to regions of polarized growth in vegetative cells. Mutants lacking Kel1p exhibit defects in cell fusion that are exacerbated by loss of FUS1, FUS2, or FPS1, or by expression of PKC1-R398P. kellΔ mutants are also elongated and heterogeneous in shape, indicating that Kel1p has a role not only in cell fusion but also in morphogenesis.

KEL1 and KEL2 are present in duplicated regions of the genome, encoding proteins that are approximately 44% identical. The function of Kel2p is related to that of Kel1p in several respects: overexpression of Kel2p suppresses the mating defect caused by an activated form of Pkc1p, localization of Kel2p is indistinguishable from that of Kel1p, and Kel2p is in a complex with Kel1p. In addition, when Kel1p or Kel2p are overexpressed, a fraction of cells display an aberrant morphology that is strikingly similar in both cases. Despite their considerable homology, Kel1p and Kel2p exhibit some differences. First, Kel2p is unable to localize in the absence of Kel1p, suggesting that Kel1p, but not Kel2p, can interact with at least one factor at the cell cortex. Second, only kellΔ mutants have detectable phenotypes. In an otherwise wild-type cell, Kel2p is not required for cell fusion or normal cellular morphology, nor does loss of KEL2 significantly affect the fusion ability of fus1Δ, fus2Δ, fps1Δ, or PKC1-R398P mutants. Kel1p and Kel2p may have diverged such that Kel2p does not play a role in these processes. Another possibility is that Kel1p and Kel2p have a similar function but that Kel1p is the major contributor. Coimmunoprecipitation and two-hybrid analyses demonstrate that Kel1p can associate with itself and with Kel2p (Fig. 5). If there are distinct Kel1p–Kel1p and Kel1p–Kel2p complexes, then loss of Kel1p would disrupt both types of complexes. In the absence of Kel2p, Kel1p–Kel1p complexes would remain, perhaps explaining the lesser role that Kel2p plays in cell fusion and morphogenesis.

Kel1p and Kel2p Are Members of the Kelch Family of Proteins

Kelch repeats, a motif of approximately fifty amino acids, are found in two to seven copies in proteins from diverse organisms including poxviruses, Drosophila, C. elegans, and mouse. Based upon sequence similarity to a superfamily of proteins that includes galactose oxidase (Ito et al., 1994) and neuraminidase (Varghese and Colman, 1991), these repeats are thought to form the blades of a β-propeller structure (Bork and Doolittle, 1994). Several kelch-containing proteins are implicated in actin interactions. For example, the Drosophila protein Kelch localizes to ring canals, actin-containing structures that separate nurse cells in the developing egg chamber. In the absence of Kelch, actin in the ring canals becomes disorganized (Xue and Cooley, 1993; Robinson and Cooley, 1997). The Limulus protein α-scrin bundles actin filaments in the acrosomal process of sperm, and is thought to be important at fertilization when the filaments undergo a conformational change (Sanders et al., 1996). Additional kelch-containing proteins implicated in actin interactions include the actin-fragmin kinase from Physarum (Eichinger et al., 1996), SPE-26 from C. elegans (Varkey et al., 1995), and ENC-1 from the mammalian nervous system (Hernandez et al., 1997).

It is not clear whether all kelch domains mediate interaction with actin. β-scrin from Limulus and calcin from bovine sperm are thought to be localized to regions of the cell that lack actin structures (Way et al., 1995; von Bulow et al., 1995). Tea1p, the closest homologue to Kel1p and Kel2p, influences microtubule dynamics rather than actin dynamics and is required in S. pombe cells for proper rod-like morphology (Mata and Nurse, 1997). In the absence of Tea1p or when Tea1p is overexpressed, cells exhibit bent and T-shaped morphologies. Kel1p is also required for proper cell shape, but there is little evidence in S. cerevisiae that microtubules play a role in morphology, whereas actin is thought to be important (for review see Botstein et al., 1997). Hence, it is currently unclear whether Kel1p interacts with the actin or microtubule cytoskeleton, or whether it is involved in some other process.

Relationship of Kel1p to the Pkc1p Pathway

The observation that overexpression of Kel1p can partially reverse the fusion defect caused by hyperactive Pkc1p suggests that Kel1p functions in opposition to Pkc1p to activate cell fusion, perhaps by acting upstream of Pkc1p (where it might affect Rho1p or other potential regulators of Pkc1p; see Kamada et al., 1996; Gray et al., 1997; Zarzov et al., 1996) or in parallel to the Pkc1 pathway. Kel1p also plays a role in vegetative cells that has some functional connection to the Pkc1 pathway. A role in vegetative cells is inferred from the observation that loss of either KEL1 or KEL2 exacerbates the growth defect of mpk1Δ mutants, which is suppressed by 1 M sorbitol. Formally, these results indicate that both Kel1p (or Kel2p) and Mpk1p promote cell integrity. Although various explanations are possible for the reduced viability of the mpk1Δ kellΔ and mpk1Δ ke12Δ strains, one possibility is that cells respond to loss of KEL1 or KEL2 by increasing Mpk1p activity, which is necessary to maintain cell integrity under these conditions. A notable feature of this explanation is that Kel1p would act in vegetative cells as it...
is proposed to act in mating cells, antagonistically to the Pkc1p pathway.

Toda et al. (1993) made the striking observation that overexpression of *pkc2*”, which codes for a Pkc1-like protein of *S. pombe*, results in production of branched cells similar to those seen in *teal* mutants (Mata and Nurse, 1997). This relationship is analogous to what we have observed for cell fusion in budding yeast: hyperactivation of protein kinase C leads to a phenotype similar to that due to loss of a kelch protein (Kel1p). Kelch proteins in other organisms may also function along with protein kinase C pathways. The relationship between other kelch proteins and protein kinase C could be explored in organisms without facile genetics using dominant negative forms of the kelch proteins and dominant activated forms of protein kinase C.

The Role of Kel1p in Cell Fusion

Kel1p belongs to a class of proteins including Spa2p and Fps1p that is required for cell fusion during mating (Dorer et al., 1997; Gammie et al., 1998; Philips and Herskowitz, 1997) and that also functions during vegetative growth for morphogenesis and cell integrity (Snyder, 1989; Snyder et al., 1991; Evangelista et al., 1997; Luyten et al., 1995). The defect in cell fusion exhibited by mutants lacking Fps1p, Spa2p, or Kel1p could result from increased activity of the Pkc1p pathway. Our observation that overexpression of *KEL1* partially restored mating ability to spa2Δ, fps1Δ, and *PKC1-R398P* mutants raises the possibility that spa2Δ and fps1Δ mutants have elevated Pkc1p activity, which may account for their defect in cell fusion. Additional data support this possibility. In particular, we have proposed earlier that the fusion defect of fps1Δ mutants results from high levels of intracellular glycerol, which activates the Pkc1p pathway (Philips and Herskowitz, 1997). Several observations suggest that the Pkc1p pathway may be activated in spa2Δ and kel1Δ mutants as well. Loss of Spa2p results in increased phosphorylation of Swi6p (Sheu et al., 1998), an apparent substrate of Mpk1p (Madden et al., 1997). Spa2p exhibits two-hybrid interactions with components of the Pkc1p pathway (Mkk1p/Mkk2p; Sheu et al., 1998). Loss of Spa2p as well as Fps1p or Kel1p exacerbates the growth defect of mutants defective in the Mpk1p MAP kinase pathway (Costigan et al., 1992; Philips and Herskowitz, 1997; Fig. 7 A). These observations lead us to suggest that Spa2p, Kel1p, and Fps1p negatively regulate Pkc1p activity. In their absence, we propose that the Pkc1p pathway becomes activated, which may be essential for cells to remain viable and which leads to inhibition of cell fusion during mating. We suggest that during mating of wild-type cells, Pkc1p monitors the osmotic and morphological integrity of the cell. If cells are not osmotically stable, as in fps1Δ mutants, or exhibit disrupted morphology, as in spa2Δ and kel1Δ mutants, Pkc1p inhibits cell fusion.

It is also possible that Kel1p does not act by inhibiting the Pkc1p pathway but may act, for example, on the actin cytoskeleton. Kel1p, as well as proteins such as Spa2p and Bni1p, might be important for directing or maintaining vesicles at a cell fusion zone through interactions with the cytoskeleton (Evangelista et al., 1997; Gehring and Snyder, 1990; Sheu et al., 1998). Consistent with such a possibility, EM analysis of mating cells shows the presence of clustered vesicles at the zone of cell fusion (Osumi et al., 1974; Gammie et al., 1998), which in the absence of Spa2p are more dispersed than in wild-type strains (Gammie et al., 1998). Cytoskeletal components play a possible role in myoblast fusion as well. In *Drosophila*, mutants defective in *myoblast city* (*mbc*) exhibit defects in cytoskeletal organization and myoblast fusion (Rushton et al., 1995; Erickson et al., 1997), failing to localize paired vesicles to the site of cell fusion (Doberstein et al., 1997). Similarly, overexpression of mutant forms of Drac1, a small GTPase known to affect the actin cytoskeleton, disrupts myoblast fusion and morphology (Luo et al., 1994; Doberstein et al., 1997). In vertebrates, cytochalasin B, which disrupts actin filaments, interferes with myoblast fusion (Sanger et al., 1971). It is unclear in myoblast fusion, as in yeast fusion, exactly what role the cytoskeleton plays in the fusion process. As more components become identified, we are in a better position to determine how proteins involved in cell fusion interact with cytoskeletal machinery, and the ways in which the protein kinase C pathway governs fusion.

We thank M. Peter and members of our laboratory, in particular L. Huang, M. Maxon, S. O’Rourke, and R. Tabtiang, for assistance and valuable discussion. We thank R. Tabtiang, A. Gammie, M. Maxon, M. Peter, S. O’Rourke, and L. Huang for helpful comments on the manuscript. We thank Y. Takai and E. O’Shea for plasmids, P. O’Farrell for anti-GFP antibodies, Chris Botka and Herve Recipon for assistance with sequence analysis, and J. Carminati, A. Gammie, V. Brizzio, and M. Snyder for communicating results before publication.

This work was supported by Research and Program Project Grants from the National Institutes of Health to I. Herskowitz. J. Philips was supported by the Julius Krevans Graduate Research Fellowship and by the Sussman Fund, supplemented by the Herbert W. Boyer Fund.

Received for publication 10 March 1998 and in revised form 2 September 1998.

References

Adames, N., K. Blundell, M.N. Ashby, and C. Boone. 1995. Role of yeast insulin-degrading enzyme homologues in propheromone processing and bud site selection. *Science*. 270:464–467.

Alpay, F. Cao, and N. Klocner. 1987. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. *Genetics*. 116:541–545.

Bardwell, J., J.G. Cook, C.J. Inouye, and J. Thorner. 1994. Signal propagation and regulation in the mating pheromone response pathway of the yeast *Saccharomyces cerevisiae*. *Dev. Biol*. 166:363–379.

Bork, P., and R.F. Doolittle. 1994. *Drosophila* kelch motif is derived from a common enzyme fold. *J. Mol. Biol*. 236:1277–1282.

Botstein, D., D. Amberg, and J. Mulholland. 1997. The yeast cytoskeleton. In *The Molecular and Cellular Biology of the Yeast *Saccharomyces*: Cell Cycle and Cell Biology. J.R. Pringle, J.R. Broach, and E.W. Jones, editors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 1–90.

Bricio, V., A.E. Gammie, G. Nijbroek, S. Michaelis, and M.D. Rose. 1996. Cell fusion during yeast mating requires high levels of a-factor mating pheromone. *J. Cell Biol*. 135:1727–1740.

Brizzio, V., A.E. Gammie, and M.D. Rose. 1998. Rvs161p interacts with Fus2p to promote cell fusion in *Saccharomyces cerevisiae*. *J. Cell Biol*. 141:567–584.

Bucher, B.M., and B. Errede. 1994. Cooperation of the mating and cell integrity mitogen-activated protein kinase pathways in *Saccharomyces cerevisiae*. *Mol. Cell. Biol*. 15:6517–6525.

Cormack, B.P., R.H. Valdivia, and S. Falkow. 1996. FACS-optimized mutants of the green fluorescent protein (GFP). *Gene*. 173:33–38.

Costigan, C., S. Gehring, and M. Snyder. 1992. A synthetic lethal screen identifies *SLK1*, a novel protein kinase homologue implicated in yeast cell morphogenesis and cell growth. *Mol. Cell. Biol*. 12:1162–1178.

Cross, F.R., and A.H. Tinkelenberg. 1991. A potential positive feedback loop controlling *CLN1* and *CLN2* gene expression at the start of the yeast cell cycle. *Cell*. 65:875–883.

Davenport, K.R., M. Sohaskey, Y. Kamada, E.D. Levin, and M.C. Gustin. 1995.

Philips and Herskowitz *Kel1p and Kel2p in Yeast*
sion during yeast conjugation: evidence for a pheromone-induced surface protein. Mol. Cell Biol. 7:2316–2328.

Varghese, J.N., and P.M. Colman. 1991. Three-dimensional structure of the neuraminidase of influenza virus A/Tokyo/3/67 at 2.2 Å resolution. J. Mol. Biol. 221:473–486.

Varkey, J.P., P.J. Muhlrad, A.N. Minniti, B. Do, and S. Ward. 1995. The Caenorhabditis elegans spe-26 gene is necessary to form spermatids and encodes a protein similar to the actin-associated proteins kelch and scruin. Genes Dev. 9:1074–1086.

von Bulow, M., H. Head, H. Hess, and W.W. Franke. 1995. Molecular nature of calcin, a major basic protein of the mammalian sperm head cytoskeleton.

Exp. Cell. Res. 219:407–413.

Way, M., M. Sanders, M. Chafel, Y.H. Tu, A. Knight, and P. Matsudaira. 1995. β-Scruin, a homologue of the actin crosslinking protein scruin, is localized to the acrosomal vesicle of Limulus sperm. J. Cell. Sci. 108:3155–3162.

Wolfe, K., and D. Shields. 1998. Yeast Gene Duplications. http://acer.gen.tcd.ie/zkhwolfe/yeast/topmenu.html

Xue, F., and L. Cooley. 1993. kelch encodes a component of intercellular bridges in Drosophila egg chambers. Cell. 72:681–693.

Zarzov, P., C. Mazzoni, and C. Mann. 1996. The SLT2 (MPK1) MAP kinase is activated during periods of polarized cell growth in yeast. EMBO (Eur. Mol. Biol. Organ.) J. 15:83–91.