Expression of an activated extracellular signal-regulated kinase 1 (ERK1) construct in yeast cells was used to examine the conservation of function among mitogen-activated protein (MAP) kinases. Sequence alignment of the human MAP kinase ERK1 with all Saccharomyces cerevisiae kinases reveals a particularly strong kinship with Kss1p (invasive growth promoting MAP kinase), Fus3p (pheromone response MAP/ERK kinase), and Mpk1p (cell wall remodeling MAP kinase). A fusion protein of constitutively active human MAP kinase ERK1 (MEK) and human ERK1 was introduced under regulated expression into yeast cells. The fusion protein (MEK/ERK) induced a filamentation response element promoter and led to a growth retardation effect concomitant with a morphological change resulting in elongated cells, bipolar budding, and multicell chains. Induction of filamentous growth was also observed for diploid cells following MEK/ERK expression in liquid culture. Neither haploids nor diploids, however, showed marked penetration of agar medium. These effects could be triggered by either moderate MEK/ERK expression at 37 °C or by high level MEK/ERK expression at 30 °C. The combination of high level MEK/ERK expression and 37 °C resulted in cell death. The deleterious effects of MEK/ERK expression and high temperature were significantly mitigated by 1 M sorbitol, which also enhanced the filamentous phenotype. MEK/ERK was able to constitutively activate a cell wall maintenance reporter gene, suggesting misregulation of this pathway. In contrast, MEK/ERK effectively blocked expression from a pheromone-responsive element promoter and inhibited mating. These results are consistent with MEK/ERK promoting filamentous growth and altering the cell wall through its ability to partially mimic Kss1p and stimulate a pathway normally controlled by Mpk1p, while appearing to inhibit the normal functioning of the structurally related yeast MAP kinase Fus3p.

Human ERK1 Induces Filamentous Growth and Cell Wall Remodeling Pathways in Saccharomyces cerevisiae*

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Mitogen-activated protein (MAP) kinases constitute a family of enzymes that control a variety of functions in eukaryotic cells. In response to an extracellular stimulus, particular MAP kinases become activated following the sequential triggering of upstream kinases, producing a kinase cascade. This results in the phosphorylation of substrates involved in both short term and long term (e.g. transcription-mediated) cell changes. As their name implies, activation of some MAP kinases can lead to a proliferative response. However, it is now clear that members of this family of kinases can initiate a variety of other cell fate pathways, such as differentiation and/or cell cycle arrest. In some cases, the activation of a given MAP kinase can lead to divergent cell fates depending upon the initiating stimulus or the cell type (1, 2). The regulation of MAP kinases and their signaling specificity are controlled at multiple levels (reviewed in Refs. 3 and 4). The association of some MAP kinases and upstream cascade components with scaffold proteins appears to be one way of orchestrating both incoming activation signals and outgoing substrate phosphorylation signals (reviewed in Ref. 5). Subcellular localization is another point that is utilized for regulating the accessibility of MAP kinases to their substrates. In particular, most MAP kinases have both cytoplasmic and nuclear substrates, necessitating the use of nuclear import mechanisms to direct signaling (6). Signal attenuation through the intervention of appropriate phosphatases (reviewed in Ref. 7) and feedback phosphorylation (8) are also involved. Incorporated within these regulatory mechanisms is the ability to modulate both the intensity and duration of phosphorylation signals, directly influencing the cell response (1). Once a MAP kinase is activated and properly localized, the final outcome is presumably dictated by substrate availability in a given cell type and by the intrinsic substrate specificity of each MAP kinase.

As with other isozyme families, it is likely that the striking levels of sequence identity among the MAP kinases reflects both an evolutionary kinship of the genes encoding these enzymes and a structural constraint dictated by a shared catalytic mechanism. Conversely, the sequence variations among MAP kinases presumably highlight residues not strictly required for core enzyme function and should include determinants of isozyme-specific properties, such as substrate preference.

The yeast Saccharomyces cerevisiae encodes six MAP kinases (9) that share extensive sequence identity among themselves and with mammalian MAP kinases. For most of the yeast MAP kinase enzymes, activating extracellular stimuli, upstream kinase cascades, and phenotypic outcomes have been characterized (reviewed in Ref. 10). In particular, Fus3p and Kss1p have been studied in great detail and have served as paradigms for the study of MAP kinase regulation and function. In wild type cells, Fus3p responds to pheromone binding, and its activation produces a mating response that includes adoption of an elongated morphology (shmoo). Kss1p acts to control invasive (filamentous) growth, which results from dif-
ferent cues in haploid and diploid cells. And Kss1p has been implicated in the maintenance of cell wall integrity during vegetative growth (11). The Fus3 and Kss1 proteins share striking sequence and function relatedness (12, 13). Both Fus3p and Kss1p respond to the same upstream activating kinases (Ste11p and Ste7p), and gene deletion experiments have indicated that Kss1p can functionally substitute for Fus3p in the pheromone response, although at lower efficiency (12, 14). In addition, both Fus3p and Kss1p activate promoters that utilize the Ste12p transcription activator. Pathway discrimination is achieved in part through the intervention of the scaffold protein Ste5p, which preferentially recruits Fus3p into a pheromone-responsive kinase cascade (5) and through the intervention of Ste12p-collaborating transcription factors, such as Tec1p, which is believed to be filamentous growth-specific. The distinct properties of Fus3p and Kss1p are also likely to reflect differences in substrate specificity dictated by sequence variation.

S. cerevisiae haploids normally propagate as spherical, axial budding, individual cells in culture. Filamentous growth represents a differentiated state that requires the coordinated induction of cell elongation, bipolar budding, incomplete cell separation, and invasion (e.g. of agar medium). Similar changes can occur in diploid cells. Kss1p is a principal regulator controlling the switch to filamentous growth in both haploids and diploids (12, 15). These phenotypes also result in the remodeling of the cellular cytoskeleton and the cell wall and are likely mediated by multiple pathways (16–18).

A key regulator of cell wall integrity is the MAP kinase Mpk1p. The activity of this kinase is itself controlled by the upstream kinases Mkk1/2p and Bck1p. This MAP kinase cascade is regulated by Pck1p, which appears to respond to upstream signaling from Rho1p in response to a membrane localized sensor of cell integrity encoded by the WSC1/HC577/SLG1 gene (19–22). This pathway must also be recruited for the organized remodeling of the cell wall in the transition to maturing or filamentous morphologies (23–25). Hallmarks of disruption in this signaling pathway include cell lysis that is exacerbated by high temperature and mitigated by isosomic growth media (e.g. media with 1 M sorbitol) and by heightened sensitivity to caffeine (20, 26, 27).

We used inducible expression of an activated human ERK1 in yeast cells to examine issues of MAP kinase substrate specificity controlling cellular responses. Primary sequence alignments have previously suggested a close relationship between mammalian ERK1 and particular yeast MAP kinases. We present experimental data and a modified alignment approach to address structure/function conservation.

Changes in cell morphology and specific reporter gene activation reported here suggest that human ERK1 functions as an activator of filamentous morphology and cell wall remodeling pathways normally regulated by the yeast kinases Kss1p and Mpk1p and inhibits signals normally channeled through the highly related kinase Fus3p. The inducible system described here may also prove useful for the isolation of human MAP kinase interaction partners and regulators.

**EXPERIMENTAL PROCEDURES**

**Strains and Growth Assays**—Escherichia coli strain DH5α was used for the construction and propagation of plasmids. Yeast strains used in this work are listed in Table I. Transformations were performed using a modified lithium acetate method (28). Cells expressing MEK/ERK or a control plasmid (pYH1) were grown in synthetic complete medium without uracil or leucine, unless otherwise indicated. Growth assays were carried out by inoculating 10⁶ cells from a log phase culture into 10 ml of selective medium with or without Cu²⁺ (0.5 mM) or sorbitol (1 M) and at 30 or 37 °C. Quantitative matings were carried out essentially as described previously (29) using 10⁶ tester cells mixed with 10⁷ partner cells in YPD diluted 10-fold with water prior to plating on synthetic complete-histidine medium. Cells were examined using a Nikon Diaphot 200 with a ×100 objective and a phase condenser.

**Plasmas**—The MEK/ERK plasmid was constructed from human MEK1 and human ERK1 clones generously provided by Natalie Ahn and Melanie Cobb, respectively. The constitutively active mutant MEK1 (AN5/S218/S222DD) was polymersase chain reaction-amplified with the primers TCAGGTCGACCGGAGTTGGAAGCGCGTTA and TACGAGGTGTCAGGCCGAGCCTGTTTTTA and initially cloned into the SaII and HindIII sites of pBlueScript KS (Stratagene). This construct (pKS-MEK/ERK) was then digested with Nhel and NotI followed by ligation with the Nhel to NotI ERK1 fragment of pCEP4Erk1. The MEK1/ERK1 fusion fragment was then released with SaI and NotI and cloned directly into pYH1, a form of pYEP (AMRAD Biotech) that had been previously modified to change the unique EcoRI site to NotI and to include an HA epitope sequence between the BamHI and SaI sites. To create the MEK1 only construct, the pKS-MEK/ERK plasmid was cut with Nhel and NotI to release the ERK1 sequences then ligated in the presence of adaptor oligonucleotides. The resulting SaI to NotI fragment of MEK1 was then moved to the modified pYEX described above. Similarly, to make the only construct, MEK1 sequences were released from the fusion construct by digestion with SaI plus Nhel followed by ligation in the presence of appropriate adaptor oligonucleotides. The PRE(FUS1) and FRY(ty1) reporter constructs were provided by the laboratory of Dr. Gerald Fink (Massachusetts Institute of Technology, Cambridge, MA). The FK52 reporter was provided with HA monoclonal antibody (12CA5 hybridoma, Baltimore, MD). All three reporter plasmids originally contained URA3 marker genes, and each was changed to HIS3 by homologous recombination.

**Immunoblots, Protein Purification, and Kinases Assays**—HA-MEK/ERK fusion protein was expressed in SP1. Cultures were grown to logarithmic phase and induced with CuSO₄ to a final concentration of 0.5 mM for 3 h. Cultures were resuspended in phosphate-buffered saline phosphate, peptatin, and phenylmethylsulfonyl fluoride. They were lysed as described previously (28). 50 μg of total extract was loaded on a gel and transferred to nitrocellulose. Membrane was probed with HA monoclonal antibody (12CA5, 1 μl of ascites from 12CA5 hybridoma, Baltimore, MD). All three reporter plasmids originally contained URA3 marker genes, and each was changed to HIS3 by homologous recombination.

**Gene Expression Assays**—Liquid β-galactosidase assays for PRE and FRE reporters were performed and quantified by established techniques (31). Liquid β-galactosidase assays for the FK52 reporter were performed by another method (32) due to the difficulty of lysing cells grown at 39 °C. The enzyme activity units for each method are distinct.

**Liquid β-Galactosidase Assays**—β-Galactosidase activities were assayed in triplicate in 20 μl of reaction mixture containing 40 μM of 1-methyl-β-D-thiogalactopyranoside and 100 μM of nitrophenyl-β-D-galactopyranoside per ml of liquid culture. Acceptable variability is ±25% at the 95% confidence level. Assays were initiated by the addition of 100 μM of 1-methyl-β-D-thiogalactopyranoside. Reactions were allowed to proceed for 30 min at 30 °C with shaking. Reactions were stopped by the addition of 50 μl of 1 N HCl, and 100 μl of 1 M sodium citrate, pH 5.0 was added to stop the reaction. The reactions were filtered through 0.45 μm filter paper, and 100 μl of the sample was added to 5 ml of 4-methylumbelliferyl-β-D-galactopyranoside in 100 mM sodium citrate buffer, pH 5.0, 0.5 mM β-mercaptoethanol, and 100 μg of toluene. The reactions were allowed to proceed for 30 min at 30 °C, and the products were separated by SDS-polyacrylamide gel electrophoresis.

**Results**—Liquid β-galactosidase assays for PRE and FRE reporters were performed and quantified by established techniques (31). Liquid β-galactosidase assays for the FK52 reporter were performed by another method (32) due to the difficulty of lysing cells grown at 39 °C. The enzyme activity units for each method are distinct and not comparable.
This analysis is an extension of the previously described evolutionary construction and searches were run locally using the PFTOOLS package. Relatedness matrices were obtained by applying ClustalW (33). Generalized profile sequence alignments—All data base searches were performed with version 2.0. Evolutionary surface patch analysis was used to identify functional surface patches in ERK1 and the yeast MAP kinases. In relatedness between sequences A and B can be viewed as a minimum conservation difference score between two data sets (including or excluding B). A shift in local sequence similarities was used to identify regions of the protein in which the “local phylogeny” differs from that of the full sequence. The contribution of specific proteins to this “shift score” can be evaluated as described above for the conservation difference score. This analysis is an extension of the previously described evolutionary tracing analysis (34, 35) and will be discussed in detail elsewhere.2

RESULTS

Constitutively Active Human ERK1 Causes Growth Arrest in Yeast—We created an active MAP kinase by connecting human ERK1 sequences to a constitutively active mutant form of human MEK1 (37). The resulting fusion protein, MEK/ERK, was also expected to show constitutive ERK1 kinase activity resulting from the steady state phosphorylation of this enzyme. MEK/ERK, which carries an HA epitope tag, was detectable in yeast extracts and appeared to have approximately the predicted molecular weight (Fig. 1). The expression vector used included both URA3 and leu2d selectable markers and employed a CUP1 promoter for Cu2+-inducible expression. Growth in medium without leucine results in selection for high plasmid copy number and gave rise to a significant increase of protein levels, presumably due to basal expression from the CUP1 promoter. The addition of Cu2+ produced a further increase in the amount of protein (Fig. 1), that was maximal after 1–3 h (data not shown).

Enzymatic activity was demonstrated by kinase assays of immunoprecipitated MEK/ERK protein from yeast cell lysates. MEK/ERK immunoprecipitated from yeast showed autophosphorylation and was capable of phosphorylating purified mammalian ELK1 protein, a well characterized ERK1 substrate (Fig. 2). This was not true for activated MEK alone. Immunopurified ERK appeared to be phosphorylated in the kinase reaction, perhaps reflecting the presence of a co-purified endogenous yeast MEK-type kinase, and this resulted in relatively weak kinase activity for ELK1. However, a mixture of MEK and ERK extracts resulted in substantial ERK phosphorylation and high level ELK1 phosphorylation. These results confirmed that, when expressed in yeast, the combination of constitutively activated human MEK with wild type human ERK, whether separate or covalently attached as a fusion protein, still shows normal substrate reactivity. Indeed, a similar construct fusing MEK1 with ERK2 has been reported to function as a constitutively active kinase in mammalian cells (38).

MEK/ERK Causes Growth Retardation and Morphological Changes in Yeast—We examined the growth properties of haploid (3A) and diploid (3B) yeast cells expressing the MEK/ERK fusion and observed severe growth suppression. When grown at 30 °C, this phenotype was strongest following induction of the CUP1 promoter. Cells expressing MEK or ERK constructs alone had no discernible growth defect (data not shown).

The effect of elevated temperature on cell growth was also examined. High temperature is a well studied stress condition that triggers a variety of cell responses, including cell wall remodeling (21, 39). When cultured at 37 °C, cells expressing MEK/ERK showed a marked long term reduction in growth compared with cells expressing vector only or MEK/ERK cells grown at 30 °C (Fig. 3). In the presence of CuSO4, which induces higher levels of MEK/ERK expression, the negative ef-

Table II. Pheromone induction was assayed in W303-1A cells using pSB234, a 2-μ based PRE(FUS1)::lacZ reporter construct. This construct was transformed into yeast cells containing pYNH or pYME. Transformants were grown overnight in selective medium, switched to YPD, and treated with α-Factor (Sigma) to a final concentration of 2.5 μg/ml for 2.5 h. Cultures were then lysed and assayed. Similar results were obtained in the SP1 strain background. Filamentous induction was assayed in SP1 cells using a modified pLG669Z, a 2-μ based FRE(Ty1)::lacZ reporter construct. Similar results were obtained for this reporter in the W303-1A strain background. Induction of the cell wall remodeling pathway was assayed in SP1 cells using an FKS2(706)::lacZ reporter construct.

Sequence Alignments—All data base searches were performed with ClustalW (33). Generalized profile construction and searches were run locally using the PFTOOLS package, version 2.0. Evolutionary surface patch analysis was used to identify functional surface patches in ERK1 and the yeast MAP kinases. In short, conservation difference scores are calculated for windows of positions, with each window representing a profile of residues adjacent in three-dimensional space rather than primary sequence. Relatedness between sequences A and B can be viewed as a minimum conservation difference score between two data sets (including or excluding B). A shift in local sequence similarities was used to identify regions of the protein in which the “local phylogeny” differs from that of the full sequence. The contribution of specific proteins to this “shift score” can be evaluated as described above for the conservation difference score. This analysis is an extension of the previously described evolutionary tracing analysis (34, 35) and will be discussed in detail elsewhere.2

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effects of high temperature were exacerbated. Under these conditions, the MEK/ERK-expressing cells were unable to grow and showed no recovery after transfer to fresh medium without CuSO₄ at 30 °C (data not shown).

We next examined the effect of 1 M sorbitol on growth of MEK/ERK-expressing cells. This condition has been shown to rescue a variety of yeast cell wall integrity mutations, presumably by providing an isoosmotic environment in which cell wall defects can be tolerated. We observed that sorbitol rescued the growth defect of MEK/ERK-expressing cells with CuSO₄-induced levels at 30 °C as well as uninduced (basal) levels at 37 °C. The combined effects of high MEK/ERK expression and elevated temperature, however, could not be rescued by sorbitol (Fig. 3).

An examination of MEK/ERK-expressing cells under various growth conditions showed several morphological alterations (Fig. 4A). These included an elongated cell morphology and short linear chains indicative of polar cell division without separation. The changes were most pronounced when cells were grown in sorbitol and were enhanced by induction of high level MEK/ERK expression. These features are reminiscent of cells undergoing invasive growth, a filamentous phenotype normally controlled by an endogenous yeast kinase cascade utilizing the Kss1p MAP kinase.

Diploid cells can normally undergo a related type of filamentous growth producing pseudohyphae cells (40). In response to nitrogen starvation conditions, cells become elongated, switch to a unipolar budding pattern, do not fully separate leading to cell chains, and exhibit agar invasive properties. The switch to pseudohyphae growth, like filamentous growth in haploids, is controlled in part by Kss1p. We expressed MEK/ERK in diploid cells and observed a striking shift to predominantly elongated cells with a high proportion of cells found in short chains and clumps (Fig. 4A). It should be noted that this apparent induction of filamentous growth took place in liquid medium with normal levels of ammonium as nitrogen source, conditions not normally conducive to this growth conversion. As with haploids, 1 M sorbitol enhanced these effects, whereas elevated MEK/ERK expression appeared to produce a somewhat distorted cell shape.

**Fig. 3.** Growth suppression by MEK/ERK in haploid and diploid cells. A, the haploid strain SP1 was transformed with the indicated plasmid, and equal inoculums were cultured in selective medium (see under “Experimental Procedures”) with or without CuSO₄ and sorbitol. Parallel cultures at 30 °C and 37 °C were analyzed after 48 h growth. *A_600* is indicated at the left. Data are from a representative experiment. B, the diploid strain SP1/DC124 was transformed and treated as described above. Data presented are mean values from an experiment carried out in triplicate.
We also investigated the effects of elevated temperature on MEK/ERK-expressing cells. Growth at 37 °C produced an apparent heightening of the degree of morphological alterations (Fig. 4B). This was especially evident in cells that were not induced (recall that uninduced growth conditions gave rise to MEK/ERK expression levels only a few fold below what was seen for CuSO₄ treated cells (Fig. 1)). Incubation of cultures in CuSO₄ medium at 37 °C led to widespread cell disruption, although surviving cells had relatively minor alterations in shape (Fig. 4B).

Although they displayed the visible signs of conversion to a filamentous growth pattern, neither haploid nor diploid cells expressing MEK/ERK showed an enhanced capacity to invade agar plates.

**MEK/ERK Can Activate an Invasive Growth Promoter**—The transcriptional activation events triggered by invasive growth signals and Kss1p activation are mediated by a Ste12p/Tec1p transcription factor heterodimer binding at FREs located in the promoters of genes, the induction of which is required for a full invasive response (41–43). Cells expressing the MEK/ERK fusion protein showed induced levels of an FRE-driven lacZ reporter (Fig. 5A). When MEK/ERK expression was increased following copper induction (see Fig. 1 for relative expression levels), there was a further increase in reporter gene activity. The observation that induced (CuSO₄-treated) MEK/ERK expression showed both a higher level of FRE promoter induction (Fig. 5A) and a more pronounced filamentous-like morphology (Fig. 4) is consistent with a direct role for MEK/ERK-mediated transcription in this phenotype.

We considered whether MEK/ERK might work through direct phosphorylation of Ste12p. We were unable to detect MEK/ERK phosphorylation of either GST-Ste12p or GST-Far1p, known substrates of the yeast MAP kinases Kss1p and Fus3p (44, 45), using *in vitro* kinase assays (data not shown).

**Involvement of Endogenous Pathways**—We also examined the effect of MEK/ERK expression in cells mutant for Ste12p or...
Tec1p, proteins that regulate transcription from FRE promoters. Although induction of the FRE reporter construct was eliminated in the ste12 strain (Fig. 5), significant morphological changes still resulted following MEK/ERK expression (Fig. 4). Unlike wild type cells, however, the cell shape deviated considerably from classic filamentous morphology even in the presence of sorbitol. In addition, either CuSO₄ or 37 °C were required for any effect. These results suggest that some morphological alterations resulting from high level MEK/ERK expression are not strictly dependent on the multipurpose transcription factor Ste12p. Similar results were obtained in a tec1 strain expressing MEK/ERK (Fig. 4). These findings are consistent with a model that some aspects of the MEK/ERK expression phenotype involve the Ste12p-Tec1p heterodimer transcription factor working at FRE type promoters. However, because significant morphological changes are induced by MEK/ERK in the absence of either Ste12p or Tec1p, other factors must be mediating this response.

**MEK/ERK Disrupts Cell Wall Integrity**—ERK1 has been categorized within the same subgroup of MAP kinases as Kss1p, Fus1p, and Mpk1p (9, 46). Alignment of the full catalytic domain of the ERK1 protein (344 residues) with all six _S. cerevisiae_ MAP kinases showed a particularly strong relationship with Kss1p and Fus3p. When alignment analysis was

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**Fig. 5.** MEK/ERK expression differentially regulates MAP kinase pathway reporters. Wild type haploid cells (see under “Experimental Procedures”) transformed with vector or MEK/ERK constructs, together with a FRE::lacZ reporter (A), FKS2::lacZ reporter (B), or FRE::lacZ reporter (C), were grown under the indicated conditions and assayed for lacZ expression. Different lacZ units (A and C versus B) reflect different assay techniques used (see under “Experimental Procedures” and “Results”).
restricted to the 63-amino acid active site domain directly implicated in substrate specificity (including the P1, lip, and L13 regions) (47, 48), a close similarity between ERK1 and Mpk1p was also revealed (Fig. 6). The "docking site" of a MAP kinase is an important determinant for interaction with regulators and substrates (49). In this sequence, ERK1 shows clear relatedness to Kss1p, Fus3p, and Mpk1p. We also performed evolutionary surface patch analysis to identify amino acid positions associated with MAP kinase isozyme specificity. This method uses a reference protein structure (ERK1) to study conservation/divergence of neighbor residues that may not be contiguous in the primary sequence. The 63-amino acid active site domain showed the highest conservation scores among data sets including or excluding Kss1p and Mpk1p (data not shown). Shift scores were then used to highlight regions of divergent phylogeny that are dependent on Kss1p and Mpk1p inclusion in the data set. This revealed contiguous surface patches representing putative Kss1p/Mpk1p functional specificity regions that are also closely related to ERK1. These results support a structural basis for the observed Kss1p-like phenotypes resulting from ERK1 expression in yeast. They also suggested a possible functional relatedness between ERK1 and Mpk1p.

We therefore considered the possible involvement of cell wall integrity pathways in the phenotypes resulting from MEK/ERK expression. The observations that elevated temperature and 1 M sorbitol enhanced the filamentous morphology of MEK/ERK-expressing yeast cells suggested that the requisite remodeling of the cell wall was not taking place efficiently. Another hallmark of cell wall maintenance defects is heightened caffeine sensitivity (20, 26, 27). We observed a marked increase in caffeine sensitivity in MEK/ERK-expressing cells (Fig. 7), a result consistent with cell wall disruption but perhaps attributable to nonspecific effects of MEK/ERK expression. To examine this further, we utilized a reporter construct derived from the FKS2 (50) promoter known to be responsive to this pathway following activation by high temperature conditions (39). At 25 °C the expression of MEK/ERK produced a 4-fold increase in reporter gene activity (Fig. 5B). At elevated levels of MEK/ERK, there was a 20-fold difference, correlating MEK/ERK expression directly with activation of this promoter. Incubation of vector transformed cells at 39 °C resulted in a strong induction of the reporter, as expected. This level was not increased by MEK/ERK, suggesting that the conditions used may have yielded maximum reporter expression.

**MEK/ERK Expression Inhibits Mating**—In contrast to the FRE and FKS2 promoter activation results, we observed that the MEK/ERK fusion protein was unable to induce expression from the FUS1 promoter, which includes a pheromone response element (Fig. 5C). This reporter is strongly induced by treatment of cells with pheromone (α-factor), which initiates a kinase cascade that terminates with activation of the MAP kinase Fus3p. Expression of MEK/ERK had a potent inhibitory effect on the induction of this reporter by pheromone. The magnitude of the pheromone signal suppression, like those of the FRE and FKS2 promoter signal activation, was directly related to MEK/ERK expression levels. Expression of MEK alone or ERK1 alone, which is mostly inactive (Fig. 2), did not cause reduction in the pheromone-induced reporter signal (data not shown).

The mating competence of MEK/ERK-expressing cells was also severely compromised (Table II). MATα cells (SP1) expressing MEK/ERK showed a greater than 50-fold reduction in mating efficiency with a MATα tester strain (DC17). This effect was also seen when MEK/ERK was expressed in MATα cells (DC124) and the block was greater in matings between strains.

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**Fig. 6.** Sequence alignment of human ERK1 and the six *S. cerevisiae* MAP kinases (Kss1p, Fus3p, Mpk1p, Hog1p, Mlp1p, and Smk1p). The regions presented (catalytic core (top panel) and substrate interaction/docking domain (bottom panel)) were chosen based on minimal conservation difference scores. Columns indicate the "shift" or divergent phylogeny score uncorrected (white) or corrected (black) for surface exposure. Structural domains (L, loop; α, α-helix) are designated according to established nomenclature (47). Amino acid positions for each sequence are given at the left. Gaps are indicated by dashes. Black bars denote regions identified by this analysis as signature sequences for Kss1p and Mpk1p.
that each expressed MEK/ERK. An increase in MEK/ERK levels (CuSO₄ medium) did not lead to significant further decreases in mating efficiencies, however, suggesting that the expression levels without induction (Fig. 1) are sufficient for the full effect on mating, as assayed here. There was no detectable mating between MEK/ERK-expressing MATα cells and a MATα tester strain (DC14), indicating no loss in mating partner discrimination.

FIG. 7. MEK/ERK expression causes caffeine sensitivity, a cell wall defect-associated phenotype. Yeast cells (SP1) transformed with vector or MEK/ERK were streaked on selective media with or without caffeine (4 mM) and CuSO₄ (0.5 mM) as indicated, and incubated at 30 °C for 3 days.

DISCUSSION

The human ERK1 protein shares extensive sequence identity with all six MAP kinases from S. cerevisiae. Alignments focusing specifically on key structural elements involved in substrate interactions (catalytic core and docking region), together with surface patch analysis, placed ERK1 in a subgroup with Kss1p, Fus3p, and Mpk1p. Although previous studies have noted the close sequence relationship between ERK1 and Kss1p (46, 51, 52), this work represents a comprehensive analysis with all yeast MAP kinase sequences and reveals a functional correlation. Integration of experimental data and structure-based analysis, as described here, should aid in developing useful approaches to the study of isozymes in general and MAP kinases in particular.

Expression of MEK/ERK, a constitutively active kinase, triggered phenotypes reminiscent of filamentous growth, suggesting that MEK/ERK can at least partially mimic activated Kss1p. Similar changes were induced by MEK/ERK expression in diploid cells, in which filamentous growth normally requires nitrogen starvation. Because neither constitutively active MEK nor wild type ERK alone produced these results, we conclude that this filamentous behavior is dependent on the activity of ERK stimulated by constitutively active MEK.

Characterization of the observed phenotypes as filamentous is supported by the ability of MEK/ERK to induce an FRE reporter construct. This activity was absent in ste12 cells, consistent with the requirement for Ste12p to activate this promoter. However, the ste12 mutant cells expressing MEK/ERK still displayed some changes in morphology. This suggests the involvement of other pathways and is consistent with previous genetic analysis of the filamentous response (16). Indeed, ste12 mutants have a greatly reduced, but not absent, response to invasive growth signals (16, 53). In addition, filamentous growth induced by overexpression of Whi2p and Phd1p or by activation of PKA have also been shown to be mostly Ste12p independent (16, 18, 54). It should be noted that Ste12p, which is also a component of the mating signal pathway, is not essential for some pheromone-induced morphological changes (23). We observed that tec1 mutant cells showed similar abnormal morphologies in response to MEK/ERK expression. Tec1p partners with Ste12p in the control of at least some filamentous response element promoters (16, 41, 55, 56). The observation that ste12 and tec1 mutants respond similarly to MEK/ERK expression, with either mutation blocking the cell elongation seen in wild type cells, is consistent with the known properties of the Ste12p/Tec1p heterodimer. Indeed, these mutations appear to have similar, though not identical, effects on gene expression (57).

Kss1p and Fus3p have a special relationship; each is more akin to the other than to any of the remaining yeast MAP kinases, and each can influence responses normally controlled by the other. Kss1p, for example, can block the Fus3p induction of pheromone-responsive genes (58). This effect has been postulated to require the inactive form of Kss1p in the stabilization of PRE complexes (59). MEK/ERK can also block pheromone-responsive gene transcription as well as mating. Because a constitutively active construct was required, however, this inhibitory effect may indicate a different type of repression from that mediated by Kss1p.

The fact that MEK/ERK can activate an FRE-driven reporter but strongly inhibits a PRE-driven reporter may reflect both the influence of specific components (e.g., Tec1p) and differences in the context of Ste12p (59, 60) that alter its interaction with MEK/ERK. Also, the persistent expression of MEK/ERK (even without copper) might lead to down-regulation of pathway components.

Our data indicate that MEK/ERK expression results in disruption of cell wall integrity, a pathway regulated by Pck1p and the MAP kinase Mpk1p in response to stress and differentiation signals. Incubation at 37 °C, a condition known to induce cell wall remodeling, heightened both the growth retardation and morphological changes resulting from MEK/ERK expression. In addition, 1M sorbitol not only ameliorated the growth retardation resulting from MEK/ERK expression but also enhanced the filamentous-like phenotype. Taken together with the induction of the FKS2 reporter, these alterations strongly indicate cell wall disruptions resulting from activated ERK. The slow growth, heat sensitivity, and caffeine sensitivity phenotypes associated with MEK/ERK may result from overexpression of genes that are only transiently induced during a normal response to heat or the adoption of new morphologies in mating or a switch to invasive growth. Indeed, although most studies of this pathway have employed inactivating mutations, overactivation of the homologous pathway in Schizosaccharomyces pombe leads to aberrant cell morphology and cell growth defects (61, 62) similar to what we have described. Whether MEK/ERK works by recognizing and phosphorylating Mpk1p substrates or by acting, like Kss1p, through a parallel cell wall pathway that also regulates FKS2 expression in vegetative cells (11) is not yet clear.

Divergence between human ERK1 and yeast Kss1p should represent sequences not important for basic catalytic function. These would include amino acids required for interfacing with upstream activation or downstream signal attenuation proteins. Conversely, residues that are conserved between ERK1 and Kss1p should include those involved directly or indirectly in catalysis. Indeed, of the 12 residues found mutated in defective Kss1p alleles and predicted to play a direct role in catalytic function (12), all are identical in ERK1. Amino acids involved in the substrate specificity characteristics shared between ERK1 and either Kss1p or Mpk1p may also be conserved.
Despite the parallel between structural and functional relatedness it is clear that ERK1 can not provide complete functional redundancy with either Kss1p or Mpk1p. This is in part due to the unregulated activity of our constructs: ERK1 alone is inactive and unresponsive to endogenous yeast activators, and MEK/ERK is constitutively active and unresponsive to endogenous yeast attenuators. More fundamentally, the kinship of the enzyme with Kss1p and Mpk1p likely extends to only a subset of target proteins.

Our findings clearly demonstrate that MEK/ERK can induce multiple cytoplasmic and cytoskeletal alterations requisite for filamentous growth. MEK/ERK expression also results in aberrant activation of cell wall remodeling, a process that is normally regulated to accompany cell shape changes. The filamentous morphology enhancement observed in 1 M sorbitol indeed suggests that this growth program is principally dictated by cytoskeletal changes that are then accommodated by cell wall modifications. Similarly, hog1 and pbs2 mutants of yeast show phenome pathway activation and apparent mating projections, but only in the presence of 1 M sorbitol (63). MEK/ERK might act directly on Spa2p and/or SphIp, two regulators of polarized morphogenesis that interact with Mpk1p and its upstream activating kinases Mkk1p and Mkk2p (64, 65).

Numerous mammalian oncoproteins are known to activate MAP kinases, including ERK1 and ERK2. These signals have been directly associated with the induction of transformation and differentiation pathways (38), each of which involves extensive cytoskeletal remodeling and morphological changes, and ERK kinase pathways have been implicated in the control of metastasis (migration and tissue invasion) in tumor cells (36, 66). The inducible model system described here may provide the basis for genetic isolation of mammalian ERK1 regulators that could affect these functions.

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