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Genetics

A dominant truncation allele identifies a gene, *STE20*, that encodes a putative protein kinase necessary for mating in *Saccharomyces cerevisiae*

(pheromone response/signal transduction/sterile genes/mating response pathway)

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ABSTRACT **This work reports the identification, characterization, and nucleotide sequence of *STE20*, a newly discovered gene involved in the *Saccharomyces cerevisiae* mating response pathway, to date one of the best understood signal transduction pathways. *STE20* encodes a putative serine/threonine-specific protein kinase with a predicted molecular mass of 102 kDa. Its expression pattern is similar to that of several other protein kinases in the mating response pathway. Deletion of the kinase domain of *STE20* causes sterility in both haploid mating types. This sterility can be partially suppressed by high-level production of *STE12* but is not suppressible by high levels of *STE4* or a dominant *STE11* truncation allele. A truncation allele of *STE20* was isolated that can activate the mating response pathway in the absence of exogenous mating pheromone. This allele causes dominant growth arrest that cannot be suppressed by deletions of *STE4*, *STE5*, *STE7*, *STE11*, or *STE12*. The allele is able to suppress the mating defect of a strain in which the *STE20* kinase domain has been deleted, but not the mating defects of strains carrying mutations in *STE4*, *STE5*, *STE7*, *STE11*, or *STE12*.**

The budding yeast *Saccharomyces cerevisiae* can exist as either a haploid or a diploid. In the haploid state, there are two mating types, **a** and α, that secrete diffusible peptide pheromones, **a** and α factors. Exposure to pheromone from the opposite cell type effects changes that include cell-cycle growth arrest, morphological alterations ("shmoo" formation), cell–cell fusion, and, finally, nuclear fusion to form an **a**/α diploid. The pheromone response pathway responsible for this cascade of events represents perhaps one of the best understood signal transduction pathways to date (reviewed in ref. 1). Each cell type expresses at its surface a receptor for mating factor of the opposite cell type. Binding of pheromone activates a heterotrimeric guanine nucleotide binding protein (G protein). At least four protein kinases—products of the *STE7*, *STE11*, *KSS1*, and *FUS3* genes—are involved in transmitting the signal. The pathway culminates with activation of a transcription factor, the product of the *STE12* gene, that binds upstream of pheromone-inducible genes required for mating. These pheromone-inducible genes contain at least two *STE12* binding sites in their promoter regions (2, 3). One such gene, *FUS1*, whose product is required for cell–cell fusion, has four *STE12* binding sites (4, 5). Its transcription is very rapidly and strongly induced in response to pheromone (4, 5) and, consequently, *FUS1* transcription is used as a measure of primary intracellular response to pheromone. A *FUS1* promoter fusion to the *Escherichia coli lacZ* gene encoding β-galactosidase, whose activity can be monitored visually, is commonly used as a reporter for activation of the endogenous *FUS1* gene.

Interestingly, high-level production of some components can activate the pathway even in the absence of exogenous mating pheromone. *STE4*, the activating β subunit of the G protein, and *STE12*, the transcription factor, cause cell cycle arrest, shmoo formation, and increased transcription of pheromone-inducible genes when overexpressed from the strong *GAL1* promoter (6–9). Truncation of the *STE11* gene product causes similar phenotypes (10, 11). A library designed for high-level gene expression from a heterologous yeast promoter has recently been described (10). This library was screened for dominant activators of the yeast mating response pathway (10). This work reports the identification, characterization, and nucleotide sequence of a newly discovered gene, *STE20*, encoding a putative protein kinase, which was isolated from that screen.*

MATERIALS AND METHODS

Strains, Plasmids, and Media. CRY1, CRY2, and CRY3 are isogenic **a**, α, and **a**/α derivatives of W303-1A with the following relevant markers: *his3-15 leu2-3,112 ura3-1 trp1-1 ade2-1 can1-100* (R. Fuller, Stanford University). Isogenic strains lacking individual components of the mating response pathway were used for epistasis studies (11). Plasmids pL19 (8), pGU-STE11ΔN (11), and pGalSTE12 (11) were used for galactose-dependent overexpression of *STE4*, *STE11ΔN*, and *STE12*, respectively. Plasmid pUZ4 (11) was used as a reporter for *FUS1–lacZ* expression. Standard media for yeast and *E. coli* were used as described (12, 13).

Yeast Strain Construction. A *ste20* deletion/insertion mutation (*ste20-Δ1*) was created in diploid strain CRY3 by one-step gene disruption, replacing a 780-base-pair (bp) *Hin*dIII fragment of *STE20* with a 2212-bp *Xho* I/*Sal* I fragment containing the *LEU2* gene. Stable LEU^+ transformants were selected and shown to be heterozygous for the gene disruption marker by Southern hybridization analysis (14). Transformants were sporulated and tetrads dissected as described (12).

Assays of Pheromone Sensitivity. For short-term exposure assays, cells were grown in liquid medium to early logarithmic phase, pelleted, and resuspended in the same volume of fresh medium (pH 4.0) containing α factor (3 mg/ml) (Sigma). Samples were incubated at 30°C with shaking for 3 hr and then lightly sonicated and examined microscopically. For long-term exposure, a halo assay was used (15). Disks of Whatman 3MM paper containing 0.5, 1.0, 2.5, and 5.0 mg of α factor were placed on lawns of 2×10^5 logarithmically growing cells.

Mating Assays. Patch-mating and quantitative-mating assays were performed as described (15). For samples requiring

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Abbreviation: YPD, yeast extract/peptone/dextrose.
*The sequence reported in this paper has been deposited in the GenBank data base (accession no. L04655).

galactose induction, mating was allowed to occur for 6 hr on yeast extract/peptone/galactose rather than yeast extract/peptone/dextrose (YPD).

β-Galactosidase Assays. Strains containing pUZ4 were grown to midlogarithmic phase in SD-Trp (12). Some samples underwent a 3-hr exposure to α factor (3 mg/ml). Cells were harvested and assayed for β-galactosidase activity as described (10).

RNA Analysis. Cultures were grown to midlogarithmic phase at 30°C in YPD. Where indicated, samples underwent a 3-hr exposure to α factor (3 mg/ml) before harvesting. Total RNA was prepared and Northern analysis was performed according to standard methods (12, 13).

RESULTS

Isolation of a Gene Involved in Pheromone Response. Transcriptional activation of a *FUS1–lacZ* fusion was used to identify genes that, when highly expressed, promoted activation of *STE12*-dependent gene expression independent of exogenous mating pheromone (10). A regulatable genomic DNA expression library in which transcription can be driven from the strong, inducible yeast *GAL1* promoter was screened for dominant activators of mating response. Positive clones were partially sequenced. Several components of the mating response pathway were isolated, as were a number of other known genes that had not previously been implicated in mating response (10). In addition, a genomic DNA fragment was isolated whose sequence did not match any known gene. The clone containing this gene caused a 50-fold induction of *FUS1–lacZ* expression. Southern analysis of chromosomes separated by CHEF gel electrophoresis (Bio-Rad) maps this gene to chromosome VIII (data not shown).

Preliminary sequence data suggested that this clone might not contain a full-length gene. A continuous open reading frame extends to the 5′ end of the genomic DNA insert in this clone. The ATG nearest the vector-borne *GAL1* promoter is in-frame with this open reading frame, occurring 99 bases downstream from the point of fusion. Thus, translation putatively initiates at an internal in-frame ATG and results in production of a protein lacking its amino terminus. Northern analysis of strains containing this clone showed the presence of a plasmid-dependent, galactose-dependent message of ≈1300 bp, whereas preliminary Northern analysis of the endogenous message suggested a much larger transcript (data not shown).

In addition to its ability to stimulate *FUS1* transcription, expression of the truncated protein is deleterious to growth. No colonies formed when cells carrying the plasmid containing the truncation allele were grown on galactose medium (inducing conditions), whereas colonies grew up at wild-type rates on glucose medium (repressing conditions).

Disruption of the Pheromone Response Gene Causes Sterility. A disrupted allele of the gene was created by replacing a 780-bp *Hin*dIII fragment representing approximately half of the coding sequence present on the original clone with *LEU2*. One copy of the gene was disrupted in the diploid strain CRY3. The diploid was sporulated, and four viable spores were obtained from each tetrad. The meiotic progeny were tested for the ability to mate. In each tetrad, only two spores were capable of mating, and leucine prototrophy segregated $2^+:2^-$ with sterility. Therefore, the newly discovered gene has been named *STE20* and its disruption allele is *ste20-Δ1*. Both *MAT***a** and *MATα ste20-Δ1* spores were recovered, indicating that the effect was not mating-type specific (Fig. 1).

Quantitative mating assays were performed to assess the severity of the mating defect. Both **a** and α strains carrying a *ste20-Δ1* mutation mate at a frequency of $\approx 3 \times 10^{-7}$, similar to the mating frequencies of strains carrying mutations in other *STE* genes (16).

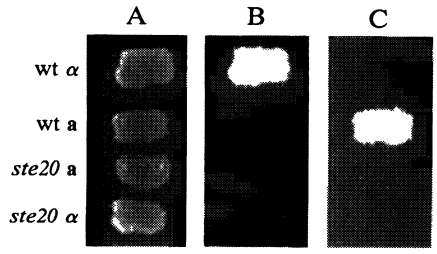


FIG. 1. Disruption of the kinase domain of *STE20* causes sterility in both **a** and α cells. Meiotic progeny of a diploid heterozygous for disruption of *STE20*. wt α, *MATα STE20*; wt **a**, *MAT***a** *STE20; ste20* **a**, *MAT***a** *ste20-Δ1; ste20* α, *MATα ste20-Δ1*. (*A*) Patches were grown on nonselective YPD plates, replica-plated to YPD plates containing lawns of **a** or α cells, allowed to mate for 4 hr, and then replica-plated to medium selective for diploids. (*B*) Cells mated to **a** tester cells. (*C*) Cells mated to α tester cells.

The *ste20-Δ1* strains were tested for growth on plates at a variety of temperatures (19°C, 30°C, and 37°C) and were found to grow at rates indistinguishable from congenic wild-type strains at all temperatures tested. Thus, the defect caused by *ste20-Δ1* appears to be specific for mating response.

Disruption of *STE20* Causes an Inability to Respond to Pheromone and Decreases Both Basal and Induced Levels of Pheromone-Dependent Gene Transcription. A hallmark of classical *ste* mutants is their inability to respond to mating pheromone (16). *ste20-Δ1* cells were tested for the ability to respond to α factor. Like other *ste* mutants, *MAT***a** *ste20-Δ1* strains were insensitive to pheromone. In liquid medium containing α factor, the mutant cells failed to arrest in G_1 and did not form shmoos under conditions in which wild-type cells did (data not shown). In halo assays, which measure the sensitivity of a strain to the growth inhibitory effects of mating factor, the mutant cells were unable to form a zone of inhibition around filters saturated with α factor, indicating that they were unable to arrest their growth in response to mating pheromone (Fig. 2). These results suggest that the mating defect caused by disruption of *STE20* occurs in the signal transduction cascade itself and not at some later point such as cell or nuclear fusion.

To test directly whether the *ste20-Δ1* mutation could affect pheromone-inducible gene transcription, the effects of the *ste20-Δ1* mutation on basal and pheromone-induced levels of *FUS1* transcription were examined. *STE12*-dependent activation of transcription was monitored with a β-galactosidase reporter plasmid, pUZ4 (11), containing the *E. coli lacZ* gene fused to a hybrid yeast promoter that makes β-galactosidase production pheromone inducible. Both basal and induced levels of *FUS1–lacZ* transcription were dramatically but not equally reduced in the mutant background (Table 1).

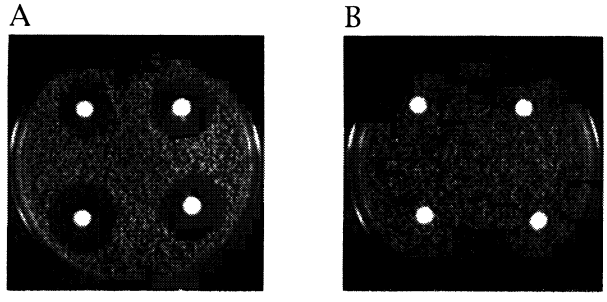


FIG. 2. Halo assay for pheromone responsiveness. Lawns of cells to be tested were overlaid in soft agar onto YPD plates. Filter disks contained (clockwise from top left) 0.5, 1.0, 2.5, and 5.0 mg of α factor. (*A*) Wild-type *STE20 MAT***a** strain. (*B*) *ste20-Δ1 MAT***a** strain.

Table 1. Effect of α factor on *FUS1–lacZ* activation

Strain	Units of β-galactosidase
Wild type induced with α factor	386
Wild type uninduced	9.4
ste20-Δ1 induced with α factor	3.2
ste20-Δ1 uninduced	0.8

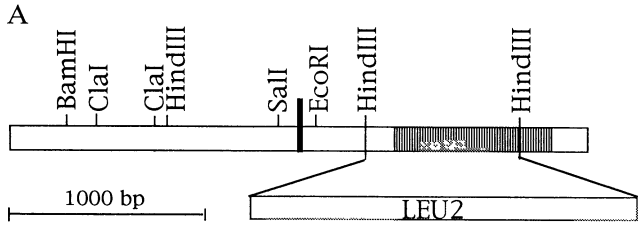
(Wild type induced)/(wild type basal) = 40; (*ste20Δ* induced)/(*ste20Δ* basal) = 4. Units of β-galactosidase are $1000(A_{420} - 1.75A_{550})$/time (min) per mg of protein in assay.

Cloning of *STE20*. The full-length *STE20* gene was cloned by complementation of the *ste20* mating defect. A *MAT***a** *ste20-Δ1* strain was transformed with a pYES-based *S. cerevisiae* genomic DNA library (10). Two clones that suppressed the *ste20-Δ1* mating defect were found among ≈50,000 URA$^+$ transformants screened. These clones had overlapping inserts of 4000–4500 bp that hybridized to a probe prepared from the originally isolated clone (pSTE20ΔN). Both clones contained >2000 bp of genomic DNA upstream of the 5′ end of the insert DNA in pSTE20ΔN.

***STE20* Encodes a Putative Serine/Threonine-Specific Protein Kinase.** Sequence analysis of the full-length *STE20* gene revealed an open reading frame encoding a protein of 939 amino acids (calculated size, 102 kDa) (Fig. 3). The predicted protein was compared with protein sequences in the Protein Identification Resource and Swiss-Prot data bases using BLAZE (D. L. Brutlag, personal communication) and was found to be similar to numerous protein kinases. The deduced amino acid sequence of the *STE20* gene contains each of the 15 invariant residues found in all protein kinases (17). The lysine at residue 741 in the protein kinase signature domain (subdomain VI) suggests that *STE20* has serine/threonine specificity (17).

The kinase domain of STE20, extending from amino acid 618 to amino acid 874, is 31% identical to the kinase domains of the *Drosophilia melanogaster* NinaC long and short protein kinases and the *S. cerevisiae* CDC15 and SLK1/BCK1/SSP31 protein kinases. Over the entire length of the protein, however, the *STE20* gene-encoded protein is <10% identical to any known protein. The predicted protein is not a member of the mitogen-activated protein kinase family of protein kinases as are KSS1 (18) and FUS3 (19) but is more similar to the other two kinases in the pheromone response pathway, STE11 (20) and STE7 (21) (26% identical to each in the kinase domain) (Fig. 4).

The 780-bp *Hin*dIII fragment deletion that was made to create *ste20-Δ1* removes amino acids 583–844, thus eliminating 90% of the kinase domain of the protein (Fig. 3*A*).



B

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                                                                                                                M  S  N  D  P     5
TCTGCTGTATCGGAACTACCAGACAAGGACAGTCTTGATAGCGGTATCAGCAATGACAATGAAAGGGCCATGGGCGGCAATGGCGATGGCGGCGATGGATTACGATTACCAAGGACCACT   240
S  A  V  S  E  L  P  D  K  D  S  L  D  S  G  I  S  N  D  N  E  R  A  M  G  G  N  G  D  G  G  D  G  L  R  L  P  R  T  T    45
GGAACTTTGAACGTCAATGCCTTACAAAAAGGCACTAATGCTGCCCATGAAGCTGGTGGATACAAATCCATGGATCCTGCGAAGAACGCGGAGACAACCAATGATGATGACAATAATGTC   360
G  T  L  N  V  N  A  L  Q  K  G  T  N  A  A  H  E  A  G  G  Y  K  S  M  D  P  A  K  N  A  E  T  T  N  D  D  D  N  N  V    85
GTTTCACTAGATGACCCTATTCAATTTACCCGGGTATCTTCCTCCTCTGTCATCAGTGGAATGTCTTCATCCATGAGTCCTCATTCTAACATCGATGAAACCAAATCTCTAGAAGCAGTC   480
V  S  L  D  D  P  I  Q  F  T  R  V  S  S  S  S  V  I  S  G  M  S  S  S  M  S  P  H  S  N  I  D  E  T  K  S  L  E  A  V   125
ACTCCAAACATAAATACCAGCAATATGACCCCGGATCATTCTGCTGACAACACATTTTCTACCATAAATGCGTCCGAGTCAGATCACCAGTTTAATGACACTTTACTATCAAAACTGTCG   600
T  P  N  I  N  T  S  N  M  T  P  D  H  S  A  D  N  T  F  S  T  I  N  A  S  E  S  D  H  Q  F  N  D  T  L  L  S  K  L  S   165
TTAACAGATTCTACAGAAACTATAGAAAATAACGCGACAGTGAAGCACCAGCAGCCAGTTGCATCTTCCACAGTAAACTCGAATAAGAGCTCCACTGATATACGAAGGGCTACACCAGTG   720
L  T  D  S  T  E  T  I  E  N  N  A  T  V  K  H  Q  Q  P  V  A  S  S  T  V  N  S  N  K  S  S  T  D  I  R  R  A  T  P  V   205
TCCACTCCCGTTATCTCTAAACCATCGATGACAACCACGCCAAGACAGATCAATTCAGCTTCCCATTCGCTTTCGAACCCTAAGCATAAGCAACATAAACCAAAAGTTAAACCGTCCAAG   840
S  T  P  V  I  S  K  P  S  M  T  T  T  P  R  Q  I  N  S  A  S  H  S  L  S  N  P  K  H  K  Q  H  K  P  K  V  K  P  S  K   245
CCTGAAGCAAAAAGTAAACCGGTTTCTGTGAAAAAAAGCTTTCCTTCGAAAAATCCTTTAAAAAACTCCTCTCCATCTAAAAAGCAAACAGAAAAATCGTATTATTCTTCCTCTTCGAAA   960
P  E  A  K  S  K  P  V  S  V  K  K  S  F  P  S  K  N  P  L  K  N  S  S  P  S  K  K  Q  T  E  K  S  Y  Y  S  S  S  S  K   285
AAAAGGAAAAGCGGTTCAAATAGTGGTACACTAAGAATGAAAGATGTCTTTACGTCCTTTGTACAGAATATAAAGAGAAATTCTCAGGATGATAAAAGGGCCTCATCGTCGTCCAATAAT  1080
K  R  K  S  G  S  N  S  G  T  L  R  M  K  D  V  F  T  S  F  V  Q  N  I  K  R  N  S  Q  D  D  K  R  A  S  S  S  S  N  N   325
TCTTCCTCATCTTCTATAACCACCGCTTTGAGGATATCTACGCCATACAATGCCAAGCATATCCACCATGTGGGCGTGGACTCCAAGACTGGTGAGTACACAGGTTTGCCGGAGGAATGG  1200
S  S  S  S  S  I  T  T  A  L  R  I  S  T  P  Y  N  A  K  H  I  H  H  V  G  V  D  S  K  T  G  E  Y  T  G  L  P  E  E  W   365
GAAAAATTGTTGACTTCTAGTGGTATTTCCAAAAGAGAACAACAGCAAAACATGCAAGCAGTCATGGATATTGTCAAATTCTATCAGGATGTCACGGAAACAAACGGTGAAGATAAAATG  1320
E  K  L  L  T  S  S  G  I  S  K  R  E  Q  Q  Q  N  M  Q  A  V  M  D  I  V  K  F  Y  Q  D  V  T  E  T  N  G  E  D  K  M   405
TTCAAGACTTTCAACACAACCACAGGATTGCCGGGAAGTCCTCAAGTTTCAACACCGCCTGCAAACTCATTCAATAAATTTCCTCCGTCGACAAGTGATTCGCACAATTACGGTTCCAGA  1440
F  K  T  F  N  T  T  T  G  L  P  G  S  P  Q  V  S  T  P  P  A  N  S  F  N  K  F  P  P  S  T  S  D  S  H  N  Y  G  S  R   445
ACAGGTACACCAATGTCCAATCACGTCATGTCTCCAACCTTAAATACAGATTCTAGTTCAGCAAACGGGAAATTCATACCAAGTAGACCGGCTCCTAAGCCCCCATCTTCTGCGTCCGCT  1560
T  G  T  P  M  S  N  H  V  M  S  P  T  L  N  T  D  S  S  S  A  N  G  K  F  I  P  S  R  P  A  P  K  P  P  S  S  A  S  A   485
TCAGCTCCAATTATAAAATCACCCGTCATGAATTCTGCCGCCAATGTTTCGCCCTTGAAGCAGACTCATGCACCTACAACTCCGAACAGAACCAGCCCAAACAGGTCCTCAATATCAAGA  1680
S  A  P  I  I  K  S  P  V  M  N  S  A  A  N  V  S  P  L  K  Q  T  H  A  P  T  T  P  N  R  T  S  P  N  R  S  S  I  S  R   525
AATGCCACTTTAAAAAAAGAGGAGCAGCCACTACCACCAATACCTCCAACCAAATCCAAAACGTCTCCAATCATCTCCACAGCTCACACACCACAGCAAGTTGCTCAATCGCCAAAAGCG  1800
N  A  T  L  K  K  E  E  Q  P  L  P  P  I  P  P  T  K  S  K  T  S  P  I  I  S  T  A  H  T  P  Q  Q  V  A  Q  S  P  K  A   565
CCGGCGCAAGAGACGGTAACGACACCTACTTCGAAGCCAGCTCAAGCAAGAAGCTTGTCTAAAGAATTAAATGAGAAAAAGAGAGGAAAGGGAAAGACGTAAAAAACAACTATATGCC  1920
P  A  Q  E  T  V  T  T  P  T  S  K  P  A  Q  A  R  S  L  S  K  E  L  N  E  K  K  R  E  E  R  E  R  R  K  K  Q  L  Y  A   605
AAATTGAACGAAATTTGCTCAGACGGTGACCCAAGTACAAAATATGCCAATTTAGTAAAAATTGGTCAAGGTGCATCAGGTGGTGTTTATACTGCTTATGAAATAGGTACGAATGTCTCA  2040
K  L  N  E  I  C  S  D  G  D  P  S  T  K  Y  A  N  L  V  K  I  G  Q  G  A  S  G  G  V  Y  T  A  Y  E  I  G  T  N  V  S   645
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V  A  I  K  Q  M  N  L  E  K  Q  P  K  K  E  L  I  I  N  E  I  L  V  M  K  G  S  K  H  P  N  I  V  N  F  I  D  S  Y  V   685
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L  K  G  D  L  W  V  I  M  E  Y  M  E  G  G  S  L  T  D  V  V  T  H  C  I  L  T  E  G  Q  I  G  A  V  C  R  E  T  L  S   725
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E  M  I  E  G  E  P  P  Y  L  N  E  T  P  L  R  A  L  Y  L  I  A  T  N  G  T  P  K  L  K  E  P  E  N  L  S  S  S  L  K   845
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K  F  L  D  W  C  L  C  V  E  P  E  D  R  A  S  A  T  E  L  L  H  D  E  Y  I  T  E  I  A  E  A  N  S  S  L  A  P  L  V   885
AAGTTAGCAAGATTGAAGAAAGTAGCTGAGAACATGGATGCTGATGAAGATAATGACGACGATAACGACAACGAGCATATTAATAAGACAAACAATTGTGACGACAATAACGATAGCAAA  2880
K  L  A  R  L  K  K  V  A  E  N  M  D  A  D  E  D  N  D  D  D  N  D  N  E  H  I  N  K  T  N  N  C  D  D  N  N  D  S  K   925
GAAACCGTAAATTTGGACGTAACTGAAGATGATAAACAAAAG
E  T  V  N  L  D  V  T  E  D  D  K  Q  K
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FIG. 3. Structure and sequence of *STE20*. (*A*) Diagram of *STE20* coding region. Heavy bar, *GAL1* promoter–*STE20* fusion junction for dominant truncation allele *STE20ΔN*; striped box, kinase domain. Position of deletion/*LEU2* insertion in *ste20-Δ1* is also shown. (*B*) Nucleotide sequence and deduced amino acid sequence of *STE20*. First nucleotide after *GAL1* promoter–*STE20* fusion junction in *STE20ΔN* is in boldface. The ATG putatively used for initiation of translation of the truncated STE20ΔN protein is underlined.

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                          I                   II                  III
STE20  618  TKYANLVKIG QGASGGVYTA YEIGTNVSVA IKQMNLEKQP KK.......E LIINEILVMK GS........ .......... .......... .........K
STE11  413  KNWLKGACIG SGSFGSVYLG MNAHTGELMA VKQVEIKNNN IGVPTDNNKQ ANSDENNEQE EQQEKIEDVG AVSHPKTNQN IHRKMVDALQ HEMNLLKELH
STE7   189  QDLVQLGKIG AGNSGTVVKA LHVPDSKIVA KKTIPVEQNN ST.....IIN QLVRELSIVK NVK....... .......... .......... .........P
FUS3    11  SDFQLKSLLG EGAYGVVCSA THKPTGEIVA IKKIEPFDKP LF......AL RTLREIKILK HF........ .......... .......... .........K
KSS1    11  SQYKLVDLIG EGAYGTVCSA IHKPSGIKVA IKKIQPFSKK LF......VT RTIREIKLLR YFH....... .......... .......... .........E
            ---------G -G--G-V--- ---------A -K-------- ---------- ----E----- ---------- ---------- ---------- ----------

                  IV                  V                                       VI
STE20  674  HPNIVNFIDS YVLK...GDL WVIMEYMEGG SLTDVVTHCI .......... .....LTEGQ IGAVCRETLS GLEFLHSKGV LHRDIKSDNI LLSMEGDIKL
STE11  513  HENIVTYYGA SQEG...GNL NIFLEYVPGG SVSSMLNNYG P......... .....FEESL ITNFTRQILI GVAYLHKKNI IHRDIKGANI LIDIKGCVKI
STE7   248  HENIITFYGA YYNQHINNEI IILMEYSDCG SLDKILSVYK RFVQRGTVSS KKTWFNELTI SKIAYGVLNG LDHLYRQYKI IHRDIKPSNV LINSKGQIKL
FUS3    68  HENIITIFNI QRPDSFENFN EVYIIQELMQ T.DLHRVIST QM........ .....LSDDH IQYFIYQTLR AVKVLHGSNV IHRDLKPSNL LINSNCDLKV
KSS1    69  HENIISILDK VRPVSIDKLN AVYLVEELME T.DLQKVINN QNSGFST... .....LSDDH VQYFTYQILR ALKSIHSAQV IHRDIKPSNL LLNSNCDLKV
            H-NI------ ---------- ---------- ---------- ---------- ---------- ---------- ---------- -HRD-K--N  L-------K-

              VII                   VIII                  IX                                  X
STE20  756  TDFGFCAQIN ELNL...... ....KRTTMV GTPYWMAPEV V.SRKEYGPK VDIWSLGIMI IEMIEGEPPY LNE...TPLR ALYLIATNGT PKLKEPEN..
STE11  596  TDFGISKKLS PLNK...... .KQNKRASLQ GSVFWMSPEV V.KQTATTAK ADIWSTGCVV IEMFTGKHPF PDF...SQMQ AIFKIGTNTT PE.IPSW...
STE7   348  CDFGVSKKLI NSIAD..... .......TFV GTSTYMSPER I.QGNVYSIK GDVWSLGLMI IELVTGEFPL GGHN...DTP DGILDLLQRI VN.EPSPRLP
FUS3   154  CDFGLARIID ESAADNSEPT GQQSGMTEYV ATRWYRAPEV MLTSAKYSRA MDVWSCGCIL AELFLRRPIF PGRDYRHQLL LIFGIIGTPH SDNDLRCIES
KSS1   160  CDFGLARCLA SSSD...SRE TLVGFMTEYV ATRWYRAPEI MLTFQEYTTA MDIWSCGCIL AEMVSGKPLF PGRDYHHQLW LILEVLGTPS FE.DFNQIKS
            -DFG------ ---------- ---------- -------PE- ---------- -D-WS-G--- -E-------- ---------- ---------- ----------

                                          XI
STE20  840  .......... .......... ......LSSS LKKFLDWCLC VEPEDRASAT ELLHDEYITE IAEANSSLAP LVKLARLKKV AENMDADEDN DDDNDNEHIN
STE11  680  .......... .......... ......ATSE GKNFLRKAFE LDYQYRPSAL ELLQHPWLDA HII*...... .......... .......... ..........
STE7   430  KDRI...... .......... ......YSKE MTDFVNRCCI KNERERSSIH ELLHHDLIMK YVSPSKDDKF RIWCRKIKSK IKEDKRIKRE ALDRAKLEKK
FUS3   254  PRAREYIKSL PMYPAAPLEK MFPR..VNPK GIDLLQRMLV FDPACRITAK EALEHPYLQT YHDPNDEPEG EPIPPSFFEF DHHKEALTTK DLKKLIWNEI
KSS1   256  KRAKEYIANL PMRPPLPWET VWSKTDLNPD MIDLLDKMLQ FNPDKRISAA EALRHPYLAM YHDPSDEPEY PPLNLDDEFW KLDNKIMRPE EEEEVPIEML
            ---------- ---------- ---------- ---------- -----R---- E-L------- ---------- ---------- ---------- ----------
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FIG. 4. Alignment of STE20 deduced protein sequence with the other identified protein kinases in the mating response pathway. STE20 was aligned by eye with the alignment of Rhodes *et al.* (20) of STE11, STE7, FUS3, and KSS1. Amino acids that are conserved in at least 62 protein kinases are underlined in the consensus. Amino acids that are conserved among the five kinases of the mating response pathway are also indicated.

Conversely, the originally isolated truncation allele, now called *STE20ΔN*, contains the entire kinase domain of the protein, effectively separated from most of the rest of the protein (Fig. 3*A*).

STE20* Is Regulated Like *STE7*, *STE11*, and *KSS1*.** Cell-type and pheromone responsiveness of the *STE20* message was examined (Fig. 5). Northern analysis revealed a 3000-bp message that is constitutively transcribed during vegetative growth in **a**, α, and **a**/α cells. Its expression is not induced by α factor in *MATa** cells. This pattern of expression is identical to what is seen for STE7, STE11, and KSS1 (18, 20, 22), three of the four other known protein kinases in the mating response pathway. The other kinase, FUS3, is pheromone inducible and is not expressed in diploids (19).

Interactions of *STE20* with Other Components of the Mating Response Pathway. Two complementary sets of epistasis experiments were undertaken to attempt to determine where *STE20* might function in the mating response pathway. First, the ability of the dominant *STE20ΔN* allele to restore mating in a variety of strains lacking functional components of the mating response pathway was examined. The allele was unable to improve the mating efficiency of strains carrying deletions in any of *ste4*, *ste5*, *ste7*, *ste11*, or *ste12* (mating efficiency, $<10^{-7}$ in each case). These data suggest that *STE20* may be acting near the beginning of the pathway.

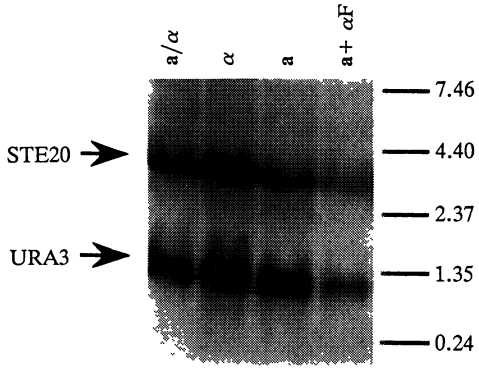

FIG. 5. Expression pattern of *STE20* message. Lanes: **a**/α, diploid CRY3; α, haploid CRY2; **a**, haploid CRY1; **a** + αF, haploid CRY1 exposed to α factor (3 mg/ml). *STE20* probe was 1207-bp internal *Eco*RV fragment. Numbers on right are positions of RNA markers of the indicated sizes in kilobases.

However, mutations in these genes did not alleviate the growth arrest caused by *STE20ΔN*. *STE20ΔN* was partially able to restore mating in the *ste20-Δ1* mutant to a level that is 600-fold higher than the mutant alone. This level represents 0.06% of wild-type levels.

In a second set of experiments, dominant alleles of some of the other components of the pathway that can activate the mating response when overproduced in a wild-type background were examined in a *ste20-Δ1* background. Overexpression of *STE4*, *STE12*, and some alleles of *STE11* can cause pheromone-independent activation of the mating response pathway that is similar to that caused by *STE20ΔN* (6–11). The ability of these genes to restore mating in a *ste20-Δ1* mutant was examined. The *ste20-Δ1* mating defect is not suppressed by overproduction of *STE4* or a dominant *STE11* truncation product (mating efficiency, $<10^{-7}$). However, overexpression of *STE12* causes a 20- to 30-fold increase in mating efficiency, suggesting that *STE20* functions prior to *STE12* in the mating response pathway.

DISCUSSION

Although the yeast pheromone response pathway has been very well studied, it is clear that there are still major molecular components of the system that have not been identified. This work reports the identification of one such gene, *STE20*. In a pathway whose purpose is rapid signal amplification that is already known to involve at least four protein kinases, it is perhaps not surprising that *STE20* also encodes a putative protein kinase.

The four previously identified protein kinases in the mating response pathway fall into two broad groups. FUS3 and KSS1 are small proteins composed almost entirely of protein kinase catalytic domains. They belong to a large family of protein kinases known as mitogen-activated protein kinases or ERK kinases (23). STE7, STE11, and STE20 appear to form a second group of much larger proteins that have long amino-terminal regions upstream of their kinase domains. The truncated STE20ΔN product that was originally isolated in this screen contains the entire kinase domain of the protein and lacks most of the amino-terminal region. The dominant phenotype displayed by this allele suggests that the missing amino-terminal portion of the gene product may have a regulatory function. It remains to be determined whether

full-length STE20 will show a similar FUS1 activation phenotype when overproduced.

Several models can account for the mechanism of activation of the mating response pathway by *STE20ΔN*. High levels of STE20 may cause an increase in phosphorylation of the STE20 substrate with consequent increased noise in the normal, unstimulated pathway. In this case, high levels of full-length STE20 would be expected to show the same phenotype as does STE20ΔN. Alternatively, the truncated STE20ΔN may have a higher affinity for its substrate than STE20, perhaps because of the absence of a negative regulatory domain. The truncated product may thus phosphorylate its substrate under unstimulated conditions where full-length STE20 is normally completely inactive. Finally, the specificity of STE20ΔN may be altered so that the truncated kinase is phosphorylating additional protein(s) rather than just its conventional substrate.

The observation that the toxic effects of overexpressing *STE20ΔN* cannot be ameliorated by mutations in other genes in the mating response pathway, particularly not even by deletion of the *STE12* transcription factor, suggests that *STE20ΔN* is exerting its toxic effects independently of the mating response pathway. One mechanism for doing so would be phosphorylation of inappropriate substrate(s) due to altered specificity. Alternatively, STE20 may have a *bona fide* substrate outside the mating response pathway in addition to the one(s) within it, as does the FUS3 kinase (19).

The results of the mating epistasis experiments were not sufficient to allow precise localization of STE20 in the pathway. It should be noted that requiring STE20ΔN to restore mating ability to strains that lack other components of the pathway is a very stringent requirement. Since STE20ΔN only partially restores mating to a *ste20-Δ1* strain, it is clear that the amino-terminally deleted protein cannot completely substitute for the full-length protein. It is possible that STE20ΔN will prove capable of restoring some subset of mating response functions (e.g., transcriptional activation) in some of the mutant backgrounds. Alternatively, STE20 may act at more than one place in the pathway, making epistatic analysis very difficult.

It is interesting to note that *STE20* resembles the classical *STE* genes in every characteristic examined to date. Strains carrying the *ste20-Δ1* kinase domain deletion behave exactly like the original *ste* genes identified in genetic screens by MacKay and Manney (24) and Hartwell (16). Thus, it is not at all clear why *STE20* was not identified in these earlier screens. However, its isolation in this screen underscores the importance of multifaceted genetic and biochemical approaches to further understanding this model signal transduction pathway.

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