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RAM2, an essential gene of yeast, and *RAM1* encode the two polypeptide components of the farnesyltransferase that prenylates a-factor and Ras proteins

(CAAX motifs/membrane targeting/post-translational modification/extragenic suppressors)

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ABSTRACT **In the yeast *Saccharomyces cerevisiae*, mutations in either of two unlinked genes, *RAM1* or *RAM2*, abolish the farnesyltransferase activity responsible for prenylation of Ras proteins and the a-factor mating pheromone. Here we report that the function of *RAM1* and *RAM2* genes is required for the membrane localization of Ras proteins and a-factor. The *RAM2* gene was sequenced and can encode a 38-kDa protein. We examined the functional interaction of *RAM2* and *RAM1* by expressing the genes in *Escherichia coli*. Extracts derived from an *E. coli* strain that coexpressed *RAM1* and *RAM2* efficiently farnesylated a-factor peptide and Ras protein substrates. In contrast, extracts derived from *E. coli* strains that expressed either *RAM* gene alone were devoid of activity; however, when the latter extracts were mixed, protein farnesyltransferase activity was reconstituted. These results indicate that the yeast farnesyl-protein transferase is comprised of Ram1 and Ram2 polypeptides. Although Ram1 is a component of the enzyme, disruption of the *RAM1* gene in yeast was not lethal, indicating that the Ram1-Ram2 farnesyltransferase is not essential for viability. In contrast, disruption of *RAM2* was lethal, suggesting that Ram2 has an essential function in addition to its role with Ram1 in protein farnesylation.**

The post-translational modification of proteins by the covalent attachment of isoprenoid groups plays an important role in the membrane targeting of various proteins (1–5). Three classes of prenylated proteins in eukaryotic cells have been described. The first class, represented by certain fungal mating pheromones, Ras proteins, and nuclear lamins, is initially synthesized with a C-terminal CAAX sequence, where X = Ser, Cys, Met, or Ala, and is modified by thioether linkage of a C_{15} farnesyl group to the cysteine residue (6–9). The second class of prenylated proteins, represented by the γ subunits of certain heterotrimeric G proteins and various Ras-like proteins, is initially synthesized with different C-terminal CAAX sequences, in which X = Leu or Phe, and is modified by the attachment of a C_{20} geranylgeranyl group to the cysteine residue (10–12). Members of both of these classes of prenylated proteins are subsequently processed by proteolytic removal of the three terminal amino acids and methylation of the newly exposed carboxyl group of the prenyl-cysteine (6, 10–15). The third class of prenylated proteins, represented by the Ypt1/Sec4 (Rab) family of Ras-like GTPases that terminate with the sequence CC or CXC, is also geranylgeranylated at a C-terminal cysteine residue(s) (16–18).

The mammalian $p21^{ras}$ protein farnesyltransferase is a heterodimeric enzyme (19–21) that appears to be responsible for the modification of all proteins that have the first type of CAAX sequence noted above (22, 23). Geranylgeranyltransferase type I (GGTase I), which modifies proteins that terminate with the second type of CAAX sequence (23–25), is also a heterodimeric enzyme that appears to share a common α subunit with the $p21^{ras}$ protein farnesyltransferase (21, 23). A third enzyme has been identified, GGTase II, which geranylgeranylates the Ypt1/Sec4 class of proteins (21).

Genetic studies in yeast have identified three genes, each of which uniquely affects one type of prenyltransferase reaction. One of these genes, *RAM1* (also called *DPR1*), was identified as a suppressor of $RAS2^{val19}$, a mutationally activated *RAS* allele (26, 27). This same gene was identified based on its involvement in a-factor production (26, 28, 29) and as a suppressor of G protein function (30). *ram1* extracts are defective in a-factor and Ras protein farnesyltransferase activity but not in other prenyltransferase activities (21, 23, 31, 32). Furthermore, Ram1 protein produced in *Escherichia coli*, although lacking enzyme activity on its own, biochemically complements *ram1* extracts for a-factor peptide farnesyltransferase activity (31). This result suggests that the *RAM1* gene product is a necessary but insufficient component of the protein farnesyltransferase. Mutants harboring lesions in *CDC43/CAL1*, a gene that shows homology to *RAM1* (33), are specifically defective in GGTase I activity (21, 23, 34). Another yeast gene that is homologous to *RAM1*, called *BET2*, affects the membrane localization of Ypt1 and Sec4 proteins (35) and specifically affects yeast GGTase II activity (21).

In this report we characterize another yeast gene, *RAM2*, that was identified as an extragenic suppressor of $RAS2^{val19}$. Previous reports have shown that *ram2* mutants, like *ram1* mutants, are defective in Ras protein farnesyltransferase activity (23, 32). Here we report the DNA sequence of *RAM2* and present direct evidence that *RAM1* and *RAM2* encode the two essential subunits of yeast farnesyl-protein transferase.

MATERIALS AND METHODS

Yeast Strains and Materials. The *ram2-1* mutants used in this study were derived from the original mutant STS39 by backcrosses to RS16-4C (26). Other yeast strains have been described (26, 29, 36). Recombinant $p21^{ras}$ was purified as described (37). Biotinylated KTSCVIM peptide (38) was obtained from Multiple Peptide Systems (San Diego). The a-factor peptides YIIKGVFWDPAC and YIIKGVFWDPACVIA were synthesized by P. Shenbagamurthi (Johns

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Abbreviations: CAAX, protein sequence where C = cysteine, A = usually an aliphatic amino acid, and X = any amino acid; FPP, farnesyl pyrophosphate; GGTase I, geranylgeranyltransferase type I; GGTase II, GGTase type II.

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Hopkins). [^{3}H]Farnesyl pyrophosphate ([^{3}H]FPP; 20 Ci/mmol; 1 Ci = 37 GBq) was purchased from NEN.

Plasmid Constructions and Gene Disruptions. The integrative mapping plasmid pBH43 contains the 1.85-kilobase-pair (kbp) *Sac* I–*Pst* I *RAM2* fragment inserted into pRS305 (39). p*ram2*::*LEU2* was constructed by insertion of the 1.5-kbp *Pst* I–*Bgl* II *RAM2* fragment into pBH43 and gene replacements were generated by digestion with *Pst* I and transformation into the diploid strain TTSD1 (40). p*ram1*::*URA3* (pSM353) contains the 4.6-kbp *Bam*HI–*Bgl* II fragment of the *RAM1* locus inserted into the vector pUC-4k, with the *URA3* *Hin*dIII fragment in place of the *RAM1* *Hin*dIII fragment, such that codons 1–322 are deleted (41). Gene replacements were generated by digestion with *Eco*RI and transformation into the diploid strain SM1060 (36). To express *RAM2* in *E. coli*, a *RAM2* coding sequence cassette was generated by PCR utilizing two oligonucleotide primers that overlapped either the initiation codon or the termination codon shown in Fig. 3. The relevant sequence of the 5′ portion of the cassette is CTCGAGGAAAATG, where the underlined ATG corresponds to the initiation codon of *RAM2*. The 3′ portion of the cassette contained an artificial *Bam*HI site following the termination codon. This cassette was inserted into the chloramphenicol-resistance plasmid pBC-KS+ (Stratagene) that had been cleaved with *Xho* I and *Bam*HI, to generate pBH57, which utilizes the *lac* promoter to direct the synthesis of an N-terminal *lacZ–RAM2* fusion protein. To express *RAM1* in *E. coli*, the plasmid pBH63 was constructed by inserting a *Sal* I–*Kpn* I *RAM1* coding sequence cassette into the ampicillin-resistance plasmid pUC119. The *RAM1* cassette was generated by PCR utilizing two oligonucleotide primers that overlapped either the initiation codon or the termination codon reported for *DPR1* (41). The relevant sequence of the 5′ portion of the cassette is GTCGACTATG, where the underlined ATG indicates the initiation codon of *RAM1*.

Preparation of *E. coli* Extracts for Farnesyltransferase Assays. *E. coli* extracts were prepared by culturing JM110 double transformants to mid-logarithmic phase followed by induction with 0.5 mM isopropyl β-D-thiogalactoside and incubation for 1 hr. Cells were collected, washed in extraction buffer (50 mM sodium Mes, pH 6.5/0.1 mM EGTA/0.1 mM $MgCl_2$/5% glycerol/2 mM phenylmethylsulfonyl fluoride), transferred to 0.5-ml tubes, pelleted, and lysed in 200 μl of extraction buffer with glass beads and Vortex mixing. Soluble fractions were prepared by centrifugation at 50,000 × *g* for 1 hr.

Other Methods. Fractionation of yeast and detection of a-factor and Ras2 proteins by immunoprecipitation were performed as described (26, 36). Yeast extracts were prepared as described (32). Farnesyltransferase assays using recombinant $p21^{ras}$ as substrate were performed and analyzed as described (32). Assays using synthetic a-factor peptides were carried out with 250 pmol of peptide essentially as described (31).

EXPERIMENTAL RESULTS

Identification and Characterization of the *ram2* Mutation. Several yeast mutations suppress $RAS2^{val19}$, including *ram1*, *cyr1*, and *cap1*/*srv2* (26, 27, 42, 43). Among the set of $RAS2^{val19}$ extragenic suppressor mutants derived by heat-shock selection from the *MAT***a** *ras1*$^-$ $RAS2^{val19}$ strain PT1-6 (26), some recessive mutations do not fall into these three known complementation groups. One of these suppressors, STS39, displayed two nonselected phenotypes: an inability to grow well at high temperatures (37°C) and a significant mating defect. The mating defect was determined to be *MAT***a**-specific and correlated with defective a-factor production. Based on cosegregation of all three of these phenotypes following a cross to a wild-type strain and subsequent analysis of 10 complete tetrads, these three phenotypes resulted from a single mutation (*ram2-1*). *ram2-1* has been previously demonstrated to be unlinked to *ram1* (32).

***ram2* Mutants Are Defective in the Farnesylation and Membrane Targeting of Both Ras Proteins and the a-Factor Mating Pheromone.** It has been shown that extracts prepared from *ram2-1* mutants fail to farnesylate bacterially produced Ras proteins *in vitro* (23, 32). We determined that *ram2-1* extracts, like *ram1* extracts, are also defective in farnesylating a-factor *in vitro* (data not shown). To determine whether the lack of farnesylation measured *in vitro* was reflected *in vivo* by a defect in membrane targeting, we radiolabeled wild-type and mutant yeast cells and examined soluble and particulate fractions by immunoprecipitation using antibodies that recognized Ras proteins or a-factor (Fig. 1). In the wild-type strain, Ras2 was localized in the particulate fraction as an apparent 40-kDa species. In contrast, in the *ram2-1* and the *ram1* mutants, Ras2 was localized to the soluble fraction and migrated more slowly (Fig. 1*A*). Fig. 1*B* shows that a-factor was detected in the particulate fraction of wild-type cells as two precursor species (P_1 and P_2), whereas a-factor synthesized in *ram1* or *ram2-1* mutants was predominantly localized in the soluble fraction as a slower migrating form (P_0). Thus, *ram2-1*, like mutations in *RAM1*, blocks the membrane targeting of Ras proteins and a-factor.

Cloning and Sequence Analysis of *RAM2*. We utilized the Ts$^-$ phenotype of *ram2-1* mutants to clone the wild-type *RAM2* gene by complementation. A *ram2-1* mutant strain was transformed with a yeast genomic library and two transformants were found that exhibited plasmid-dependent Ts$^+$ growth. Plasmids recovered from these two transformants restored normal growth and mating properties to *ram2* mutant strains. Restriction enzyme mapping revealed that the two plasmids contained overlapping inserts with a common region of DNA of >11 kbp (Fig. 2). To determine whether the gene we cloned corresponded to the locus identified by the *ram2-1* mutation, we integrated a *LEU2*$^+$-marked plasmid (pBH43) containing a fragment of the cloned DNA into a *RAM2*$^+$ strain. Integration was targeted to the chromosomal locus corresponding to the cloned fragment by digestion with *Sph* I (see Fig. 2), and correctly targeted integration was

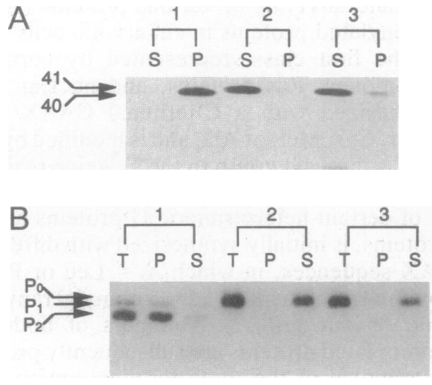

FIG. 1. Localization of Ras proteins and a-factor in *ram* mutants. (*A*) SDS/PAGE analysis of immunoprecipitated Ras2 proteins from soluble (S) and particulate (P) fractions of extracts prepared from [^{35}S]methionine-labeled wild-type and mutant yeast strains, all of which contained high-copy *RAS2* plasmid (26). Lanes: 1, wild-type (SP1); 2, *ram2-1* (STS39); 3, *ram1-1* (STS11). The soluble precursor form of Ras2 (41 kDa) and the particulate mature form of Ras2 (40 kDa) are marked by arrows. (*B*) SDS/PAGE analysis of immunoprecipitated a-factor from total extracts (T) and particulate (P) and soluble (S) fractions of total extracts prepared from [^{35}S]cysteine-labeled wild-type and mutant yeast strains, all of which contained high-copy *MFa1* plasmid (29). Lanes: 1, wild-type (SM1229); 2, *ram1*::*URA3* (SM1866); 3, *ram2-1* (SM1863). Three of the intracellular forms of a-factor are marked by arrows (P_0, P_1, P_2).

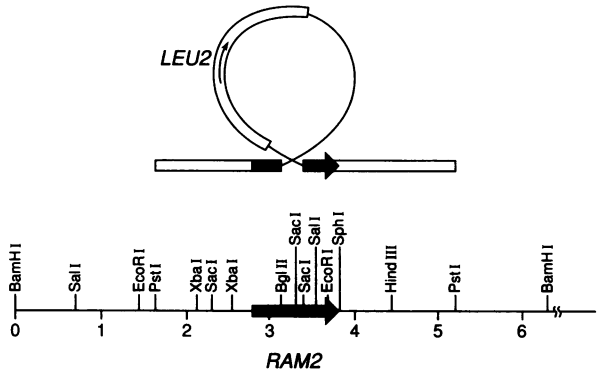

FIG. 2. Restriction map of the *RAM2* locus and the *ram2::LEU2* gene disruption construct. The restriction map of the *RAM2* locus is shown. Both of the original library plasmid isolates contained additional regions of DNA (≈6 kbp) that are not shown in this map. Shown above the map is the γ-disruption vector p*ram2::LEU2*, which contains two noncontiguous fragments from the *RAM2* region inserted in a head-to-tail orientation into the yeast integration vector pRS305 (39). Integration of the plasmid into the *RAM2* locus results in the deletion of the *RAM2* coding region that resides between the indicated *Bgl* II and *Sac* I restriction sites.

confirmed by DNA-blot hybridization for the transformant BHYDC1. In a cross of a *ram2-1 leu2* strain with BHYDC1, the segregation pattern of *LEU2*$^+$ and *ram2-1* indicated complete linkage (no recombinants in 25 tetrads), and we therefore conclude that the cloned gene is indeed *RAM2*.

Subclones of the two original plasmids were constructed and based on their ability to complement the *ram2* mutation we determined that the entire *RAM2* gene (or at least a functional segment) resided within the 1.4-kbp *Xba* I–*Sph* I fragment shown in Fig. 2. The DNA sequence of this region was determined for both strands and it contains an open reading frame that can encode a protein of 316 amino acids if the first ATG is used to initiate translation (Fig. 3). The predicted molecular mass of the Ram2 protein is 38 kDa.

Reconstitution of Farnesyltransferase Activity. To investigate the possibility that Ram1 and Ram2 proteins are indeed structural subunits of the yeast farnesyl-protein transferase, we expressed the *RAM1* and *RAM2* genes in *E. coli* and assayed extracts for farnesyltransferase activity. Using a substrate peptide corresponding to the C-terminal sequence of K-*rasB*, control extracts and extracts prepared from bacterial strains that expressed either *RAM1* or *RAM2* gene alone were completely inactive (Table 1). In contrast, extracts prepared from a strain that coexpressed *RAM1* and *RAM2* exhibited high levels of farnesyltransferase activity (Table 1). We have determined that the specific activity of such *E. coli* extracts is at least 100-fold higher than that of similarly prepared wild-type yeast extracts (data not shown).

Extracts prepared from *E. coli* cells that coexpressed *RAM1* and *RAM2* also efficiently farnesylated synthetic **a**-factor substrate. We observed by SDS/PAGE analysis that control extracts derived from *E. coli* carrying plasmids without *RAM* inserts produced an unidentified product from [^{3}H]FPP (Fig. 4*A*, lane 3) that is not related to **a**-factor since it was also observed in reactions in which the peptide was absent (Fig. 4*A*, lane 9). In contrast, using extracts from cells that coexpressed *RAM1* and *RAM2*, a reaction product was observed (Fig. 4*A*, lane 1) that was not found in reactions utilizing extracts prepared from *E. coli* that expressed either the *RAM1* or *RAM2* gene alone (Fig. 4*A*, lanes 4 and 5). This product comigrated with authentic mature **a**-factor produced *in vivo* (Fig. 4*B*) and was not detected in control reactions containing a synthetic **a**-factor peptide from which the terminal-AAX residues (of the CAAX sequence) were missing (Fig. 4*A*, lane 8).

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-444  TCTAGAGTATAATTATTTTGAATGAGTTTCGTTGACATTTTCAGTTCATGCGATCATCTT
-384  ACTTTCATGGTTGCTCCTTGCCGTTAAAATTGAAACGAAAAAAGCGGGAATACAACAATG
-324  TCACCTTACACGAGCAGAGAAAAGGGCAGCAATAGCGACAATAACCGATAATGCCAGGCC
-264  TTCCAGAGCCCTATGCTGACCTATATTCATCATGACGCTATCCATATGCAACTAGAGACC
-204  TGCTGGGAAGCGCTCTCTCATTTTTGAAGGTTGCAGCAGCTTGCGCGCCGGTTTTCGATT
-144  GGGCCTCGTGATAAAACACATCAGCAAAGTTTCGCTTTTGACATTCTGTTAAATTGGTCC
 -84  TTAACTGTTGGTGCAGACCTACTGCAAGACTGTTAATTATGGTATATTATATATCAAGTG
 -24  CTGATACAACGTACACCATCGAAAATGGAGGAGTACGATTATTCAGACGTTAAACCTTTG
                      1   M  E  E  Y  D  Y  S  D  V  K  P  L

  36  CCCATTGAGACAGACTTGCAGGATGAACTGTGCAGGATTATGTATACCGAGGATTATAAG
  12  P  I  E  T  D  L  Q  D  E  L  C  R  I  M  Y  T  E  D  Y  K

  96  CGGTTGATGGGACTCGCAAGGGCTCTGATCAGCCTTAACGAACTGTCACCCAGGGCACTA
  32  R  L  M  G  L  A  R  A  L  I  S  L  N  E  L  S  P  R  A  L

 156  CAGCTAACAGCCGAAATTATCGACGTGGCGCCAGCCTTCTACACCATATGGAACTACCGA
  52  Q  L  T  A  E  I  I  D  V  A  P  A  F  Y  T  I  W  N  Y  R

 216  TTCAATATCGTCAGGCACATGATGAGTGAATCCGAAGACACTGTCTTGTACCTGAACAAG
  72  F  N  I  V  R  H  M  M  S  E  S  E  D  T  V  L  Y  L  N  K

 276  GAATTAGACTGGCTAGATGAAGTTACGCTGAATAATCCAAAGAACTATCAGATCTGGTCC
  92  E  L  D  W  L  D  E  V  T  L  N  N  P  K  N  Y  Q  I  W  S

 336  TATAGACAGTCTCTTTTGAAGCTACATCCGTCTCCTTCCTTCAAAAGAGAGCTGCCTATC
 112  Y  R  Q  S  L  L  K  L  H  P  S  P  S  F  K  R  E  L  P  I

 396  TTAAAACTGATGATTGATGATGATTCCAAGAATTATCACGTTTGGTCGTACAGAAAGTGG
 132  L  K  L  M  I  D  D  D  S  K  N  Y  H  V  W  S  Y  R  K  W

 456  TGCTGTTTGTTCTTCAGTGACTTTCAACATGAGCTCGCCTACGCCAGCGACCTCATCGAG
 152  C  C  L  F  F  S  D  F  Q  H  E  L  A  Y  A  S  D  L  I  E

 516  ACAGACATTTATAACAACAGCGCATGGACTCATAGGATGTTTTACTGGGTGAACGCTAAA
 172  T  D  I  Y  N  N  S  A  W  T  H  R  M  F  Y  W  V  N  A  K

 576  GATGTCATTTCAAAAGTGGAATTGGCCGACGAGCTCCAGTTCATTATGGACAAGATTCAA
 192  D  V  I  S  K  V  E  L  A  D  E  L  Q  F  I  M  D  K  I  Q

 636  TTGGTTCCGCAGAACATCAGTCCGTGGACCTACCTCCGTGGTTTCCAAGAGCTATTCCAT
 212  L  V  P  Q  N  I  S  P  W  T  Y  L  R  G  F  Q  E  L  F  H

 696  GATAGGCTACAGTGGGATAGCAAAGTAGTCGACTTCGCCACAACCTTCATCGGTGACGTA
 232  D  R  L  Q  W  D  S  K  V  V  D  F  A  T  T  F  I  G  D  V

 756  TTGTCACTTCCAATTGGCTCACCAGAGGATTTGCCCGAGATCGAGTCCTCATATGCCCTG
 252  L  S  L  P  I  G  S  P  E  D  L  P  E  I  E  S  S  Y  A  L

 816  GAATTCCTGGCATATCACTGGGGGGCAGACCCTTGTACCCGAGACAACGCTGTTAAGGCC
 272  E  F  L  A  Y  H  W  G  A  D  P  C  T  R  D  N  A  V  K  A

 876  TATAGCTTGCTAGCAATCAAATACGATCCTATTAGAAAAAACTTGTGGCACCACAAAATA
 292  Y  S  L  L  A  I  K  Y  D  P  I  R  K  N  L  W  H  H  K  I

 936  AATAATCTGAACTGAATATATGTAAGCACTCTATTTGTACGAATATATTTACGTGTGTAC
 312  N  N  L  N  *

 996  GTGCATGC
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FIG. 3. Nucleotide sequence of the *RAM2* region and predicted amino acid sequence of the Ram2 protein.

We tested whether farnesyltransferase activity could be reconstituted by mixing *E. coli* extracts containing Ram1 alone with extracts containing Ram2 alone. The results shown in Table 1 indicate that these extracts, when mixed, do indeed exhibit farnesyltransferase activity. The activity produced by the mixed extract was ≈25-fold lower than that observed with *E. coli* extracts from cells that coexpressed

Table 1. Farnesyltransferase activity in extracts from *E. coli*

E. coli transformant	*RAM* gene expressed		[^{3}H]Farnesyl transferred, pmol per tube
	RAM1	*RAM2*	
BHE-1	−	−	<0.006
BHE-2	+	−	<0.004
BHE-3	−	+	<0.002
BHE-4	+	+	5.5 ± 0.1
Mixed extract	+	+	0.19 ± 0.02

The *E. coli* host JM110 was transformed sequentially with two different plasmids to generate the strains used in this experiment. Each assay (25 μl) was performed in duplicate and contained 50 mM potassium phosphate (pH 7.4), 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.2% Tween 20, 50 μg of soluble extract from the indicated *E. coli* transformant, 90 pmol of biotinylated KTSCVIM peptide, and 25 pmol of [^{3}H]FPP. After incubation for 30 min at 30°C, the amount of [^{3}H]farnesyl transferred to peptide was measured as described (38). For the mixed extract, 50 μg of BHE-2 and BHE-3 *E. coli* extracts was mixed in a microcentrifuge tube and left on ice for 30 min before performing the assay. In a separate experiment, BHE-4 extract transferred 1.6 ± 0.1 pmol of [^{3}H]farnesyl onto bacterially produced p21^{ras}, whereas control extracts listed transferred <6 fmol to Ras substrate.

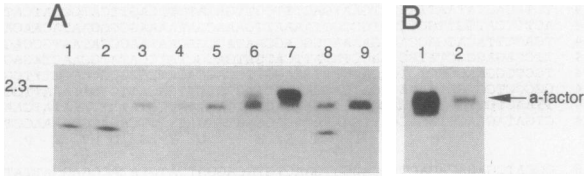

FIG. 4. SDS/PAGE analysis of a-factor farnesyltransferase activity in *E. coli* extracts. (*A*) Farnesylation assays with the a-factor quindecapeptide substrate were performed (see text) and analyzed by SDS/PAGE and fluorography. Unless otherwise stated, all reaction mixtures contained buffer, [^{3}H]FPP, the a-factor 15-mer substrate, and an *E. coli* extract. Mock reactions are shown in which the *E. coli* extract and peptide substrate (lane 1) or *E. coli* extract only (lane 2) is absent. Lanes 3–7 display complete reactions containing extract from the control *E. coli* strain BHE-1 (lane 3), extract from BHE-2, which expresses *RAM1* alone (lane 4), extract from BHE-3, which expresses *RAM2* alone (lane 5), a mixture of extracts from BHE-2 and BHE-3 (lane 6), and extract from BHE-4, which coexpresses *RAM1* and *RAM2* (lane 7). Lanes 8 and 9 represent control reactions identical to that shown in lane 7 but with substitution with the 12-mer a-factor substrate (lane 8) or no peptide substrate (lane 9). (*B*) Comigration of *in vitro* farnesylated a-factor and authentic a-factor. Lane 1, same reaction mixture as in lane 7 of *A*. Lane 2, a-factor that was immunoprecipitated from the culture supernatant of a *MATa* strain that had been radiolabeled with [^{35}S]cysteine (36, 44).

RAM1 and *RAM2* genes (Table 1). The lower activity of mixed extracts was also observed in experiments utilizing Ras proteins as substrate (data not shown) and in reactions utilizing the quindecapeptide a-factor as substrate (Fig. 4*A*, lane 6).

Effect of Disruption of *RAM1* and *RAM2* Genes in Yeast. To examine whether the Ram1-Ram2 farnesyl-protein transferase was essential for yeast viability, we made plasmids that contained deletion alleles of *RAM1* and *RAM2*. By DNA transformation, we constructed diploids that were heterozygous for one of the disrupted *RAM* alleles. Analysis of tetrads from a *ram2*/*RAM2*$^+$ diploid indicated that *ram2* null mutants were nonviable; 2:2 segregation of viability was observed in 30 tetrads, and all 60 viable colonies were Leu$^-$, indicating that they contained a wild-type *RAM2* gene. By microscopic examination, we observed that *ram2*$^-$ spores occasionally germinated and divided up to four times but then ceased growing.

To test for the effects of disrupting the *RAM1* gene, we analyzed tetrads derived from a *ram1*$^-$/*RAM1*$^+$ diploid. We observed that at 30°C *ram1*$^-$ spores germinated and slowly formed colonies, indicating that *ram1* null mutants are viable (Fig. 5). Further analysis indicated that *ram1* null mutants were significantly defective for growth at low temperatures and completely unable to grow at 37°C (Fig. 5).

The viability of *ram1* null mutants is in marked contrast to the lethal effects of disrupting the *RAM2* gene. This is not due to genetic variations in the strains employed since disruption of *RAM1* was not lethal in the strains used to test the effects of *RAM2* disruption (data not shown). To explore the possibility of residual protein farnesyltransferase activity in *ram1* null mutants, we prepared soluble extract from a *ram1* null mutant and assayed for farnesyltransferase activity. No reaction product was observed using the a-factor quindecapeptide substrate, even after long exposures of the fluorogram (data not shown). The amount of [^{3}H]farnesyl transferred to the K-*rasB* peptide using the *ram1* null mutant extract was not significantly higher than blank values (<2 fmol), whereas parallel reactions with extract prepared from the wild-type strain SP1 transferred 284 ± 50 fmol. Since we did not detect residual protein farnesyltransferase activity in *ram1* null mutants, we conclude that this activity is not required for yeast viability. This raises a paradox, since *RAS* function is essential in yeast. Possible resolution of the paradox, and explanations for the disparate effects of disrupting *RAM1* and *RAM2*, are discussed below.

DISCUSSION

We have characterized a newly identified yeast gene, *RAM2*, and have shown by expression in *E. coli* that *RAM1* and *RAM2* encode the two required components of the yeast enzyme responsible for farnesylation of a-factor and Ras proteins. The finding that Ram1 and Ram2 proteins are required for reconstitution of protein farnesyltransferase activity parallels the finding that the mammalian Ras protein farnesyltransferase is comprised of two different polypeptide subunits (19–21). It is also noteworthy that both components of the mammalian enzyme, the β subunit and the α subunit, are structurally homologous to Ram1 and Ram2, respectively (21, 45, 46).

RAS function is essential for yeast, and mutant Ras proteins that cannot be farnesylated cannot supply essential *RAS* function unless significantly overexpressed (47–49). It is therefore somewhat surprising that *RAM1*, which is absolutely required for farnesyl-protein transferase activity, is not an essential gene. One possible explanation, based on the ability of CAAX peptides that are efficient substrates for the farnesyl-protein transferase to serve as less efficient substrates for GGTase I, is that in *ram1*$^-$ mutant cells a small but sufficient amount of Ras proteins is geranylgeranylated by yeast GGTase I (22, 23, 25). Accordingly, we propose that

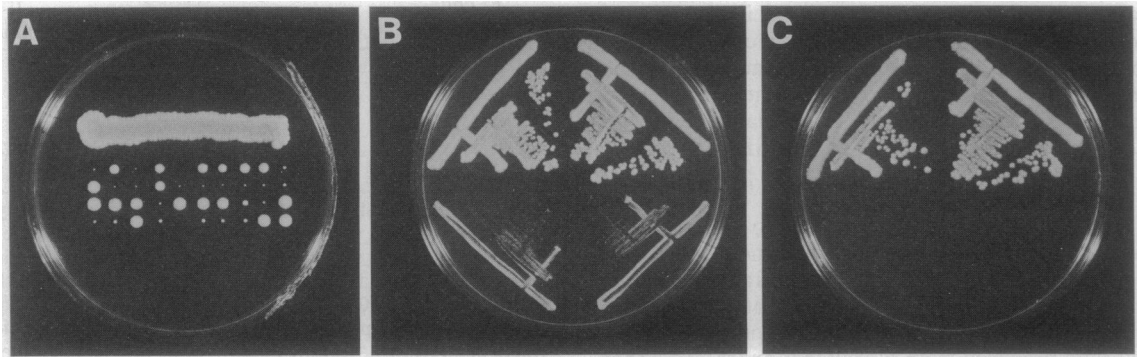

FIG. 5. Tetrad analysis of *ram1*$^-$/*RAM1*$^+$ diploids and the growth phenotype of the segregants. (*A*) Tetrads after sporulation and dissection of the diploid SM1589 (*ram1*::*URA3*/*RAM1*), derived from SM1060 (36), were grown on YEPD plates for 6 days at 30°C. Nine of 10 tetrads displayed 2:2 segregation of slow growth. All slow-growing colonies were determined to be Ura$^+$, indicating that they contained the *ram1*::*URA3* allele. (*B*) All four colonies from a single tetrad were streaked onto a YEPD plate and incubated at 30°C for 3 days. The two *ram1*::*URA3* segregants grew much slower than the two wild-type segregants. (*C*) All four colonies from a single tetrad were streaked onto a YEPD plate and incubated at 37°C for 3 days. The two *ram1*::*URA3* segregants did not grow at all.

ram2$^-$ null mutants are nonviable as a result of not only deficient farnesyltransferase activity but also deficient GGTase I activity. This proposal is based in part on the finding that the mammalian α subunit of the farnesyl-protein transferase, which is homologous to Ram2, also appears to be a structural subunit of GGTase I (21, 24). In addition, *ram2-1* mutants have been shown to be somewhat defective in GGTase I activity (23). Further analysis will be required to fully understand the role of Ram2 in other prenyltransferase reactions.

Two of the three yeast enzymes postulated to be involved in the post-translational modification of Ras proteins and a-factor have now been definitively assigned to particular genes in *Saccharomyces cerevisiae* and have been reconstituted by expression in *E. coli*. In addition to the Ram1-Ram2 farnesyl-protein transferase, it has been shown that *STE14* encodes the methyltransferase that modifies a-factor and Ras proteins (36). In the absence of *STE14* function, prior steps in Ras protein processing, presumably prenylation or proteolytic removal of the three C-terminal amino acids, were significantly delayed. This finding suggests that different steps in the modification of Ras proteins, although biochemically separable, may somehow be coupled *in vivo*.

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