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Farnesyl Cysteine C-Terminal Methyltransferase Activity Is Dependent upon the *STE14* Gene Product in *Saccharomyces cerevisiae*

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Membrane extracts of sterile *Saccharomyces cerevisiae* strains containing the a-specific *ste14* mutation lack a farnesyl cysteine C-terminal carboxyl methyltransferase activity that is present in wild-type a and α cells. Other a-specific sterile strains with *ste6* and *ste16* mutations also have wild-type levels of the farnesyl cysteine carboxyl methyltransferase activity. This enzyme activity, detected by using a synthetic peptide sequence based on the C-terminus of a *ras* protein, may be responsible not only for the essential methylation of the farnesyl cysteine residue of a mating factor, but also for the methylation of yeast *RAS1* and *RAS2* proteins and possibly other polypeptides with similar C-terminal structures. We demonstrate that the farnesylation of the cysteine residue in the peptide is required for the methyltransferase activity, suggesting that methyl esterification follows the lipidation reaction in the cell. To show that the loss of methyltransferase activity is a direct result of the *ste14* mutation, we transformed *ste14* mutant cells with a plasmid complementing the mating defect of this strain and found that active enzyme was produced. Finally, we demonstrated that a similar transformation of cells possessing the wild-type *STE14* gene resulted in sixfold overproduction of the enzyme. Although more complicated possibilities cannot be ruled out, these results suggest that *STE14* is a candidate for the structural gene for a methyltransferase involved in the formation of isoprenylated cysteine α-methyl ester C-terminal structures.

Eucaryotic polypeptides synthesized with the C-terminal sequence -Cys-Xaa-Xaa-Xaa (where Xaa is any amino acid) can be posttranslationally modified by proteolytic, lipidation, and methylation reactions. The resulting structures, first found in the peptidyl mating factors from the jelly fungi *Tremella mesenterica* and *Tremella brasiliensis*, contain C-terminal cysteine residues that are modified by both an *S*-isoprenyl group in a thioether linkage and α-methyl esterification (21, 29). Additional examples include the **a** mating factor (1, 3) and *RAS* proteins (10, 13, 30) from *Saccharomyces cerevisiae* and the mammalian Ras proteins (4, 8, 17, 18, 30), large G-proteins (14), cyclic GMP phosphodiesterase (26), and nuclear lamins (5, 6, 11, 20, 33, 36). The Ras proteins can also be palmitylated at a nearby cysteine residue (7, 9, 18). Although the physiological roles of these modifications are unclear, it has been proposed that the lipidation and methylation reactions may guide at least some of these polypeptides to their functional sites on membrane surfaces (8, 10, 11, 14, 17, 18, 20, 26, 33). Additionally, they may participate in the signal transduction roles common to most of these proteins (8, 10, 14, 17, 18, 26, 30).

We have been interested in the genetic and molecular basis of these modifications in the synthesis of **a** factor pheromone, a peptide necessary for mating between haploid **a** and α cells of the yeast *S. cerevisiae* (19, 25). At least three genes have been associated with its posttranslational maturation, which results in a C-terminal *S*-farnesyl cysteine α-methyl ester residue. One of these genetic loci, alternatively named *STE16* (35), *DPR1* (12, 16, 32), and *RAM* (28), has been characterized. Mutations here result in the defective maturation of both **a** factor and the *RAS* proteins. From sequence analysis, it has been tentatively suggested that this gene may encode the protease that removes the C-terminal three amino acids that follow the modifiable cysteine residue (16). Two other genes have been identified whose loss results in defective **a** mating factor production: *STE6* (23, 24; J. D. Rine, Ph.D thesis, University of Oregon, Eugene, 1979) and *STE14* (L. C. Blair, Ph.D. thesis, University of Oregon, Eugene, 1979). It has recently been proposed that the *STE6* protein is a transmembrane translocator that exports peptides by a route independent of the classical secretory pathway (23, 24). To date, no function for the *STE14* gene product has been established, although DNA sequence analysis has suggested that it is a membrane protein (S. Sapperstein, C. Berkower, and S. Michaelis, Abstr. Cold Spring Harbor Yeast Biol. Meet., Cold Spring Harbor, N.Y., 1989, p. 139). Our present study explores the possibility that the *STE14* gene is required for the methylation of the C-terminal farnesyl cysteine residue in the production of active **a** factor and other polypeptides.

MATERIALS AND METHODS

Yeast strains and media. All strains of *S. cerevisiae* used appear in Table 1. Strain designations are ABYS1 (*STE*), C39-U3 (*STE'*), K43-4c (*ste16*), K39A-3b (*ste6*), HR129-2d (*ste14*), and XBH38-3a (*ste14'*). Complete medium (YPD) contained 1% (wt/vol) yeast extract (Difco Laboratories), 2% (wt/vol) Bacto-Peptone (Difco), and 2% (wt/vol) D-glucose (Fisher Scientific Co.). Synthetic minimal medium (SD) contained 0.5% ammonium sulfate, 0.17% yeast nitrogen base without amino acids and ammonium sulfate (Difco), and 2% (wt/vol) D-glucose. When necessary, SD medium was supplemented with L-leucine (30 mg/liter) and L-methionine (20 mg/liter) (SDLM) in order to maintain plasmids selectively. For plates, 2% (wt/vol) Bacto-Agar (Difco) was

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TABLE 1. *S. cerevisiae* strains

Strain	Relevant genotype	Source
ABYS1	*MATa pra1 prb1 prc1 cps1 ade*	D. Meyer
C39-U3	*MATα ura3-52 met6 ubi*	C. Clarke
K43-4c	*MATa ste16-1 cryR leu2-3 leu2-112 ura3 trp1*(Am) *tyr1*(Oc) *his3 his4 met*	I. Herskowitz
K39A-3b	*MATa ste6-21 ade6 his4 lys2 can1 leu2-3 leu2-112*	I. Herskowitz
HR129-2d	*MATa ste14-1 leu2-3 leu2-112 ade5 ho met can1*	I. Herskowitz
XBH38-3a	*MATa ste14-1 leu2-3 leu2-112 met ura3-52*	L. C. Blair
GX3087	*MATα arg1 tsm11*	C. Clarke

included. Luria broth (LB) contained 0.5% (wt/vol) yeast extract (Difco), 1% (wt/vol) Bacto-Tryptone (Difco), and 1% (wt/vol) NaCl.

Preparation of crude membrane and cytosolic fractions. Single colonies of each strain were used to inoculate 10-ml cultures in YPD or SDLM medium and were grown overnight with constant shaking at 30°C. Larger cultures (250 or 500 ml) were inoculated with 2 or 5 ml of the small-scale culture and grown at 30°C with constant shaking. Cells were harvested at an OD_{600} of 0.7 to 1.2 by centrifugation at 1,400 × *g* for 10 min at 4°C and were washed twice with H_2O under the same conditions. All subsequent steps were performed at 4°C. Two milliliters of cold sorbitol buffer (0.3 M sorbitol, 0.1 M NaCl, 5 mM Tris hydrochloride [Tris-HCl, pH 7.4], 0.06 mg of phenylmethylsulfonyl fluoride per ml of buffer) was added per g of cells, and the cells were subsequently broken by French press treatment at 1,200 lb/in^2. The broken cells were centrifuged at 1,600 × *g* for 10 min, and the supernatant (whole-cell extract) was spun at 100,000 × *g* for 120 min. The supernatant was stored at −20°C, and the pellet (crude membrane fraction) was suspended in 1 ml of cold glycerol buffer (20% glycerol, 10 mM Tris-HCl [pH 7.4]).

Purification of membranes by sucrose gradient centrifugation. Membranes were purified by a modification of the sucrose gradient centrifugation procedure previously described by Serrano (31). Gradients were established in 4.4-ml polyallomer centrifuge tubes with 0.95 ml of 53.5% (wt/wt) sucrose and 1.80 ml of 43.5% (wt/wt) sucrose. Suspended 1-ml samples (crude membrane fractions) were layered on top, and the gradients were spun at 310,000 × *g* for 3 h in a Beckman SW60 swinging-bucket rotor. Membrane banding occurred at the interface between the two sucrose layers. The band was drawn off and washed in 20 ml of sorbitol buffer (120,000 × *g*, 120 min) to remove the sucrose. Each pellet was suspended in sorbitol buffer to a concentration of about 10 mg of protein per ml. Protein concentrations were assayed by a modification of the Lowry procedure (2) after precipitation with 1 ml of 10% (wt/vol) trichloroacetic acid.

Synthetic peptides. Both L-Leu–L-Ala–L-Arg–L-Tyr–L-Lys–L-Cys (LARYKC) and L-Leu–L-Ala–L-Arg–L-Tyr–L-Lys–*S-trans,trans*-farnesyl–L-Cys (*S*-farnesyl-LARYKC) were provided by Robert Stephenson in this laboratory. Their synthesis and characterization have been described elsewhere (31a).

C-terminal methyltransferase assay. Enzyme activity was measured in yeast extracts with *S*-adenosyl-L-[^{14}C-*methyl*] methionine (ICN; 50 mCi/mmol) in both the presence and absence of 1 nmol of synthetic peptide methyl acceptors. Peptides were dried in 1.5-ml polypropylene microcentrifuge tubes and suspended in 20 μl of 100 mM Tris-HCl (pH 7.50). Incubation mixtures generally contained 3 to 10 μl of purified membrane preparation, 20 μl (1 nmol) of *S*-adenosyl-L-[^{14}C-*methyl*]methionine in the Tris buffer, and Tris buffer to give a final volume of 60 μl and a final *S*-adenosyl-L-[^{14}C-*methyl*] methionine concentration of 16.7 μM. After a timed incubation at 37°C generally from 30 to 60 min, the reaction was stopped by the addition of 50 μl of 1 M NaOH–1% (wt/vol) sodium dodecyl sulfate. The mixture was vortexed immediately, and 100 μl was spotted on pleated filter paper (1.5 by 8 cm; Bio-Rad Laboratories, catalog no. 165-0962). The paper was placed in the neck of a 20-ml scintillation vial containing 10 ml of scintillation fluid (Amersham ACSII), and the vial was capped. After 2 to 3 h, the filter paper was removed, and radioactivity was counted in a liquid scintillation counter. Volatile [^{14}C]methanol released by the base-catalyzed cleavage of methyl esters diffuses into the scintillant, while unreacted *S*-adenosyl-L-[^{14}C-*methyl*]methionine and other labeled materials remain on the filter paper (27).

Isolation of plasmids and transformation. Plasmid pSL646 is a YEp24-based plasmid carrying the 5-kilobase *Bam*HI fragment containing the entire *STE14* gene. This plasmid, constructed by George Sprague and kindly provided by Ira Herskowitz, was propagated in *Escherichia coli* MH6. Bacterial cells were grown in LB-ampicillin (100 μg/ml), and the plasmid was isolated by the QIAGEN maxipreparation procedure as described by the manufacturer (QIAGEN Inc., Studio City, Calif.).

Yeast transformations were performed by a modification of the lithium acetate method (22). XBH38-3a (*ste14'*) or C39-U3 (*STE'*) cells were grown in 50 ml of YPD to an OD_{600} of 0.45. Cells were harvested by centrifugation at 1,100 × *g* for 10 min and suspended in 10 ml of 0.1 M lithium acetate. Cells were incubated at 30°C for 45 min and harvested again at 1,100 × *g*. The supernatant was removed, and the pellet was suspended in 400 μl of 0.1 M lithium acetate. Each transformation reaction mix contained 50 μl of cells, 4 μg of calf thymus DNA as a carrier, and either 1 to 2 μg of *STE14*-containing plasmid or the YEp24 vector alone. After a 30-min incubation at 30°C, 130 μl of 50% polyethylene glycol 4000 in 10 mM Tris-HCl (pH 7.5) was added, and the incubation was continued for an additional 60 min at 30°C. The transformation mixtures were then heated at 42°C for 5 min and diluted with 1 ml of YPD medium. The cells were pelleted by centrifugation in a microcentrifuge, suspended in 1 ml of YPD, and allowed to incubate at 30°C for 1 to 5 h. The cells were again pelleted and then suspended in 100 μl of SD medium. Transformants were selected on SDLM plates.

The transformants were able to mate when crossed with a complementary α strain (GX3087) on SDL medium. No mating was observed with the nontransformed *ste14'* mutant cells. We further confirmed the identity of the plasmid in the transformed cells by demonstrating the presence of a 4.8-kilobase insert upon digestion of the plasmid DNA with *Bam*HI.

RESULTS

Identification of a C-terminal carboxyl methyltransferase activity in *S. cerevisiae*. An in vitro assay has recently been developed to detect the methyltransferase activity responsible for the C-terminal α-carboxyl methylation of lipidated cysteine residues in mammalian cells. This assay makes use of the synthetic methyl-accepting peptide *S*-farnesyl-LARYKC that is recognized by the membrane-bound enzymes that form C-terminal methyl esters on cyclic GMP phosphodiesterase and other proteins (31a). We decided to

TABLE 2. Methyltransferase activity in purified membrane fractions of various strains

Strain	Relevant genotype	Methyltransferase sp act[a] (pmol/min per mg) ± SD
ABYS1	*STE MATa*	1.34 ± 0.10 (5)
C39-U3	*STE' MATα*	5.70 ± 1.12 (4)
K43-4c	*ste16*	1.47 ± 0.59 (3)
K39A-3b	*ste6*	2.86 ± 1.46 (13)
HR129-2d	*ste14*	0.003 ± 0.007 (6)
XBH38-3a	*ste14'*	0.001 ± 0.005 (10)

[a] Activities from control incubations lacking peptide have been subtracted. The number of determinations is shown in parentheses.

use this assay to try to identify a similar enzyme activity in the yeast *S. cerevisiae*, where the formation of carboxyl-terminal cysteinyl α-methyl esters has been found in a mating factor (1) and the RAS 2 protein (10). The amino acid sequence of this peptide, derived from a *Drosophila melanogaster ras* sequence, does not correspond to that of either yeast a factor or the RAS1 or RAS2 protein. However, there is little sequence similarity in the residues immediately preceding the conserved cysteine residue of the expanding group of proteins which appear to be isoprenylated and methyl esterified (8). This peptide does contain, nevertheless, the farnesylated C-terminal cysteine residue that appears to form the methylation site in these molecules.

We detected C-terminal methyltransferase activity in the purified membrane fraction of various strains of *S. cerevisiae* at specific activities similar to those found in preparations of rat liver, kidney, spleen, and brain membranes (Table 2) (31a). The methyltransferase activity was linearly dependent upon incubation time (Fig. 1) and enzyme concentration (Fig. 2). The activity measured was dependent upon the presence of the *S*-farnesyl peptide; control incubations lacking the peptide showed only background methyl ester formation (Fig. 2; Table 3). We also found that the LARYKC

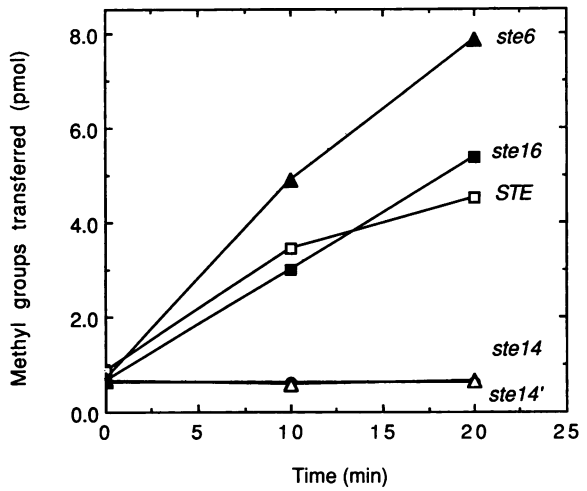

FIG. 1. Time course analysis of the methyltransferase activity of nonsterile (*STE*) and a-specific sterile yeast strains. Incubations included 1 nmol of *S*-farnesyl LARYKC, 20 μl of 50 μM *S*-adenosyl-L-[^{14}C-*methyl*]methionine, 34 μl of 100 mM Tris-HCl (pH 7.50), and 6 μl of membrane preparation (30 mg of protein per ml). Strains are represented as follows: K39A-3b (*ste6*) (▲), K43-4c (*ste16*) (■), ABYS1 (*STE*) (□), HR129-2d (*ste14*) (●), and XBH38-3a (*ste14'*) (△).

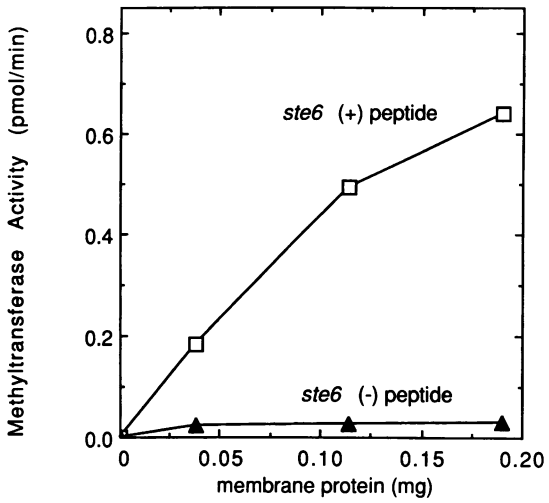

FIG. 2. Dependence of methyltransferase activity on peptidyl methyl acceptor. The a-specific mutant strain K39A-3b (*ste6*) was incubated with (□) or without (▲) the *S*-farnesyl-LARYKC peptide substrate in reaction mixes containing 20 μl of 50 μM *S*-adenosyl-L-[^{14}C-*methyl*]methionine, 20 μl of 100 mM Tris-HCl (pH 7.50), 0, 1, 3, or 5 μl of membranes (38 mg/ml), and buffer to a total volume of 20 μl. Samples were incubated for 30 min at 37°C.

peptide lacking the *S*-farnesyl modification was not a methyl-accepting substrate (Table 3), suggesting that the methylation reaction is preceded by the farnesylation reaction in vivo. Little or no activity was detected in the cytosolic fraction (100,000 × *g*) of any of the strains listed in Table 2 (Table 3; data not shown).

***ste14* mutant strains lack C-terminal methyltransferase activity.** While enzyme activity was detected in the purified membrane fraction of a and α wild-type strains and *ste16* and *ste6* mutant strains, essentially no activity was found in two *ste14* mutant strains (Table 2, Fig. 1). The lack of activity in these strains did not appear to be the result of the presence of an inhibitor in the membrane preparation. Mixing experiments were performed, demonstrating that the methyltransferase activities in the various strains were additive. For example, when 3 μl of membranes from *ste14* mutant strains were mixed with 3 μl of membranes from wild-type strains, the activity was approximately half of that seen for 6 μl of

TABLE 3. Farnesylated peptide substrate is required for methyltransferase activity

Enzyme source[a] (μg of protein)	Methyl-accepting substrate[b]	Methyl groups transferred[c] (pmol)
Membranes (64)	None	1.3
	LARYKC	1.3
	S-Farnesyl-LARYKC	12.5
	S-Farnesyl-LARYKC + LARYKC	12.2
Cytosol (56)	None	1.2
	LARYKC	1.3
	S-Farnesyl-LARYKC	1.4
	S-Farnesyl-LARYKC + LARYKC	1.4

[a] Purified membranes and cytosol were prepared from XBH38-3a (*ste14'*) cells transformed with the *STE14*-containing plasmid pSL646.

[b] One nanomole of LARYKC or *S*-farnesyl-LARYKC or both was present in each incubation mix.

[c] Values represent averages from duplicate 45-min incubations.

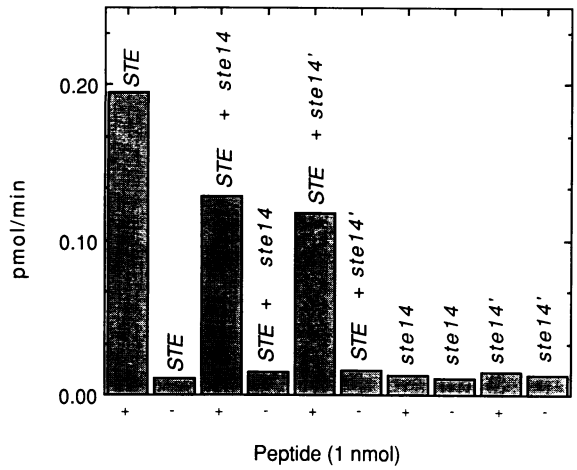

FIG. 3. Methyltransferase activity is not inhibited in the presence of membrane preparations of *ste14* mutant strains. Incubations with (+) or without (−) the *S*-farnesyl-LARYKC peptide substrate included 20 μl of 50 μM *S*-adenosyl-L-[^{14}C-*methyl*]methionine, 34 μl of 100 mM Tris-HCl (pH 7.50), and a total of 6 μl of enzyme from ABYS1 (*STE*), HR129-2d (*ste14*), and XBH38-3a (*ste14'*) membrane preparations. Mixed incubations contained 3 μl of each of the membrane preparations. The protein concentrations of these strains were 24 (*STE*), 42 (*ste14*), and 37 (*ste14'*) mg of protein per ml. Samples were incubated for 45 min at 37°C.

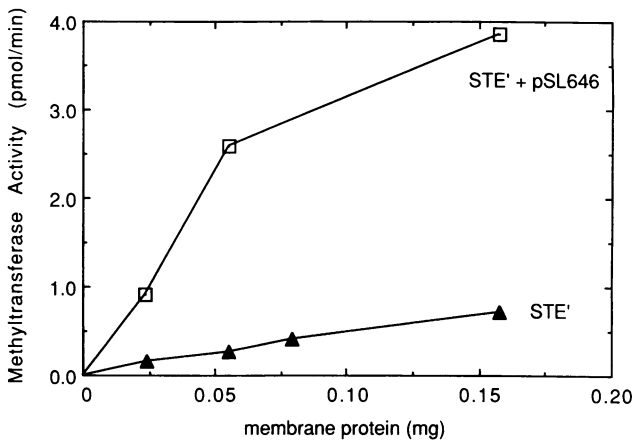

FIG. 4. Transformation of wild-type cells with the *STE14*-containing plasmid results in a sixfold overproduction of the methyltransferase activity. Incubation mixes with or without peptide substrate included 1 nmol of *S*-farnesyl-LARYKC, 20 μl of 50 μM *S*-adenosyl-L-[^{14}C-*methyl*]methionine, 0 to 20 μl of C39-U3 (*STE'*) membranes (7.9 mg/ml) (▲) or 0 to 20 μl of C39-U3(pSL646) membranes (3.4 mg/ml) (□), and 100 mM Tris-HCl (pH 7.50), to a total volume of 30 μl. Samples were incubated for 35 min at 37°C.

wild-type membranes (Fig. 3). Additionally, when membranes containing the active methyltransferase were mixed with the cytosolic fraction from the *ste14* mutant strains, no inhibition of activity was found (data not shown). This latter result suggests the absence of a cytosolic inhibitor in the *ste14* mutant cells.

Transformation of wild-type cells and *ste14* mutant cells with a plasmid containing the *STE14* gene results in the overproduction of active C-terminal methyltransferase. In order to clearly demonstrate the linkage between the defect in the *ste14* mutant strains and the identified methyltransferase activity, the *ste14'* mutant was transformed with a plasmid containing the *STE14* gene in a YEp24 vector as described in Materials and Methods. We found that this transformation resulted in an overproduction of active methyltransferase (Table 4). No detectable methyltransferase activity was observed in nontransformed cells or in cells obtained from control transformations with the YEp24 vector alone (Table 4). An α strain (C39-U3) containing the wild-type *STE14* gene was also transformed with the plasmid pSL646. This resulted in a sixfold overproduction of active methyltransferase above the endogenous level (Fig. 4).

TABLE 4. Plasmid complementing the *ste14* mutation leads to production of an active C-terminal methyltransferase in the purified membrane fraction

Strain	Methyltransferase sp act[a] (pmol/min per mg) ± SD
XBH38-3a (*ste14'*)	0.00 ± 0.01 (4)
XBH38-3a(YEp24)	0.02 ± 0.04 (4)
XBH38-3a(pSL646)	55.3 ± 8.00 (5)

[a] Activities from control incubations lacking peptide have been subtracted. The number of determinations is shown in parentheses.

DISCUSSION

Our results show that the *STE14* gene product is required for the expression of a farnesyl cysteine C-terminal methyltransferase and suggest that any **a** factor produced in *ste14* mutant strains lacks the C-terminal methyl ester modification. The physiological role of methylation of **a** factor is not yet clear. Anderegg et al. demonstrated that chemically demethylated **a** factor showed little or no activity under the conditions of their assay (1). In *T. brasiliensis*, the unmethylated peptide mating factor, tremerogen *A*-9291-VIII, is 200 times less active than the methylated form, *A*-9291-I (21). Additionally, it is possible that methylation may be required for the secretion of **a** factor. Because we detected no methyl-accepting activity of the nonfarnesylated form of this peptide, we propose that the methyl esterification reaction follows the isoprenylation reaction and may represent the final C-terminal posttranslational modification in the maturation of **a** factor.

This enzyme may also modify other proteins in yeast cells containing a similar C-terminal-modified cysteine residue. The substrate for the methyl esterification reaction is a peptide containing the *S*-farnesyl C-terminal cysteine residue found in **a** factor but is otherwise unrelated in sequence. Additionally, the enzyme activity is present in α mating type cells. Thus, the same enzyme responsible for the methylation of **a** factor may also recognize the *RAS* products (10, 13), although it is not yet clear whether the *ste14* mutation affects *RAS* function in a similar fashion as the *ste16* mutation (28). This methyltransferase may also be responsible for the C-terminal modification of other yeast proteins initially synthesized with the -Cys-Xaa-Xaa-Xaa sequence (8). Candidates for this type of methylation reaction include the nuclear lamin analogs (5, 6, 15) and the *STE18* gene product encoding the γ-subunit of a G-protein complex coupled to the mating pheromone receptors (14, 34).

Although the variation in enzyme activity measured in a given membrane preparation is small, we found a fourfold difference in the specific activity of preparations of membranes from non-*ste14* strains (Fig. 1, Table 2). It is unclear

whether these differences reflect actual changes in methyltransferase levels in these strains of differing genetic backgrounds or reflect differences in growth conditions and/or membrane preparations.

What can we say about the nature of the *STE14* gene? An attractive hypothesis is that it represents the structural gene for the methyltransferase. Alternatively, the *STE14* gene product may encode an essential activator protein required either for the expression of the methyltransferase gene or for the action of the methyltransferase enzyme itself. The fact that we observed a sixfold overproduction of enzyme by cells containing a wild-type *STE14* gene transformed with a plasmid carrying multiple copies of *STE14* suggests that methyltransferase activity is related to gene copy number. This result is consistent with the structural-gene hypothesis, but we cannot rule out other possibilities at present.

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LITERATURE CITED

1. **Anderegg, R. J., R. Betz, S. A. Carr, J. W. Crabb, and W. Duntze.** 1988. Structure of *Saccharomyces cerevisiae* mating hormone a-factor: identification of *S*-farnesyl cysteine as a structural component. J. Biol. Chem. **263**:18236–18240.
2. **Bailey, J. L.** 1967. Techniques in protein chemistry, p. 340–341. Elsevier Publishing Co., New York.
3. **Betz, R., J. W. Crabb, H. E. Meyer, R. Wittig, and W. Duntze.** 1987. Amino acid sequences of a-factor mating peptides from *Saccharomyces cerevisiae*. J. Biol. Chem. **262**:546–548.
4. **Casey, P. J., P. A. Solski, C. J. Der, and J. E. Buss.** 1989. p21^{ras} is modified by a farnesyl isoprenoid. Proc. Natl. Acad. Sci. USA **86**:8323–8327.
5. **Chelsky, D., J. F. Olson, and D. E. Koshland, Jr.** 1987. Cell cycle-dependent methyl esterification of lamin B. J. Biol. Chem. **262**:4303–4309.
6. **Chelsky, D., C. Sobotka, and C. L. O'Neill.** 1989. Lamin B methylation and assembly into the nuclear envelope. J. Biol. Chem. **264**:7637–7643.
7. **Chen, Z.-Q., L. S. Ulsh, G. DuBois, and T. Y. Shih.** 1985. Posttranslational processing of p21^{ras} proteins involves palmitylation of the C-terminal tetrapeptide containing cysteine 186. J. Virol. **56**:607–612.
8. **Clarke, S., J. P. Vogel, R. J. Deschenes, and J. Stock.** 1988. Posttranslational modification of the Ha-*ras* oncogene protein: evidence for a third class of protein carboxyl methyltransferases. Proc. Natl. Acad. Sci. USA **85**:4643–4647.
9. **Deschenes, R. J., and J. R. Broach.** 1987. Fatty acylation is important but not essential for *Saccharomyces cerevisiae RAS* function. Mol. Cell. Biol. **7**:2344–2351.
10. **Deschenes, R. J., J. B. Stimmel, S. Clarke, J. Stock, and J. R. Broach.** 1989. *RAS2* protein of *Saccharomyces cerevisiae* is methyl esterified at its carboxyl terminus. J. Biol. Chem. **264**:11865–11873.
11. **Farnsworth, C. C., S. L. Wolda, M. H. Gelb, and J. A. Glomset.** 1989. Human lamin B contains a farnesylated cysteine residue. J. Biol. Chem. **264**:20422–20429.
12. **Fujiyama, A., K. Matsumoto, and F. Tamanoi.** 1987. A novel yeast mutant defective in the processing of *ras* proteins: assessment of the effect of the mutation on processing steps. EMBO J. **6**:223–228.
13. **Fujiyama, A., and F. Tamanoi.** 1990. RAS2 protein of *Saccharomyces cerevisiae* undergoes removal of methionine at N terminus and removal of three amino acids at C terminus. J. Biol. Chem. **265**:3362–3368.
14. **Fung, B. K.-K., H. K. Yamane, I. M. Ota, and S. Clarke.** 1990. The γ-subunit of brain G-proteins is methyl esterified at a C-terminal cysteine. FEBS Lett. **260**:313–317.
15. **Georgatos, S. D., I. Maroulakou, and G. Blobel.** 1989. Lamin A, lamin B, and lamin B receptor analogues in yeast. J. Cell Biol. **108**:2069–2082.
16. **Goodman, L. E., C. M. Perou, A. Fujiyama, and F. Tamanoi.** 1988. Structure and expression of yeast *DPR1*, a gene essential for the processing and intracellular localization of *ras* proteins. Yeast **4**:271–281.
17. **Gutierrez, L., A. I. Magee, C. J. Marshall, and J. F. Hancock.** 1989. Post-translational processing of p21^{ras} is two-step and involves carboxyl-methylation and carboxy-terminal proteolysis. EMBO J. **8**:1093–1098.
18. **Hancock, J. F., A. I. Magee, J. E. Childs, and C. J. Marshall.** 1989. All Ras proteins are polyisoprenylated but only some are palmitoylated. Cell **57**:1167–1177.
19. **Herskowitz, I.** 1989. A regulatory hierarchy for cell specialisation in yeast. Nature (London) **342**:749–757.
20. **Holtz, D., R. A. Tanaka, J. Hartwig, and F. McKeon.** 1989. The CaaX motif of lamin A functions in conjunction with the nuclear localization signal to target assembly to the nuclear envelope. Cell **59**:969–977.
21. **Ishibashi, Y., Y. Sakagami, A. Isogai, and A. Suzuki.** 1984. Structures of tremerogens *A*-9291-I and *A*-9291-VIII: peptidyl sex hormones of *Tremella brasiliensis*. Biochemistry **23**:1399–1404.
22. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
23. **Kuchler, K., R. E. Sterne, and J. Thorner.** 1989. *Saccharomyces cerevisiae STE6* gene product: a novel pathway for protein export in eukaryotic cells. EMBO J. **8**:3973–3984.
24. **McGrath, J. P., and A. Varshavsky.** 1989. The yeast *STE6* gene encodes a homologue of the mammalian multidrug resistance P-glycoprotein. Nature (London) **340**:400–404.
25. **Michaelis, S., and I. Herskowitz.** 1988. The a-factor pheromone of *Saccharomyces cerevisiae* is essential for mating. Mol. Cell. Biol. **8**:1309–1318.
26. **Ong, O. C., I. M. Ota, S. Clarke, and B. K.-K. Fung.** 1989. The membrane binding domain of rod cGMP phosphodiesterase is posttranslationally modified by methyl esterification at a C-terminal cysteine. Proc. Natl. Acad. Sci. USA **86**:9238–9242.
27. **Ota, I. M., and S. Clarke.** 1989. Enzymatic methylation of 23–29-kDa bovine retinal rod outer segment membrane proteins. Evidence for methyl ester formation at carboxyl-terminal cysteinyl residues. J. Biol. Chem. **264**:12879–12884.
28. **Powers, S., S. Michaelis, D. Broek, S. Santa Anna-A., J. Field, I. Herskowitz, and M. Wigler.** 1986. *RAM*, a gene of yeast required for a functional modification of *RAS* proteins and for production of mating pheromone a-factor. Cell **47**:413–422.
29. **Sakagami, Y., M. Yoshida, A. Isogai, and A. Suzuki.** 1981. Peptidal sex hormones inducing conjugation tube formation in compatible mating-type cells of *Tremella mesenterica*. Science **212**:1525–1527.
30. **Schafer, W. R., R. Kim, R. Sterne, J. Thorner, S.-H. Kim, and J. Rine.** 1989. Genetic and pharmacological suppression of oncogenic mutations in *RAS* genes of yeast and humans. Science **245**:379–385.
31. **Serrano, R.** 1983. In vivo glucose activation of the yeast plasma membrane ATPase. FEBS Lett. **156**:11–14.

31a. **Stephenson, R. C., and S. Clarke.** 1990. Identification of a C-terminal protein carboxyl methyltransferase in rat liver membranes utilizing a synthetic, farnesyl cysteine-containing peptide substrate. J. Biol. Chem. **265**:14791–14797.

32. **Tamanoi, F., E. C. Hseuh, L. E. Goodman, A. R. Cobitz, R. J. Detrick, W. R. Brown, and A. Fujiyama.** 1988. Posttranslational modification of *ras* proteins: detection of a modification prior to fatty acid acylation and cloning of a gene responsible for the modification. J. Cell Biochem. **36**:261–273.
33. **Vorburger, K., G. T. Kitten, and E. A. Nigg.** 1989. Modification of nuclear lamin proteins by a mevalonic acid derivative occurs in reticulocyte lysates and requires the cysteine residue of the C-terminal CXXM motif. EMBO J. **8**:4007–4013.
34. **Whiteway, M., L. Hougan, D. Dignard, D. Y. Thomas, L. Bell, G. C. Saari, F. J. Grant, P. O'Hara, and V. L. MacKay.** 1989. The *STE4* and *STE18* genes of yeast encode potential β and γ subunits of the mating factor receptor-coupled G protein. Cell **56**:467–477.
35. **Wilson, K. L., and I. Herskowitz.** 1987. *STE16*, a new gene required for pheromone production by a cells of *Saccharomyces cerevisiae*. Genetics **155**:441–449.
36. **Wolda, S. L., and J. A. Glomset.** 1988. Evidence for modification of lamin B by a product of mevalonic acid. J. Biol. Chem. **263**:5997–6000.