

# The *Saccharomyces cerevisiae* *STE14* gene encodes a methyltransferase that mediates C-terminal methylation of a-factor and RAS proteins

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**Post-translational processing of a distinct group of proteins and polypeptides, including the a-factor mating pheromone and RAS proteins of *Saccharomyces cerevisiae*, results in the formation of a modified C-terminal cysteine that is S-isoprenylated and  $\alpha$ -methyl esterified. We have shown previously that a membrane-associated enzymatic activity in yeast can mediate *in vitro* methylation of an isoprenylated peptide substrate and that this methyltransferase activity is absent in *ste14* mutants. We demonstrate here that *STE14* is the structural gene for this enzyme by expression of its product as a fusion protein in *Escherichia coli*, an organism in which this activity is lacking. We also show that a-factor, RAS1 and RAS2 are physiological methyl-accepting substrates for this enzyme by demonstrating that these proteins are not methylated in a *ste14* null mutant. It is notable that cells lacking *STE14* methyltransferase activity exhibit no detectable impairment of RAS function or cell viability. However, we did observe a kinetic delay in the rate of RAS2 maturation and a slight decrease in the amount of membrane localized RAS2. Thus, methylation does not appear to be essential for RAS2 maturation or localization, but the lack of methylation can have subtle effects on the efficiency of these processes.**

**Key words:** a-factor mating pheromone/C-terminal farnesyl cysteine methyltransferase/methyl esterification/RAS processing/yeast *STE14* gene

## Introduction

A group of eukaryotic proteins and polypeptides that undergo post-translational modification are synthesized with a C-terminal sequence, -Cys-Xaa-Xaa-Xaa (where -Xaa is any amino acid). This sequence motif, designated the CXXX motif, serves as a signal for three ordered modification events including isoprenylation of the cysteine sulfhydryl, proteolysis of the terminal three residues, and  $\alpha$ -methyl esterification of the newly exposed carboxyl group (Clarke *et al.*, 1988; Hancock *et al.*, 1989; Rine and Kim, 1990; Stimmel *et al.*, 1990). Examples include fungal mating pheromones (Ishibashi *et al.*, 1984; Anderegg *et al.*, 1988; Akada *et al.*, 1989), RAS proteins (Gutierrez *et al.*, 1989), nuclear lamins (Farnsworth *et al.*, 1989) and the  $\gamma$ -subunit

of trimeric G proteins (Yamane *et al.*, 1990). All of these polypeptides are membrane associated, or pass through a membrane. While isoprenylation has been shown to play an essential role in membrane localization, the role of the methylation reaction has not been established.

Genetic analysis in the yeast *Saccharomyces cerevisiae* has provided a means for identifying cellular components that mediate these post-translational processing events. The yeast mating pheromone a-factor precursor terminates in a CXXX motif (Brake *et al.*, 1985; Powers *et al.*, 1986; Michaelis and Powers, 1988). Mature bioactive a-factor is a secreted dodecapeptide with a C-terminal cysteine that is farnesylated and methyl esterified (Anderegg *et al.*, 1988). Because defects in the synthesis of a-factor result in a readily detectable *MATa* cell-specific sterile phenotype, it has been possible to identify four mutants, *ste6* (Rine, 1979), *ste14* (Blair, 1979), *ram1* (Powers *et al.*, 1986; Wilson and Herskowitz, 1987) and *ram2* (Goodman *et al.*, 1990), that are impaired in a-factor secretion or processing. *STE6* encodes a membrane transporter responsible for the export of a-factor (McGrath and Varshavsky, 1989; Kuchler *et al.*, 1989). *RAM1*, alternatively named *DPR1*, was originally identified on the basis of its necessity for both RAS and a-factor activity (Powers *et al.*, 1986; Fujiyama *et al.*, 1987; Michaelis and Powers, 1988). In *ram1* mutants, the RAS and a-factor precursors fail to become membrane localized and remain in the cytosol (Powers *et al.*, 1986; Schafer *et al.*, 1990; P.Chen and S.Michaelis, unpublished observations). Recently, the *RAM1* gene was shown to encode a component of the farnesyltransferase enzyme responsible for isoprenylation of RAS, a-factor and the  $\gamma$ -subunit of a yeast G protein (Goodman *et al.*, 1988, 1990; Finegold *et al.*, 1990; Schafer *et al.*, 1990). Thus, the yeast farnesyltransferase mediates modification of multiple CXXX-terminating cellular proteins. The *RAM2* gene, like *RAM1*, appears to be critical for isoprenylation of proteins in yeast (Goodman *et al.*, 1990). Farnesyltransferase activity has also been found in mammalian systems (Reiss *et al.*, 1990; Schaber *et al.*, 1990).

We have investigated the role of the *STE14* gene product in the post-translational modification of yeast proteins containing a CXXX sequence at their C-terminus. DNA sequence analysis reveals that the *STE14* product is 239 residues in length and appears to contain multiple membrane spanning domains (Sapperstein *et al.*, 1989). We have recently demonstrated, using an *in vitro* assay, that *S.cerevisiae* contains a membrane-bound C-terminal methyltransferase activity specific for a peptide substrate terminating in S-farnesyl cysteine (Hrycyna and Clarke, 1990). This methyltransferase activity is present in both *MATa* and *MAT $\alpha$*  cells and is dependent upon the presence of a wild-type *STE14* gene. A mammalian counterpart of the yeast methyltransferase has been recently described that possesses similar activity (Stephenson and Clarke, 1990).

In the present report, we further examine the *in vitro* and



*in vivo* activities promoted by STE14. We show that an extract from an *Escherichia coli* strain synthesizing a TrpE-STE14 hybrid protein is capable of promoting methylation *in vitro*. This result provides strong evidence that STE14 is the structural gene for the yeast methyltransferase enzyme. We also demonstrate that STE14 is required for the *in vivo* methylation of a-factor, RAS1 and RAS2. Thus, the STE14 gene product, like RAM1, is an enzyme responsible for the post-translational modifications of multiple yeast proteins. Interestingly, *ste14* null mutants are not detectably impaired in RAS activity, suggesting that methyl modification is not essential for RAS function. We do observe, however, that *ste14* mutants exhibit a significant kinetic delay in RAS2 maturation and a slight decrease in the amount of RAS2 that becomes membrane-bound.

## Results

### Methyltransferase activity of TrpE-STE14 hybrid proteins synthesized in *E. coli*

We recently characterized a farnesyl cysteine methyltransferase activity in membrane extracts from *S. cerevisiae* and found that the activity is absent in extracts from *ste14* mutant strains (Hrycyna and Clarke, 1990). This result raised the possibility that the STE14 gene encodes the methyltransferase enzyme. Alternatively, the STE14 product could be a rate-limiting component of this enzyme or a regulator of its synthesis. To distinguish these possibilities, we expressed STE14 as a hybrid protein in *E. coli*, an organism with no endogenous farnesyl cysteine methyltransferase activity (Figure 1). Using pATH vectors, two gene fusions were constructed, *TrpE-STE14<sub>1-239</sub>* and *TrpE-STE14<sub>102-239</sub>*, in which all or part of the STE14 coding sequence, respectively, was joined to the C-terminal end of the *E. coli* TrpE gene.

We assayed *in vitro* methyltransferase activity in membrane and cytosolic extracts from strains induced for synthesis of these fusions. A high level of methyltransferase activity was observed with the membrane fraction from a strain containing the *TrpE-STE14<sub>1-239</sub>* fusion (Figure 1) in which the entire coding sequence of STE14 is present. This activity was dependent upon the presence of a methyl-accepting substrate and product formation was linearly dependent on time. No activity was observed in membrane fractions from the parental strain which lacks a plasmid, nor in membrane fractions from a strain with the *TrpE-STE14<sub>102-239</sub>* fusion, which contains only the C-terminal portion of STE14 (Figure 1). In no case was cytosolic methyltransferase activity detected. These results indicate that the STE14 gene product is a methyltransferase and provide strong evidence that the STE14 polypeptide is the sole component of this methyltransferase. The possibility that another component required for activity might be supplied by the *E. coli* membrane fraction is unlikely, but cannot be ruled out.

### Characterization of substrate recognition by the STE14 methyltransferase

In our previous work, we showed that the farnesylated peptide, *S*-farnesyl LARYKC is an efficient *in vitro* substrate for the yeast methyltransferase (Hrycyna and Clarke, 1990). To refine our understanding of the substrate requirements

for the STE14 methyltransferase and, in particular, to learn whether specific peptide sequences are required for enzyme recognition, we examined *in vitro* methylation of a compound lacking any amino acids except for a farnesylated cysteine residue. This compound, *N*-acetyl farnesyl cysteine (*N*-AcFC), has been reported to be a methylatable substrate in a mammalian cell extract (Stock et al., 1990). We compared the efficiency of methylation of *S*-farnesyl LARYKC and *N*-AcFC substrates using membrane extracts from a wild-type strain and from two isogenic *ste14* null mutants. As shown in Table I, the wild-type extract is able to methylate both substrates with comparable efficiency. The identity of the expected product, methyl esterified *N*-AcFC, was confirmed (Figure 2). No methylation of either substrate was detected in the *ste14* membrane extracts (Table I). The

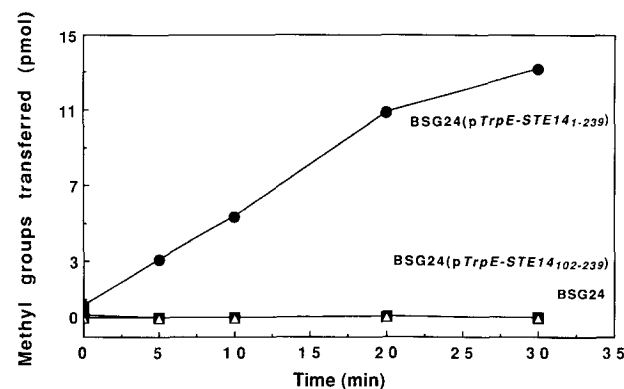


Fig. 1. Expression of the full length STE14 gene product as a TrpE fusion protein in *E. coli* generates C-terminal farnesyl cysteine methyltransferase activity. Crude membrane fractions (10  $\mu$ l) from the parent *E. coli* strain, BSG24 ( $\Delta$ ), and from transformants with a plasmid containing TrpE fused to the full length STE14 coding region, BSG24 (pTrpE-STE14<sub>1-239</sub>) ( $\bullet$ ), or to the C-terminal portion of STE14 BSG24 (pTrpE-STE14<sub>102-239</sub>) ( $\blacksquare$ ) were assayed with 20  $\mu$ l of 50  $\mu$ M [ $^3$ H-methyl]AdoMet and 20  $\mu$ l 100 mM Tris-HCl (pH 7.5) with or without the methyl-accepting substrate *N*-acetyl farnesyl cysteine (*N*-AcFC) (1 nmol). The samples were incubated for the times indicated at 37°C. The protein concentrations of the BSG24, BSG24 (pTrpE-STE14<sub>1-239</sub>) and BSG24 (pTrpE-STE14<sub>102-239</sub>) samples were 15.5 mg/ml, 17.8 mg/ml and 19.5 mg/ml respectively. Methyl esters were detected as described in Materials and methods. The small amount of methyl esters formed (less than 0.1 pmol) in the absence of *N*-AcFC were subtracted to give the values presented. Similar results were obtained when *S*-farnesyl LARYKC was used as a substrate.

Table I. Methyltransferase activity in isogenic strains

Strain	Relevant genotype	Methyltransferase activity (pmol/min/mg protein) <sup>a</sup>
<b>Substrate: <i>S</i>-farnesyl-LARYKC</b>		
SM1058 (STE14 <sup>+</sup> )	STE14 <sup>+</sup>	0.94 ( $\pm$ 0.20)
SM1188 ( <i>ste14</i> )	<i>ste14</i>	0.003 ( $\pm$ 0.008)
SM1639 ( <i>ste14</i> )	<i>ste14</i>	0.0006 ( $\pm$ 0.0005)
SM1639 (pSM344)	<i>ste14</i> (2 $\mu$ STE14 LEU2)	4.42 ( $\pm$ 0.02)
<b>Substrate: <i>N</i>-acetyl <i>S</i>-farnesyl cysteine</b>		
SM1058 (STE14 <sup>+</sup> )	STE14 <sup>+</sup>	0.43 ( $\pm$ 0.01)
SM1188 ( <i>ste14</i> )	<i>ste14</i>	0.011 ( $\pm$ 0.002)
SM1639 ( <i>ste14</i> )	<i>ste14</i>	0.005 ( $\pm$ 0.003)
SM1639 (pSM344)	<i>ste14</i> (2 $\mu$ STE14 LEU2)	8.40 ( $\pm$ 0.05)

<sup>a</sup> Activities from control incubations lacking farnesylated substrate have been subtracted. Each value represents duplicate incubations  $\pm$  the observed range.

**Table II.** Incorporation of [<sup>3</sup>H]methyl esters and [<sup>35</sup>S]cysteine in immunoprecipitated RAS1 and RAS2 in *STE14*<sup>+</sup> and *ste14* strains

Strain	Plasmid	[ <sup>3</sup> H]Methyl esters in RAS1 or RAS2 (c.p.m.) <sup>a</sup>	[ <sup>35</sup> S]Cysteine in RAS1 or RAS2 (c.p.m.) <sup>a</sup>	Relative extent of methylation ([ <sup>3</sup> H]c.p.m./[ <sup>35</sup> S]c.p.m. × 10)
SM1058 ( <i>STE14</i> <sup>+</sup> )		17.3	285.8	0.61 <sup>b</sup>
SM1188 ( <i>ste14</i> )		0.9	192.2	<0.05
SM1058 ( <i>STE14</i> <sup>+</sup> )	(YE <i>p-RAS2-4</i> )	148.8	1120.8	1.3
SM1188 ( <i>ste14</i> )	(YE <i>p-RAS2-4</i> )	0.1	1563.8	<0.0006
SM1058 ( <i>STE14</i> <sup>+</sup> )	( <i>pADH-RAS2</i> )	114.8	1574.6	0.73
SM1188 ( <i>ste14</i> )	( <i>pADH-RAS2</i> )	1.4	1720.5	<0.008
SM1058 ( <i>STE14</i> <sup>+</sup> )	(YE <i>p-RAS1</i> )	115.8	2259.2	0.51
SM1639 ( <i>ste14</i> )	(YE <i>p-RAS1</i> )	0.1	1182.4	<0.0008

<sup>a</sup>Background radioactivity from adjacent gel slices have been subtracted.

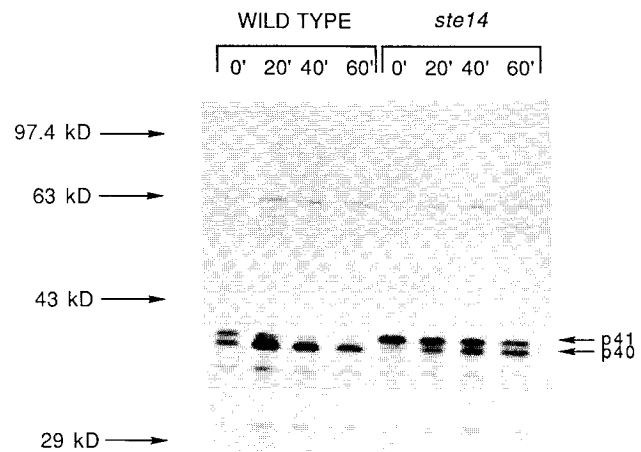
methyl]AdoMet, and RAS proteins were examined after immunoprecipitation and SDS-PAGE. Gel slice vapor assays were performed to detect [<sup>3</sup>H-methyl]esters, and [<sup>35</sup>S]cysteine incorporated into proteins was monitored by autoradiography and direct counting of gel slices. Figure 4 shows the data obtained with strains containing the *RAS2* plasmid. In the autoradiograph shown in Figure 4 (top), a 40 kd [<sup>35</sup>S]cysteine-labelled band corresponding to *RAS2* is present at similar levels in both wild-type and *ste14* strains (lanes 1 and 3). In the *ste14* mutant, a doublet was consistently observed at this position (see below). The identity of the 40 kd species and doublet bands as *RAS2* was confirmed by their disappearance in control immunoprecipitations performed with a competitor peptide recognized by the Y13-259 *RAS* monoclonal antibody (Figure 4, top, lanes 2 and 4).

To determine the level of *RAS2* methylation, the dried gels shown at the top of Figure 4 were examined by gel slice vapor assays (Figure 4, lower panels A-D). In the wild-type strain (Figure 4, panel A), a methyl ester peak is apparent in gel slice number 13, which corresponds to *RAS2*, as evidenced by its disappearance with addition of the blocking peptide (Figure 4, panel B). Strikingly, no methyl esters are detected at the *RAS2* position in immunoprecipitates from the *ste14* mutant (Figure 4, panels C and D). These results indicate that *RAS2* remains unmethylated in the *ste14* mutant and demonstrate that *RAS2* is a physiological substrate for the *STE14* methyltransferase. These data are quantified in Table II, together with similar results from experiments using a different *ste14* null mutant.

We also examined methylation of *RAS1* in wild-type and *ste14* strains containing a high copy number *RAS1* plasmid. The results we obtained were analogous to those described above for *RAS2* (Table II). Taken together, the results in Figure 4 and Table II establish that *STE14* is responsible for methylation of both the *RAS1* and *RAS2* proteins. Since it is known that functional *RAS* proteins are required for viability in yeast (Powers *et al.*, 1984; Tatchell *et al.*, 1984), and since *ste14* mutants are not compromised for viability, our results point to the possibility that methylation is not essential for *RAS* function, at least under normal conditions.

#### Maturation of *RAS2* in strains lacking the *STE14* methyltransferase

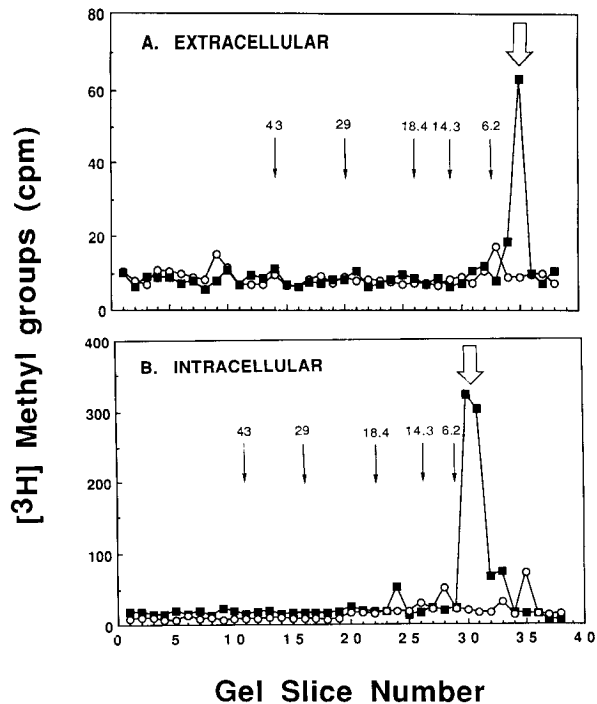
We were interested in understanding the significance of the *RAS2* doublet detected in the *ste14* mutant, above (Figure 4, upper panel, lane 3). The upper band of the doublet has a



**Fig. 5.** Kinetics of *RAS2* processing in wild-type and *ste14* strains. Wild-type (SM1058) and *ste14* (SM1188) strains containing the plasmid *pADH-RAS2* were labelled with [<sup>35</sup>S]Translabel for 2 min at 30°C, and chased for the indicated times. Total cell extracts prepared as described in Materials and methods were immunoprecipitated with anti-ras monoclonal antibody Y13-259 and immunoprecipitates were analyzed by SDS-PAGE using a 10% acrylamide gel.

mobility characteristic of the *RAS2* precursor (p41), which migrates slightly above authentic *RAS2* (p40) (Fujiyama *et al.*, 1987; Tamanoi *et al.*, 1988). Normally, maturation of the *RAS2* precursor occurs quite rapidly and p41 can only be detected in pulse-labelled wild-type strains or in mutants, such as *ram1*, in which isoprenylation is blocked (Powers *et al.*, 1986). Thus, the persistence of p41 here suggested that the rate of *RAS2* maturation might be altered in strains lacking the *STE14* methyltransferase.

To examine this possibility, we performed pulse-chase analysis of *RAS2* in wild-type and isogenic *ste14* strains. Cells were labelled briefly with a mixture of [<sup>35</sup>S]cysteine and [<sup>35</sup>S]methionine (<sup>35</sup>S-Translabel), chased with non-isotopically labelled amino acids for varying lengths of time, and *RAS2* proteins were immunoprecipitated and analyzed by SDS-PAGE (Figure 5). Since processing in the wild-type strain is extremely rapid, we observe that even before the chase is initiated, 50% of the *RAS2* protein has already undergone processing to p40 (Figure 5). By 20 minutes, 80% of *RAS2* is converted to the p40 form, and by 40 minutes, conversion to p40 is essentially complete. In

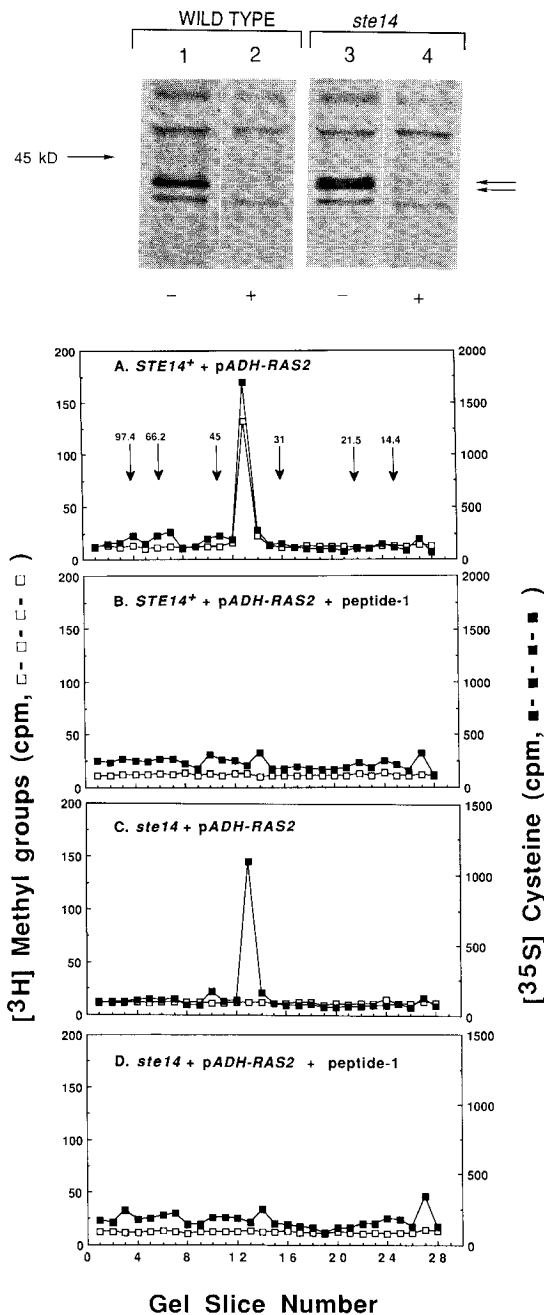


**Fig. 3.** Comparison of *a*-factor methylation *in vivo* in wild-type and *ste14* strains of *S. cerevisiae*. A wild-type strain, SM1058 (■) and *ste14* mutant, SM1188 (○) containing the *a*-factor plasmid pSM219 were labelled separately with [<sup>35</sup>S]cysteine or [<sup>3</sup>H-methyl]AdoMet at 30°C and processed as described in Materials and methods. Extracellular and intracellular fractions were immunoprecipitated using *a*-factor antiserum. Immunoprecipitates from the extracellular (A) and intracellular (B) fractions were subjected to SDS-PAGE using a 12.5% acrylamide gel. The lanes of the dried gel containing the [<sup>3</sup>H-methyl]AdoMet samples were cut into 3 mm slices and each slice assayed for methyl esters as described in Materials and methods. The distribution of base hydrolyzed [<sup>3</sup>H]methyl groups released from extracellular (A) and intracellular (B) proteins is shown. The portion of the gel containing [<sup>35</sup>S]cysteine-labelled samples was subjected to autoradiography to visualize *a*-factor. The open arrow indicates the migration position of the mature (A) and precursor (B) *a*-factor species that were detected. Positions of the pre-stained molecular weight markers are marked.

as has also been previously observed (Marr *et al.*, 1990; S.Sapperstein and S.Michaelis, in preparation). In addition, the lack of methylation greatly reduces *a*-factor biological activity; as described in Materials and methods, we determined by halo dilution assays of concentrated culture fluid that the unmethylated species of *a*-factor secreted by the *ste14* mutant appears to be at least 200-fold less active than *a*-factor produced by wild-type cells.

#### STE14-mediated methylation of RAS1 and RAS2

The RAS proteins of yeast, like *a*-factor, are farnesylated and carboxyl methyl esterified (Deschenes *et al.*, 1989; Stimmel *et al.*, 1990). Since the isoprenylation of RAS proteins and *a*-factor is carried out by a common mechanism involving the *RAM1* and *RAM2* gene products (Powers *et al.*, 1986; Goodman *et al.*, 1990) and since STE14 activity is not confined to cells of the *MATa* mating type, we were interested in determining whether RAS and *a*-factor are also methylated by a common enzyme. To test whether STE14 is responsible for methylation of RAS proteins, we compared *in vivo* methylation of RAS in wild-type and *ste14* mutant strains. Cells containing a high copy number *RAS1* or *RAS2* plasmid were double-labelled with [<sup>35</sup>S]cysteine and [<sup>3</sup>H-



**Fig. 4.** Comparison of RAS2 methylation in wild-type and *ste14* strains. Wild-type (SM1058) and *ste14* (SM1188) cells containing pADH-RAS2 plasmids were double-labelled with [<sup>35</sup>S]cysteine and [<sup>3</sup>H-methyl]AdoMet at 30°C and total cell extracts were prepared and immunoprecipitated with rat anti-pan-ras monoclonal antibody Y13-259 as described in Materials and methods. Immunoprecipitates were fractionated by SDS-PAGE on a 10% acrylamide gel. The dried gel was subjected to autoradiography to allow detection of [<sup>35</sup>S]cysteine incorporation (top). To examine methylation, the lanes were sliced into 3 mm slices after autoradiography (lower panels A-D). The slicing was designed using the autoradiogram above as a guide, such that the band corresponding to RAS2 was fully contained in gel slice number 13 and the non-specific background band which appears immediately below RAS2 in all lanes was fully contained in gel slice number 14. These slices were assayed for methyl esters as base-volatile [<sup>3</sup>H]methanol radioactivity (panels A and C) (□). Controls with the blocking peptide are shown in panels B and D. Subsequently, the same slices were quantitatively assayed for total [<sup>35</sup>S]cysteine incorporation into the RAS2 protein (■) as described in Materials and methods and shown in Table II. Migration positions of the low molecular weight standards are indicated.

independent isolates of SM1823 (*ras2<sup>val19</sup> ste14*) were tested and gave results that are indistinguishable from their *ras2<sup>val19</sup> STE14* parent, TK161-R2V (data not shown). Thus, the *ste14* mutation does not appear to reverse the heat shock sensitivity or lack of iodine staining of *ras2<sup>val19</sup>*. Apparently, although lack of methylation can lead to subtle defects in RAS processing and localization, there are no measurable consequences for RAS activity under the conditions tested here.

## Discussion

Methyl esterification appears to be the final step in formation of C-terminal farnesyl and geranylgeranyl cysteine methyl esters in peptides and proteins. The results presented here demonstrate that this methylation reaction is catalyzed by the product of the *STE14* gene in *S. cerevisiae*. We have also shown that the a-factor mating pheromone, RAS1 and RAS2 lack detectable C-terminal methylation in a *ste14* null mutant, and thus are physiological substrates of the STE14 methyltransferase. Our results strengthen the biochemical link between RAS and a-factor that was first suggested by studies of *RAM1* (Powers *et al.*, 1986), and extend the notion that a common machinery mediates the series of three modifications (isoprenylation, proteolytic cleavage, and methylation) proposed to occur on proteins that terminate with a CXXX motif, where the penultimate residue is aliphatic (Stimmel *et al.*, 1990).

Where in the cell does C-terminal methylation of isoprenylated proteins occur? Interestingly, inspection of the *STE14* DNA sequence reveals multiple potential membrane spanning domains, indicative of integral membrane proteins (Sapperstein *et al.*, 1989). Moreover, biochemical studies clearly indicate that STE14 methyltransferase enzyme activity is found in the membrane fraction (Hrycyna and Clarke, 1990). It will be interesting to determine whether methylation is carried out at the plasma membrane of yeast or on an intracellular membrane such as the cytoplasmic face of the endoplasmic reticulum. One attractive hypothesis is that the three reactions involved in maturation of CXXX-terminating proteins are carried out by a higher order complex of several polypeptides. Our observation here that a defect in methylation can impede the preceding steps of isoprenylation and proteolytic processing of RAS proteins (see below) supports the idea of a processing complex. However, it should be noted that while the STE14 methyltransferase is membrane-bound, the RAM1 component of the isoprenyltransferase is apparently soluble (Schäfer *et al.*, 1990; Goodman *et al.*, 1990) though a weak membrane association might not be easily detectable.

### Methylation of a-factor by STE14

Mutants in the *STE14* gene were originally isolated on the basis of the inability of *MATa* cells to mate, due to a defect in a-factor production (Blair, 1979; Wilson and Herskowitz, 1987). We show here that methylation of a-factor fails to occur in the *ste14* mutant. The absence of methylation has dramatic consequences for a-factor. We have observed that the non-methylated a-factor produced by a *ste14* mutant is at least 200-fold less active than a-factor made by a wild-type strain. In addition, others have shown that a-factor lacking its methyl group has severely decreased biological activity (Anderegg, 1988; J. Becker, personal

communication). Thus the methyl ester on a-factor appears to be necessary either for binding of a-factor to its receptor, STE3, on the surface of MAT $\alpha$  cells (Nakayama *et al.*, 1985; Hagen *et al.*, 1986), or for the subsequent activation step that leads to G<sub>1</sub> arrest and mating. As observed here and elsewhere (Marr *et al.*, 1990; S. Sapperstein and S. Michaelis, in preparation) a second consequence of the lack of methylation is a reduced amount of a-factor secretion by *ste14* mutants. The basis for this apparent defect in a-factor export remains to be established.

### Modification of RAS proteins by the STE14 methyltransferase

The profound methylation defect we observe for RAS1 and RAS2 in a *ste14* null mutant (Figure 4 and Table II) suggests that STE14 may be the major, if not the only yeast methyltransferase that can modify the C-terminus of RAS proteins. It is surprising, however, that while mutations that eliminate RAS function cause a major disruption in cell growth and survival (Kataoka *et al.*, 1984; Tatchell *et al.*, 1984), the apparent lack of methylation in *ste14* mutants has no impact on cell viability, nor on the more subtle phenotypes of heat shock sensitivity and low starch levels conferred by a *ras2<sup>val19</sup>* mutation. In comparison with *ram1* and *ram2* mutants, which are defective in farnesylation of RAS and exhibit a severe growth defect (Powers *et al.*, 1986), the *ste14* mutant grows normally under all conditions that we have examined. Although we cannot rule out the possibility that the methylation of an undetectable fraction of RAS proteins by an as yet unidentified enzyme could preserve their function, we stress that we detect < 1% of the wild-type methylation level of RAS1 and RAS2 in *ste14* mutants (Table II). In addition, methyltransferase activity is essentially undetectable in *in vitro* assays using membranes derived from *ste14* null mutants (Table I). In view of the essential nature of RAS function in yeast, the viability of the *ste14* mutant suggests that RAS proteins retain function in the absence of methylation. Alternatively, function may be somewhat impaired, but if the amount of RAS present in cells is actually far above saturating levels, then even a dramatic decrease in RAS activity would go unnoticed under normal laboratory conditions. If this were the case, it might be possible to identify a particular carbon source, temperature or ionic condition under which cell growth becomes dependent on a functional STE14 methyltransferase activity.

### Maturation and membrane localization of RAS2 in a ste14 mutant

Despite the lack of an obvious effect on cell viability, we have observed several intriguing differences in RAS2 maturation between wild-type and *ste14* mutant strains. Biogenesis of RAS proteins is a complex process. In both yeast and mammalian cells, RAS is initially synthesized as a soluble precursor with a C-terminal CXXX motif. Maturation has been proposed to involve two steps (Hancock *et al.*, 1989; Fujiyama and Tamanoi, 1990): Step 1 includes isoprenylation, proteolytic cleavage and methylation, resulting in a cytosolic species whose gel mobility differs slightly from that of the precursor. Methylation is thought to occur last in this series of reactions. In Step 2, RAS becomes membrane localized and palmitoylated. In *ste14* cells we observe a striking kinetic