

Ras membrane targeting is essential for glucose signaling but not for viability in yeast

(RAS2/farnesylation/palmitoylation/immunofluorescence/cAMP)

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ABSTRACT Ras proteins are small GTP binding proteins that serve as critical relays in a variety of signal transduction pathways in eukaryotic cells. Like most metazoan Ras proteins, yeast Ras is post-translationally modified by addition of a farnesyl and a palmitoyl moiety, and these modifications are required for targeting the protein to the cytoplasmic face of the plasma membrane and for biological activity of the protein. We have constructed mutants of the yeast (*Saccharomyces cerevisiae*) Ras that are farnesylated *in vivo* but are not palmitoylated. These mutant proteins are not localized to the plasma membrane but function in the cell as well as the wild-type protein. Such mutants are viable but fail to induce a transient increase in intracellular cAMP concentration in response to glucose addition, although this deficiency does not yield a marked growth phenotype. These results are consistent with the hypothesis that the essential role of the farnesyl moiety on yeast Ras is to enhance productive interaction between Ras and its essential downstream target, adenylyl cyclase, rather than to localize Ras to the plasma membrane.

All Ras proteins undergo a series of post-translational modifications of their carboxyl termini, including farnesylation and in most cases palmitoylation, that are required for partitioning the proteins to the cytoplasmic face of the plasma membrane (1–10). In metazoans, this localization appears to be essential for Ras's role in signal transduction, since signaling through the pathway relies on repartitioning the Ras activating protein, Sos, and the Ras effector protein, Raf, to the membrane compartment at which Ras resides as the means of completing the signaling circuit (11–17).

Yeast Ras proteins participate in a signal transduction pathway that appears to connect glucose availability to the metabolic activity of the cell. The two Ras proteins, encoded by *RAS1* and *RAS2*, stimulate adenylyl cyclase to yield increased cAMP levels, which in turn activates the cAMP-dependent protein kinase (A kinase) (18, 19). Phosphorylation of a number of target proteins by activated A kinase results in enhanced glycolysis, mobilization of energy reserves, and specific transcriptional activation. The precise biological signal that activates Ras is not known, but addition of glucose to starved cells yields a transient increase in cAMP levels that is dependent on Ras and on the Ras guanine nucleotide exchange factor, Cdc25p (20–24).

Previous observations have established a correlation between post-translational processing of Ras, its membrane localization, and its biological activity. Like metazoan Ras proteins, yeast Ras proteins are post-translationally modified by farnesylation, palmitoylation, proteolytic removal of the terminal three amino acids, and carboxyl methylesterification (2, 6, 9). Yeast Ras proteins are located predominantly in the cell in a membrane compartment, as are other components of the signal transduction pathway with which Ras interacts,

including adenylyl cyclase and Cdc25p (M. Jacquet, personal communication; refs. 25 and 26). Mutation of the site of farnesylation in Ras blocks all post-translational processing, abolishes its biological activity, and renders the protein predominantly cytoplasmic (27). This has prompted models suggesting that post-translational modification of Ras is essential for its biological activity because it allows colocalization of Ras with other components of the signal transduction apparatus.

In this report we describe mutants of Ras in *Saccharomyces cerevisiae* that separate membrane localization from biological activity. We have found that mutations of the palmitoylation site of Ras block palmitoylation but not other post-translational processing steps. In contrast to wild-type Ras protein, these mutant proteins are not localized to the plasma membrane and do not support a glucose-mediated transient induction of cAMP. However, these mutant proteins perform their essential function in the cell as well as the wild-type protein. Thus, these results clearly separate membrane localization and glucose signaling from the essential role of Ras in yeast and support the hypothesis that the primary role of post-translational modification of Ras is in direct protein-protein interaction.

MATERIALS AND METHODS

Plasmids and Strains. Strain KP-2 (*MATa trp1-289 leu2-3, 112 his3Δ ura3-52 ade8 can1 ras2::URA3*) was constructed by transforming strain SP-1 (18) to Ura⁺ with *ras2::URA3* (19). Strain Y294 (*MATα his3Δ1 leu2-3,112 ura3-52 trp1-289 GAL⁺*), used for immunofluorescence, carried either the wild-type *RAS2* or the mutant *RAS2*^{C318S} or *ras2*^{C319S} alleles on plasmid YEPRAS2-1. Strain Y1810 (*MATa/MATα leu2/leu2 his3/his3 ura3/ura3 trp1/trp1 ras1::HIS3/ras1::HIS3 ras2*^{C319S}/*RAS2::URA3*) was obtained by crossing Y768 [designated SGP-4 in Deschenes and Broach (27)] with strain Y1809 (*MATa leu2 his3 ura3 trp1 ras1::HIS3 ras2*^{C319S} [pTLC1-*RAS2*]) and then retrieving a diploid isolate that had lost the pTLC1-*RAS2* plasmid. Strain Y1809 was obtained as a segregant from a cross between Y525 [designated RJS-1 in Deschenes and Broach (27)] and Y651 (*MATα his3 leu2 ura3 trp1 ade8 ras1::HIS3 ras2::URA3* [pTLC1-*RAS2*]). Strain Y1808 (*MATa/MATα leu2/leu2 his3/his3 ura3/ura3 trp1/trp1 ade8/ade8 ras1:TRP1/ras1::HIS3 RAS2*^{C318S}/*RAS2::URA3*) was obtained by crossing strain Y768 with a *ras1::TRP1 RAS2*^{C318S} segregant from a cross between ST103 (*MATa leu2 ura3 trp1 leu2 ade8 ras1::TRP1 ras2::ADE8* [YE24-*RAS2*]) and L1-D-1 (*MATα ura3 trp1 leu2 his3 RAS2*^{C318S}). Strains 1808-3A (*RAS2*⁺) and 1808-3C (*RAS2*^{C318S}) were segregants obtained by sporulating diploid Y1808. Isogenic derivatives of strain RS62-22A (*MATα his3 leu2 ura3 trp1 ade2 ade8 ras2::URA3*) containing the indicated *RAS2* alleles were obtained by a modified form of the counterselection method for allele replacement (28) by cotransforming linear fragments containing the *RAS2* allele (29) along with plasmid pHV1 (30)

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selecting His⁺ transformants and screening them for Ura⁻. Allele replacement was confirmed by PCR analysis.

The 2 μ plasmids YEp51 GAL10-RAS2 and YEp51 GAL10-RAS2^{C319S} have been described (27). Plasmid YEP-RAS2-1 (29) consists of the ADH1 promoter on a *LEU2*-2 μ vector and was used to express wild-type or mutant *RAS2* alleles.

Growth Media. Yeast were grown either in rich medium (YEPD: 1% yeast extract, 2% Bacto-Peptone, and 2% glucose) or in synthetic medium [0.67% yeast nitrogen base without amino acids, supplemented with the appropriate amino acid(s) to satisfy auxotrophic requirements and the indicated carbon source added to 2%].

Palmitate Labeling. Strains were grown to 10⁷ cells per ml in synthetic medium with raffinose (2%) as sole carbon source. Synthesis of the wild-type or mutant Ras2p was induced by addition of galactose to 2%, the cultures were split, and cells in one half of each culture were labeled with [³H]palmitate. Cells were harvested from all cultures after 2 hr and extracts were prepared as described (31). For immunoblotting, 50- μ g extracts from the unlabeled cultures were fractionated by SDS gel electrophoresis, transferred to nitrocellulose, and probed with anti-Ras antibody, Y13-259 (32). For autoradiography, equal amounts of labeled extract ($\approx 5 \times 10^7$ total cpm) from the two cultures were immunoprecipitated with Y13-259 antibody. The precipitates were dissolved in sample buffer and fractionated by SDS/PAGE, and the gel was subjected to autoradiography for 3 weeks.

Immunofluorescence Analyses. Cells were grown to 10⁷ cells per ml in synthetic complete medium lacking leucine and then fixed and prepared for immunofluorescence as described (33). The primary anti-Ras2p monoclonal antibody, γ -RAS2-3G6, was used as undiluted culture supernatant; the goat anti-mouse secondary antibody (Zymed) was conjugated with biotin and visualized with fluorescein isothiocyanate-conjugated streptavidin (Zymed) as described (34). Images were obtained with a Bio-Rad MRC600 confocal imaging head, equipped with an ILT argon/krypton laser, mounted on a Nikon Optiphot II microscope using a 60 \times planapo objective (Nikon; numerical aperture, 1.40; oil immersion objective; zoom factor, 2). While optimal results for visualization of Ras protein localization were obtained with strains in which Ras protein was moderately overexpressed, identical results to those shown in Fig. 2 were obtained with *RAS2* expressed under its own promoter on an intermediate-copy level plasmid, pUV2, and on a low-copy plasmid, pRS316. These constructs were also tested in a *ras1 ras2* strain with identical results.

Iodine Assay and Heat Shock Analysis. Strains were grown in YEPD plates and exposed to iodine vapors as described (18). Heat shock analyses were performed by incubating YEPD plates with the relevant strains at 55°C for the indicated times and then shifting to 30°C for 24 hr (35).

RESULTS

Palmitoylation Is Required for Localization of Ras2p to the Plasma Membrane. To evaluate the role of post-translational modification of Ras in yeast, we introduced a series of mutations into *RAS2*, the major Ras isoform in the cell, that ablated different steps in the post-translational modification program. We then evaluated the subcellular localization and the biological activity of the resultant mutant proteins. Ras2p is normally modified by a thioether linkage of farnesyl to cysteine 319, a thioester linkage of palmitate to cysteine 318, proteolytic removal of the carboxyl-terminal three amino acids, and methyl esterification of the revealed carboxyl-terminal cysteine 319. One mutation, C318S, prevented palmitoylation of the protein without affecting farnesylation, proteolytic cleavage, or methyl esterification. A second mutation, C319S, blocked farnesylation of the protein and, since farnesy-

lation is required for all subsequent modifications, precluded palmitoylation, cleavage, and methyl esterification as well (4). The structures of the carboxyl termini of the wild-type Ras2p and the two mutant proteins are summarized in Table 1. The inability of Ras2p(C318S) to undergo palmitoylation is documented in Fig. 1. The absence of any modification of Ras2p(C319S) has been previously shown (27, 36, 37).

We examined the subcellular localization of wild-type Ras2p and the mutant Ras2p proteins by indirect immunofluorescence using confocal microscopy. As evident in Fig. 2, wild-type Ras2p resides primarily on the plasma membrane. In contrast, Ras2p(C318S), which lacks only the palmitoyl moiety, and Ras2p(C319S), which lacks the palmitoyl and farnesyl groups, are localized predominantly to the cytoplasm, with no apparent enrichment in the plasma membrane. The patterns of staining of the two mutant proteins are indistinguishable. Identical results are obtained with strains carrying the *RAS2* alleles on low-copy *CEN* plasmids, indicating that <10% of the Ras2p(C318S) is localized to the plasma membrane (data not shown). Results from subcellular fractionation studies of the wild-type and mutant proteins are consistent with those obtained by immunofluorescence (data not shown). The requirement for membrane localization on palmitoylation is also observed with two other mutant proteins, Ras2(Δ 308-317) and Ras2(Δ 308-318). The former lacks the 10 amino acids immediately upstream from the palmitoylation site and exhibits a wild-type localization pattern; in contrast, the latter mutant lacks the same 10 amino acids as well as the palmitoylation site and shows no localization to the membrane (Fig. 2 Lower). Thus, membrane localization of Ras2p requires palmitoylation in addition to farnesylation; farnesylation alone is not sufficient to yield stable association of Ras2p with the plasma membrane. Similar results are observed for mammalian Ras proteins (38, 39).

Ras Biological Activity Requires Farnesylation But Not Palmitoylation. Despite the fact that Ras2 protein lacking the palmitoyl moiety alone and Ras2 protein lacking the palmitoyl and farnesyl moieties show identical subcellular localization, the biological activities of these two proteins are quite distinct. The first demonstration of the difference in function of the two mutant proteins is in cell viability. Wild-type yeast contain two *RAS* genes, *RAS1* and *RAS2*, and require at least one functional *RAS* gene for mitotic growth (19). As shown in Fig. 3 Upper, *ras1 RAS2*^{C318S} spores show normal viability, whereas *ras1 ras2*^{C319S} spores fail to yield colonies. The slightly smaller spore clones from *ras1 RAS2*^{C318S} versus *ras1 RAS2* strains may reflect a slight delay in germination of the former cells, since single *ras1 RAS2*^{C318S} cells give rise to the same-sized colonies as do *RAS2* cells on restreaking (Fig. 3 Lower). We conclude that Ras2p(C318S) supports normal mitotic growth of yeast and thus fulfills the essential function of yeast Ras in the cell. In contrast, Ras2p(C319S) does not support mitotic growth and shows no biological activity by this assay.

The second measure of the biological activity of Ras2p(C318S) is intragenic suppression of activated alleles of

Table 1. Modification pattern of Ras2 proteins used in this study

Allele	Amino acid sequence*			Modification	
	Positions 308-317	Position 318	Position 319	Farnesyl [†]	Palmitate [‡]
<i>RAS2</i>	SEASKSGSGG	C	C	+	+
C318S	SEASKSGSGG	S	C	+	-
C319S	SEASKSGSGG	C	S	-	-
Δ 307-317	Δ	C	C	+	+
Δ 307-318	Δ	Δ	C	+	-

*Alleles were constructed by site-directed mutagenesis.

[†]Ref. 36.

[‡]Ref. 27 and Fig. 1.

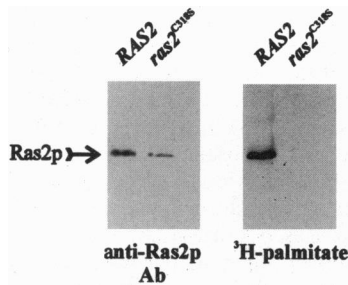


FIG. 1. Palmitate labeling of Ras2 proteins. (Right) Autoradiogram of SDS/PAGE fractionated extracts of strain KP-2 harboring a YEp51 GAL10-RAS2 plasmid or YEp51 GAL10-RAS2^{C318S} plasmid, prepared after labeling cells *in vivo* for 2 hr with [³H]palmitate. (Left) Immunoblot of SDS/PAGE fractionation of the same extracts as in Right, probed with anti-Ras antibody.

Ras. Strains containing dominant mutations of *RAS2*, such as G19V, that lock the protein in its activated state exhibit a variety of distinct phenotypes. These include diminished accumulation of glycogen, as monitored by staining colonies with iodine vapors, and increased sensitivity to heat shock (18, 35). Second site mutations introduced into the *RAS2*^{G19V} gene that diminish the function of the protein attenuate these activated phenotypes. Thus, the degree to which a mutation inserted into *RAS2*^{G19V} gene reverses the activated phenotype of strains carrying it is a measure of the degree to which that mutation abolishes the biological activity of the Ras2 protein. As shown in Fig. 4 Upper, *RAS2*^{G19V} strains and *RAS2*^{G19V,C318S} strains show the same diminished level of iodine staining, compared to *RAS2* strains. In contrast, *RAS2*^{G19V,C319S} strains show the same intense level of staining as do *ras2* strains. Thus, by this assay the C318S mutation maintains full biological activity, whereas the C319S mutation completely abolishes activity. A similar situation is observed with heat shock: the *RAS2*^{G19V,C318S} strain exhibits heat shock sensitivity similar to that of the *RAS2*^{G19V} strains, whereas the *RAS2*^{G19V,C319S} strain behaves like a *ras2* strain. In this assay, though, the *RAS2*^{G19V,C318S} strain is slightly more resistant than the *RAS2*^{G19V} strains, suggesting that the C318S mutation is not completely innocuous. Nonetheless, these results substantiate the conclusions from the previous assays that the C319S mutation severely compromises the function of the Ras2 protein whereas the C318S mutation has only a minor effect.

Palmitoylation Is Required for Ras-Mediated Transient Induction of Intracellular cAMP Levels by Glucose. Whereas loss of Ras2p palmitoylation has only a minor effect on the essential functions of Ras2p, strains carrying this mutation are

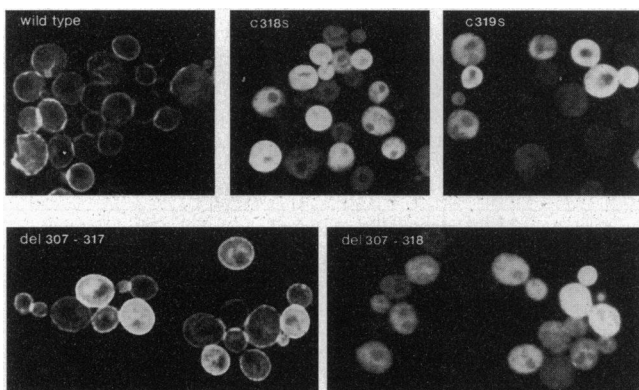


FIG. 2. Immunofluorescence localization of mutant Ras2 proteins in yeast. Confocal micrographs of strain Y294 carrying the indicated *RAS2* alleles. Ras proteins were visualized by indirect immunofluorescence using a monoclonal antibody (yRAS2-3G6) raised to yeast Ras2p.

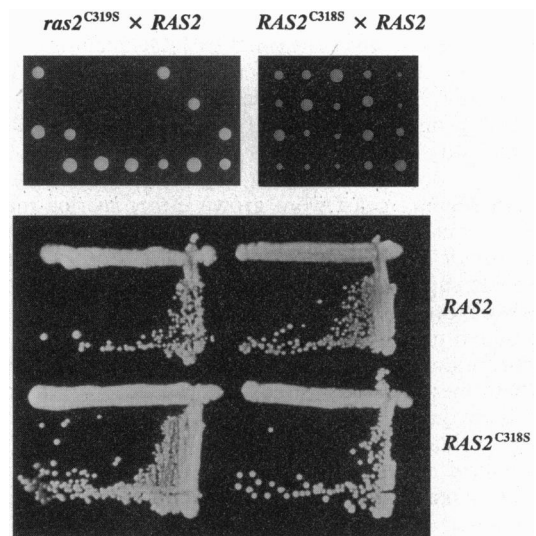


FIG. 3. Palmitoylation is not required for Ras-dependent mitotic growth in yeast. (Upper) Strains Y1808 and Y1810, which are *ras1/ras1 RAS2::URA3/RAS2*^{C318S} and *ras1/ras1 RAS2::URA3/ras2*^{C319S}, respectively, were sporulated, dissected on YEPD plates, and grown at 30°C. The four spores from each tetrad are arrayed vertically. As determined by subsequent tests for uracil auxotrophy, the two smaller spore clones in each tetrad from strain Y1808 are *RAS2*^{C318S} and the two spore clones in each tetrad from strain Y1810 that failed to grow are *ras2*^{C319S}. (Lower) The four clones from a single tetrad from strain Y1808 were restreaked on YEPD and grown at 30°C. The relevant *RAS2* genotype is indicated.

severely compromised for glucose-induced transient activation of adenylyl cyclase. As has been previously reported and as shown in Fig. 5, addition of glucose to a *ras1 RAS2* strain yields a rapid but transient increase in the intracellular concentration of cAMP (20–22, 40). In contrast, addition of glucose to a *ras1 RAS2*^{C318S} strain yields absolutely no transient increase in cAMP accumulation. Thus, loss of Ras2p palmitoylation prevents rapid activation of adenylyl cyclase to external stimuli, presumably as a consequence of diminished membrane localization of Ras2p. However, this deficiency does not yield a substantive cellular phenotype.

DISCUSSION

Our results have shown that farnesylation of Ras protein, but not its membrane targeting, is required for Ras to fulfill its essential function in the cell—that is, regardless of the sub-cellular location of Ras, productive interaction between Ras and one or more of the components of the Ras signal transduction pathway is dependent on the farnesyl moiety. In its essential role in yeast, Ras serves as a substrate for Cdc25p, a guanine nucleotide exchange factor, and stimulates adenylyl cyclase, the single essential effector target of Ras in the cell (18, 24, 41). *In vitro* analysis suggests that Cdc25p-catalyzed exchange of guanine nucleotide is not affected by the presence of the farnesyl residue on Ras (42). However, Kuroda *et al.* (43) have shown that farnesylated Ras2p had a significantly higher kinetic affinity for adenylyl cyclase *in vitro* than did unmodified Ras2p. Our results are consistent with the hypothesis that the farnesyl group plays a direct role in the interaction between Ras2p and adenylyl cyclase and documents that this role of the farnesyl residue is critical for the biological activity of Ras *in vivo*.

We have previously shown that, although a single copy of *ras2*^{C319S} does not impart essential RAS function to the cell, *ras1 ras2* strains carrying multiple copies of *ras2*^{C319S} are viable. Thus, overexpression of Ras2p(C319S) can compensate for the reduced activity of the mutant protein. This is consistent with

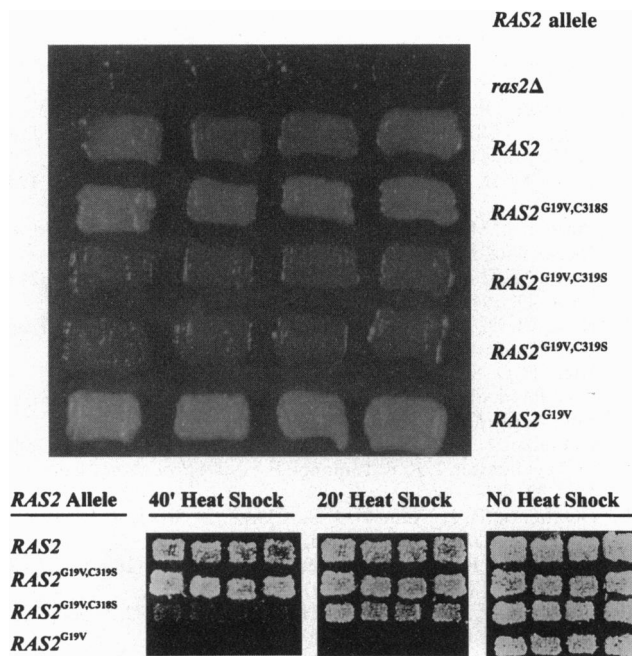


FIG. 4. Intragenic suppression of $RAS2^{G19V}$ by mutations affecting post-translational modification. (Upper) $RAS2^{G19V}$ activity as measured by glycogen accumulation. Isogenic RS62-22A strains containing the indicated single and double mutations in $RAS2$ present at single copy were grown on YEPD plates and treated with iodine vapors. Each row represents different isolates of the same genotypes. The extent of staining correlates inversely with the degree of activation of the RAS/cAMP pathway: strains with low Ras activity stain dark brown, strains with wild-type activity stain light brown, and strains with high activity show no staining. (Lower) $RAS2^{G19V}$ activity as measured by heat shock sensitivity. Strains containing the indicated single or double mutations in $RAS2$ were incubated on plates at 55°C for the indicated time and then shifted to 30°C for 24 hr. Strains with high Ras activity are heat shock sensitive and those with low Ras activity are heat shock resistant.

the hypothesis that farnesylation of Ras2p facilitates interaction with adenylyl cyclase, since overexpression of a mutant Ras2p protein with a lowered affinity for its target would be expected to compensate for the reduced affinity. This was observed by Kuroda *et al* (43), who showed that increased concentrations of nonfarnesylated Ras2p yielded increased cyclase activity in their *in vitro* assays.

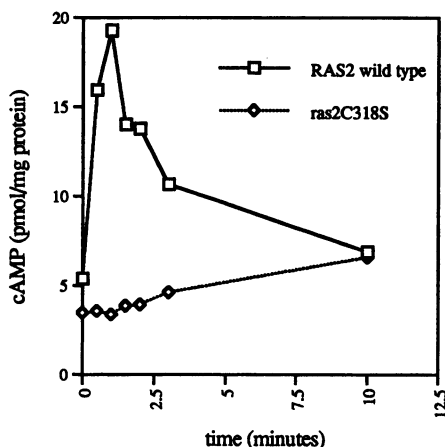


FIG. 5. Time-dependent *in vivo* accumulation of cAMP following glucose addition. Strains Y1808-3A ($RAS2$) and Y1808-3C ($RAS2^{C318S}$) were grown in YEPD to stationary phase and at time 0 glucose was added to 2%. Samples were removed at the indicated times and the intracellular level of cAMP was determined as described (40). Similar results were obtained with strains grown in synthetic medium.

Ras2p that is farnesylated but not palmitoylated, and thus not targeted to the plasma membrane, promotes cell growth and, thus by inference, is still capable of stimulating adenylyl cyclase *in vitro*. One possible explanation for this result is that a small fraction of this nonpalmitoylated Ras2p actually resides in the membrane and provides sufficient stimulation of the membrane-localized cyclase to yield viable levels of cAMP in the cell. However, we find that strains containing only one or two copies of the $RAS2$ mutation defective for palmitoylation exhibit $<10\%$ plasma membrane localization as judged by immunofluorescence. Since other $RAS2$ mutations with less severe reductions in biochemical activity show more dramatic loss of function phenotypes, we do not believe that residual membrane localization accounts for the wild-type phenotype of the $RAS2^{C318S}$ mutants. Rather, we suspect that, since adenylyl cyclase resides in the plasma membrane and cytoplasmic compartments in the cell (25, 26), cytoplasmic Ras protein, as long as it is farnesylated, can activate the cytoplasmic adenylyl cyclase.

Ras protein that is farnesylated but delocalized, though capable of providing sufficient stimulation of adenylyl cyclase for viability, is not capable of mediating a rapid induction of cyclase activity in response to stimulation of the cell by glucose. Thus, membrane targeting is clearly required for Ras to mediate this signaling event. Restriction of Ras2p to the plasma membrane may facilitate efficient interaction with Cdc25p, which is membrane bound and required for transient activation of cyclase by glucose (M. Jaquet, personal communication; refs. 23 and 44). However, our results indicate that this signaling pathway is not required for viability in yeast—a result suggested by previous analysis of rapid exchange mutants of Ras2p (23). Nonetheless, loss of this rapid response pathway may underlie some of the more subtle phenotypes—such as delayed germination—of $RAS2^{C318S}$ strains. By cleanly separating signaling from viability, these mutant proteins should help resolve the biological role of this Ras-mediated signaling pathway.

The results on localization and function of yeast Ras contrast with those obtained for mammalian Ras. Buss *et al* (45) showed that the farnesyl moiety could be replaced by a different membrane targeting signal—myristoylation of the amino terminus—to yield a protein with normal or near-normal activity. Thus, farnesylation *per se* is not required for Ras function in mammalian cells. More recently, Hancock *et al* (38) showed that elimination of the palmitoylation site of Ha-ras or the carboxyl polybasic region of Ki-ras caused loss of membrane localization. However, in this case the mislocalized protein retained transformation potential and the ability to mediate activation of mitogen-activated protein kinase, as long as the Ras protein was farnesylated (39). Thus, the mammalian Ras protein requires either farnesylation or membrane localization for biological activity.

Alternatively, as we suggest for yeast Ras, farnesylation may play a critical role in promoting protein-protein interaction of mammalian Ras, for which some evidence exists (10, 42, 43, 46), but the myristoyl moiety may effectively substitute for farnesyl in this capacity. If this is the case, then the role of post-translational modification in Ras-mediated signal transduction may prove to be the same in yeast and mammals.

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1. Buss, J. E. & Sefton, B. M. (1986) *Mol. Cell. Biol.* 6, 116–122.
2. Clarke, S., Vogel, J. P., Deschenes, R. J. & Stock, J. (1988) *Proc. Natl. Acad. Sci. USA* 85, 4643–4647.
3. Gutierrez, L., Magee, A. I., Marshall, C. J. & Hancock, J. F. (1989) *EMBO J.* 8, 1093–1098.

4. Hancock, J. F., Magee, A. I., Childs, J. E. & Marshall, C. J. (1989) *Cell* **57**, 1167–1177.
5. Casey, P. J., Solski, P. A., Der, C. J. & Buss, J. E. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8323–8327.
6. Fujiyama, A. & Tamanoi, F. (1990) *J. Biol. Chem.* **265**, 3362–3368.
7. Clarke, S. (1992) *Annu. Rev. Biochem.* **61**, 355–386.
8. Giannakouros, T. & Magee, A. I. (1992) in *Lipid Modifications of Proteins*, ed. Schlesinger, M. J. (CRC, Boca Raton, FL), pp. 135–162.
9. Schafer, W. R. & Rine, J. (1992) *Annu. Rev. Genet.* **30**, 209–237.
10. Marshall, C. J. (1993) *Science* **259**, 1865–1866.
11. Egan, S. E., Giddings, B. W., Brooks, M. W., Buday, L., Sizeland, A. M. & Weinberg, R. A. (1993) *Nature (London)* **363**, 45–51.
12. Clark, S. G., Stern, M. J. & Horvitz, H. R. (1992) *Nature (London)* **356**, 340–344.
13. Rozakis-Adcock, M., Fernley, R., Wade, J., Pawson, T. & Bowtell, D. (1993) *Nature (London)* **363**, 83–85.
14. Li, N., Batzer, A., Daly, R., Yajnik, V., Skolnik, E., Chardin, P., Bar-Sagi, D., Margolis, B. & Schlessinger, J. (1993) *Nature (London)* **363**, 85–88.
15. Stokoe, D., MacDonald, S. G., Cadwallader, K., Symons, M. & Hancock, J. F. (1994) *Science* **264**, 1463–1467.
16. Leever, S. J., Paterson, H. F. & Marshall, C. J. (1994) *Nature (London)* **369**, 411–414.
17. Buday, L. & Downward, J. (1993) *Cell* **73**, 611–620.
18. Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K. & Wigler, M. (1985) *Cell* **40**, 27–36.
19. Kataoka, T., Powers, S., McGill, C., Fasano, O., Strathern, J., Broach, J. & Wigler, M. (1984) *Cell* **37**, 437–445.
20. Purwin, C., Leidig, F. & Holzer, H. (1982) *Biochem. Biophys. Res. Commun.* **107**, 1482–1489.
21. Mazon, M. J., Gancedo, J. M. & Gancedo, C. (1982) *Eur. J. Biochem.* **127**, 605–608.
22. Mbonyi, K., Beullens, M., Detremere, K., Geerts, L. & Thevelein, J. M. (1988) *Mol. Cell. Biol.* **8**, 3051–3057.
23. Van Aelst, L., Boy-Marcotte, E., Camonis, J. H., Thevelein, J. M. & Jacquet, M. (1990) *Eur. J. Biochem.* **193**, 675–680.
24. Jones, S., Vignais, M. L. & Broach, J. R. (1991) *Mol. Cell. Biol.* **11**, 2641–2646.
25. Engelberg, D., Simchen, G. & Levitzki, A. (1990) *EMBO J.* **9**, 641–651.
26. Mitts, M. R., Grant, D. B. & Heideman, W. (1990) *Mol. Cell. Biol.* **10**, 3873–3883.
27. Deschenes, R. J. & Broach, J. R. (1987) *Mol. Cell. Biol.* **7**, 2344–2351.
28. Rothstein, R. (1991) *Methods Enzymol.* **194**, 281–301.
29. Powers, S., Kataoka, T., Fasano, O., Goldfarb, M., Broach, J. & Wigler, M. (1984) *Cell* **36**, 607–612.
30. Rose, M. D. & Broach, J. R. (1991) *Methods Enzymol.* **194**, 195–230.
31. Powers, S., Michaelis, S., Broek, D., Santa-Anna, A. S., Field, J., Herskowitz, I. & Wigler, M. (1986) *Cell* **47**, 413–422.
32. Furth, M., Davis, L. J., Fleurdeleys, B. & Scolnick, E. M. (1982) *J. Virol.* **43**, 294–304.
33. Rose, M. D., Winston, F. & Hieter, P. (1990) *Methods in Yeast Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
34. Page, B. D. & Snyder, M. (1992) *Genes Dev.* **6**, 1414–1429.
35. Sass, P., Field, J., Nikawa, J., Toda, T. & Wigler, M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9303–9307.
36. Goodman, L. E., Judd, S. R., Farnsworth, C. C., Powers, S., Gelb, M. H., Glomset, J. A. & Tamanoi, F. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9665–9669.
37. Deschenes, R. J., Stimmel, J. B., Clarke, S., Stock, J. & Broach, J. R. (1989) *J. Biol. Chem.* **264**, 11865–11873.
38. Hancock, J. F., Paterson, H. & Marshall, C. J. (1990) *Cell* **63**, 133–139.
39. Cadwallader, K. A., Paterson, H., MacDonald, S. G. & Hancock, J. F. (1994) *Mol. Cell. Biol.* **14**, 4722–4730.
40. Nakafuku, M., Obara, T., Kaibuchi, K., Miyajima, I., Miyajima, A., Itoh, H., Nakamura, S., Arai, K. I., Matsumoto, K. & Kaziro, Y. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1374–1378.
41. Broek, D., Toda, T., Michaeli, T., Levin, L., Birchmeier, C., Zoller, M., Powers, S. & Wigler, M. (1987) *Cell* **48**, 789–799.
42. Porfiri, E., Evans, T., Chardin, P. & Hancock, J. F. (1994) *J. Biol. Chem.* **269**, 22672–22677.
43. Kuroda, Y., Suzuki, N. & Kataoka, T. (1993) *Science* **259**, 683–686.
44. Munder, T. & Kuntzel, H. (1989) *FEBS Lett.* **242**, 341–345.
45. Buss, J. E., Solski, P. A., Schaeffer, J. P., MacDonald, M. J. & Der, C. J. (1989) *Science* **243**, 1600–1602.
46. Itoh, T., Kaibuchi, K., Masuda, T., Yamamoto, T., Matsuura, Y., Maeda, A., Shimizu, K. & Takai, Y. (1993) *J. Biol. Chem.* **268**, 3025–3028.