Substrate specificity determinants in the farnesyltransferase β-subunit

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ABSTRACT Protein prenyltransferases catalyze the covalent attachment of isoprenoid lipids (farnesyl or geranylgeranyl) to a cysteine near the C terminus of their substrates. This study explored the specificity determinants for interactions between the farnesyltransferase of Saccharomyces cerevisiae and its protein substrates. A series of substitutions at amino acid 149 of the farnesyltransferase β-subunit were tested in combination with a series of substitutions at the C-terminal amino acid of CaaX protein substrates. Efficient prenylation was observed when oppositely charged amino acids were present at amino acid 149 of the yeast farnesyltransferase β-subunit and the C-terminal amino acid of the CaaX protein substrate, but not when like charges were present at these positions. This evidence for electrostatic interaction between amino acid 149 and the C-terminal amino acid of CaaX protein substrates leads to the prediction that the C-terminal amino acid of the protein substrate binds near amino acid 149 of the yeast farnesyltransferase β-subunit.

Biological activity of various proteins, including Ras, lamin B, and yeast α-factor mating pheromone, requires covalent attachment of a 15 carbon prenyl lipid (farnesyl) by protein farnesyltransferase. A related enzyme, protein geranylgeranyltransferase-I, transfers a 20 carbon prenyl lipid (geranylgeranyl) to numerous proteins, including the Ras-related Rho, Rac, and Cdc42 proteins. Both farnesyltransferase and geranylgeranyltransferase-I function as heterodimers; they share an a-subunit but have distinct b-subunits, which are only 33% identical. Both enzymes catalyze the attachment of a prenyl lipid to a cysteine four amino acids from the C-terminal of the protein substrate. The preferred substrates of the mammalian and yeast farnesyltransferases have serine, methionine, cysteine, glutamine, or alanine in the C-terminal position (1–3) and are often referred to as CaaX proteins, where C is cysteine, a is usually an aliphatic amino acid, and X is the C-terminal amino acid. The preferred substrates of the mammalian and yeast geranylgeranyltransferase-I usually have leucine in the C-terminal position (1–3) and are often referred to as CaaL proteins. Farnesyltransferase and geranylgeranyltransferase-I exhibit a degree of cross-specificity for both lipid (3, 9–11) and protein (3, 6, 7, 12–14) substrates.

The discovery that the Ras oncprotein required farnesylation for function (15, 16) prompted intensive studies of farnesyltransferase, in large part because inhibitors of farnesyltransferase may prove to be effective for anti-cancer therapy (17). Despite the recent observation that both farnesyltransferase and geranylgeranyltransferase-I can efficiently prenylate K-rasB in vitro (14), and presumably in vivo, farnesyltransferase-specific inhibitors slow growth of K-rasB tumors in mice (18–24). Surprisingly, geranylgeranyltransferase-I-specific inhibitor (25) can also reverse the transformed phenotypes conferred by activated K-rasB. These promising observations suggest that reducing prenylation of Ras, without completely blocking it, may be sufficient to inhibit uncontrolled growth of cancer cells. Alternatively, additional proteins that depend on farnesylation or geranylgeranylation for function may be required for cancer cell growth. For example, RhoB prenylation appears to be required for Ras-mediated transformation (26, 27).

A more complete understanding of how farnesyltransferase and geranylgeranyltransferase-I bind their protein and lipid substrates will reveal the molecular basis of the substrate specificity and cross-specificity of these enzymes. Genetic (28–32) and biochemical (33–35) studies have provided evidence that both the α- and β-subunits are involved in protein substrate binding and that the active site is likely to be at the interface of the two subunits. The recently published structure of rat farnesyltransferase reveals a hydrophilic cleft at the junction of the α- and β-subunits, which has been proposed to bind the CaaX protein, and a hydrophobic cleft within the α-α barrel structure of the β-subunit, which has been proposed to bind the farnesyl diphosphate substrate (36). Previous studies had strongly implicated cysteine-299 of the β-subunit in both zinc binding and catalysis (31, 37, 38), and the structural data demonstrate that cysteine-299 coordinates with a zinc ion and resides at the junction of the hydrophobic and hydrophilic clefts (36). However, the currently available data do not provide a definitive view of protein or lipid substrate binding or the mechanism of catalysis.

We report here the identification of mutant forms of the yeast farnesyltransferase β-subunit (Ram1p) with altered protein substrate specificity. Our genetic evidence strongly suggests that the C-terminal amino acid of the protein substrate is in close proximity to amino acid 149 of the β-subunit when the protein substrate is productively bound to the farnesyltransferase. In conjunction with the rat farnesyltransferase structural data (36), our observations lead to a prediction of the general orientation for binding of the CaaX sequence to the farnesyltransferase.

MATERIALS AND METHODS

Plasmids. A series of plasmids with substitutions at the last codon of the α-factor structural gene were constructed by inserting a PCR product (encoding the last 7 amino acids of the α-factor precursor and the 3′-flanking sequence) between the BamHI and EcoRI sites of YCplMFAI1, a CEN LEU2 plasmid that carries the MFA1 promoter and coding sequence for the first 29 amino acids of the α-factor precursor inserted as a 605 bp BglII–BamHI fragment into the BamHI site of YCplac111 (39). The BamHI site in the MFA1 gene was created by site-directed mutagenesis of nucleotide 90 from C to T (M. Ashby, unpublished work). The two oligonucleotides used in a PCR with Perkin–Elmer Taq polymerase were C-TGG-GAT-CCA-GCA-TGT-GTT-ATT-NNN-TAG-

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TTT-C, which contained a BamHI site (underlined) and a mixture of all four nucleotides at the last codon (NNN), and TCA-CTG-TAT-ACG-GAA-TTC-TCA-GC, which contained an EcoRI site (underlined) and was complementary to the 3' flanking sequence of the MFA1 gene except for the C in bold.

A series of YcpU-RAS2val19 plasmids with substitutions at the last codon was constructed in pJR1056, a URA3-CEN plasmid containing RAS2val19-BMS, a mutated RAS2 gene with a valine codon in place of glycine codon 19 and with BamHI, MfuI, and SpH1 sites in place of the 3' end of the gene. The RAS2val19-BMS gene was constructed by site-directed mutagenesis (40) of the RAS2val19 gene (1.9 kb Clat-HindIII fragment) in vector pRS306 (41), with a mutagenic oligonucleotide RAS2-SMB (5'-GGC-CTT-CTA-CCA-CTT-TTG-GCA-TGC-TAC-ACG-CGT-TAG-GAT-CCG-CTC-CTG-GAG-GC), which replaced 33 nucleotides of RAS2 (from 945 to 977) with CTAAAGGCTGTCAGATGC. The SpH1, MfuI, and BamHI sequences in the RAS2-SMB oligonucleotide are underlined. pJR1044 was sequenced to confirm the presence of the RAS2val19-BMS gene.

To create a library of RAS2val19 variants with all possible substitutions at the C-terminal or X position of the CaaX sequence, oligonucleotides were inserted between the BamHI and SpH1 sites of pJR1056, a derivative of YCP106 (41) that lacks the polylinker BamHI site and has a 1.9 kb Clat-HindIII fragment carrying RAS2val19-BMS (from pJR1044) inserted into the polylinker. The RAS2-CTA oligonucleotide (5'-CTT-GGC-ATG-CTA-CTA-NNN-TAT-AAT-ACA-ACA-ACC-GCA-TGG-ATC) was annealed to the RAS2-BM primer (5'-TAT-GGA-TCC-GGT-GGC-TG) and incubated with Klenow fragment of T4 polynucleotide kinase and 


gene with

Table 1. Strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or ref.</th>
</tr>
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<tbody>
<tr>
<td>JRY3443</td>
<td>mata MATa sst2-t (ochre) trpl his3 ura3 can1 possibly cys1</td>
<td></td>
</tr>
<tr>
<td>JRY5312</td>
<td>mata dlp hmlap hmnap ade2 leu2 lys2 ura3 mfa1:hisG mfa2:hisG sst2 D GAL1-STE3:HI3</td>
<td>M. Ashby</td>
</tr>
<tr>
<td>JRY5388</td>
<td>mata dlp hmlap hmnap ade2 leu2 lys2 ura3 mfa1:hisG mfa2:hisG sst2 D GAL1-STE3:HI3 ram1-102</td>
<td>V. Boyartchuk (3)</td>
</tr>
<tr>
<td>JRY5389</td>
<td>mata dlp hmlap hmnap ade2 leu2 lys2 ura3 mfa1:hisG mfa2:hisG sst2 D GAL1-STE3:HI3 ram1-103</td>
<td>V. Boyartchuk (43)</td>
</tr>
<tr>
<td>JRY5590</td>
<td>mata dlp hmlap hmnap ade2 leu2 lys2 ura3 mfa1:hisG sst2 D GAL1-STE3:HI3 [pJR157 (MATa URA3)]</td>
<td>This study</td>
</tr>
<tr>
<td>JRY5591</td>
<td>mata dlp hmlap hmnap ade2 leu2 lys2 ura3 mfa1:hisG sst2 D GAL1-STE3:HI3 [pJR157 (MATa URA3)]</td>
<td>This study</td>
</tr>
<tr>
<td>JRY5592</td>
<td>mata dlp hmlap hmnap ade2 leu2 lys2 ura3 mfa1:hisG sst2 D GAL1-STE3:HI3 [pJR157 (MATa URA3)]</td>
<td>This study</td>
</tr>
<tr>
<td>JRY5593</td>
<td>mata dlp hmlap hmnap ade2 leu2 lys2 ura3 mfa1:hisG sst2 D GAL1-STE3:HI3 [pJR157 (MATa URA3)]</td>
<td>This study</td>
</tr>
</tbody>
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RESULTS

A genetic selection designed to isolate mutants defective in processing of the mating pheromone a-factor led to the discovery of two genes, AFC1 and RCE1, encoding proteins involved in proteolytic processing of prenylated proteins (43). Although the selection was intentionally biased toward the identification of genes encoding the CaaX proteases, it also provided the opportunity to find mutations that alter the protein substrate specificity of farnesyltransferase. Briefly, the selection identified mutations that decrease the ability of yeast cells to produce and export fully processed a-factor, particularly when the CaaX sequence at the C terminus of a-factor was changed from CVLA to CAMQ. A strain expressing a-factor–CAMO as the only source of a-factor was mutagenized and 92 mutants that produced very little or no functional a-factor were isolated. After transformation with a wild-type a-factor gene, 24 of these 92 mutants produced a substantial amount of functional a-factor. Among the 24 mutants that were much more defective in the processing of a-factor–CAMO than wild-type a-factor, mutants M1 and M10 were complemented by the wild-type RAG1 gene on a plasmid (Fig. 1). These results suggested that a ram1 mutation was responsible for the a-factor production defect in the M1 and M10 mutant strains.
RAM1 Alleles That Affected Substrate Specificity of Farnesyltransferase. To determine whether mutations were present in the RAM1 genes of the M1 and M10 mutant strains, the RAM1 genes from these two strains (JRY5388 and JRY5389) and from the wild-type parental strain (JRY5312) were amplified, with PCR, and sequenced. The sequence from the parental strain (JRY5312) was identical with GenBank entries for RAM1, whereas the M1 and M10 mutant strains each had a single nucleotide substitution of an A for G at nucleotide 446 in the ORF (Fig. 2). This mutation changed codon 149 from a glycine (GGA) to a glutamic acid (GAA) codon. Because M1 and M10 were both isolated from the same pool of mutagenized cells and have identical sequences, they are likely to be clonal.

The level of Ram1p in the M1 and M10 mutant strains was indistinguishable from the level in the parental strain as determined by immunoblot (data not shown). Thus, the defect in a-factor production in the mutants could not be explained by an effect of the GI49E substitution on the steady-state Ram1p concentration.

To determine whether or not the mutation that changed glycine-149 to glutamate (G149E) altered the specificity of the farnesyltransferase for its protein substrates, we assayed the in vivo substrate specificity of the wild-type and mutant farnesyltransferases. The ram1G149E strain (JRY5389) and the RAM1 parental strain (JRY5312) were transformed with mutated MFA1 plasmids encoding a-factor proteins with substitutions at the C-terminal (or X) position of the CaaX sequence. The relative levels of active a-factor produced from each of the altered MFA1 genes was determined by halo assay (Fig. 3). As expected, the RAM1 strain produced a large halo when a-factor terminated with the wild-type alanine (CVIA), or with cysteine (CVIC) or glutamine (CVIQ), which are the C-termini of amino acids of the known farnesyltransferase substrates, Ras1p (CVIC) and Ydi1p (CASO), respectively. A large halo was also observed when a-factor terminated with glycine (CVIG). Small halos were observed when a-factor terminated with glutamate (CVIE), aspartate (CVID), tryptophan (CVIR), and lysine (CVIK); and virtually no halo was observed with glutamate (CVIG). Small halos were observed when a-factor terminated with glutamate (CVIE), aspartate (CVID), tryptophan (CVIR), and lysine (CVIK); and virtually no halo was observed with a-factor terminating with arginine (CVIR). For seven of the nine a-factor variants (a-factor terminating with A, C, G, Q, E, D, or W), the halo produced by the ram1G149E strain was smaller than the halo produced by the RAM1 strain, which implied that the Ram1G149E protein could simply have a reduced effectiveness relative to the wild-type Ram1 protein. However, the differences in the relative halo sizes produced by wild-type farnesyltransferase than by wild-type farnesyltransferase. With a-factor–CVIR, the halo produced by wild-type farnesyltransferase was larger than that produced by wild-type farnesyltransferase. With a-factor–CVIR, the halo produced by the ram1G149E strain was larger than the halo produced by the RAM1 strain, indicating that the Ram1G149E protein could be farnesylated more effectively by wild-type farnesyltransferase than by wild-type farnesyltransferase.

These halo assay results suggested that substitution of glutamate for glycine-149 of Ram1p affected the region of the farnesyltransferase responsible for interaction with the protein substrate. The positive charges at the C termini of a-factor–CVIR and a-factor–CVIK may be accommodated better by ram1G149E–farnesyltransferase because of a charge attraction between the positively charged arginine or lysine and the negatively charged glutamate 149. If this hypothesis were correct, then glycine-149 would be in, or very near, the protein substrate binding site of farnesyltransferase.

Interactions Between Farnesyltransferase Variants and Ras Protein Variants. To test the hypothesis of direct interaction between the positively charged arginine or lysine and the negatively charged glutamate 149, we used a GST–farnesyltransferase because of a charge attraction between the positively charged arginine or lysine and the negatively charged glutamate 149. If this hypothesis were correct, then glycine-149 would be in, or very near, the protein substrate binding site of farnesyltransferase.

![Fig. 1. RAM1 plasmid restored a-factor processing and export to the M1 and M10 mutants, as determined by a-factor halo assay. A CEN LEU2 RAM1 plasmid (YCpL-RAM1) or the CEN LEU2 vector (YCplac11) were transformed into the M1 and M10 mutant strains (JRY5388 and JRY5389) and the RAM1 parental strain (JRY5312 transformed with UR43 CEN MFA1-CAMQ plasmid, pJR1556). These MATa strains, which expressed a-factor–CAMQ as the only source of mating pheromone, were assayed for a-factor production by halo assay (see Materials and Methods). The indicated MATa strains were spotted onto a lawn of MATa sst2 cells and grown for 3 days; biologically active a-factor exported from the MATa strains arrested growth of the MATa sst2 cells, forming a zone of growth inhibition (halo) that is proportional to the amount of a-factor produced.](https://example.com/1.png)

![Fig. 2. M1 and M10 mutants have mutations in codon 149 of the RAM1 gene. (A) Nucleotides 424–468 of the RAM1 gene from wild-type (JRY5312) and M1 and M10 mutant strains (JRY5388 and JRY5389). The sequences of the ram1–102 and ram1–103 alleles, in M1 and M10, respectively, were identical and differed from the wild-type RAM1 sequence in having an A at nucleotide 446 rather than a G, which changed codon 149 to GAA (a glutamate codon, underlined) from human farnesyltransferase β-subunit (human FTβ; ref. 32) and tomato farnesyltransferase β-subunits (tomato FTβ; ref. 54). The GI49E amino acid substitution in the ram1–102 and ram1–103 mutants (underlined) was in a highly conserved position in farnesyltransferase.](https://example.com/2.png)
between amino acid 149 of Ram1p and the C-terminal amino acids of the CaaX substrate, mutant forms of the CaaX protein Ras2val19 were used for the general feature of farnesyltransferase interaction with its subunit. Ram1p variants with different amino acids at the C terminus. These observations indicate that mutant forms of farnesyltransferase with substitutions of D, E, R, K, or A for Ram1p glycine-149 were able to prenylate and produce active Ras2val19p, when the C terminus of Ras2val19p was wild type (CIIS). In contrast, only the Ram1p variants with a negatively charged amino acid acid 149 (G149D and G149E) were able to effectively farnesylate Ras2val19p variants with positively charged C-terminal amino acids (CIIR and CIIK, Fig. 4B and C). Moreover, the Ram1p variants with a positively charged amino acid acid 149 (G149R and G149K) were most effective in farnesylating Ras2val19p variants with the negatively charged aspartate (CIID) as the C-terminal amino acid, whereas the Ram1p variants with a negatively charged amino acid were the least effective (Fig. 4D). In strains expressing the nonprenylatable Ras2val19p-CIIS protein in combination with any Ram1p variant, the viability upon starvation was similar to the viability of strains lacking Ram1p and expressing either Ras2val19p-CIIS protein (Fig. 4E). Ras2val19p-CIIS protein or any other Ras2val19p variant. Thus, the viability differences observed in Fig. 4B-D were due to differences in the efficiency of Ras2val19p prenylation. The particular amino acid present at position 149 of Ram1p clearly influenced the ability of the enzyme to prenylate Ras2val19p variants with different amino acids at the C terminus. These data were most simply explained by a direct interaction between the charged amino acids at position 149 of Ram1p and the charged amino acids at the C terminus of Ras2val19p. Based on these data, we concluded that glycine-149 was very near the protein substrate recognition site of the farnesyltransferase.

**Ram1 Farnesyltransferase Variants Influence Prenylation of Other Substrates.** Because mutant forms of farnesyltransferase with substitutions at amino acid 149 have altered substrate specificity, they may fail to prenylate adequately one or more of the normal farnesyltransferase substrates in vivo. Indeed, ram1G149E-farnesyltransferase appears to be deficient in prenylation of Ste18p, the γ-subunit of the heterotrimeric G protein involved in the mating pheromone response pathway. *MATa ram1G149E* cells were less sensitive to arrest by α-factor than *MATa RAM1* cells (data not shown), strongly suggesting that ram1G149E-farnesyltransferase does not prenylate Ste18p well. In addition, our observation that α-factor halos produced by *MATA ram1G149E* strains were generally smaller than the α-factor halos produced by *MATa RAM1* strains (Fig. 1) is consistent with a Ste18p prenylation defect that results in decreased a-factor induction by α-factor. Moreover, halo assays revealed that an α-factor variant terminating with the C-terminal sequence of Ste18p (CTLM) was poorly prenylated by ram1G149E-farnesyltransferase compared with wild-type α-factor, whereas α-factor-CTLM and wild-type α-factor were prenylated to a similar extent by wild-type farnesyltransferase (data not shown). Together these data led us to conclude that ram1G149E-farnesyltransferase does not farnesylate Ste18p as well as wild-type farnesyltransferase.

Ram1p variants with basic or acidic amino acids at position 149 were not able to support growth at high temperatures, suggesting that they may be defective in prenylation of Ydj1p, a DnaJ homolog with CASQ at the C terminus that is required for growth at 37°C, but not at 30°C (48). Overproduction of Ydj1p from plasmid pAV5 (A. Caplan, unpublished work) partially suppressed the growth defect of ram1G149D, ram1G149E, and ram1G149R strains at 37°C. Moreover, a-factor, CVSA, which is similar to Ydj1p in having a serine at the a2 position of the CaaX sequence, was an adequate substrate for wild-type farnesyltransferase but not for ram1G149E-farnesyltransferase (data not shown).

**DISCUSSION.**

The CaaX sequence is necessary and sufficient for substrate recognition by farnesyltransferase (2) and the identity of the
C-terminal amino acid strongly influences substrate selection (1-3), but specific interactions between the protein substrate and the farnesyltransferase β-subunit have not been previously defined. We have presented strong genetic evidence for electrostatic interactions between Ramlp variants with charged amino acids at position 149 and CaaX substrate variants of α-factor and Ras2p with charged amino acids at the X position. Specifically, Ramlp, with a negatively charged amino acid substituted for glycine-149, was more competent than wild-type Ramlp in prenylation of CaaX substrates with a positively charged amino acid at the C-terminus and was much less competent in prenylation of CaaX substrates with a negatively charged amino acid at the C-terminus. Similarly, Ramlp, with a positively charged amino acid substituted for glycine-149, was more competent than wild-type Ramlp in prenylation of CaaX substrates with a positively charged amino acid at the C-terminus and was much less competent in prenylation of CaaX substrates with a positively charged amino acid at the C-terminus. Therefore, our genetic data predict that Ramlp amino acid 149 is in very close proximity to the C-terminal amino acid of the CaaX sequence, providing evidence about the location of the protein substrate binding site of the farnesyltransferase β-subunit.

Glycine-149 is in a region of Ramlp that has high sequence similarity to other farnesyltransferase β-subunits (Fig. 2); a glycine is found in the analogous position of all published sequences [rat (50), bovine (51), human (32), pea (52), Arabidopsis (53), and tomato (54)]. The sequence surrounding glycine-149 lacks charged amino acids and includes a hydrophobic phenylalanine, which may explain why CaaX substrates with charged C-terminal amino acids are poor substrates for wild-type farnesyltransferase and why peptidomimetics with charged C-terminal amino acids are poor substrates for a variety of reasons, including the β turn conformation that had been previous proposed (18, 60). Our prediction that the C-terminal amino acid of the CaaX substrate resides near glycine-142 of the rat farnesyltransferase β-subunit, which corresponds to glycine-149 of the yeast farnesyltransferase β-subunit, is located approximately 2 nm from the α carbon of cysteine-299. Therefore, if the cysteine of the CaaX sequence were near cysteine-299 and the CaaX peptide were in an extended conformation, the C terminus of the CaaX sequence could reside near glycine-142. Evidence from farnesyltransferase inhibitor studies (58, 59) is consistent with an extended conformation of the CaaX peptide, rather than the β turn conformation that had been previous proposed (18, 60).

Our prediction that the C-terminal amino acid of the CaaX substrate resides near glycine-142 of rat farnesyltransferase disagrees with the binding orientation of the CaaX substrate proposed by Park et al. (36). Their proposal is based on the supposition that the binding of the C-terminal tail of an adjacent β-subunit in the crystal lattice mimics some aspects of normal CaaX peptide binding. The binding of this tail may differ from the binding of CaaX substrates for a variety of reasons, including the fact that the sequence of the tail (proline–alanine–threonine–aspartate) differs significantly from the CaaX sequence and the fact that binding of the farnesyl diphosphate substrate, which is not present in the crystal, precedes binding of the CaaX substrate (61) and could affect enzyme or peptide substrate conformation. The genetic data presented here imply either that the binding of the C-terminal tail from an adjacent β-subunit in the crystal lattice does not accurately mimic the position of CaaX sequence binding or that the CaaX sequence binds farnesyltransferase in a manner that is not consistent with the crystal structure.
more than one position during the course of the farnesylation reaction.

Other amino acid substitutions that affect protein substrate recognition have been identified in farnesyltransferase β-subunits (29, 30, 32), but it is unclear whether or not the amino acids identified are directly involved in substrate binding. All of these amino acid substitutions are in a repeated sequence, either in the glycine-rich region or in the 10 amino acids following the glycine-rich region, which correspond to the inner α-helices of the α-α barrel. Interestingly, the sequences in the inner helices are the most highly homologous regions (67%–73%) among the farnesyltransferase β-subunits from different species. Mutations in the yeast geranylgeranyltransferase-β subunit gene that appear to affect protein substrate specificity (62) are also found in the repeated sequences.

In our studies, mutation of codon 149 resulted in farnesyltransferase variants that differentially affected prenylation and function of various CaaX proteins, including Ras2p, a-factor, Ste11p, and Ydj1p. Prenylation of Ras2val19p (CIIS) did not appear to be affected by substitutions at position 149 of Ram1p, whereas prenylation of Ste11p (CTLM) and Ydj1p (CASQ) by the Ram1p-G149E variant was reduced enough to partially or completely compromise the functions of these proteins. In general, the results obtained with a-factor and Ras2val19p variants terminating with a positively or negatively charged amino acid at the C terminus were similar, but there were some differences. The Ras2val19p variants terminating with arginine and lysine and a-factor terminating with arginine were prenylated very poorly by wild-type farnesyltransferase.

However, a-factor terminating with lysine was prenylated to a significant extent. We propose that sequences outside the CaaX sequence of a-factor and Ras2val19p were responsible for differential interaction with farnesyltransferase. There is no sequence similarity between a-factor and Ras2p beyond the CaaX sequence and, although CaaX tetrapeptides can be prenylated in vitro by protein prenyltransferases, sequences outside the CaaX region can influence the prenylation efficiency (14).

Analysis of mutant enzymes has often led to insights on enzyme mechanisms. The data presented here provided a genetic test of a structure-based proposal for a protein substrate binding site.

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