Structural Homology among Mammalian and Saccharomyces cerevisiae Isoprenyl-protein Transferases*

(Received for publication, July 3, 1991)

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Farnesyl-protein transferase (FTase) purified from rat or bovine brain is an α/β heterodimer, comprised of subunits having relative molecular masses of approximately 47 (α) and 45 kDa (β). In the yeast Saccharomyces cerevisiae, two unlinked genes, RAM1/DPR1 (RAM1) and RAM2, are required for FTase activity. To explore the relationship between the mammalian and yeast enzymes, we initiated cloning and immunological analyses. cDNA clones encoding the 329-amino acid COOH-terminal domain of bovine FTase α-subunit were isolated. Comparison of the amino acid sequences deduced from the α-subunit cDNA and the RAM2 gene revealed 30% identity and 58% similarity, suggesting that the RAM2 gene product encodes a subunit for the yeast FTase analogous to the bovine FTase α-subunit. Antiseras raised against the RAM1 gene product reacted specifically with the β-subunit of bovine FTase, suggesting that the RAM1 gene product is analogous to the bovine FTase β-subunit. Whereas a ram1 mutation specifically inhibits FTase, mutations in the CDC43 and BET2 genes, both of which are homologous to RAM1, specifically inhibit geranylgeranyl-protein transferase (GGTase) type I and GGTase-IL, respectively. In contrast, a ram2 mutation impairs both FTase and GGTase-I, but has little effect on GGTase-II. Antiseras that specifically recognized the bovine FTase α-subunit precipitated both bovine FTase and GGTase-I activity, but not GGTase-II activity. Together, these results indicate that for both yeast and mammalian cells, FTase, GGTase-I, and GGTase-II are comprised of different but homologous β-subunits and that the α-subunits of FTase and GGTase-I share common features not shared by GGTase-II.

Site-specific farnesylation or geranylgeranylation of cellular polyisoprenylated proteins and the γ-subunit of some heterotrimeric GTPase proteins and the γ-subunit of some heterotrimeric GTPase proteins and the γ-subunit of some heterotrimeric GTPase proteins and the γ-subunit of some heterotrimeric GTPase proteins and the γ-subunit of some heterotrimeric GTPase proteins.

Three different enzymes in mammalian cells that catalyze the isoprenylation of these proteins have been identified and chromatographically resolved: a farnesyl-protein transferase (FTase,1 see Ref. 1) and two geranylgeranyl-protein transferases (GGTase-I and GGTase-II, Refs. 3-6). These enzymes each have different specificities for the protein substrate and the isoprenoid disphosphate utilized in the catalytic reaction. FTase preferentially farnesylates proteins having the COOH-terminal sequence CaaX, where a is usually an aliphatic amino acid and X is Ala, Ser, Gly, Cys, or Met (3-6). GGTase-I geranylgeranylates proteins having the COOH-terminal sequence CaaX where X is Leu (3-6). For FTase and GGTase-I, the major determinants for interaction between the enzyme and protein substrate reside within the CaaX sequence as demonstrated by selective isoprenylation of tetrapeptides (6). GGTase-II modifies proteins having the COOH-terminal sequence Gly-Gly-Cys-Cys (6). The interaction between GGTase-II and its substrates is complex as peptides of the modification site neither compete nor serve as substrates for geranylgeranylation.

FTase has been purified to homogeneity from rat brain by Reiss et al. (7). The enzyme is a heterodimer of approximately 100 kDa as determined by gel filtration chromatography on Superdex-75 (4). The α-subunit of approximately 49 kDa is immunologically distinct from the β-subunit of 46 kDa (4, 8). The β-subunit functionally binds the protein substrate as determined in cross-linking experiments (8). FTase and GGTase-I appear to be related because antisera raised against peptides from the FTase α-subunit recognize a similar molecular size polypeptide in GGTase-I and immunoedelete both activities (4).

FTase, GGTase-I, and GGTase-II activities are also detected in the yeast Saccharomyces cerevisiae (6, 9-11). For FTase, two unlinked genes, RAM1/DPR1 (RAM1) and RAM2, are essential for activity (9, 10). Mutations in ram1 are specific for FTase and do not affect GGTase-I or GGTase-II (6, 11). In contrast, mutations in ram2 result in decreased activity for both FTase and GGTase-I, suggesting that the yeast enzymes share a common genetic feature (6, 11). More recently, CDC43 has been shown to be essential for GGTase-I but not FTase activity (11). Cdc4 and Ram1 proteins share

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) M74083.

1 The abbreviations used are: FTase, farnesyl-protein transferase; GGTase, geranylgeranyl-protein transferase; CaaX, where a is Cys, a any aliphatic amino acid, and X, other amino acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography.
were immunized with antigen in Freund's complete adjuvant. Rabbits were injected once a week for 4 weeks. A week after the last injection, the rabbits were bled by heart puncture and the serum was collected. The IgG fraction was isolated by precipitation with 1 volume of 1 M ammonium sulfate followed by dialysis against 0.02 M Tris-Cl, pH 8, concentrated by centrifugation (Centricon-30, Amicon), and stored at 4 °C in phosphate-buffered saline.

For immunoblot analyses, either purified bovine brain FTase holoenzyme or resolved subunits were fractionated on 7.5% SDS-polyacrylamide gels. To separate the subunits, purified FTase from bovine brain (100 µg) was resolved on a 7.5% SDS-polyacrylamide gel alongside an aliquot of holoenzyme and the proteins transferred to Immobilon-P (Millipore). Membranes were incubated with protein A-purified antibody at 4 °C overnight, washed, and incubated with horseradish peroxidase-labeled anti-rabbit antiserum (Amersham; 1:50,000 dilution). Proteins were visualized by the ECL detection system (Amersham).

For immunoprecipitations, bovine isoprenyl-protein transferases were partially purified and resolved as described (6) with an additional HPLC step using a Bio-Gel Phenyl-5-PW column (Bio-Rad). Each enzyme was separately incubated with protein A-purified antibody for 3 h at 0 °C in phosphate-buffered saline. Protein A-Sepharose CL-4B beads (100 µl of a 50% slurry) were added for 1 h at 4 °C and subsequently washed with phosphate-buffered saline. Assay reaction mixture (70 µl) was added to the packed beads and incubated for 20 min at 30 °C. The clarified supernatant was then analyzed for isoprenylated substrate (6).

Yeast Strains—The following S. cerevisiae strains were used: RS36-1C, strain YF1594 carrying the ORF1 gene; RS16-4C, strain YF1594 containing the ORF2 gene; and YF1594, strain YS34 carrying the plasmid pYS104 which contains a wild-type ORF2 gene; and YF1894, strain YS34 carrying the plasmid pYS135 containing a temperature-sensitive allele ORF2-1 as the sole copy of the gene (see Refs. 13 and 14).

RESULTS

We have previously reported the purification of FTase from bovine brain (6). The purified bovine enzyme consists of two subunits which co-migrate with rat FTase (purified as in Ref. 7) and have apparent molecular weights of 47,100 (a) and 45,600 (b) on denaturing gels; the holoenzyme is approximately 90 kDa by gel filtration on Superdex-75 (data not shown). To learn more about the structure of FTase, we initiated efforts to clone the cDNAs for both subunits of the bovine enzyme.

Following reduction and carboxymethylation, the purified holoenzyme was digested with Lys-C, and peptides were isolated by reversed-phase HPLC. The amino acid sequences obtained from analysis of the resulting peptides are shown in Fig. 1. Oligonucleotides complementary to the FTase messenger RNA sequence predicted from the peptide sequence data were synthesized and used to screen amplified bovine brain and bovine liver cDNA libraries. All of the five hybridizationpositive clones from the brain library and the one clone from the liver library cross-hybridized. Sequence analysis of four of the brain clones and the liver clone revealed an open reading frame of 987 base pairs, bounded on the 5’ end by an in-frame initiation codon and on the 3’ end by an in-frame termination codon (Fig. 1). This open reading frame would encode a 329-amino acid polyepitope in which the sequences of all but one of the peptide fragments derived from the purified enzyme were found. The predicted M, of this polypeptide is 39,502, which is less than the apparent M, observed for either of the subunits on SDS-PAGE. Although this difference might be explained by post-translational modification of the protein isolated from bovine brain, it seems likely that the clone does not contain the complete coding region. The proposed initiation codon is preceded by only 35
FIG. 1. Nucleotide and deduced amino acid sequence of the partial bovine FTase subunit cDNA. The nucleotide numbering begins with the first base of the first in-frame initiation codon and is indicated on the left of each line. Amino acid numbering begins with the first amino acid in the in-frame methionine and is indicated on the right of each line. The amino acid sequences for peptides derived from the DNA sequence of the bovine FTase subunit are given in capital letters. The parameters for gap weight and gap length weight were done using the BESTFIT program of the University of Wisconsin package (22).

Fig. 2. The predicted amino acid sequences of the bovine FTase subunit cDNA and RAM2 are homologous. The deduced amino acid sequence of the bovine FTase subunit is given in capital letters on the top line and that of full-length Ram1 in lower case letters on the bottom line. Vertical lines indicate identical amino acids at a given position, dots correspond to conservative substitutions. The comparison was done using the BESTFIT program of the University of Wisconsin package (22). The parameters for gap weight and length weight were 3.00 and 0.10, respectively.

The significance of the homologies is discussed in the text. The deduced amino acid sequence of the bovine FTase subunit was compared with the cDNA sequence of the Ram1 gene product (11) and Ram2 gene product (12) of Saccharomyces cerevisiae, which encodes a yeast FTase (31). The deduced sequences of the Ram1 and Ram2 genes encode proteins of 76 and 75 amino acids, respectively, which have a size of 38 kDa by SDS-PAGE (Fig. 2). The two proteins have 30% amino acid identity and 58% amino acid similarity. As shown in Fig. 2, the homology extends over the entire Ram2 protein and nearly all of the bovine protein.

We used immunoblot analysis to determine whether the protein product of the cloned cDNA corresponded to the α-subunit of the purified bovine brain FTase. Antisera against a synthetic peptide corresponding to the sequence of one of the products of the Lys-C digestion of purified FTase and which was found in the amino acid sequence deduced from the cDNA (see Materials and Methods). Anticipating that Ram1 might encode a structural polypeptide of FTase (10) which would share homology with bovine FTase, we also prepared antisera against the Ram1 protein (rabbit 114) to a bacterially expressed CheY-Ram1 fusion protein. Fig. 3A shows immunoblots of purified bovine FTase. Antibody 793 reacted only with the α-subunit, and antibody 114 reacted only with the β-subunit. To confirm this assignment, the two subunits of the purified enzyme were resolved on a SDS-polyacrylamide gel, separately excised from the gel, and fractionated on a second SDS-polyacrylamide gel. As shown in Fig. 3B, antibody 793 specifically reacted with the isolated α-subunit and antibody 114 with the isolated β-subunit. These results demonstrate that the cloned cDNA corresponds to the α-subunit and suggest that the bovine β-subunit shares homology with the Ram1 gene product.
FIG. 3. Immunoblot analysis of bovine FTase subunits. A, purified bovine FTase (about 400 ng) was subjected to 7.5% SDS-PAGE and transferred to Immobilon-P. The filters were incubated with antibody 793 derived from a peptide sequence within the cDNA or both antibodies were fractionated in separate lanes on a 7.5% SDS polyacrylamide gel and transferred to Immobilon-P. One portion of the membrane was incubated with antibody 793 (Anti-α) and another portion with antibody 11 (Anti-RAMI). Aberrant banding patterns in the α and β lanes are due to nonideal protein electroelution from the gel slices in the loading wells. Immunoreactive proteins were visualized using the ECL detection system following incubation with the gel slices in the loading wells. Immunoreactive proteins were recovered in the pellet was proportional to the activity depleted from the supernatant (not shown).

GGTase-II, we performed the immunoprecipitation using pools of the partially purified bovine GGTases. Following incubation with either preimmune antibody or antibody 793, protein α-agarose beads were added and the isoprenyl-protein transferase activity present in the pellet was determined. Whereas no isoprenyl-protein transferase activity was detected in pellets incubated with preimmune antibody, a significant amount of FTase and GGTase-I but not GGTase-II activity was present in pellets incubated with 793 (Table I). The amount of activity increased with increasing amounts of antibody 793, suggesting that the activity was due to precipitation by the antibody. This result indicates that the polypeptide encoded by the cDNA is required for FTase activity and demonstrates that the α-subunits of bovine FTase and GGTase-I share a common epitope for immunoprecipitation that is not shared by GGTase-II.

Since the cloning and immunological analyses suggested that RAM1 and RAM2 encode the subunits of *S. cerevisiae* FTase, we evaluated the activities of isoprenyl-protein transferases in *S. cerevisiae* strains having defects in the genes cdc43 and bet2 which are homologous to but distinct from RAM1. As has been shown previously, RAM1 is not required for GGTase-I or GGTase-II activity in yeast (Table II; Refs. 6 and 11). In contrast, a mutation in ram2 inhibited not only FTase but also GGTase-I, but did not significantly inhibit GGTase-II activity (Table II; Refs. 6 and 11). Recently, the protein product of the *S. cerevisiae CDC43* gene was shown to be required for GGTase-I activity in yeast (11). To determine if CDC43 was also required for GGTase-II activity, we analyzed crude soluble extract from yeast having a mutation in this gene for the ability to geranylgeranylate Ypt1. As shown in Table II, the cdc43 mutant cell extract was defective for GGTase-I activity and showed 1.4-fold greater GGTase-II activity than the corresponding wild-type control, suggesting that Cdc43 was not a component of GGTase-II. In contrast, the BET2 mutant extract contained wild-type levels of FTase and GGTase-I but was devoid of GGTase-II activity. To further evaluate the relationship between Bet2 and GGTase-II, cell extract was assayed from a yeast strain containing another allele of bet2, the temperature-sensitive orf2-1 mutant. The orf2-1 cell extract was defective for GGTase-II activity when it was prepared from cells incubated at the nonpermissive temperature but wild-type when prepared from cells incubated at the permissive temperature (0.06 ± 0.01 and 0.24 ± 0.03 pmol/min/mg of protein at 37 and 25 °C, respectively).

**DISCUSSION**

We report here the isolation of a partial cDNA encoding the carboxyl-terminal portion of the α-subunit of bovine FTase. The cloning strategy was based on obtaining partial protein sequence from purified holoenzyme. Assuming random distribution of lysine residues between the two subunits,
the peptides obtained from the Lys-C digest should have derived from both subunits because the two subunits of the enzyme appear to be present in equimolar amounts. However, 10 of the 11 peptide sequences obtained were from the α-subunit. A possible explanation may lie in our observation that the β-subunit degrades readily. The remaining peptide derives from the β-subunit.

The deduced amino acid sequence of the bovine FTase α-subunit shares significant sequence homology with the predicted protein product of the full-length S. cerevisiae RAM2 gene. This homology extends over the entire Ram2 polypeptide and over most of the cloned FTase α-subunit, suggesting that the two proteins might be functionally similar. Studies to determine whether this hypothesis is correct are underway. If this hypothesis were correct, it would indicate that the functional domain of the mammalian FTase α-subunit lies within the cloned region.

Mutation of RAM2 affects not only FTase activity but also GGTase-I activity, indicating that the two yeast enzymes share an identical α-subunit. Since the ram2 mutation completely inhibits S. cerevisiae FTase, partially inhibited GGTase-II by 67%, and did not significantly inhibit GGTase-II activity (Table II), it is possible that at least one other polypeptide having functional properties of an α-subunit might be a component of S. cerevisiae GGTases. However, we cannot exclude the possibility that the partial inhibition of GGTase-I is specific for the mutant allele of ram2 tested. RAM2 and CDC43 (12), but not RAM1 (Refs. 9 and 10), are essential genes in S. cerevisiae. If the yeast cell requires either FTase or GGTase-I activity for viability, it is not surprising that the isolated ram2 allele allows some GGTase-I activity. We have shown that an antibody raised against a peptide from the bovine FTase α-subunit is able to precipitate bovine FTase and GGTase-I activity but not GGTase-II activity, suggesting that at least one additional α-subunit polypeptide might be similarly present in mammalian cells. These results corroborate the immunological data of Seabra et al. (4) who showed that antisera to rat FTase α-subunit cross-reacts with a similar size polypeptide in a GGTase-I preparation resolved from FTase and quantitatively immunodepletes both activities. Together, these data suggest that a common α-subunit may be a general property of eukaryotic FTase and GGTase-I. These three isoprenyl-protein transferases are all likely to be α/β heterodimers because mammalian FTase, GGTase-I, and GGTase-II have a similar size by gel filtration chromatography (data not shown and Refs. 4 and 23).

We have also observed that the β-subunit of bovine FTase is immunologically similar to Ram1 (Fig. 3), suggesting that Ram1 is a functionally equivalent β-subunit for S. cerevisiae FTase. Reiss et al. (8) have shown that the β-subunit binds protein substrate. By analogy, it seems likely that Cdc43 and Bet2, which are 30% homologous to Ram1, comprise the protein substrate-binding β-subunit of GGTase-I and GGTase-II, respectively. This hypothesis would be consistent with the absolute specificity that mutations in RAM1, CDC43, and BET2 have for FTase, GGTase-I, and GGTase-II, respectively (Table II). Indeed, the temperature sensitive phenotype observed with the orf2-1 allele indicates that BET2/ORF2 encodes a structural polypeptide of GGTase-II. These data suggest, then, that mammalian isoprenyl-protein transferases will have different but homologous β-subunits. Structure-function studies should identify the regions of these proteins which confer the high substrate specificity observed for the enzymatic reactions.

Acknowledgments—We thank the following people for their contributions to this work: M. Marshall for help constructing the CheY-RAM1 expression plasmid, V. Garsky for synthesis of peptides, M. Sardana and J. Rodkey for identification of one of the peptide sequences, and D. Martinez for helping in generating antibodies. We are grateful to A. Oliff, C. Omer, and J. Shafer for helpful discussions and to P. Friedman and E. Scollnick for their support.

Note Added in Proof—After submission of this manuscript, Chen et al. (24) reported that the FTase β-subunit cloned from a rat cDNA library shares 37% amino acid identity with S. cerevisiae Ram1. We have cloned the bovine FTase β-subunit cDNA and have found it to be 98% identical to the rat FTase β-subunit.

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