**COQ2 Is a Candidate for the Structural Gene Encoding para-Hydroxybenzoate:Polyprenyltransferase**

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Coenzyme Q functions as a lipid-soluble electron carrier in eukaryotes. In Saccharomyces cerevisiae, the enzymes responsible for the assembly of the polyprenoid side chain and subsequent transfer to para-hydroxybenzoate (PHB) are encoded by the nuclear genes COQ1 and COQ2, respectively. Yeast mutants defective in coenzyme Q biosynthesis are respiratory defective and provide a useful tool to study this non-sterol branch of the isoprenoid biosynthetic pathway. We isolated a 5.5-kilobase genomic DNA fragment that was able to functionally complement a coq2 strain. Additional complementation analyses located the COQ2 gene within a 2.1-kilobase HindIII-BglIII restriction fragment. Sequence analyses revealed the presence of a 1,116-base pair open reading frame coding for a predicted protein of 372 amino acids and a molecular mass of 41,001 daltons. The amino acid sequence exhibits a typical amino-terminal mitochondrial leader sequence and six potential membrane-spanning domains. Primer extension and Northern analyses indicate the gene is transcriptionally active. Transformation of a coq2 strain with the 2.1-kilobase HindIII-BglIII genomic restriction fragment facilitated the study of coenzyme Q biosynthesis and function.

The majority of coenzyme Q biosynthesis takes place in the mitochondria (reviewed in Olson and Rudney, 1983); however, coenzyme Q synthesis in rat liver endoplasmic reticum-Golgi subcellular fractions has been observed (Kalen et al., 1987, 1990). The biosynthesis of sterols, dolichol, and coenzyme Q share a common synthetic pathway up to the formation of IPP. The first steps unique to coenzyme Q biosynthesis involve the synthesis from IPP of an all-trans polyrenyl dihydroxybenzoate where the chain length is a species-specific phenomenon (Ramasarma, 1985). Subsequently, the polyrenyl group is transferred to PHB to form 3-polyrenyl-4-hydroxybenzoate. Winrow and Rudney (1969) first described the synthesis of 3-polyrenyl-4-hydroxybenzoate from PHB and preformed polyrenyl phosphates in a cell-free rat tissue preparation. The authors designated the enzyme that catalyzes this reaction PHB:polyrenyltransferase. Mitochondria from rat liver (Momose and Rudney, 1972) and yeast (Casey and Threlfall, 1978) contain all the enzymes necessary for the synthesis of polyrenyl hydroxybenzoate from IPP and PHB. These include IPP isomerase, farnesyl-diphosphate synthetase, polyrenyl-diphosphate synthetase, and PHB:polyrenyltransferase.

The availability of yeast respiratory mutants has greatly facilitated the study of coenzyme Q biosynthesis and function (Tzagoloff et al., 1975; De Kok and Slater, 1975; reviewed in Tzagoloff and Dieckmann, 1990). The COQ2 gene encoding the coenzyme Q biosynthetic enzyme 3,4-dihydroxy-5-hexaprenylbenzoate methyltransferase has recently been isolated and characterized (Clarke et al., 1991). Identification of the defect in two yeast coenzyme Q mutants designated coq1-1 and coq2-1 has been described in addition to the DNA sequence of COQ1 (Ashby and Edwards, 1990). Cells possessing coq1-1 or coq2-1 mutant alleles are defective in hexaprenyl-diphosphate synthetase or PHB:polyrenyltransferase activities, respectively. In this report we describe the molecular
cloning and characterization of COQ2, a candidate for the yeast structural gene encoding PHB:polyprenyltransferase.

**EXPERIMENTAL PROCEDURES**

**Materials**—The prenyltransferase assay substrates geranyl diphosphate and isopentenyl diphosphate were a generous gift of Dr. H. C. Rilling (University of Utah). [U-"C"]~tyrosine by the alkaline fusion method of Parsons and Rudney (1964) with some modifications (Ashby and Edwards, 1990). Unlabeled PHB was purchased from Kodak. [r-32P]dCTP (>1,000 Ci/mmol) was obtained from Amersham Corp. [a-125I]BN was purchased from ICN Radiochemicals. Thin layer chromatography was performed on Adsorbosil "H plates (No. 16385) acquired from Alttech. Restriction enzymes and other enzymes employed in subcloning were purchased from Bethesda Research Laboratories.

**Yeast Strains and Growth Media**—The strains of Saccharomyces cerevisiae used in this study are described in Table I. The coq mutants E3-71 and C33 are part of a large number of pet mutants derived from the respiratory competent haploid yeast strain D273-10B and D273-10B/A1, respectively, by mutagenesis with ethylthene sulfonate (Tzagoloff et al., 1975). The following media were used for growth of yeast: YPD (2% glucose, 2% peptone, 1% yeast extract); YEPG (5% glycerol, 2% ethanol, 2% peptone, 1% yeast extract); YPGal (2% galactose, 2% peptone, 1% yeast extract); VO (2% glucose, 0.87% yeast nitrogen base without amino acids from Difco). Where required media were supplemented with auxotrophic requirements at 20 μg/ml. Solid media contained 2% agar. Yeast cells that are respiatory deficient (Tzagoloff et al., 1975) were analyzed with computer programs from the University of Wisconsin Computer Group (Devereux et al., 1984). Hydropathy calculations were performed with the program PEPPLOT which is based on the algorithm of Kyte and Doolittle (1982). Predictions of α-helices and β-sheets were determined as described (Chou and Fasman, 1978). Potential transmembrane regions were found by the method of Eisenberg (1984). This involves calculating the mean hydrophobic moment over a moving 11-amino acid window through regions of the protein sequence which exceed a predetermined average hydrophobicity. The various mean hydrophobic maxima are then plotted against the mean hydrophobicity for the same window to assess transmembrane potential.

**RESULTS**

**Molecular Cloning of COQ2**—Nine separate complementation groups of yeast coenzyme Q mutants have been previously isolated that are respiratory deficient (Tzagoloff et al., 1975; Tzagoloff and Dieckmann, 1990). Two of these mutants were shown to be defective in the first two committed steps of coenzyme Q biosynthesis (Ashby and Edwards, 1990). The two mutants, C296 (coq1) and C33 (coq2), are defective in hexaprenyl-diphosphate synthetase and PHB:polyprenyltransferase activities, respectively. Yeast cells that are respiratory deficient are unable to utilize the nonfermentable

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**Table I**

| Genotypes and sources of S. cerevisiae strains |
|-----------------------------------------------|
| **Strain** | **Genotype** | **Sources** |
| D273-10B/A1 | α p+ met6 | Tzagoloff et al., 1976 |
| W303-1A | α p+ ade2-1 his3-1,15 leu2-3,112 | * |
| W303 COQ2 | α p+ ade2-1 his3-1,15 leu2-3,112 | This study |
| E3-71 | α p+ cog2-1 | Tzagoloff et al., 1975 |
| aE3-71/U1 | α p+ ura3-1 coq2-1 | E3-71 x W303-1A |
| C33 | α p+ met6 coq2 | This study |
| C33/U1 | α p+ ura3-1 coq2 | C33 x W303-1A |

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carbon source glycerol. To facilitate studies on the COQ2 product, the gene was cloned by complementing the respiratory deficient phenotype of aE3-71/U1. Transformation of this mutant with a yeast genomic library yielded several respiratory competent clones that were also complemented for uracil auxotrophy. Vegetative growth of the transformants on nonselective rich glucose medium followed by replica plating onto glycerol or minimal glucose media indicated that the Gly" and Ura" phenotype cosegregated. This established that respiratory competence and uracil prototrophy were functions of the presence of an autonomously replicating plasmid. Four independent Gly", Ura" transformants carried a plasmid containing identical genomic inserts. Plasmid DNA (pG10/T1) prepared from one of the clones when back-transformed into aE3-71/U1 rescues the respiratory defect of the mutant.

The plasmid pG10/T1 contains a genomic DNA insert approximately 5.5 kb in length. To locate the COQ2 gene various regions of the 5.5-kb genomic fragment were subcloned into YEp352 (Hill et al., 1986) and were tested for their ability to complement aE3-71/U1 and restore growth on glycerol-containing media (Fig. 1). These experiments indicate the COQ2 gene must be located within a HindIII-BglII restriction fragment. The entire 2,195-bp HindIII-BglII fragment was sequenced in both directions by the strategy shown in Fig. 1. The nucleotide sequence of this region is shown in Fig. 2. Two open reading frames (ORFs) are present. The first unidentified ORF is oriented from right to left in Fig. 1 and by the double-stranded DNA sequence in Fig. 2. This unidentified ORF begins with an ATG at nucleotide 583 and continues to the HindIII site (nucleotide 1) without encountering a termination codon. The second ORF is oriented from left to right shown schematically in Fig. 1. This ORF begins with an ATG at nucleotide 819 and ends with a termination codon at nucleotide 1,937 (Fig. 2). The predicted translation product of this ORF is a protein of 372 amino acids with a molecular mass of 41,001 daltons.

Construction of a COQ2 Disruption Allele—To confirm that the COQ2 gene had been correctly identified, the chromosomal copy of the gene was disrupted by the one-step gene replacement procedure of Rothstein (1983) in the respiratory competent haploid strain W303-1A. The construction of the disrupted gene is shown in the upper part of Fig. 3. The plasmid ST4 containing the 3-kb BglII insert was digested with XbaI to yield a 2.2-kb fragment containing the entire COQ2 coding region. One of the XbaI sites is inside the BglII fragment and the other XbaI site is in the multiple cloning region of the YEp352 vector. This DNA fragment was transferred to the XbaI site of YEp352X (this plasmid is similar to YEp352 except that the multiple cloning region of the latter plasmid has been replaced with a unique XbaI site). The resultant plasmid (pG10/ST9) was opened at the unique PstI site and ligated to a 1,125-bp PstI fragment containing the yeast HIS3 gene to yield the disrupted allele, coq2::HIS3.

A linear 3.3-kb XbaI fragment containing the disrupted gene with 5'- and 3'-flanking sequences was isolated from pG10/ST9 and used to transform W303-1A. Clones were selected on minimal glucose medium supplemented with all the auxotrophic requirements of W303-1A except histidine. Most of the histidine-independent transformants were respiratory defective and were complemented by a tester but not by C33, an independent coq2 mutant. The results of these crosses imply a genetic linkage of the disrupted coq2::HIS3 allele to the coq2 mutation. As verification of this allelism, linkage analyses were performed on the diploid product of the mating of the cross between C33 and W303/VCOQ2 (data not shown). To facilitate sporulation the C33/W303/VCOQ2 diploid strain was transformed with the COQ2-containing plasmid pG10/ST6. Twenty-two independent tetrads were dissected. After propagation on nonselective media (YPD), the segregants were replica plated onto media containing 5-fluoroorotic acid (Boeke et al., 1987) to select for uracil auxotrophy and, hence, loss of pG10/ST6. None of the resulting haploid strains exhibited respiratory competence, as judged by growth on YEPG, confirming the allelism between the cloned COQ2 gene and an authentic coq2 mutation.

Southern hybridization analysis of the genomic DNA from one of the Gly" His" transformants (W303/VCOQ2) confirmed the successful substitution of the wild-type gene by the disrupted allele. In this analysis, wild-type and mutant DNAs were digested with a combination of EcoRI and KpnI and hybridized to a nick-translated 2.2-kb XbaI-BglII fragment containing the entire COQ2 gene and flanking sequences. The probe detects a 3-kb band in the genomic digest of wild-type DNA and two coincident bands of approximately 2.1 kb in the mutant DNA digest (Fig. 3). The sizes of these fragments are consistent with the restriction maps of the wild-type COQ2 and the coq2::HIS3 allele.

Primer Extension and DNA Sequence Analyses—Both the

![Fig. 1. Strategy employed to clone and sequence COQ2.](image)
gene disruption and linkage analysis confirm that the full-length gene in the BglII-HindIII fragment corresponds to COQ2. To map the start site of transcription of COQ2, a 35S leader RNA was synthesized from the plasmid ST4 to restore PHB:polyprenyltransferase activity of the transformant C33/U1/ST4. Hexaprenyl-diphosphate (HPP) synthetase and PHB:polyprenyltransferase activities were assayed as described by Casey and Threlfall (1978). In this assay the allylic isoprenoid geranyl diphosphate is elongated to hexaprenyl diphosphate through the action of the COQ1 gene product, hexaprenyl-diphosphate synthetase. In this assay the allylic isoprenoid geranyl diphosphate is activated. In this case the enzyme will accept other allylic isoprenoid substrates such as geranyl diphosphate.

The COQ2 Gene Restores PHB:Polyprenyltransferase Activity to a Coenzyme Q Mutant—The pet mutant C33 has been shown to lack detectable PHB:polyprenyltransferase activity (Ashby and Edwards, 1990). Therefore, we tested the ability of the plasmid ST4 to restore PHB:polyprenyltransferase activity. Mitochondrial membrane-enriched fractions were isolated from wild-type, from the coq2 mutant C33/U1, and from the transformant C33/U1/ST4. Hexaprenyl-diphosphate (HPP) synthetase and PHB:polyprenyltransferase activities were assayed as described by Casey and Threlfall (1978). In this assay the allylic isoprenoid geranyl diphosphate is elongated to hexaprenyl diphosphate through the action of the COQ1 gene product, hexaprenyl-diphosphate synthetase. Subsequently, PHB:polyprenyltransferase prenylates C4 of PHB with the 30-carbon HPP. At the magnesium concentration used in these assays (33 mM), PHB:polyprenyltransferase is activated. In this case the enzyme will accept other allylic isoprenoid diphosphate substrates such as geranyl diphosphate. Thus, the production of 3-diprenyl-4-hydroxybenzoate requires the activity of only PHB:polyprenyltransferase, while the synthesis of 3-hexaprenyl-4-hydroxybenzoate requires the activity of both HPP synthetase as well as PHB:polyprenyltransferase.

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PHB:Polypropenyltransferase

Fig. 3. Disruption of the COQ2 gene. The upper part of the figure shows the restriction maps of the wild-type COQ2 and disrupted coq2:HIS3 allele. The 1,125-bp fragment of DNA with the HIS3 gene is shown by the dashed lines. The coding regions of COQ2 and HIS3 are depicted by solid and open bars, respectively, and the direction of transcription by the arrows. The locations of the KpnI (K), XbaI (X), PstI (P), BglII (G), and EcoRI (E) sites are marked on the two maps. The lower part of the figure shows the results of the Southern analysis of wild-type and mutant genomic DNA. Nuclear DNA was prepared from the HIS3 transformant, W303 VCOQ2, and from the respiratory competent strain W303-1A. The two DNAs were digested with a combination of EcoRI and PstI and separated by electrophoresis on a 1% agarose gel. After transfer to nitrocellulose, the blot was hybridized to the nick-translated 2.2-kb XbaI-RglII fragment (probe) shown in the upper part of the figure. The migration of known size standards is shown in the left-hand margin.

Fig. 4. Primer extension. A synthetic oligonucleotide 35-mer complementary to the coding strand and corresponding to nucleotides 917–883 in Fig. 2 was 5’-end-labeled and hybridized to yeast RNA (+), or RNA was omitted (–). Primer extension and analysis were performed as described under “Experimental Procedures.” Sequencing reactions for M13 mp18 are shown (A, C, G, T). The arrows indicate the major primer extension products, and the length of these extensions in bases is indicated.

Both diprenyl- and hexaprenyl-hydroxybenzoate species. In contrast, a similar protein fraction from C33 is unable to form any polypropenyl hydroxybenzoate products reflecting the absence of any detectable PHB:polypropenyltransferase activity (Fig. 5B). Transformation of C33 with the plasmid ST4 results in the restoration of respiratory competence, as judged by growth on YEPG (Fig. 1). Additionally, mitochondrial membranes from the transformed cells have the restored ability to synthesize diprenyl- and hexaprenyl-hydroxybenzoate coenzyme Q intermediates (Fig. 5C). These results indicate the COQ2 gene is able to restore respiratory competence and PHB:polypropenyltransferase activity to the coenzyme Q mutant C33.

The substrate PHB of PHB:polypropenyltransferase is biosynthetically derived from and structurally closely resembles tyrosine. Because of the biological significance of protein prenylation, we examined whether tyrosine might also serve as a substrate for PHB:polypropenyltransferase. To address this question we performed a competition experiment with mitochondrial membranes from D273–10B exactly as in Fig. 5A except that 1 mM L-tyrosine was included in the incubation. As can be seen in Fig. 5D, even at a 500-fold molar excess tyrosine does not affect the activity of PHB:polypropenyl transferase and, therefore, is not likely a substrate of this enzyme.

**COQ2 Amino Acid Sequence Analyses**—The translation product of COQ2 is a protein 372 amino acids in length with a molecular mass of 41,001 daltons (Fig. 6A). The protein possesses a typical mitochondrial leader sequence as judged by a preponderance of basic and hydroxylated residues and a predicted tendency to form α-helices (Hartl et al., 1989). Moreover, the amino terminus contains a three-amino-acid proteolytic recognition sequence found in mitochondrial proteins that are processed in two steps (Hendrick et al., 1989; Fig. 6A). This consensus sequence is defined by an arginine at position –10 (relative to the mature amino terminus), a hydrophobic residue (Leu, Val, Ile, or Phe) at position –8, and a serine, threonine, or glycine at position –5. The present...
The unidentified ORF is shown in Fig. 6B. The net result of this processing would be a particular sequence to become transmembrane or globular, depending upon where the value plots (Eisenberg, 1984). Based on these criteria, the PHB:polyprenyltransferase amino acid sequence revealed only limited global similarities; however, three highly conserved domains were identified (Ashby and Edwards, 1990). Two of these domains (I, II) possess a direct repeat of the consensus sequence (I, L, or F)xDDxxD and were proposed to represent substrate binding structures. Three, highly conserved domains were identified (Ashby and Edwards, 1990). Two of these domains (I, II) possess a direct repeat of the consensus sequence (I, L, or F)xDDxxD and were proposed to represent substrate binding structures. At least nine functionally distinct enzymes have been arranged into six groups (A–F) based upon functional similarities. The consensus sequence in Fig. 8 was derived from amino acids that were present in three or more functionally distinct enzymes.

**DISCUSSION**

PHB:polyprenyltransferase catalyzes the prenylation of para-hydroxybenzoate with an all-trans polypropenyl group, the second committed step in the coenzyme Q biosynthetic pathway (Winrow and Rudney, 1969). The respiratory deficient strain of *S. cerevisiae* C33, previously assigned to complementation group G10, is defective in PHB:polyprenyltransferase activity (Ashby and Edwards, 1990; Fig. 5). Mitochondria isolated from C33 as well as other mutants from this complementation group have a normal composition of cytochromes and dehydrogenases but are unable to oxidize NADH unless supplemented with coenzyme Q (Tzagoloff et al., 1975). Taken together, the two observations suggest that the genetic lesion responsible for the respiratory defect of this group of mutants is either in the gene coding for the transferase or in a factor responsible for the expression of the gene. This gene has been designated COQ2 to indicate a deficiency in coenzyme Q.

In this report we describe the molecular cloning of a yeast genomic DNA fragment containing the wild-type COQ2 gene by complementation of a coq2 mutant. Sequence analysis of the smallest subclone capable of complementing coq2 mutants and restoring their PHB:polyprenyltransferase activity disclosed the presence of two ORFs. One of the ORFs codes for the amino-terminal region of a protein whose derived primary sequence is not homologous to any entries in the current databases. The second ORF is full-length and codes for a predicted primary translation product with a molecular weight of 41,001. This reading frame was confirmed by *in situ* disruption and allelism tests to be the COQ2 gene. Disruption of COQ2 elicits regions of hydrophobicity. The first two hydrophobic regions each encompass two potential membrane spanning domains while the third and fourth hydrophobic regions each contain single potential transmembrane domains. The potential membrane spanning regions are indicated by the solid bar in Fig. 7B.

Previous alignments of the amino acid sequences of rat, human, and yeast FPP synthetase with yeast HPP synthetase amino acid sequence revealed only limited global similarities; however, three highly conserved domains were identified (Ashby and Edwards, 1990). Two of these domains (I, II) possess a direct repeat of the consensus sequence (I, L, or F)xDDxxD and were proposed to represent substrate binding sites. Fig. 8 shows an alignment of amino acid sequences from several different proteins (from multiple species, in some cases) that share the common property of employing (poly)isoprenyl diphosphate substrates. At least nine functionally distinct enzymes have been arranged into six groups (A–F) based upon functional similarities. The consensus sequence in Fig. 8 was derived from amino acids that were present in three or more functionally distinct enzymes.

**FIG. 7. Hydrophobic and structural predictions of the presumptive PHB:polyprenyltransferase polypeptide.** A, hydrophobicity profile of the COQ2 protein. Hydrophobicity values appear in the right margin. Positive values indicate hydrophobic regions while negative values represent hydrophilic regions. Amino acid numbers (1–372) are shown beneath the plot. B, Chou-Fasman plot. Regions which are predicted to form α-helices (solid line) and β-structures (dashed line) are shown.
respiratory incompetence and loss of detectable PHB:polyprenyltransferase activity. The mutant construct with the disrupted copy of COQ2 is complemented by a p-tester strain but not by coq2 mutants. Furthermore, only respiratory deficient meiotic progeny were produced from a cross of the disruption strain to a coq2 mutant.

Even though definitive proof that COQ2 is the structural gene for PHB:polyprenyltransferase will require biochemical characterization of the purified enzyme, all the available data are most consistent with this conclusion. In addition to the already mentioned dependence of transferase activity on the presence of the wild-type COQ2 allele, the primary sequence of those found in other enzymes known to bind isoprenoid substrates suggest a mitochondrial membrane protein with a putative polyprenyl diphosphate-binding domain similar to itself suggests a mitochondrial membrane protein with a putative polyprenyl diphosphate-binding domain similar to those found in other enzymes known to bind isoprenoid substrates. The predicted amino acid sequence of the COQ2 protein was shown to bind all-trans polyprenyl diphosphate substrates. The predicted amino acid sequence of the COQ2 product displays a typical mitochondrial leader sequence with the three-amino-acid consensus found in precursors that are processed by two consecutive proteolytic cleavages (Hendrick et al., 1989). The protein also has a hydrophobic character with six potential membrane-spanning domains suggestive of a membrane localization. Both attributes would be expected of the transferase which has been shown to be associated with the mitochondrial fraction of yeast (Casey and Threlfall, 1978) and rat liver (Momose and Rudney, 1972) mitochondria. It is interesting that recent evidence points to a second localization of PHB:polyprenyltransferase in endoplasmic reticulum. Ka\lem et al. (1987) reported a greater extent of labeling of respiratory deficient meiotic progeny were produced from a cross of the disruption strain to a coq2 mutant. The sequence in question is located between residues 134-156 of the amino acid sequence. The amino acid sequences are arranged in the following functionally similar groups: A, polyprenyl synthetases that catalyze a 1'-4 condensation reactions between the homoallylic substrate IPP and an elongating prenyl diphosphate; B, unidentifed; C, proposed to be phytoene synthetase; D, polyprenyl transferases catalyzing 1'-ary condensation; E, enzymes catalyze head-to-head condensation of two allylic polyprenyl diphosphate substrates; F, enzymes prenylate amino group of adenosine. Numbers of the first and last amino acids in the region shown are listed in parentheses. The source or reference for the protein sequences are shown. Amino acids which appear in three or more functionally distinct enzymes are boxed and represented in the consensus at the bottom of the figure. Species abbreviations are as follows: Nc, Neurospora crassa; Sc, S. cerevisiae; Hs, Homo sapiens; Rr, Ratus ratus; Ec, E. coli; Cp, Cyanophora paradoxa; Rc, Rhodobacter capsulatus; Eh, Eruvicia herbicola; Lc, Lycopersicon esculentum; Ac, Agrobacterium tumeifaciens; Ps, Pseudomonas sauvastnii.

**Fig. 8. Putative allylic polyprenyl diphosphate-binding site.** Amino acid sequences are arranged in the following functionally similar groups: A, polyprenyl synthetases that catalyze a 1'-4 condensation reactions between the homoallylic substrate IPP and an elongating prenyl diphosphate; B, unidentified; C, proposed to be phytoene synthetase; D, polyprenyl transferases catalyzing 1'-ary condensation; E, enzymes catalyze head-to-head condensation of two allylic polyprenyl diphosphate substrates; F, enzymes prenylate amino group of adenosine. Numbers of the first and last amino acid in the region are shown are listed in parentheses. The source or reference for the protein sequences are shown. Amino acids which appear in three or more functionally distinct enzymes are boxed and represented in the consensus at the bottom of the figure. Species abbreviations are as follows: Nc, Neurospora crassa; Sc, S. cerevisiae; Hs, Homo sapiens; Rr, Ratus ratus; Ec, E. coli; Cp, Cyanophora paradoxa; Rc, Rhodobacter capsulatus; Eh, Eruvicia herbicola; Lc, Lycopersicon esculentum; Ac, Agrobacterium tumefaciens; Ps, Pseudomonas sauvastnii.
region of PHB:polyprenyltransferase is most similar to the corresponding region in several enzymes that catalyze the head-to-head condensation of allylic polyprenyl diphosphate substrates such as squalene synthetase.

The amino acid sequence alignment in Fig. 8 reveals a significant amount of sequence identity within functionally similar groups; however, some interesting similarities exist between distinct groups. For instance, FPP synthetase (Escherichia coli, Fig. 8A) and CrtE (Rhodobacter capsulatus, Fig. 8C) share 10 of 23 identical amino acids. Additionally, CrtE (Eruinia herbicola, Fig. 8C) and MOD5 (S. cerevisiae, Fig. 8F) share 9 of 23 identical amino acids. ORF323 is encoded in cyanelle DNA from the alga Cyanophora paradoxa (Fig. 8B). The identity of the ORF323 gene product is unknown; the protein shows 29% sequence identity with the CrtE gene product from Rhodobacter (Michaowski et al., 1991). ORF323 actually shows considerably more sequence identity with the polyprenyl synthetase enzymes in group A. Alignment of ORF323 with HPP synthetase reveals 45% identity over 163 amino acids (not shown). Additionally, ORF323 is 70% (16/23) identical with Domain I of HPP synthetase (not shown). Domain I is another highly conserved region among polyprenyl synthetase enzymes (Ashby et al., 1990).

The enzymes depicted in Fig. 8E catalyze the head-to-head condensation of polyprenyl diphosphate substrates. Consistent with this reaction mechanism CrtB and squalene synthetase both possess a second region with some identity to Domain II that may represent a binding site for the second polyprenyl diphosphate substrate (data not shown). The identity of the pTOM5 protein is unknown. The gene encoding pTOM5 from tomato was isolated on the basis of its induced expression during fruit ripening and may represent the plant homolog of CrtB.

A significant amount of information exists about the interaction of farnesyl diphosphate synthetase with its substrates. Holloway and Popjak (1967) found that the binding of the homoallylic and allylic substrates to the enzyme is ordered with the allylic substrate binding first. Studies, for example, avian liver farnesyl diphosphate synthetase indicated that, in the presence of magnesium, each subunit possesses a single binding site for the homoallylic substrate isopentenyl diphosphate and a single binding site for the allylic polyprenyl substrates dimethylallyl diphosphate, geranyl diphosphate, or farnesyl diphosphate (Reed and Rilling, 1976). These observations have been extended by King and Rilling (1977) who found that the presence of 1 mM MnCl₂ or MgCl₂ greatly enhances the binding of geranyl diphosphate and isopentenyl diphosphate to the enzyme. In addition, binding of Mn²⁺ to farnesyl diphosphate synthetase is apparently nonspecific only in the absence of substrate, whereas in the presence of substrate Mn²⁺ binds to two specific sites in the enzyme. These data imply that homoallylic and allylic substrates bind as their magnesium salts. Gotoh et al. (1988) suggested both substrates bind as magnesium salts, but, once bound, magnesium is captured by the enzyme from isopentenyl diphosphate. The magnesium bound to the allylic substrate would be expected to render the pyrophosphate a better leaving group. This mechanism predicts that once condensation has taken place magnesium captured by the enzyme is released together with the newly formed allylic diphosphate product.

Popjak and co-workers (1969) suggested that interaction of prenyltransferases with isoprenoid diphosphates is likely to be articulated through the highly charged phosphates. Therefore, assuming Domains I and II represent substrate binding sites, interaction of the substrates with the enzyme could be facilitated by salt bridges between the carboxyl groups of the aspartic acid side chains and magnesium (Ashby and Edwards, 1990). There is precedence for this type of interaction. For example, x-ray crystallographic studies have shown that the carboxylate group of Asp⁸ in EF-Tu was shown to form a magnesium salt bridge with the β-phosphate group of GDP (Jurnak, 1985). Likewise, Asp⁷ of ras (p21) was shown to interact with the β-phosphate of GDP in an analogous manner (McCormick et al., 1985).

Of significance in evaluating the possible role of magnesium in PHB:polyprenyltransferase function is the observation of Casey and Threlfall (1978) as well as that of our own laboratory (Ashby and Edwards, 1990) that the specificity of the yeast polyprenyltransferase can be manipulated by altering magnesium concentrations in the assay. At low magnesium concentration (3 mM), PHB:polyprenyltransferase utilizes predominantly hexaprenyl diphosphate, its natural substrate. When the concentration of magnesium is raised to 33 mM the enzyme becomes activated and loses its specificity as evidenced by its utilization of any available allylic polyprenyl diphosphate as a substrate. Similarly, preliminary experiments with the DPR1-dependent yeast protein: farnesyltransferase have shown that the divalent cation requirements are substantially different depending on whether this enzyme employs farneyl diphosphate or geranylgeranyl diphosphate as a substrate.⁴

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PHB:Polyisoprenyltransferase