Flavokinase catalyzes the transfer of the γ-phosphoryl group of ATP to riboflavin to form the flavoenzyme FMN. Consistent patterns of sequence similarities have identified the open reading frame of unknown function YDR236c as a candidate to encode flavokinase in Saccharomyces cerevisiae. In order to determine whether the product of this gene corresponds to yeast flavokinase, its coding region was amplified from genomic DNA by polymerase chain reaction and expressed in Escherichia coli. The purified form of the expressed recombinant protein efficiently catalyzed the formation of FMN from riboflavin and ATP. In contrast to bifunctional prokaryotic flavokinase/FAD synthetase enzymes, the yeast enzyme did not show accompanying FAD synthetase activity. Deletion of YDR236c produced yeast mutants unable to grow on rich medium; however, the growth of the ydr236cΔ mutants could be rescued by the addition of FMN to the medium. Overexpression of YDR236c caused a 50-fold increase in flavokinase specific activity in yeast cells. These findings demonstrate that YDR236c corresponds to the gene encoding a monofunctional flavokinase in yeast, which we propose to be designated as FMN1. The FMN1 gene codes for a 25-kDa protein with characteristics of signals for import into mitochondria. By immunoblotting analysis of Saccharomyces subcellular fractions, we provide evidence that the Fmn1 protein is localized in microsomes and in mitochondria. Analysis of submitochondrial fractions revealed that the mitochondrial form of Fmn1p is an integral protein of the inner membrane exposing its COOH-terminal domain to the matrix space. A similarity search in the data base banks revealed the presence of sequences homologous to yeast flavokinase in the genome of several eu-karyotic organisms such as Schizosaccharomyces pombe, Arabidopsis thaliana, Drosophila melanogaster, Caenorhabditis elegans, and humans.

Riboflavin (vitamin B2) serves as a precursor of the flavin-nucleotide cofactors riboflavin monophosphate (FMN) and flavin adenine dinucleotide (FAD). The formation of FAD depends on the sequential utilization of two molecules of ATP in reactions that first involve the riboflavin (flavokinase, EC 2.7.1.26) kinase-phosphorylation of riboflavin to form FMN and then FAD (EC 2.7.7.2) synthetase-catalyzed adenyllylation of the latter to form FAD. The enzymes responsible for catalyzing the two steps have been purified from several sources (1–9). In Corynebacterium ammoniagenes and Bacillus subtilis, flavokinase and FAD synthetase co-purify and are present in a single, bifunctional flavokinase/FAD synthetase enzyme (7, 10). To date, a large number of homologs to the bifunctional flavokinase/FAD synthetase ribC gene of B. subtilis have been identified in archaea and eubacteria, indicating that this type of gene organization is common in prokaryotes. In contrast, both enzymatic activities have been purified separately in eukaryotic organisms (1, 6, 8). In mammalian tissues, the smaller flavokinase (1) is readily separable from the larger FAD synthetase (3). In the yeast Saccharomyces cerevisiae, the gene encoding a monofunctional FAD synthetase (FAD1) has recently been cloned as an extragenic suppressor of a respiratory deficient pet mutant (11). However, the gene encoding flavokinase in yeast as well as in the rest of eukaryotic organisms remains to be identified.

The formation of holo-flavoproteins, by binding of the coenzyme to the apo-protein, depends on the availability of the cognate flavoenzyme. It is known that most cell flavoproteins are located in the mitochondria but little is known about the subcellular distribution of the enzymes involved in flavin metabolism. It has been described that flavokinase is a cytosolic enzyme in plant and mammalian tissues (4, 6) and the synthesis of FMN and FAD occurs in hepatocyte cytosol (12). However, the synthesis of both flavoenzymes from externally added riboflavin by isolated mitochondria from rat liver has been also reported (13). Whereas it has been determined that the synthesis of riboflavin occurs in the cytosol of S. cerevisiae cells (14, 15), the exact location of the synthesis of FMN and FAD and their compartmentation is controversial in this organism. It has been reported that yeast mitochondria do not contain FAD synthetase and that a specific mitochondrial carrier protein, Flx1p, is involved in flavin transport across the mitochondrial membrane (16). However, it has also been described that FMN and FAD synthesis occurs in isolated yeast mitochondria (17) and that yeast cells overexpressing the FAD1 gene show mitochondrial FAD synthetase activity (11).

Homeostasis of flavin coenzymes is achieved by an intricate interplay of the different enzymes in the pathway responding to regulators (18), by organelle compartmentation (11, 19), and by altered susceptibility of holo- and apo-flavoproteins to proteolytic digestion (20). Since yeast and other eukaryotes share the same flavin coenzyme biosynthetic pathway, study of flavin coenzyme synthesis in yeast should provide a system amenable for study by basic and biochemical techniques of classic and molecular genetics. The present work addresses the identifica-
and characterization of the structural gene encoding flavokinase in *S. cerevisiae* as well as the subcellular localization of the enzyme.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains, Media, and Growth Conditions**—The yeast strains used in this study are congeneric to the BY4743 background (21). Gene replacements were performed as described (22) using PCR-derived cassette containing the geneticin resistance marker *kanMX*. The following primers were used to replace YDR236c codons 2-218 (23): CCGGTCAAGCTTGGAATTCCGGATCC (YDR236c-S1) and GGGTCGACCTATAATTGTTTGAATAC (YDR236c-E1) and cloned into the same sites of pVT101L (28), to obtain pVT101L-FMN1. The coding region was amplified by high fidelity PCR from yeast genomic DNA using the primers GGAATTCCATATGGACAAGCTTCTAG (YDR236c-S2) and TGCAGGTCGAC (YDR236c-R1). All probes were used to replace YDR236c codons 2-218 (23); CCCGCGTCCATGATTCTGAC (YDR236c-S1) and GTTAAGAAAAACTACTAGCCCTG (YDR236c-R1). All gene replacements were confirmed by PCR analysis.

Yeast strains were usually grown in rich medium (YPD, synthetic dextrose, supplemented with auxotrophic requirements) (25). Semisynthetic low sulfate medium was prepared as described (26).

**Plasmid Constructions and Overexpression of FMN1p**—For overexpression of *Fmn1p* in *E. coli*, the entire YDR236c coding sequence was amplified by high fidelity PCR from yeast genomic DNA with the primers 

GGAATTCCATATGGACAAGCTTCTAG (YDR236c-S2) and TGCAGGTCGAC (YDR236c-R1) and cloned into the same sites of pET28b (27) to obtain pET28b-FMN1. *E. coli* BL21(DE3) strain recombinant cells obtained by transformation with pET28b-FMN1 were grown on Luria-Bertani medium, supplemented with 50 mg/ml kanamycin. When the cell density in the culture reached 600 of 1.0, IPTG was added to a final concentration of 1 m M. The microsomal fraction was isolated from the postmitochondrial supernatant by centrifugation at 100,000 g for 1 h, and the supernatant was named the cytosolic fraction. The microsomal fraction was pelleted by ultracentrifugation in a 10 m Tris-HCl, pH 7.4, and cleared of residual mitochondria by centrifugation through a step 25–50% sucrose gradient (5% increment by step) in the same buffer.

**Preparation of Subcellular and Submitochondrial Fractions**—Cells were harvested by centrifugation after 1 h of induction, resuspended in 20 m M Tris-HCl, pH 7.5, 5 m M NaCl, 1 m M phenylmethylsulfonyl fluoride, and washed once with 0.6 M mannitol, 10 m M Tris-HCl, pH 7.4, and resuspended in 5 m M MES, pH 6.0, containing 0.6 m M sorbitol, 1 m M KCl, and 1 m M phenylmethylsulfonyl fluoride (breakage buffer). Mitoplasts were homogenized in a 10-ml glass Dounce homogenizer, and after unbroken cells and nuclei were removed by centrifugation at 3,000 x g, the crude mitochondrial pellet was obtained by centrifugation at 9,000 x g for 15 min. The supernatant thus obtained was named the postmitochondrial fraction. Crude mitochondria were diluted in a small volume of breakage buffer and overlaid on a continuous 20–50% sucrose gradient in 0.6 M sorbitol, 5 m M MES, pH 6.0. After 1 h of centrifugation at 100,000 x g, purified mitochondria were recovered as a light-brown band from the lower third of the tube. The solution of purified mitochondria was diluted 5-fold with breakage buffer, centrifuged for 10 min at 12,000 x g and the resulting pellet washed once with 0.6 M mannitol, 10 m M Tris-HCl, pH 7.4, and resuspended in a small volume of the same buffer. The microsomal fraction was isolated from the postmitochondrial supernatant by centrifugation at 100,000 x g for 1 h, and the supernatant was named the cytosolic fraction. The microsomal fraction was pelleted by ultracentrifugation in 10 m Tris-HCl, pH 7.4, and cleared of residual mitochondria by centrifugation through a step 25–50% sucrose gradient (5% increment by step) in the same buffer.

**Identification of the FMN1 Gene of *S. cerevisiae***—While the processes that mediate FMN biosynthesis in yeast are not defined, it seemed reasonable to surmise that a flavokinase...
could be required. Sequence similarity searches such as FASTA and BLAST (35) failed to unambiguously detect any yeast protein showing homology to the riboflavin-kinase carboxyl-terminal (COOH-terminal) domain of bifunctional prokaryotic flavokinase/FAD synthetases. Comparison of proteins encoded in seven complete genomes, including the S. cerevisiae genome, and the elucidation of consistent patterns of sequence similarities allowed the delineation of clusters of orthologous groups, each containing individual orthologous proteins from at least three different lineages (36, 37). One of these clusters (clusters of orthologous 0196) contains the predicted YDR236c gene product from S. cerevisiae in addition to flavokinase/FAD synthetases from several bacterial species. Since members of the same cluster are predicted to have the same or related function and the gene encoding FAD synthetase in yeast (FAD1) has been previously identified, we hypothesized that YDR236c would encode riboflavin kinase in yeast.

To test this hypothesis a null allele of YDR236c was constructed by the replacement of the complete open reading frame by the geneticin resistance kanMX4 marker (22) and used to make a chromosomal deletion of YDR236c in BY4743 diploid cells, which was confirmed by PCR (38). When ydr236c::YDR236c diploids were sporulated and the resulting tetrads dissected, viability segregated 2:2:2 and all viable spores were geneticin-sensitive, suggesting that YDR236c is a single copy gene that is essential for growth on YPD medium. Examination of the non-growing spores under the light microscope revealed that they gave rise to microcolonies comprising several hundred small and morphologically heterogeneous cells, suggesting that the spores had gone through germination and grown for an additional 7–10 generations before arresting. When ydr236c::YDR236c diploids were subsequently transformed to leucine prototrophy with the LEU2-marked plasmid YCPlac111-YDR0236c and then sporulated, upon tetrad dissection all four spores were viable on YPD medium, all geneticin-resistant spores were Leu-, and the geneticcin-resistance marker segregated 2:2 as expected.

Cells disrupted in the gene encoding flavokinase are predicted to be unable to grow because of their metabolic block in the biosynthesis of the essential cofactors FMN and FAD. To investigate whether YDR0236c is indeed a gene involved in the biosynthesis of FMN and the external supply of FMN or FAD cofactors is able to rescue the growth impairment of the ydr236c::mutant, cells from the microcolonies on the dissection plates were transferred to YPD plates supplemented with riboflavin, FMN, or FAD. Of the compounds examined, only FMN (minimum 1 mM) supported the growth of mutant cells (Fig. 1). Accordingly, this gene was designated FMN1.

**FMN1 Encodes a Monofunctional Flavokinase**—To facilitate the biochemical characterization of the Fmn1p product an expression system in which the protein could be rapidly isolated from a bacterial lysate was developed. The primers YDR236c-E1, containing a NdeI restriction site 5’ upstream from the translation initiation codon, and YDR236c-E2, containing a SalI restriction site downstream from the translation termination codon, were designed to amplify the FMN1 open reading frame on the basis of the published sequence (23). The polymerase chain reaction fragment obtained using S. cerevisiae genomic DNA as template was cloned into pET28b vector for expression under the control of T7 RNA polymerase in E. coli strain BL21(DE3) (27). Induction of FMN1 expression from the T7 promoter led to the production of a single 29-kDa protein, consistent with the expected molecular mass of the Fmn1p fusion protein (which contains the His-tag and the T7-tag of the poly linker of the vector at the NH2-terminal end of Fmn1p) (Fig. 2, lane 4). The 29-kDa recombinant protein was absent in uninduced extracts (Fig. 2, lane 3) and also in cells carrying the empty vector (Fig. 2, lanes 1 and 2). The 29-kDa protein was partially purified from insoluble bacterial inclusion bodies and solubilized and refolded in the presence of riboflavin as described under “Experimental Procedures” (Fig. 2, lanes 6 and 7). The biochemical properties of the partially purified Fmn1p were analyzed in vitro. Efficient phosphorylation of riboflavin to form FMN was observed in the presence of the purified Fmn1p and ATP (Fig. 3). In contrast to known prokaryotic riboflavin-kinase/FAD synthetases (9, 10), no FAD synthetase-associated enzymatic activity could be detected. A similar extract that did not contain Fmn1p, obtained from control cells transformed with the empty pET28b vector and prepared according to the Fmn1p purification protocol, showed no flavokinase activity.

To demonstrate independently that FMN1 encodes flavokinase in S. cerevisiae, the gene was overexpressed in yeast cells. In order to maximize the level of expression, a leu2Δ-based multicopy vector carrying the FMN1 open reading frame under the control of the strong ADH1 promoter was constructed (28). When this plasmid (pVT101L-FMN1) was introduced into the fmn1Δ null mutant strain, the resulting expression level of Fmn1p was elevated by approximately 50-fold as compared with that observed in the control wild-type strain, as estimated by determination of the flavokinase specific activity (Table I). Cell extracts from the nontransformed fmn1Δ mutant or fmn1Δ transformed with the empty pVT101L vector did not display any enzymatic activity. No significant increases in FAD synthetase specific activity above the level of the wild-type control were found among the studied strains.

**Analysis of the Amino Acid Sequence of the Flavokinase Protein**—A detailed analysis was made of the deduced amino acid sequence of Fmn1p. The yeast flavokinase comprises 218 amino acids with a calculated mass of 24,539 Da. This molecular mass value is lower than the range of values (i.e. 34–36 kDa) for other flavokinases of known sequence from prokaryotes. The protein is quite acidic, displaying an isoelectric point of 34–36 kDa for other flavokinases of known sequence from prokaryotes.
4.93 and a net charge of \(-6.47\), pH 7.0. Analysis of the amino-terminal residues of Fmn1p revealed that the protein contains a potential signal peptide (residues 1 to 20) with a predicted cleavage site located between positions Leu19 and Met20 (39). The NH\textsubscript{2}-terminal domain of Fmn1p resembles presequence signals for import into mitochondria. Presequences are extremely variable in length and amino acid sequence, but they do share common features such as the predominance of hydroxylated and positively charged residues and an amphiphilic structure (generally an \(\alpha\)-helix) with polar, positively charged, and apolar surfaces (40). The NH\textsubscript{2}-terminal domain of Fmn1p has a high pI (pI 9–10) and may form an amphipathic \(\alpha\)-helix.

Additionally, a signal for retention/recycling in the endoplasmic reticulum (a COOH-terminal KQLL dilysine-like motif) was noted (41).

A search of the Swiss-Prot and the GenBank data bases was carried out in order to identify other sequences that may be closely related to the yeast flavokinase (Fig. 4). The Fmn1p sequence displayed the greatest similarity to DNA sequences encoding proteins of unknown function from Schizosaccharomyces pombe (i.e. 36.36% identity over a 176-amino acid overlap; Swiss-Prot accession number O74866), Drosophila melanogaster (accession number, O76206), Arabidopsis thaliana (accession number O65412), and Caenorhabditis elegans (accession number Q21918). Partial, but nonetheless significant, similarity was observed between Fmn1p and the conceptual translation of a human DNA sequence corresponding to the q11.2-q13.2 region of chromosome 22 (32% identity over a 128-amino acid overlap; GenBank accession number NT_001454). A lesser degree of homology, restricted to a few conserved residues, was also noticed for the COOH-terminal (flavokinase) domain of bifunctional flavokinase/FAD synthetases from several prokaryotes (i.e. ribF from C. ammoniagenes, accession number Q59263).

Intracellular Location of FMN1p—To determine the intracellular location of the protein encoded by FMN1, we fused green fluorescent protein (GFP) to the carboxyl terminus of Fmn1p under the control of the ADH1 promoter (28). Episomic, high copy number plasmids encoding the Fmn1p-GFP fusion protein were transformed into fmn1D cells. The construct was able to complement the FMN-auxotrophic phenotype of the disruptant, indicating that the protein was fully functional \textit{in vivo}. Fluorescence microscopy of cells expressing Fmn1p-GFP showed that the GFP fluorescence was distributed into cytoplasmic punctate structures that resemble a mitochondrial localization (42). The signal was completely absent from cells carrying the untagged wild-type FMN1 gene. Co-staining of cells with the mitochondrial-specific dye rhodamine revealed that the GFP and the rhodamine fluorescence patterns were
FIG. 4. Comparison of the predicted amino acid sequence of Fmn1p with those of related proteins. The amino acid sequence of Fmn1p was aligned with the sequences of putative homologs from S. pombe (S.p.), D. melanogaster (D.m.), A. thaliana (A.t.), C. elegans (C.e.), humans (H.s.) and the bifunctional flavokinase/FAD synthetase from C. ammoniagenes (C.a.). Areas of identity are indicated by black boxes and gaps are indicated by dashes. Numbers on the right correspond to the amino acid residues.

Fig. 5. Indicated by black boxes and gaps are areas of identity. Numbers on the right correspond to the amino acid residues.

To determine the localization of Fmn1p independently, we constructed a fusion protein containing the entire Fmn1p and a three tandem repeats of the influenza virus hemagglutinin A epitope sequence (3HA) that serves as an antibody recognition site. The COOH-terminal, 3HA-tagged Fmn1p produced a functional protein, since it was able to complement the fmn1Δ mutant. When the fusion protein was expressed under the control of the native FMN1 promoter and the cells were fractionated, it was detected both in the mitochondrial and microsomal fractions by Western blot analysis (Fig. 6A). Although some of our microsomal fractions contained mitochondrial membranes that co-purify, as judged by the presence of a small amount of the mitochondrial membrane-specific marker porin (Fig. 6A, lane Mc), the predominant presence of Fmn1p in the microsomal fraction excluded the possibility that this could be due to mitochondrial contamination. The Fmn1p-3HA signal found in the microsomal fraction accounted for approximately 80% of the total Fmn1p-3HA cellular content. A similar distribution ratio of flavokinase between microsomes and mitochondria was also obtained from the determination of the total enzyme activity in subcellular fractions of wild-type cells (1.78 enzyme units in the microsomal fraction versus 0.20 units in the mitochondrial fraction of 100 mg of protein of crude extract). From these results we conclude that yeast flavokinase is unevenly distributed in mitochondria and in microsomes, the bulk fraction being located in microsomes.

Submitochondrial Location of FMN1p—To examine the submitochondrial location of Fmn1p, mitochondria isolated from cells harboring the 3HA-tagged protein were subfractionated and used for immunoblotting. After carbonate extraction, virally infected mitochondria (Fig. 6B). The COOH-terminal 3HA tag of Fmn1p-3HA was protease-protected when isolated mitochondria were treated with proteinase K, and also when mitoplasts, generated by selectively opening the outer membrane by incubation with hypotonic buffer, were subjected to proteinase K digestion. The COOH-terminal 3HA tag was accessible to added protease only when the inner membrane was opened with detergent (Fig. 6B). Together, these data indicate that the COOH terminus of Fmn1p is located inside the inner membrane. We conclude that the mitochondrial form of Fmn1p is an integral protein of the inner membrane, exposing its COOH-terminal domain to the matrix space.

DISCUSSION

Growing cells depend on a continuous supply of newly synthesized flavin nucleotides FMN and FAD which are required as cofactors for a large number of flavoenzymes. This class of enzymes is of crucial importance in a wide range of biological processes, including lipid, protein, and carbohydrate metabolism (43). The synthesis of FAD from riboflavin involves an initial phosphorylation of riboflavin to FMN, which then reacts with ATP to form FAD. In S. cerevisiae and other eukaryotes, monofunctional riboflavin kinase and FAD synthetase catalyze the two reactions, respectively. Whereas the gene encoding FAD synthetase in yeast, FAD1, has been cloned and characterized (11), the gene encoding flavokinase remains to be identified. In this report, we have clearly shown that FMN1 encodes a yeast monofunctional flavokinase that is present in both microsomal and mitochondrial fractions. The increased flavokinase activity of yeast cells overexpressing Fmn1p supports the notion that this gene codes either for a protein involved in the regulation of FMN biosynthesis or for the flavokinase itself. More definitive evidence supporting FMN1 as the yeast flavokinase gene was obtained by enzymatic assays with the Fmn1 protein purified from E. coli cells overexpressing FMN1. Deletion of FMN1 impairs growth on rich medium, demonstrating that, as expected, the synthesis of FYN is essential for cell viability and that no redundant flavokinase activities, other
than that encoded by FMN1, exist in yeast cells. The fact that the growth defect shown by the fmn1Δ null mutants could be rescued by supplementing the medium with FMN indicates that this phosphorylated compound may enter the yeast cells to overcome the metabolic block in flavocoenzyme synthesis. The high concentration of externally supplied FMN required to allow the growth of the mutants suggests that a passive, diffusion mechanism would be involved in the uptake of FMN by yeast cells. In contrast, flavin uptake by mammalian cells is mediated by an efficient membrane-associated carrier system (44). Although FAD may be metabolically converted into FMN by the action of a catabolic FAD pyrophosphatase (EC 3.6.1.18), the addition of FAD to the medium does not support the growth of fmn1 mutants. Two reasons can be invoked to explain this result: either the yeast cells are not permeable to external FAD, or the amount of intracellular FMN formed from FAD by the action of FAD pyrophosphatase is not sufficient or is not compartmentally available for recruiting by FMN-dependent flavoenzymes.

Comparison of the sequence of Fmn1p with proteins in data bases identifies Fmn1p as the founding member of a new family of eukaryotic monofunctional flavokinases that includes protein products from several eukaryotic organisms subjected to systematic genome sequencing projects, such as S. pombe, D. melanogaster, A. thaliana, and C. elegans. This family of proteins share a well conserved motif (VXFGGRG/S/G/(R/K/A)ELG/UC/P/AN), which is also present in the COOH-terminal domain of bifunctional flavokinase/FAD synthetases from C. ammoniagenes and other prokaryotes (Fig. 4). A human truncated hypothetical protein, showing 34.2% identity with Fmn1p, also contains part of the flavokinase signature, suggesting that this amino acid sequence belongs to the human flavokinase.

C. ammoniagenes and other prokaryotes (Fig. 4). A human truncated hypothetical protein, showing 34.2% identity with Fmn1p, also contains part of the flavokinase signature, suggesting that this amino acid sequence belongs to the human flavokinase. Most of the ATP-dependent enzymes show more or less conserved sequence motifs. The best conserved of these motifs is a glycine-rich region which typically forms a flexible loop between a β-strand and an α-helix which is generally referred to as “P-loop.” In contrast, this sequence motif is not present in the yeast flavokinase. Additionally, it has been reported that mammalian flavokinase preferentially utilizes ATP with Zn(II) instead of Mg(II), which is the preferred metal ion for classic enzymes that catalyzes phosphoryl transfers (45). Taken together, all these data indicate that flavokinase is different to most other ATP-dependent kinases as regards binding the metal-ATP complex.

At present, knowledge of subcellular localization of flavocoenzyme biosynthesis and of its compartmentation is rather poor. In yeast, flavokinase activity has been found in both mitochondria and the cytoplasm. However, the precise localization of flavokinase activity within the cell is not known. In mammalian cells, flavokinase activity is primarily localized to the cytosol, although some activity has been detected in subcellular fractions enriched for mitochondria. The localization of flavokinase activity in yeast may be different, as flavokinase activity has been found in both the cytosol and mitochondria. Further studies are needed to clarify the subcellular localization of flavokinase in yeast.
mitochondrial and extramitochondrial fractions whereas FAD synthetase appears to be restricted to the cytoplasm (11, 16). A recent study, however, has reported that isolated mitochondria are capable of synthesizing FMN and FAD from externally added riboflavin (17). In this work, HA-tagged Fmn1p was located in two subcellular fractions: mesosomes and mitochondria. In contrast to previous reports suggesting that the mitochondrial form of yeast flavokinase is present in the matrix (11, 17), the submitochondrial immunolocalization of Fmn1p shows that it is an integral protein of the inner membrane. Our results also demonstrate that the extramitochondrial form of yeast flavokinase, unlike plant and mammalian flavokinases, which have been reported as soluble cytosolic proteins, is a membrane-bound protein located in the microsomal fraction. It is likely that a peptide signal present in the yeast enzyme but absent in plant and mammalian flavokinases would be responsible for this difference (Fig. 4). In addition, preliminary results showing that the microsomal form of Fmn1p is located on the cytoplasmic face of endoplasmic reticulum membranes (data not shown) suggest that it may play the same role as the soluble, cytosolic enzyme from higher eukaryotes in supplying FMN cofactor to this compartment. The dual distribution of flavokinase in both mitochondria and cytoplasm is consistent with an enzyme catalyzing a biosynthetic reaction whose product is necessary in both compartments. The uneven subcellular distribution of Fmn1p may favor an efficient and appropriate supply of coenzyme for the formation of holo-flavoproteins at the corresponding compartments.

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Molecular Characterization of FMN1, the Structural Gene for the Monofunctional Flavokinase of Saccharomyces cerevisiae
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