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The Gene for Biotin Synthase from *Saccharomyces cerevisiae*: Cloning, Sequencing, and Complementation of *Escherichia coli* Strains Lacking Biotin Synthase

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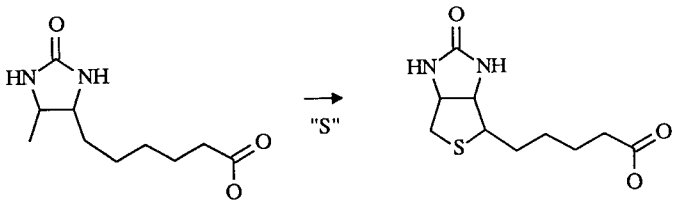
Biotin synthase catalyzes the insertion of a sulfur atom between two carbon atoms of dethiobiotin to form biotin in the last step of the biotin biosynthesis pathway. In *Escherichia coli,* biotin synthase is coded for by *bioB* gene. We report here cloning, sequencing, and initial functional characterization of the yeast gene for biotin synthase in *Saccharomyces cerevisiae.* We have named this gene *BIO2.* It consists of a 355-codon open reading frame near the *ZUO1* gene. Analysis of the yeast protein encoded by the *BIO2* gene reveals that it shares extensive homology with biotin synthases of *E. coli* and *Bacillus sphaericus.* The yeast and the two bacterial biotin synthase proteins have similar molecular weights, amino acid compositions, and hydropathies. The plasmid pUCBIO2 containing the yeast *BIO2* gene completely complements *E. coli bioB*$^-$ and Δ*bio* mutants and enables these mutants to grow on dethiobiotin. Although *BIO2* is physically linked to *ZUO1,* which encodes the putative left-handed Z-DNA binding protein zuotin, it appears to be regulated independently from it. The yeast *BIO2* and *ZUO1* genes reside near *ADE3* gene on chromosome VII. *BIO2* is the first eukaryotic gene reported from the biotin biosynthetic pathway. © 1994 Academic Press, Inc.

The last step of the biotin biosynthetic pathway is catalyzed by biotin synthase (1). Although very little is known about this enzyme, the transformation involves the addition of sulfur to dethiobiotin to form biotin as shown in Scheme I.



SCHEME I. The reaction catalyzed by biotin synthase.

This reaction has recently been reported for the first time in crude extracts (2). From a chemical point of view, the addition of sulfur to two unactivated carbon atoms appears to be a very difficult reaction, and the elucidation of the mechanism of this reaction promises to be a fascinating problem. Some work was done on the mechanism of this reaction before the availability of a cell-free system. For example, Parry (3) has shown that all the hydrogen atoms of dethiobiotin are preserved in the biotin synthase reaction, except for the two removed to allow the addition of sulfur; namely the *pro*-S hydrogen atom on the methylene carbon attached to the imidazolinone ring, and a hydrogen atom on the methyl carbon attached to the imidazolinone ring. Parry has also shown that the addition of sulfur to the methylene carbon attached to the imidazolinone ring occurs with retention of configuration.

The immediate sulfur donor for this reaction has not been identified despite considerable effort, and there is confusion in the literature on this matter. For example, in experiments with *Saccharomyces cerevisiae,* methionine sulfoxide and methionine appeared to be the most effective sulfur donors, whereas cysteine was not effective (4, 5). On the other hand, experiments with *Escherichia coli* seem to show that methionine may not be the sulfur donor; rather, it is cysteine or a compound closely related to it (6, 7). Whether there is a difference in sulfur donors for this reaction among various organisms is not clear at present.

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The gene that codes for biotin synthase has been identified in *E. coli* and has been given the designation *bioB* (1). The *E. coli bioB* gene has been cloned and sequenced (8), as has the gene coding for biotin synthase from *B. sphaericus* (9). The gene coding for biotin synthase from *R. capsulatus* has been cloned, but no sequence has been reported (10).

Although higher plants (11, 12), *S. cerevisiae* (13), and other eukaryotes (14) apparently make biotin by a pathway similar to that in *E. coli,* very little work has been done on biotin biosynthesis in eukaryotes and none of the genes involved in biotin biosynthesis in eukaryotes has been identified. *S. cerevisiae* are unusual in that they contain only part of the biotin biosynthetic pathway. *S. cerevisiae* cannot make biotin *de novo;* however, if supplemented with 7,8-diaminopelargonic acid, an intermediate in the biotin biosynthetic pathway worked out for *E. coli, S. cerevisiae* can carry out the last two steps in that pathway, i.e., the conversion of 7,8-diaminopelargonic acid to dethiobiotin, and the conversion of dethiobiotin to biotin (13). This would be possible only if yeast possesses the genes for the last two enzymes in the biotin biosynthetic pathway. We report here the cloning and sequencing of *BIO2,* the gene for biotin synthase in yeast. We also show that functional protein from this gene can be expressed in *E. coli* and phenotypically complement *E. coli bioB*$^-$ and Δ*bio* strains.

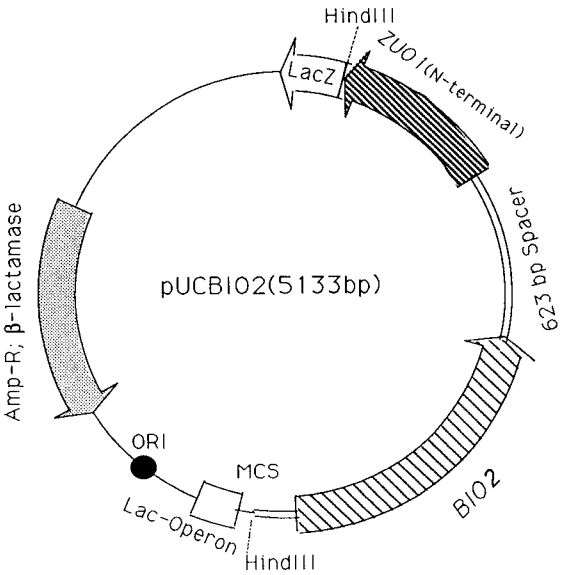

FIG. 1. Diagram of pUCBIO2. This plasmid was used for DNA sequencing and for the transformation of *E. coli bioB* mutants. The 2.4-kb yeast *Hin*dIII fragment was inserted at the *Hin*dIII site in pUC19. The *BIO2,* N-terminus portion of *ZUO1,* and Amp are indicated. Yeast DNA between the *BIO2* and the *ZUO1* genes is shown in double lines. ORI is the origin of replication. MCS is the multiple cloning site of pUC19. The *BIO2* and *ZUO1* are in the same orientation as *lac*Z. After sequencing the *Hin*dIII fragment, it was calculated that the plasmid contains 5113 base pairs.

MATERIALS AND METHODS

Strains and DNA. The yeast EMBL3A DNA library was a gift of Richard Young of the Whitehead Institute and MIT. The *E. coli* strains used in the complementation experiments were *E. coli* K12-Y10 *bioB*$^-$105 obtained from Max Eisenberg of Columbia University and KS302 Δ*bio* (biotin operon deletion strain) from Gerald Cohen of Tel Aviv University. Enzymes and plasmid pUC19 were purchased from New England Biolabs; DNA Sequenase Kit was purchased from United States Biochemicals; [γ-^{35}S]dATP (1000 Ci/mM) was purchased from NEN and Amersham. Oligonucleotides used as sequencing primers were either made internally or purchased from Oligos, ETC., Inc.

Cloning and sequencing BIO2 gene. Detailed methods used to clone the yeast *ZUO1* gene that led to our interest in the ORF[4] 5′ to this gene have been described (15). Briefly, a *S. cerevisiae* genomic phage λEMBL3A DNA library was screened using a pool of degenerative oligonucleotides corresponding to the N-terminus of zuotin. Fourteen positive phage plaques were isolated. Phage DNA from 11 clones was purified from the confluent plate lysates. DNA was then digested with several restriction enzymes and a Southern blot was performed as described (15). A 3.1-kb *Eco*RI and *Bam*HI fragment was used to sequence the *ZUO1* gene. Part of an ORF that turned out to be the *BIO2* gene was found on this fragment 5′ to the *ZUO1* gene. To sequence the complete ORF 5′ to the *ZUO1* gene, a phage clone containing a yeast 2.4-kb *Hin*dIII fragment was chosen because this fragment contained a longer piece of DNA in the direction of the ORF 5′ to the *ZUO1* gene. This fragment was subcloned into pUC19 and the new plasmid was designated pUCH2.4. The 2.4-kb *Hin*dIII fragment was sequenced on both strands by the Sanger method (16) using synthetic oligonucleotides primers to the *lac*Z region and part of the DNA in the ORF that had already been sequenced. The plasmid pUCH2.4 was renamed pUCBIO2 after we became convinced that it contains the entire yeast biotin synthase gene.

Transformation of E. coli biotin auxotrophic mutants. The plasmid pUCBIO2 containing a 2.4-kb *Hin*dIII yeast fragment was used to transform competent *E. coli* K12-Y10 *bioB*$^-$105 and KS302 Δ*bio* cells prepared using the $CaCl_2$ method (17). The transformed cells were placed on the M-9 plates with 1% glucose, 10 mM dethiobiotin, and 100 mg/ml ampicillin. Several colonies grew under these conditions.

Measurement of growth rate of the complemented E. coli cells. Single colonies of *E. coli bioB*$^-$ and Δ*bio* strains transformed with the pUCBIO2 plasmid were inoculated into flasks containing M-9 with 1% glucose and 100 mg/ml ampicillin. Biotin or dethiobiotin was added at a concentration of 20 mM to the media. The cultures were grown with shaking at 37°C and the absorbance of the cultures was measured periodically at 600 nm.

Computer analysis of sequences. The sequence analysis programs used in comparing the structures were TfastA and Bestfit from the Genetic Computer Group, Inc. (GCG) (version 6.2) and MacMolly Tetra (version 1.2) (SoftGene, Berlin, Germany).

RESULTS

Cloning and sequencing the ORF adjacent to the ZUO1 gene. While sequencing the *ZUO1* gene and the contiguous DNA, it became apparent that a peptide encoded by a partially sequenced ORF 5′ to the *ZUO1* gene shared significant homology with the C-terminal region of the peptide encoded by the *bioB* gene of *E. coli* (8). The possibility that the gene for biotin synthase in yeast had also been cloned serendipitiously while cloning the

[4] Abbreviations used: ORF, open reading frame.

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        TCC CAT ACT TGC TGA AAA TTT TTC AGA TCT TAC TCT TCT GGT AGT GGT GCC TCA GAA GAG CTA ACT AGC CGA   72
TTA TTT CAA TTT AGT GCA GTA TGA TGT CTA CTA TCT ACC GTC ATT TAT CTA CCG CTA GAC CGG CTT TAA CTA AAT ACG  150

        Met Pro Gln Leu Asn Arg Gln Leu His Pro Gln Lys Leu Val Pro Gly Cys Ser Thr Ile Cys Ile Val Phe   24
CAA CCA ATG CCG CAG TTA AAT CGA CAA CTG CAT CCT CAG AAG CTA GTA CCT GGG TGC TCT ACA ATA TGC ATT GTC TTT  228

Arg Leu Thr Lys Ser Phe Val Asp Lys Ile Ala Ile Lys Arg Asn Leu Ser Tyr Pro Thr Ala Arg Thr Tyr Ser Cys   50
AGA TTA ACC AAG TCA TTC GTG GAC AAA ATC GCA ATT AAA AGA AAT TTA TCA TAC CCC ACT GCT CGA ACT TAC TCA TGC  306

Ser His Asn Cys Ser His Arg Lys Trp His Asp Pro Thr Lys Val Gln Leu Cys Thr Leu Met Asn Ile Lys Ser Gly   76
AGT CAC AAT TGC AGT CAC AGA AAG TGG CAC GAT CCA ACC AAA GTG CAA TTG TGC ACA TTG ATG AAC ATC AAA TCT GGT  384

Gly Cys Ser Glu Asp Cys Lys Tyr Cys Ala Gln Ser Ser Arg Asn Asp Thr Gly Leu Lys Ala Glu Lys Met Val Lys  102
GGT TGT TCT GAG GAC TGT AAG TAT TGT GCG CAG TCT TCG AGA AAC GAT ACC GGT CTA AAG GCT GAG AAA ATG GTT AAA  462

Val Asp Glu Val Ile Lys Arg Gly Arg Arg Gly Cys Lys Arg Asn Gly Ser Thr Arg Phe Cys Leu Gly Ala Ala Trp  128
GTG GAT GAA GTG ATT AAA AGA GGC AGA AGA GGC TGC AAA AGA AAC GGA TCT ACT AGA TTC TGC CTA GGT GCT GCA TGG  540

Arg Asp Met Lys Gly Arg Lys Ser Ala Met Lys Arg Ile Gln Glu Met Val Thr Lys Val Asn Asp Met Gly Leu Glu  154
AGA GAC ATG AAA GGT CGT AAA TCA GCC ATG AAA AGA ATT CAG GAA ATG GTG ACC AAA GTG AAT GAT ATG GGG CTA GAA  618

Thr Cys Val Thr Leu Gly Met Val Asp Gln Asp Gln Ala Lys Gln Leu Lys Asp Ala Gly Leu Thr Ala Tyr Asn His  180
ACG TGT GTT ACT TTA GGT ATG GTT GAT CAA GAT CAA GCA AAG CAA TTG AAA GAT GCA GGT TTG ACT GCA TAC AAC CAT  696

Asn Ile Asp Thr Ser Arg Glu His Tyr Ser Lys Val Ile Thr Thr Arg Thr Tyr Asp Asp Arg Leu Gln Thr Ile Lys  206
AAC ATC GAC ACT TCC AGA GAA CAC TAT AGT AAG GTC ATC ACC ACG AGA ACC TAC GAC GAC AGG TTA CAG ACC ATC AAG  774

Asn Val Gln Glu Ser Gly Ile Lys Ala Cys Thr Gly Gly Ile Leu Gly Leu Gly Glu Ser Glu Asp Asp His Ile Gly  232
AAT GTC CAA GAA TCT GGA ATA AAA GCC TGT ACC GGT GGT ATT TTG GGT CTC GGT GAA AGC GAA GAC GAC CAT ATA GGA  852

Phe Ile Tyr Thr Leu Ser Asn Met Ser Pro His Pro Glu Ser Leu Pro Ile Asn Arg Leu Val Ala Ile Lys Gly Thr  258
TTC ATC TAC ACA TTA TCC AAT ATG TCT CCT CAT CCT GAG TCC CTA CCA ATT AAT AGA CTA GTT GCT ATC AAA GGG ACT  930

Pro Met Ala Glu Glu Leu Ala Asp Pro Lys Ser Lys Lys Leu Gln Phe Asp Glu Ile Leu Arg Thr Ile Ala Thr Ala  284
CCA ATG GCT GAG GAA CTT GCC GAT CCA AAG AGT AAA AAG TTG CAA TTC GAC GAA ATT TTG AGA ACC ATT GCC ACA GCG 1008

Arg Ile Val Met Pro Lys Ala Ile Ile Arg Leu Ala Ala Gly Arg Tyr Thr Met Lys Glu Thr Glu Gln Phe Val Cys  310
AGA ATA GTT ATG CCA AAG GCC ATT ATA AGA CTT GCC GCT GGT CGT TAT ACA ATG AAA GAA ACA GAG CAA TTT GTC TGT 1086

Phe Met Ala Gly Cys Asn Ser Ile Phe Thr Gly Lys Lys Met Leu Thr Thr Ile Tyr Asn Gly Trp Asp Glu Asp Lys  336
TTC ATG GCA GGT TGT AAC AGT ATC TTC ACC GGT AAG AAA ATG CTG ACG ACA ATA TAT AAC GGT TGG GAC GAA GAC AAG 1164

Ala Met Leu Ala Lys Trp Gly Leu Gln Pro Met Glu Ala Phe Lys Tyr Asp Arg Ser  *                          355
GCA ATG TTG GCT AAA TGG GGA TTG CAA CCT ATG GAG GCA TTT AAG TAC GAC AGA TCT TGA AGA TAG GGA TAT GTG GAT 1242

AAT TCT ACG ATT CTA ACT GTA CAT TTC TCC CTT ATT TAT TAA GAA AAC CTA TAT ATA TAT ATA TTT ACC TAT TTA TTC 1320
TGC CAT CGT TAG CTG GCG TTT TAT CTT TTA TGC ATC CAA TAT CTA ATA TTA CTT CCG ATC ACG CAT TTA GTT CTG ATT 1398
ACA GCA GAA ATC GTA GCG CGA TGA GAC ATT TCA TCA AAT GGC CTT TTT TTT TTG GGC AAT TTT TTT ATA TCT TGA AAT 1476
GAT AGT TGC CTT GTA CTT TCA ACC GTT CAT TTC ATT AAG AAC TTG ACT AAA TAT GAA CAT TTC TTA AAA AAA AAG GTT 1554
GAC ATA TAA AAA TAA TCG AAT ATA AAC GAT GGA ATT TTT ATA AAA TTA AAC ACA TAT ATA TAT ATA TAT TAA CTA TAA 1632
ATA TGT CAA AGA AAC CAT ACA ATC ATA GAT TTA TAA CTA TCT TTT GGA TGA CAT TAA TGA ACA TAA CGC TCC TAA TAC 1710
AAA TGT CAA AAA ATA TTA CCC GCA AAT ACG AAT CTT TTT TTT TTC TCA TAA ATT TTG CAA AGA GTT CGA AAT TTT TAT 1788
TTC AAG AGC TGG TAG AGA AAA TTT CAT AAG GTT TTC CTA CCG ATG CTT TTA TAA AAT ATG                  1848
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FIG. 2. Nucleotide and translated peptide sequence of yeast *BIO2* gene. *BIO2* encodes the gene for yeast biotin synthase. The sequence is shown for the entire *BIO2* gene 5′ to the *ZUO1* gene including 156 base pairs 5′ to the coding region of *BIO2*, and an intergenic spacer of 623 base pairs. The last three nucleotides, ATG, constitute the first Met codon for zuotin. The rest of the zuotin sequence has been published (15). The 5′ noncoding regions that are underlined contain (A/T)-rich segments that may serve as transcription start sites for *BIO2*. The 623-base-pair intergenic spacer between the 3′ end of the *BIO2* and the 5′ end of *ZUO1* also contains several (A/T) and $(AT)_n$ segments that may involve in regulation of *ZUO1*. Yeast *BIO2* has been deposited in EMBL Data Library under Accession No. X72701.

ZUO1 gene became apparent. This led us to complete the sequence of the ORF to see if the remaining portion encoded an amino acid sequence that was homologous to the N-terminal region of the peptide sequence derived from the *bioB* gene of *E. coli*. This was accomplished by sequencing a 2.4-kb *Hin*dIII fragment shown in Fig. 1.

By sequencing the *Hin*dIII fragment, we found that it contains the entire ORF 5′ to the *ZUO1* gene and in addition 156 base pairs 5′ to the coding region of the ORF. It also contains a 623-base-pair intergenic spacer between the ORF and the *ZUO1* gene and 170 of the N-terminal codons of *ZUO1* gene. The sequence of this fragment up to the start codon for the *ZUO1* gene along with the translated amino acid sequence of the ORF 5′ to the *ZUO1* gene is shown in Fig. 2. Since intergenic spacers are only about 500–600 base pairs in yeast (Bobby Baum, personal communication), a lesson from our experience is that se-

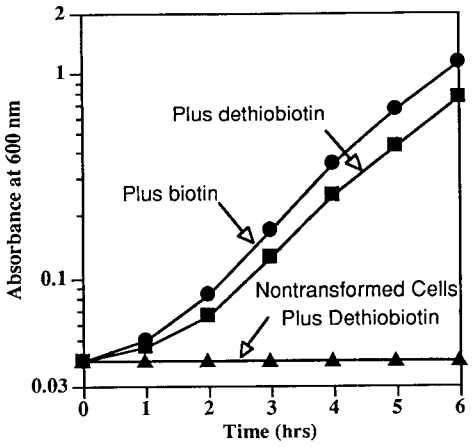

FIG. 3. The growth rates of *E. coli* KS302Δ*bio* that has been transformed with pUCBI02. *E. coli* cells were grown in M9 plus 1% glucose with or without biotin or dethiobiotin. Growth rates were measured by following the absorbance at A_{600nm}. The absorbance is plotted vs time. ●, Transformed KS302Δ*bio*::pUCBIO2 cells with 20 mM biotin added to media; ■, transformed KS302Δ*bio*::pUCBIO2 cells with 20 mM dethiobiotin added to media; ▲, nontransformed KS302Δ*bio* cells with 20 mM dethiobiotin added to media.

quencing beyond a gene of interest in yeast can lead to the discovery of other important genes.

pUCBIO2 functionally complements E. coli bioB⁻ and Δbio strains. The pUCBIO2 plasmid was transformed into two *E. coli* strains, K12-Y10 *bioB*⁻105 and KS302Δ*bio*. Both strains require biotin for growth and are unable to grow on dethiobiotin. After transforming these strains with pUCBIO2, they were able to grow on minimal media when dethiobiotin was present. The rates of growth of KS302Δ*bio*::pUCBIO2 in liquid minimal media are shown in Fig. 3.

This strain grew in the presence of biotin as expected. The pUCBIO2 transformants also grew vigorously in the presence of dethiobiotin; whereas there is no growth of this strain without pUCBIO2 in the presence of dethiobiotin as shown in Fig. 3. The growth rate of the pUCBIO2 transformants with dethiobiotin was almost equivalent to that with biotin. These results strongly suggest that the mutant cells transformed with pUCBIO2 can express the yeast biotin synthase protein which converts dethiobiotin to biotin that is absolutely required for cell growth. Similar results were obtained with the *E. coli* K12-Y10 *bioB*⁻105 pUCBIO2 cells (data not shown).

DISCUSSION

Since *E. coli bioB* mutants lacking functional biotin synthase can grow in the presence of dethiobiotin when transformed with pUCBIO2, the pUCBIO2 plasmid must contain a gene that encodes biotin synthase. We have therefore chosen to name the ORF 5′ to the *ZUO1* gene in yeast the *BIO2* gene. The number 2 was chosen rather than 1 for consistency in nomenclature since all the genes for biotin synthase in bacteria have so far been given the designation of the second letter of the alphabet.

The *S. cerevisiae* BIO2 gene encodes a protein that is functionally and physically homologous to biotin synthase from *E. coli* and *Bacillus sphaericus*. The molecular masses of the proteins encoded by the yeast *BIO2*, *E. coli bioB*, and *B. sphaericus bioB* genes are 40,020, 38,665, and 37,000 Da, respectively. There is 46% identity and 66% similarity between the proteins encoded by yeast *BIO2* and *E. coli bioB*. There is 32% identity and 55% similarity between the proteins encoded by *BIO2* and *B. sphaericus bioB*. It is surprising that there is a greater homology between the proteins encoded by *E. coli bioB* and yeast *BIO2* than there is between the proteins encoded by *E. coli* and *B. sphaericus* (34% identity and 59% similarity).

It has been reported that *E. coli* harboring conjugative plasmids can mobilize DNA transfer between *E. coli* and yeast (18). An intriguing possibility is that the *BIO2* gene in yeast was acquired from *E. coli* through a conjugation event during evolution. If the degree of homology between the yeast and *E. coli* dethiobiotin synthase is also very high, it would be consistent with both genes being acquired by conjugation.

There are approximately equal numbers of hydrophobic and hydrophilic segments in these three proteins, and the

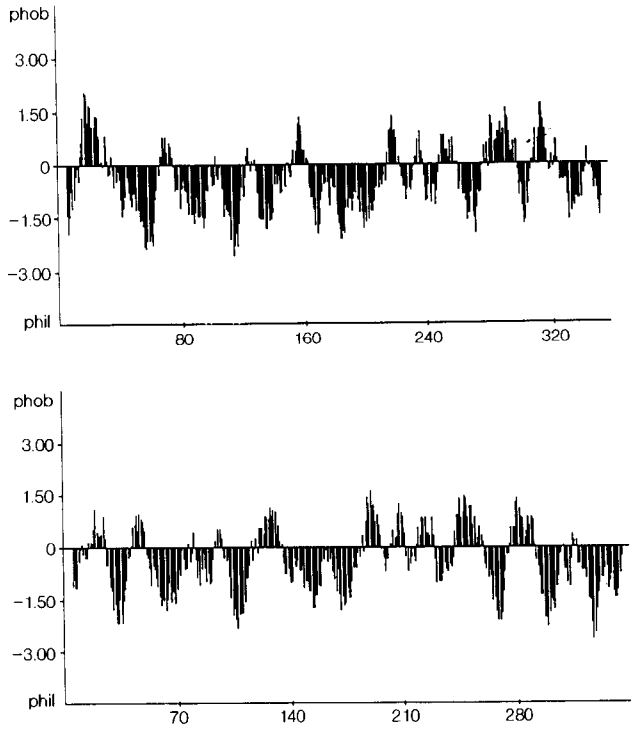

FIG. 4. Comparison of hydropathy of biotin synthases of yeast (top) and *E. coli* (bottom). The hydropathy of the proteins are remarkably similar. The profiles are nearly superimposable, implying structural similarity.

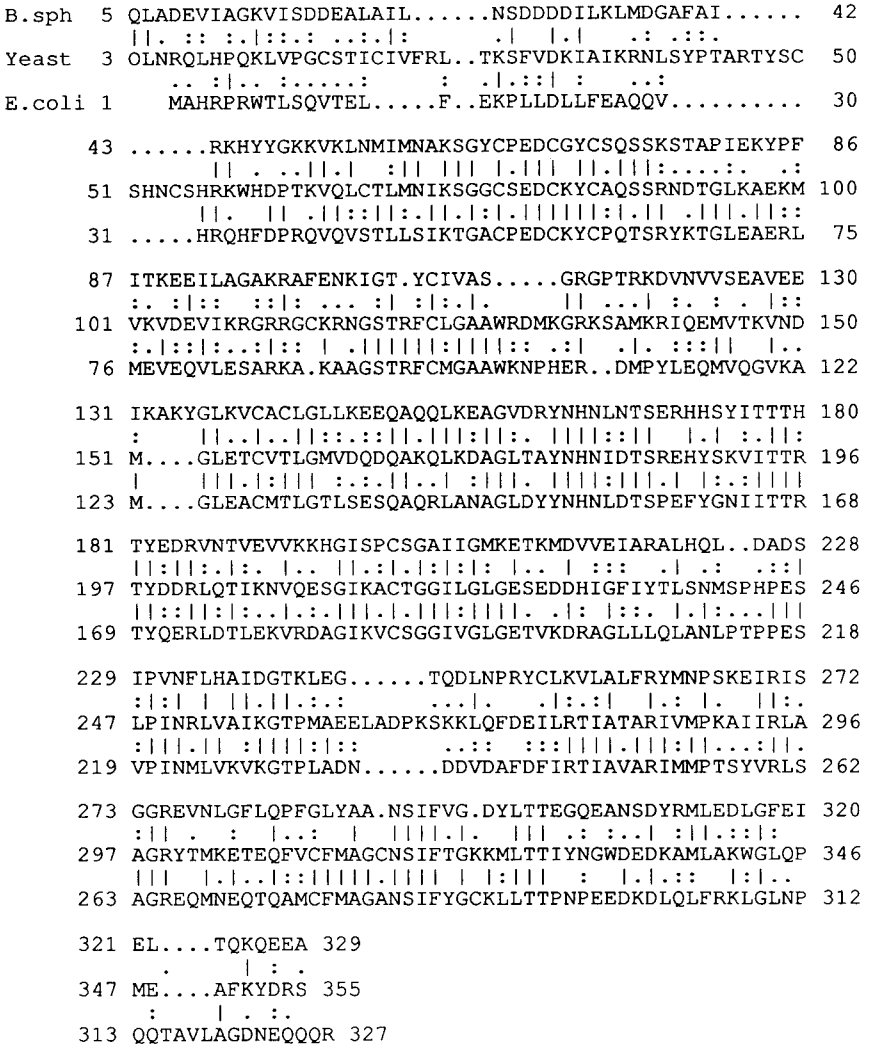

FIG. 5. The alignment of biotin synthases of *B. sphaericus bioB*, yeast *BIO2*, and *E. coli bioB*. A vertical line indicates the identity, a colon, indicates conservative amino acid change, and a single dot indicates less conservative changes. Note the conserved cysteines corresponding to yeast residues 78, 82, 85, 123, 156, and 216 as well as the conserved histidine at 180.

overall hydropathy patterns are remarkably similar. This is shown in Fig. 4 for yeast *BIO2* and *E. coli bioB*. These similarities suggest that these proteins fold into similar structures.

The aligned sequences of the proteins encoded by these three biotin synthase genes are shown in Fig. 5. There are many conserved residues. From the nature of the reaction catalyzed by biotin synthases, it seems likely that enzyme-bound metal ion(s) may participate in catalysis. In this regard, the six conserved cysteines and one conserved histidine among these three proteins are of particular interest. In the protein encoded by yeast *BIO2* the conserved cysteines occur at residues 78, 82, 85, 123, 156, and 216 and the conserved histidine occurs at residue 180. The side chains of these amino acids could be involved in metal binding. Cysteines 78, 82, and 85 are in a particularly highly conserved region with the motif Cys–X–X–X–Cys–X–X–Cys. Although this exact motif is quite rare, two of its components are found in proteins with Fe–S clusters. The Cys–X–X–Cys motif is very common in proteins with Fe–S clusters (19). The Cys–X–X–X–Cys motif occurs, for example, in 7-Fe ferredoxins, some 2-Fe ferredoxins, succinate dehydrogenase, and fumarate reductase (19–21). Among the 7-Fe ferredoxins containing the Cys–X–X–X–Cys motif, the side chains of each of the Cys are ligands for a Fe–S cluster, but they are in different clusters. In addition, in the 7-Fe ferredoxins, Glu is commonly the amino acid corresponding to the middle X (i.e., Cys–X–E–X–Cys), and this is also the case in each of the biotin synthases in Fig. 3. As far as we are aware, there are no known cases where the side chains of both Cys in a Cys–X–X–X–Cys motif are ligands to the same cluster (21).

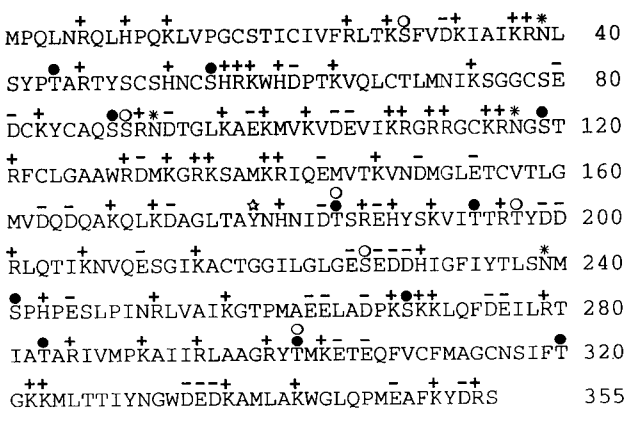

FIG. 6. Selected features of yeast *BIO2* protein. *BIO2* has 28.5% charged residues unevenly distributed in the protein as indicated by the ± on each amino acid. There are a number of potential phosphorylation and N-glycosylation sites as marked: ● protein kinase C; ○casein kinase II; ☆ tyrosine kinase; and ✳ N-glycosylation sites.

A search of the current protein and nucleic acid data bases reveals that there are only four other proteins that contain a Cys–X–X–X–Cys–X–X–Cys motif. One is encoded by the *E. coli lipA* gene. This gene encodes a protein involved in the synthesis of lipoic acid (22, 23). This is of particular interest since a reaction(s) in the biosynthesis of lipoic acid resembles the reaction of biotin synthase in that sulfur atoms are added to the unactivated carbon atoms. Three other proteins with such a motif are found in *nifB* gene product of *K. pneumoniae, B. japonicum,* and *A. vinlandii* (24). The *nifB* gene product seems to function in the synthesis of the iron molybdeum cofactor of nitrogenase.

The one conserved histidine is in the motif, Tyr–Asn–His–Asn, which is entirely conserved in all three biotin synthases. This motif occurs at residues 178 to 181 in yeast BIO2. The *lipA* protein also contains such a Tyr–Asn–His–Asn motif. It is perhaps of significance that in the *lipA* protein there are 92 amino acids separating the final cysteine (residue 61) in the Cys–X–X–X–Cys–X–X–Cys motif and the histidine (residue 153) in the Tyr–Asn–His–Asn motif (22). Likewise, in the biotin synthases of *E. coli, B. sphaericus,* and yeast, there are respectively 92, 93, and 95 amino acids separating the corresponding residues.

BIO2 and *ZUO1* reside in tandem on yeast chromosome VII near *ADE3* and toward the teleomere (15). The distance between the 3′ end of the coding region of *BIO2* and 5′ end of the coding region of *ZUO1* coding regions is 623 bp. The 5′ noncoding A/T rich region for *ZUO1* gene does not extend to the *BIO2* gene coding region. This suggests that these two genes are under separate transcriptional regulation even though they are physically in close contact.

In the case of *E. coli,* the *birA* gene product (1) acts as the transcriptional repressor of the *bio* operon, (2) catalyzes the activation of biotin to biotin-AMP, and (3) catalyzes the addition of this activated form of biotin to the proper lysine side chain in biotin-dependent carboxylases (25). A similar system may exist in yeast. The 5′ noncoding region of the *BIO2* gene does not resemble the transcriptional and translational regulatory region of the *E. coli bio* operon, so the nature of its regulation in yeast is not clear at present. Since the yeast *BIO2* gene can functionally complement *E. coli bioB* mutants, the yeast *BIO2* gene must have been correctly transcribed and translated in *E. coli.* It is not clear which part of the 5′ noncoding region of the *BIO2* gene functioned as a promoter, in *E. coli,* but there are several AT-rich regions in the 5′ noncoding region of *BIO2* that could act as *E. coli* promoters.

Additional regulation of biotin biosynthesis in yeast may take place post-translationally since there are a number of potential phosphorylation sites in *BIO2* including sites for protein kinase C, casein kinase II, and tyrosine kinase as well as sites for four potential N-glycosylation (Fig. 6). The involvement of these phosphorylation and N-glycosylation sites as well as regulation of the *BIO2* gene await further investigation.

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