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Similarity of *Escherichia coli* Propanediol Oxidoreductase (*fucO* Product) and an Unusual Alcohol Dehydrogenase from *Zymomonas mobilis* and *Saccharomyces cerevisiae*

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The gene that encodes 1,2-propanediol oxidoreductase (*fucO*) from *Escherichia coli* was sequenced. The reading frame specified a protein of 383 amino acids (including the N-terminal methionine), with an aggregate molecular weight of 40,642. The induction of *fucO* transcription, which occurred in the presence of fucose, was confirmed by Northern blot analysis. In *E. coli*, the primary *fucO* transcript was approximately 2.1 kilobases in length. The 5′ end of the transcript began more than 0.7 kilobase upstream of the *fucO* start codon within or beyond the *fucA* gene. Propanediol oxidoreductase exhibited 41.7% identity with the iron-containing alcohol dehydrogenase II from *Zymomonas mobilis* and 39.5% identity with ADH4 from *Saccharomyces cerevisiae*. These three proteins did not share homology with either short-chain or long-chain zinc-containing alcohol dehydrogenase enzymes. We propose that these three unusual alcohol dehydrogenases define a new family of enzymes.

L-Fucose can serve as a sole source of carbon and energy for *Escherichia coli* via an inducible pathway (3). Transport of L-fucose is mediated by L-fucose permease (17). L-Fucose is converted to L-fuculose through the action of L-fucose isomerase (15) and is phosphorylated by L-fuculose kinase to form L-fuculose 1-phosphate (19), the inducer of the *fuc* regulon (3). L-Fuculose 1-phosphate is enzymatically cleaved to form L-lactaldehyde and dihydroxyacetone phosphate (14). The dihydroxyacetone thus formed enters central metabolism. Under aerobic conditions, L-lactaldehyde is converted to pyruvate through the action of an inducible lactaldehyde dehydrogenase and a sequentially inducible lactate dehydrogenase (10, 35). Under anaerobic conditions, L-lactaldehyde is converted by 1,2-propanediol oxidoreductase to the fermentation product 1,2-propanediol, which is excreted into the medium (36).

The enzymes of the fucose pathway are encoded by five genes that are clustered at 62.5 min on the *E. coli* map (1): *fucP* (fucose permease), *fucI* (fucose isomerase), *fucK* (fuculose kinase), *fucA* (fuculose 1-phosphate aldolase), and *fucO* (1,2-propanediol oxidoreductase). An activator protein for the pathway, which is active in the presence of L-fuculose 1-phosphate, is encoded by *fucR* (7). These genes have been cloned and appear to constitute a regulon composed of three operons: *fucPIK*, *fucA*, and *fucO* (7).

Wild-type *E. coli* can give rise to mutant strains that acquire the ability to utilize 1,2-propanediol as a sole carbon and energy source (2, 40). The acquisition of these mutations during repeated selection for growth on 1,2-propanediol appears to occur in two stages (16). The primary mutation responsible for the new phenotype results in increased expression of *fucO*. A secondary mutation causes complex changes in the regulation of the entire *fuc* regulon. Sequence analysis of the genes of the *fuc* regulon should provide information concerning the molecular nature of these changes.

In this report the nucleotide sequence of the *fucO* gene is presented. Only a few primary structures of procaryotic proteins with significant alcohol dehydrogenase activity are available (18, 20, 25), although a large number of sequences are available for eucaryotic enzymes (21). The deduced amino acid sequence of propanediol oxidoreductase from *E. coli* was found to share significant identity with alcohol dehydrogenase II from *Zymomonas mobilis* (12) and ADH4 from *Saccharomyces cerevisiae* (38) but does not share identity with the alcohol dehydrogenase from *Alcaligenes eutrophus* (20), the methanol dehydrogenases from *Methylobacterium organophilum* (25) and *Paracoccus denitrificans* (18), or any of the eucaryotic alcohol dehydrogenases.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The strains and plasmids used in this study are summarized in Table 1. *E. coli* strains were routinely grown in Luria broth without added carbohydrate (24) at 37°C. Media were solidified by addition of agar (1.5%). Antibiotic-resistant transformants were selected by addition of ampicillin (50 mg/liter) or kanamycin (15 mg/liter). Transcription of *fucO* in *E. coli* DH5α was induced by the addition of fucose (2 g/liter) to the minimal medium of Sridhara et al. (36) as described previously (8). Plasmid clones which expressed alcohol dehydrogenase activity were detected by using aldehyde indicator plates (12).

DNA methods. Transformation, restriction mapping, and subcloning were carried out by using standard methods (26, 34). Small-scale plasmid isolations were prepared by a modification of the method of Birnboim and Doly (4) as described previously (34). Restriction enzymes and DNA-modifying enzymes were used according to the recommendations of the manufacturers. Isolation of genomic DNA from *E. coli* DH5α was performed as described previously (6).

Southern probing. Agarose gels (0.8%) to be used for Southern blotting were electrophoresed and stained with ethidium bromide by standard methods (34). Capillary transfer of the DNA to Genescreen Plus hybridization transfer

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TABLE 1. Plasmids and strains used

Strain or plasmid	Description	Source or reference
Z. mobilis CP4	Prototroph	29
E. coli		
DH5α	*fuc*$^+$	BRL[a]
TC4	*fuc*$^+$	6
Plasmids		
pUC18	*bla lacI'Z'*[b]	39
pcos2EMBL	Kan	30
pLOI340	*fucO*	This study
pLOI341	*fucO*	This study

[a] Bethesda Research Laboratories.
[b] Incomplete *lacI* and incomplete *lacZ*.

membranes and hybridization at 42°C in the presence of 50% formamide were carried out according to the protocols of the manufacturer of the membranes (New England Nuclear Corp., Boston, Mass.; catalog no. NEF-976). The hybridization probe was prepared by random primer labeling of a 0.4-kilobase (kb) *Eco*RV fragment of DNA (internal to the *fucO* reading frame) using [α-^{32}P]dATP and a kit.

Analysis of RNA. RNA was isolated as described previously (11). Fractionation of RNA through agarose-formaldehyde gels, capillary transfer to Genescreen Plus membranes, and hybridization were carried out according to technical information provided by New England Nuclear. The hybridization probe was identical to that used for Southern probing (described above). Methods for primer extension mapping experiments have been described previously (11). The oligonucleotide used for these experiments was complimentary to *fucO* and spanned base pairs (bp) 371 to 387 (sequence 5'-CCGTTTCGTTCAGAATC-3'). An Autogen 500 (Millipore Corp., Bedford, Mass.) was used for oligonucleotide synthesis.

DNA sequence analysis. The *fucO* gene from *E. coli* DH5α was sequenced in both directions by the dideoxy method (33) with a Sequenase sequencing kit. The 1,690-bp fragment from the *Bam*HI site to the *Pvu*II site of pLOI341 was sequenced by subcloning of various restriction fragments into M13mp18 and M13mp19 (39). Sequence data were analyzed by using the computer programs of Pustell and Kafatos (31). Alignment of homologous proteins was performed by using the algorithm of Wilbur and Lipman (37).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Electrophoresis and Coomassie blue staining of gels were as described previously (11).

Assay of 1,2-propanediol oxidoreductase. Cells from 3-ml overnight cultures grown in Luria broth were harvested and washed in phosphate buffer (30 mM, pH 7.0) and then disrupted as described previously (29). Enzyme assays were performed by the methods of Sridhara et al. (36). Activities of 1,2-propanediol oxidoreductase are expressed as international units per milligram of total cell protein (micromoles · minute^{-1} · milligrams of protein^{-1}). Protein was determined as described by Layne with bovine serum albumin as the standard (23).

Enzymes and chemicals. Restriction enzymes and DNA-modifying enzymes were obtained from Bethesda Research Laboratories, Inc. (Gaithersberg, Md.). The M13 universal sequencing primer was obtained from New England BioLabs, Inc. (Beverly, Mass.). Radioactive compounds were purchased from New England Nuclear. The Sequenase kit was obtained from US Biochemical Corp. (Cleveland, Ohio).

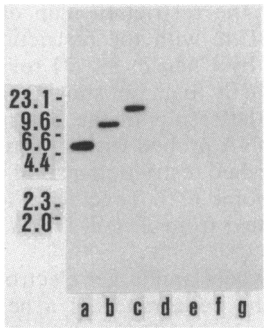

FIG. 1. Southern blot analysis to determine origin of gene cloned on pLOI340. Chromosomal DNA from *E. coli* DH5α (a through c) or *Z. mobilis* (e through g) was digested with *Hin*dIII (a, g), *Sal*I (b, f), or *Eco*RI (c, e). As a control, an undigested crude plasmid preparation of pcos2EMBL containing a *Z. mobilis* insert (described in Results) was run in lane d. DNA samples were separated on a 0.8% agarose gel and transferred to a nylon filter. The probe was a ^{32}P-labeled, 0.4-kb *Eco*RV fragment from pLOI341 (from bp 864 to 1259 in Fig. 2). Sizes of fragments are given in kilobases on the left.

The random primer labeling kit was purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.).

RESULTS

Cloning of the gene encoding 1,2-propanediol oxidoreductase. The *fucO* gene was isolated while attempting to subclone from a crude, small-scale, plasmid preparation made from *E. coli* DH5α carrying pcos2EMBL (30), which contained a 35-kb insert of *Z. mobilis* genomic DNA. The subcloning strategy involved partial digestion of the crude plasmid preparation with *Sau*3A, ligation into pUC18 (39), and transformation into *E. coli* DH5α, with selection for expression of alcohol dehydrogenase activity on an aldehyde indicator plate (12). A single transformant that expressed alcohol dehydrogenase activity was obtained from a pool of approximately 10,000 antibiotic-resistant transformants.

The plasmid carried by the alcohol dehydrogenase-positive clone, pLOI340, contained an *E. coli* DNA insert of 2.7 kb. Both of the *Bam*HI sites at the junction of the insert DNA to pUC18 were regenerated. The origin of the cloned DNA was established by Southern blot analysis (Fig. 1). Genomic DNAs from *E. coli* DH5α and from *Z. mobilis* were digested to completion with three different restriction enzymes for analysis. The DNA probe used for hybridization was made by labeling a 0.4-kb *Eco*RV fragment from the middle of the DNA insert in pLOI340. Under conditions of high stringency, the *fucO* probe did not hybridize to genomic DNA from *Z. mobilis* or to the cosmid containing *Z. mobilis* DNA but hybridized strongly to DNA from *E. coli* DH5α. The labeling of a single band in each of three total genomic digests with the three different restriction enzymes confirmed that *fucO* is present in strain DH5α as a single copy on the genome.

A portion of the DNA insert of pLOI340 from an internal *Sal*I site to a *Bam*HI site at the right-hand end of the insert in pUC18 could be deleted, leaving a 2.15-kb fragment of DNA that retained the ability to encode an alcohol dehydrogenase activity, as detected on aldehyde indicator plates. This plasmid was called pLOI341. Further attempts to shorten the DNA insert of pLOI341 with *Eco*RV or *Pst*I abolished the alcohol dehydrogenase activity in these clones.

Comparison of the restriction map of the cloned DNA fragment in pLOI340 with the restriction map of the *fuc* region published by Chen et al. (7) revealed that the two maps matched exactly (data not shown). The restriction map of pLOI340 and the results of the Southern blot of *E. coli* DH5α genomic DNA probed with a portion of *fucO* (Fig. 1) matched the published restriction map of the 62.5-min region of the *E. coli* genome (22). Together, these data suggested that the gene cloned from *E. coli* DH5α into pLOI341 was *fucO*.

Denaturing polyacrylamide gel electrophoresis was used to demonstrate the appearance of a new protein band in strain DH5α carrying pLOI341, when compared with strain DH5α carrying pUC18 (data not shown). This protein migrated with an apparent molecular weight of 38,000 to 40,000, which is equivalent to that of alcohol dehydrogenase II from *Z. mobilis* (12). This is much smaller than the size of the primary alcohol dehydrogenase from *E. coli* (D. P. Clark, personal communication). This result provided further evidence that the cloned gene was *fucO*.

Crude extracts of *E. coli* DH5α and strain DH5α carrying pLOI341 were assayed for the ability to oxidize a variety of alcohols. The strain carrying pLOI341 was able to oxidize 1,2-propanediol (specific activity, 0.31 μmol · min^{-1} · mg of $protein^{-1}$), glycerol (specific activity, 0.18 μmol · min^{-1} · mg of $protein^{-1}$), ethylene glycol (specific activity, 0.34 μmol · min^{-1} · mg of $protein^{-1}$), and ethanol (specific activity, 0.06 μmol · min^{-1} · mg of $protein^{-1}$) but not sorbitol, mannitol, or xylitol. The uninduced parent strain, DH5α, was unable to oxidize any of the alcohols tested. These data confirmed that the alcohol dehydrogenase activity specified by the clone in pLOI341 is truly that of 1,2-propanediol oxidoreductase, which is specified by *fucO*. Other alcohols oxidized by strain DH5α carrying pLOI341 were *n*-butanol (specific activity, 0.08 μmol · min^{-1} · mg of $protein^{-1}$), *n*-propanol (specific activity, 0.08 μmol · min^{-1} · mg of $protein^{-1}$), and allyl alcohol (specific activity, 0.10 μmol · min^{-1} · mg of $protein^{-1}$) but not isopropanol.

Sequence of *fucO*. The sequence of the gene that specifies 1,2-propanediol oxidoreductase is presented in Fig. 2. A ribosome-binding site and tandem methionine codons indicate the start of an open reading frame that stretches for 1,149 bp. This corresponds to a protein of 383 amino acids (including the N-terminal methionines) with an aggregate molecular weight of 40,642. Based on comparisons with the published restriction map for the *fuc* region (7) as described above, counterclockwise transcription of the *fucO* gene was confirmed by this sequence. The reading frame terminates with a single stop codon, TAA.

The 353 bp 5′ to *fucO* did not contain any sequences that matched the *E. coli* consensus promoter sequence (sigma 70). However, there was a sequence between bp 189 and 206 that closely matched the consensus sequence for sigma 60 promoters of *E. coli* (28). In addition, the sequence upstream of the *fucO* reading frame contained a second reading frame that originated 5′ to the sequenced region and terminated at a stop codon 24 bp before the start codon of *fucO*. This reading frame is indicated in Fig. 2. We found no reading frames that originated in the region upstream of *fucO* and could be transcribed in the opposite direction.

Examination of the sequences 3′ to the *fucO* reading frame did not reveal any additional downstream open reading frames. A region with a potential stem-loop structure was found at bp 1633 to 1659, 128 bp downstream from the *fucO* stop codon. This region contained an inverted repeat in which seven of eight bases were complimentary, with a loop of 11 bp separating the stem structure. This sequence was immediately followed by a string of six thymidines, giving this structure the appearance of a transcriptional terminator (32). It is not known whether this structure can serve as a terminator of *fucO* transcription. A direct repeat of 7 bp was found beginning at bp 1542, with 5 bp between the repeated sequences.

Transcription of *fucO*. Primer extension mapping was used to identify the 5′ end of the *fucO* message. The primer that was used for this experiment was complimentary to the noncoding strand (plus strand), spanning the N-terminal codons at bp 371 to 387, and the RNA was obtained from *E. coli* DH5α, which was induced by growth in the presence of L-fucose. The extension products obtained by this procedure were more than 700 bp in length, beyond the length at which a reliable determination of the 5′ end of the message could be made (data not shown). This indicated that the promoter for *fucO* is at least 700 bp upstream of the *fucO* reading frame and over 350 bp 5′ to the region sequenced. This result placed the *fucO* promoter within—or even beyond—the *fucA* gene on the restriction map of the *fuc* region published by Chen et al. (7).

In an effort to clarify the results of the primer extension experiment, a portion of the *fucO* gene was used to probe a Northern blot of RNA obtained from *E. coli* DH5α grown in the presence or absence of L-fucose (Fig. 3). No transcripts were found when strain DH5α was grown under noninducing conditions. RNA from induced strain DH5α grown in the presence of L-fucose contained three transcripts that hybridized to the *fucO* gene fragment. The major transcript was approximately 2.1 kb in length. Two smaller transcripts with lengths of 1.5 and 1.3 kb were also observed. As a control, RNA from *E. coli* TC4 (grown under noninducing conditions) was included. It is interesting to note that a single transcript of 1.5 kb was observed with strain TC4, although the relative amount of this transcript was significantly lower.

Comparison of amino acid sequence with other alcohol dehydrogenases. A comparison of the deduced amino acid sequence of the 1,2-propanediol oxidoreductase from *E. coli* with those of alcohol dehydrogenase II from *Z. mobilis* (12) and ADH4 from *S. cerevisiae* (38) is presented in Fig. 4. The *E. coli* enzyme was 41.7% identical to the *Z. mobilis* enzyme and 39.5% identical to the *S. cerevisiae* enzyme. For comparison, the *Z. mobilis* and *S. cerevisiae* enzymes are 54.6% identical. No significant homologies were found when *E. coli* propanediol oxidoreductase was compared with the primary structures of other procaryotic (18, 20, 25) or other eucaryotic (21) alcohol dehydrogenases.

The *E. coli* and *Z. mobilis* enzymes are both 383 amino acids in length, whereas the *S. cerevisiae* enzyme is 382 amino acids long. There are several stretches of amino acids that are conserved in all three sequences. Only a few short gaps were necessary to obtain an excellent fit of the three proteins.

DISCUSSION

In this study, the gene encoding the *E. coli* 1,2-propanediol oxidoreductase was sequenced. The deduced amino acid sequence is remarkably similar to alcohol dehydrogenase II from *Z. mobilis* and ADH4 from *S. cerevisiae*. These three enzymes are dissimilar to any of the 16 previously characterized eucaryotic alcohol dehydrogenases (21). The eucaryotic enzymes fall into two different classes of proteins: short- and long-chain alcohol dehydrogenases. Each of these is a zinc metalloenzyme containing one or two atoms

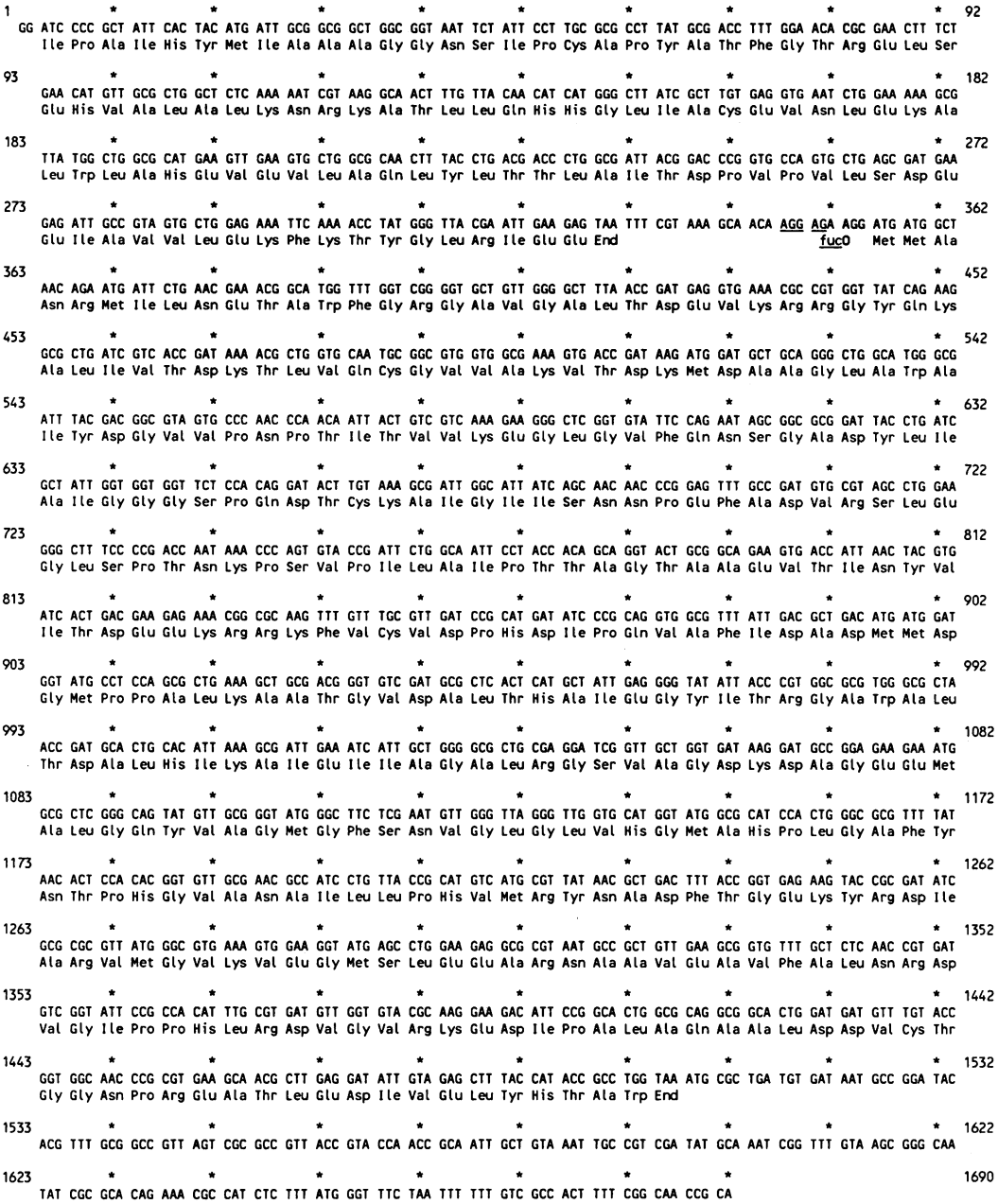

FIG. 2. Nucleotide sequence and translation of *fucO* from *E. coli* DH5α. The ribosome-binding site is underlined. The beginning of the *fucO* reading frame is indicated. Translation of a reading frame that begins upstream of the sequenced region is also given.

of zinc per protein subunit. Alcohol dehydrogenase II from *Z. mobilis* is unusual, requiring iron rather than zinc for activity (27). The data of Sridhara et al. (36) indicated that 1,2-propanediol oxidoreductase from *E. coli* is iron activated. *S. cerevisiae* ADH4 has recently been purified and shown to contain zinc ions, not ferrous ions (13). However, based on sequence data, Williamson and Paquin (38) have suggested that ADH4 and alcohol dehydrogenase II from *Z. mobilis* define a new family of alcohol dehydrogenases. The finding that 1,2-propanediol oxidoreductase from *E. coli* shares approximately 40% amino acid identity with these two enzymes indicates that the *E. coli* enzyme also belongs in this family.

The primary alcohol dehydrogenase of *E. coli* K-12 contains 891 amino acids, with a molecular weight of 96,000 (D. P. Clark, personal communication). The 2.15-kb DNA insert in pLOI341 is not large enough to code for a protein of this size. Another *E. coli* enzyme with significant ability to oxidize ethanol is 1,2-propanediol oxidoreductase, which is specified by the *fucO* gene. The fit of the restriction map of the *E. coli* DH5α genomic DNA inserted in pLOI340 to the published maps of the *fuc* region (7) and the 62.5-min region of the genome (22) suggested that the cloned gene on pLOI340 might be *fucO*. Comparison of the substrate range published for 1,2-propanediol oxidoreductase (36) with the enzyme activity expressed by strains carrying pLOI340 confirmed that the gene cloned on pLOI340 is indeed *fucO*.

Analysis of transcription of the *fucO* gene provided some

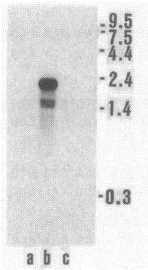

FIG. 3. Northern blot analysis of *fucO* transcription. RNA was prepared from cultures of *E. coli* DH5α grown in minimal medium in the absence (a) or presence (b) of L-fucose and a culture of *E. coli* TC4 grown in Luria broth (c). RNA samples were separated on a formaldehyde–1.0% agarose gel and transferred to a nylon filter membrane. The probe was a fragment of *fucO* (described in the legend to Fig. 1 and in the text). Each lane contains 5 μg of RNA.

unexpected results. If termination of *fucO* transcription occurred at the stem-loop structure 128 bp downstream from the *fucO* reading frame, a transcript of under 1.3 kb would be sufficient to encode the gene. However, the length of the major transcript from *E. coli* DH5α was 2.1 kb (Fig. 3). This is in keeping with the primer extension result, which suggested that the *fucO* promoter lies over 700 bp upstream of the *fucO* reading frame. This places the *fucO* promoter within or beyond the *fucA* gene. In fact, construction of *lacZ* fusions to the *fucO* promoter resulted in decreased expression of the neighboring *fucA* gene (9). The *fucO-lacZ* fusion joints were subsequently shown to map within the *fucA*

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     1       10        20        30        40        50        60
Adh2 MASST-FYIPFVNEMGEGSLEKAIKDLNGSGFKNALIVSDAFMNKSGVVKQVADLLKAQG
     :              : :            :   :::: :      :::  : :   : :
FucO MMANR-MILNETAWFGRGAVGALTDEVKRRGYQKALIVTDKTLVQCGVVAKVTDKMDAAG
     :             :: ::     :  :   : :::::::      :    :       :
YAdh MSSVTGFYIPPISFFGEGALEETADYIKNKDYKKALIVTDPGIAAIGLSGRVQKMLEERG

    61       70        80        90       100       110       120
Adh2 INSAVYDGVMPNPTVTAVLEGLKILKDNNSDFVISLGGGSPHDCAKAIALVATNG--GEV
        : :::: :::: : : :::        :  :  :::::  :  :::     :     :
FucO LAWAIYDGVVPNPTITVVKEGLGVFQNSGADYLIAIGGGSPQDTCKAIGIISNNPEFADV
     :  ::::   ::: :  :  :: :          :::::  :  :::     :
YAdh LNVAIYDKTQPNPNIANVTAGLKVLKEENSEIVVSIGGGSAHDNAKAIALLATNG--GEI

   121      130       140       150       160       170       180
Adh2 KDYEGIDKSKKPALPLMSINTTAGTASEMTRFCIITDEVRHVKMAIVDRHVTPMVSVNDP
        ::     ::  :   : :::::: : :    ::::    :   :: :  : :   :
FucO RSLEGLSPTNKPSVPILAIPTTAGTAAEVTINYVITDEEKRRKFVCVDPHDIPQVAFIDA
        ::     :   :  :: :::::: : :    :  :::  :    :    : ::  :
YAdh GDYEGVNQSKKAALPLFAINTTAGTASEMTRFTIISNEEKKIKMAIIDNNVTPAVAVNDP

   181      190       200       210       220       230       240
Adh2 LLMVGMPKGLTAATGMDALTHAFEAYSSTAATPITDACALKAASIMIKNLKTACDNGKDM
       : :::  : :::: :::::: : :    :   :::   ::  :    :       ::
FucO DMMDGMPPALKAATGVDALTHAIEGYITRGAWALTDALHIKAIEIIAGALRGSVAGDKD-
       : : :::: :::: ::::: :: :        :::   : :  :   :       ::
YAdh STMFGLPPALTAATGLDALTHCIEAYVSTASNPITDACALKGIDLINESLVAAYKDGKDK

   241      250       260       270       280       290       300
Adh2 PAREAMAYAQFLAGMAFNNASLGYVHAMAHQLGGYYNLPHGVCNAVLLPHVLAYNASVVA
      : : ::  :  ::: : :  :: :: ::: ::  :: ::::  :: :::::  :::
FucO -AGEEMALGQYVAGMGFSNVGLGLVHGMAHPLGAFYNTPHGVANAILLPHVMRYNADFTG
      :   :    : ::: : :  :: ::  ::  :: ::  ::::  :: :::::    :
YAdh KARTDMCYAEYLAGMAFNNASLGYVHALAHQLGGFYHLPHGVCNAVLLPHVQ--EANMQC

   301      310       320       330       340       350       360
Adh2 GRLKDVGVAMGLDIANLGDKEGAEATIQAVRDLAASIGIPANLTELGAKKEDVPLLADHA
         :    ::         :   :   ::  :    :::  :   :  ::: : ::  :
FucO EKYRDIARVMGVKVEGMSLEEARNAAVEAVFALNRDVGIPPHLRDVGVRKEDIPALAQAA
      :     :             :       :   :::   ::  : : ::  ::   ::  :
YAdh PKAKK--RLGEIALHCGASQEDPEETIKALHVLNRTMNIPRNLKDLGVKTEDFDILAEHA

   361      370       380
Adh2 LKDACALTNPRQGDQKEVEELFLSAF--
     : : :   :::        ::   :
FucO LDDVCTGGNPREATLEDIVELYHTAW--
       : :   ::   : :  :     :
YAdh MHDACHLTNPVQFTKEQVVAIIKKAYEY
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FIG. 4. Comparison of amino acid identity of 1,2-propanediol oxidoreductase (FucO) with alcohol dehydrogenase II from *Z. mobilis* (Adh2) and ADH4 from *S. cerevisiae* (YAdh).

gene, on the basis of Southern blot experiments (7). A hypothesis that takes these facts into account is presented below. The 1.5- and 1.3-kb *fucO* transcripts in Fig. 3 may be specific products of mRNA processing or independent transcripts that were initiated from promoters not revealed by primer extension mapping.

Placement of the *fucO* sequence on the restriction map of the *fuc* region confirmed the finding that the *fucO* gene is transcribed counterclockwise on the *E. coli* genome (7). Previous data suggested that the *fucO* and *fucA* genes are transcribed in different directions and constitute different operons (7). However, examination of the 353 bases 5′ to the *fucO* gene did not reveal a reading frame that would originate in this region and be transcribed in the clockwise direction. Instead, there is a reading frame that originates upstream of the sequenced fragment and terminates just 24 bp 5′ to *fucO*. An alternative hypothesis based on the Northern blot analysis, which indicated a transcript that is long enough to encode both genes, is that the two genes form an operon and are transcribed counterclockwise in the order *fucAO*. Further sequence data for the remainder of the *fucA* region and characterization of its promoter are necessary to clarify this point.

Wild-type *E. coli* can give rise to mutants that have acquired the ability to utilize 1,2-propanediol as a sole carbon and energy source. It is interesting to speculate that the nature of the primary mutation resulting in increased levels of 1,2-propanediol oxidoreductase could be mediated by a change of adenine-205 to a cystosine (Fig. 2). The region between bp 189 and 206 contains a near-consensus sigma 60 promoter, which would be brought to perfect identity if the aforementioned change were to occur. This hypothesis can be tested by using site-directed mutagenesis.

Acquisition of the ability to grow on 1,2-propanediol by *E. coli* serves as a model system for the study of evolution of metabolic pathways (40). Comparison of wild-type and mutant 1,2-propanediol oxidoreductase revealed that not only is increased *fucO* expression acquired during mutation to 1,2-propanediol utilization, but also the mutant enzyme itself differs with respect to K_m, maximum rate of catalysis, energy of activation, thermal stability, pH optimum, and substrate specificity (5). The difference in substrate range between the enzyme from *E. coli* and those from *Z. mobilis* (12) and *S. cerevisiae* (13) indicates that the *E. coli* enzyme evolved in a manner somewhat different than that of the other two enzymes. A study of the differences at the primary amino acid sequence level that resulted in such altered substrate specificity is warranted. This presents a molecular approach from which the evolution of metabolic pathways can be studied. The examination of 1,2-propanediol oxidoreductase and the other members of this new alcohol dehydrogenase family will be of particular interest from the standpoint of convergent evolution of this family of enzymes to catalysis of the same reaction that is carried out by the other two families of alcohol dehydrogenases.

ACKNOWLEDGMENTS

Special thanks are extended to D. P. Clark for suggesting that our cloned *E. coli* alcohol dehydrogenase might in fact be propanediol oxidoreductase. We also thank K. T. Shanmugam for helpful discussions during the course of this study.

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ADDENDUM IN PROOF

A more extensive portion of the *fucAO* region of the *E. coli* genome was recently sequenced by Y.-M. Chen, Z. Lu, and E. C. C. Lin (submitted for publication). Our sequences and conclusions regarding gene expression are in substantial agreement.

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