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The organization of the *fuc* regulon specifying L-fucose dissimilation in *Escherichia coli* K12 as determined by gene cloning

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Summary. In *Escherichia coli* the six known genes specifying the utilization of L-fucose as carbon and energy source cluster at 60.2 min and constitute a regulon. These genes include *fucP* (encoding L-fucose permease), *fucI* (encoding L-fucose isomerase), *fucK* (encoding L-fuculose kinase), *fucA* (encoding L-fuculose 1-phosphate aldolase), *fucO* (encoding L-1,2-propanediol oxidoreductase), and *fucR* (encoding the regulatory protein). In this study the *fuc* genes were cloned and their positions on the chromosome were established by restriction endonuclease and complementation analyses. Clockwise, the gene order is: *fucO-fucA-fucP-fucI-fucK-fucR*. The operons comprising the structural genes and the direction of transcription were determined by complementation analysis and Southern blot hybridization. The *fucPIK* and fucA operons are transcribed clockwise. The *fucO* operon is transcribed counterclockwise. The *fucR* gene product activates the three structural operons in *trans*.

Key words: *fuc* regulon – Genetic map – Transcription

Introduction

In *Escherichia coli* L-fucose and L-rhamnose are utilized as carbon and energy sources through parallel pathways, each mediated sequentially by a permease, an isomerase, a kinase, and an aldolase (Fig. 1; for a review see Lin 1987). The two pathways merge after the aldolases cleave their 6-carbon substrates into dihydroxyacetone phosphate (C-1 to C-3) and L-lactaldehyde (C-4 to C-6). Aerobically, lactaldehyde is converted by a common NAD-linked aldehyde dehydrogenase to L-lactate which is further oxidized to pyruvate (Cocks et al. 1974; Sridhara and Wu 1969; Chen et al. 1987a). Anaerobically, lactaldehyde is reduced by a common NADH-linked oxidoreductase to L-1,2-propanediol, which is excreted. The fermentiation product is irretrievable even when molecular oxygen becomes available (Cocks et al. 1974; Boronat and Aguilar 1979, 1981a).

There are at least six genes specifying the fucose pathway: *fucP* encoding fucose permease, *fucI* encoding fucose isomerase, fucK encoding fuculose kinase, *fucA* encoding fuculose 1-phosphate aldolase, *fucO* encoding propanediol oxidoreductase, and *fucR* encoding the activator protein (Chakrabarti et al. 1984; Skjold and Ezekiel 1982a, b; Bachmann 1983; Bartkus and Mortlock 1986). These genes cluster at 60.2 min and appear to constitute a regulon (Hacking and Lin 1976, 1977), with fuculose 1-phosphate as the true inducer (Bartkus and Mortlock 1986).

All of the *fuc* genes, including *fucO*, are induced both aerobically and anaerobically (Boronat and Aguilar 1981b; Chen et al. 1983). Reduction of lactaldehyde to propanediol during aerobic growth on fucose (or rhamnose) is prevented by the lack of catalytically active propanediol oxidoreductase (Boronat and Aguilar 1981b; Chen and Lin 1984a).

Five genes specifying the rhamnose pathway have been identified: *rhaA* encoding rhamnose isomerase, *rhaB* encoding rhamnulose kinase, *rhaD* encoding rhamnulose 1-phosphate aldolase, *rhaR* (formerly *rhaC*) encoding the major activator protein and *rhaS* encoding the minor activator protein (Power 1967; J.F. Tobin and R.F. Schleif, personal communication). These genes cluster at 87.7 min. The rhamnose system lacks its own propanediol oxidoreductase (Boronat and Aguilar 1979; Chen and Lin 1984b). Instead, anaerobic growth on rhamnose depends on the enzyme encoded by *fucO*. Mobilization of this enzyme is made possible by the cross induction of the *fuc* regulon during growth on rhamnose. It appears that in this cross induction, lactaldehyde instead of fuculose 1-phosphate acts as the effector (Chen et al. 1987b).

The fucose system has been studied as a model for experimental evolution of novel metabolic function (Lin and Wu 1984; Zhu and Lin 1986, 1987). For example, wild-type strains K12, B, ML, and W cannot grow on propanediol, but each strain can give rise to mutants that grow aerobically on the compound as the sole source of carbon and energy (Sridhara et al. 1969; Lin and Wu 1984). The acquisition of this growth ability involved the recruitment of the *fucO* product. The complex changes in the expression of the *fuc* regulon associated with the selection for growth on propanediol and the cross induction of the regulon by rhamnose in the wild-type strain prompted us to examine further the organization of this genetic system. In this report we present the relative positions of the *fuc* genes, their organization as operons, and the directions of transcription of the structural genes.

Materials and methods

Bacterial strains and vectors. The genotypes and sources of *E. coli* K12 strains and plasmid vectors used are given in Table 1. Strains ECL721 (*fucP*), ECL722 (*fucI*), and ELC723 (*fucK*) were isolated from strain ECL708 which

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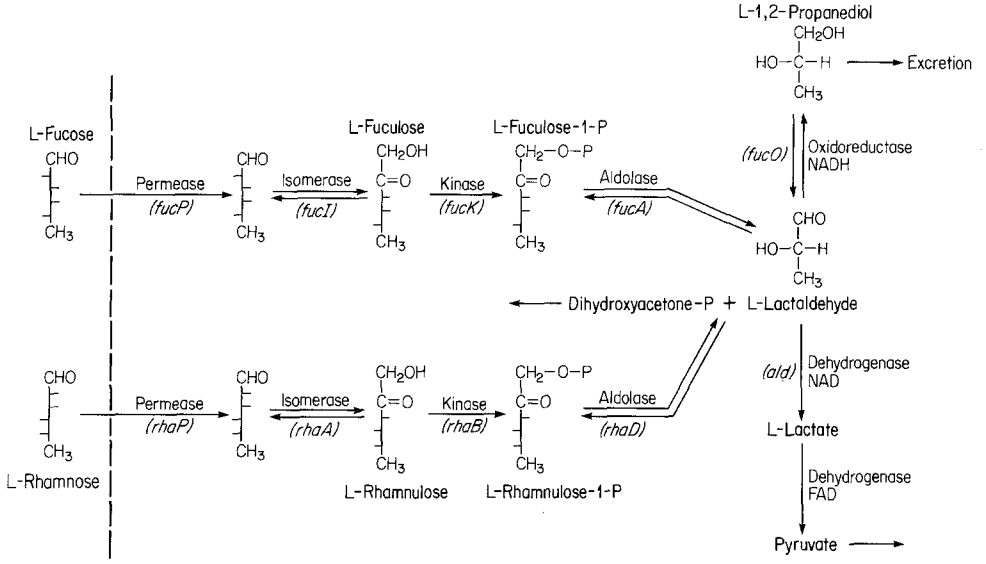

Fig. 1. Pathways of L-fucose and L-rhamnose dissimilation by *Escherichia coli*

Table 1. *Escherichia coli* K12 strains and plasmids

Strains	Genotype	Source or reference
Bacteria		
MRi7	F⁻ delta *rbs-7 araD139* delta *lacU169 relA rpsL thiA*	Lopilato et al. (1987)
MRi93	F⁻ *pcnB80 zad::*Tn*10* delta *rbs-7* delta *lacU169 araD139 relA rpsL thiA*	Lopilato et al. (1987)
ECL1	HfrC *phoA8 relA1 tonA22* T2ʳ (lambda)	Lin (1976)
ECL116	F⁻ *endA hsdR* delta *lacU169 thi*	Backman et al. (1981)
ECL326	F⁻ *fucO*-1-*lac*::lambda pl(209) *endA hsdR* delta *lacU169 thi*	Chen et al. (1983)
ECL327	F⁻ *fucO*-2-*lac*::lambda pl(209) *endA hsdR* delta *lacU169 thi*	Chen et al. (1983)
ECL357	F⁻ *argA*::Tn*10 recB21 thyA36*	Chakrabarti et al. (1984)
ECL366	F⁻ delta (*fuc-argA*)-1 *zfj3*::Tn*10 endA hsdR* delta *lacU169 thi*	Chen et al. (1987b)
ECL475	HfrC *crp-201 fucA*::Tn*10 fucPIK*(C) *fucO*(C) *phoA8 relA1 tonA22* T2ʳ(lambda)	Chakrabarti et al. (1984) Y. Zhu, to be published
ECL476	HfrC *fucA514 phoA8 relA1 tonA22* T2ʳ(lambda)	Chen et al. (1987b)
ECL477	HfrC *fucR501 phoA8 relA1 tonA22* T2ʳ(lambda)	This study
ECL608	HfrC *fucK506 phoA8 relA1 tonA22* T2ʳ(lambda)	This study
ECL708	F⁻ *fucPIK*(C) *fucA*(C) *fucO*(C) *endA hsdR* delta *lacU169 thi*	Chen et al. (1987b)
ECL721	F⁻ *fucP fucIK*(C) *fucA*(C) *fucO*(C) *endA hsdR* delta *lacU169 thi*	This study
ECL722	F⁻ *fucI fucPK*(C) *fucA*(C) *fucO*(C) *endA hsdR* delta *lacU169 thi*	This study
ECL723	F⁻ *fucK fucPI*(C) *fucA*(C) *fucO*(C) *endA hsdR* delta *lacU169 thi*	This study
Plasmids		
pBR322	*Apʳ Tcʳ*	Sutcliffe (1979)
pUC19	*Apʳ lacZ*⁺	Yanish-Perron et al. (1985)
pMLB1031	*Apʳ lacZ*⁺	Silhavy et al. (1984)
pSE100	*Apʳ rbsK*⁺	Lopilato et al. (1984)

C, constitutively expressed

expressed all of the *fuc* genes constitutively. After ethyl methanesulfonate mutagenesis and segregation, fucose-negative mutants were first identified on MacConkey-fucose agar. The specific defects of the fucose-negative mutants were determined by transport and enzyme assays. Strain ECL608, derived from wild-type strain ECL1, was identified as a *fucK* mutant by the following observations. When grown on casein hydrolysate in the presence of fucose, none of the fucose enzymes was induced. When grown on rhamnose, fucose permease and fucose isomerase were induced but fuculose kinase activity was missing.

Materials and media. Restriction endonucleases, T4 DNA ligase, and DNA polymerase I (Klenow fragment) were purchased from New England Biolabs. Calf intestinal alkaline phosphatase was from Boehringer-Mannheim Corp. Agarose and low melting point agarose were from Bethesda Research Laboratories, Inc. [^{32}P]dATP was from Amersham Corp. Nylon filter (Nytran) was from Schleicher & Schuell, Inc. L-Lactaldehyde was prepared by the reaction of ninhydrin with D-threonine (Zagalak et al. 1966). Protein molecular weight markers, L-fucose, L-rhamnose, ampicillin (Ap), and tetracycline (Tc) were from Sigma Chemical Co. Vitamin-free casein acid hydrolysate (CAA) was from ICN Nutritional Biochemicals. MacConkey agar base was from Difco Laboratories.

MacConkey-fucose agar contained 1% of the sugar. Mineral agar (Tanaka et al. 1967) was supplemented with

0.2% of a sugar and 2 μg/ml thiamine. When required, arginine was added at 40 μg/ml, Ap at 200 μg/ml, and Tc at 20 μg/ml.

Growth conditions and enzyme assays. Cells were grown to stationary phase in LB medium for the preparation of chromosomal DNA and in LB-Ap medium for the preparation of plasmid DNA. For assays of fucose permease, fucose isomerase, fuculose kinase, or fuculose 1-phosphate aldolase, the cells were grown aerobically (Chen et al. 1984). For assay of propanediol oxidoreductase, the cells were grown anaerobically (Chen and Lin 1984b). Complementation of *fucP, fucI, fucK* and *fucA* mutations on the chromosome by various constructed plasmids was detected by the red color of colonies grown on MacConkey-fucose-Ap agar and by the ability to grow on fucose-Ap agar. Complementation of *fucO* was determined by assaying the activity of propanediol oxidoreductase in the cell extract. Complementation of the *argA* mutation was determined by the ability of the cells to grow on glucose-Ap agar. To test the segregation of the plasmid, a sample cell culture was diluted and plated on the LB and LB-Ap agars.

Transduction of pcnB *mutations.* The plasmid pfuc1 contained a 21 kb chromosomal insert spanning the entire *fuc* region and *argA*. Cells harboring this multicopy plasmid gave colonies of subnormal size on LB-Ap agar and extensive segregation was observed on MacConkey-fucose-Ap agar. The detrimental effect of harboring multiple copies of the insert hampered subcloning and complementation experiments. The difficulty was overcome by introducing a *pcnB* chromosomal mutation that decreased the plasmid copy number of pBR322 and its derivatives (Lopilato et al. 1987).

The *pcnB* mutation in strain MRi93 was transduced into the desired strains by using a closely linked Tn*10* (90%) as a selected marker. Tc^r transductants were scored for inheritance of *pcnB* by the ability of their P1 lysates to give D-ribose-negative transductants of the test strain MRi7/pSE100. Strain MRi7 was deleted in the chromosomal *rbs* operon. The plasmid pSE100 contained an insert of *rbsK*$^+$ transcribed from a weak promoter. Because of the *pcnB*$^+$ background of strain MRi7, harboring this plasmid in large numbers confers a ribose-positive phenotype. The introduction of *pcnB* reduces the plasmid copy number, thereby rendering the strain ribose negative (Lopilato et al. 1987).

DNA manipulations. Chromosomal DNA of each strain was prepared from 3 ml LB culture by the lysozyme-SDS method (Mekalanos 1983). Plasmid DNA was routinely prepared from 1.5 ml of LB-AP culture by the alkaline-lysis method (Maniatis et al. 1982). For probes, plasmids were prepared by the same method from 200 ml of a culture grown on LB-AP medium and subsequently treated with chloramphenicol to amplify the plasmids. The DNA was further purified by cesium chloride-ethidium bromide gradient centrifugation (Maniatis et al. 1982). Cloning of chromosomal DNA and transformation with plasmids were according to routine procedures (Maniatis et al. 1982).

Southern blot analysis. DNA fragments to be used as probes were isolated from low melting point agarose gel (0.8%) and labeled with [^{32}P]dATP by nick translation (Maniatis et al. 1982). Chromosomal DNA (0.5 μg) was digested with restriction enzyme overnight at 37° C and fractionated by electrophoresis on a 1% agarose gel which was then stained with ethidium bromide and photographed. The DNA was transferred to a nylon filter and hybridized with the radioactive DNA probe under high-stringency conditions (Mekalanos 1983). After autoradiography, the probe DNA was removed from the nylon blots by boiling the filter in a solution of 100-fold diluted SSPE (SSPE = 0.18 M NaCl, 1 mM EDTA, 10 mM sodium phosphate, pH 7.7) and 0.5% SDS for 20 min. The treated nylon filter was autoradiographed to make sure that any small remaining amount of the probe would not interfere with subsequent hybridizations with new probes.

Results

Cloning of the fuc *region*

Chromosomal DNA from *E. coli* strain ECL116 (*fuc*$^+$) was partially digested with the restriction enzyme *Sau*3A and fractionated by centrifugation in a sucrose gradient (10%–40%). Since the *fuc* region contains at least six genes, the fractions containing 6–20 kb DNA fragments were collected and ligated to the plasmid vector pBR322 which had been digested with *Bam*HI and dephosphorylated with alkaline phosphatase. The ligation mixture (4 μg chromosomal DNA and 4 μg vector DNA in a final volume of 0.8 ml) was used to transform the competent cells of strain ECL366 which was deleted in the *fuc* region. The transformants were plated on MacConkey-fucose-Ap agar. Among 3000 Ap^r transformants, 4 fucose-positive colonies (red) were found. One of the 4 Fuc$^+$ transformants harbored a plasmid carrying a 21 kb insert. This plasmid (pfuc1), which also complemented the mutation in the nearby *argA* gene (strain

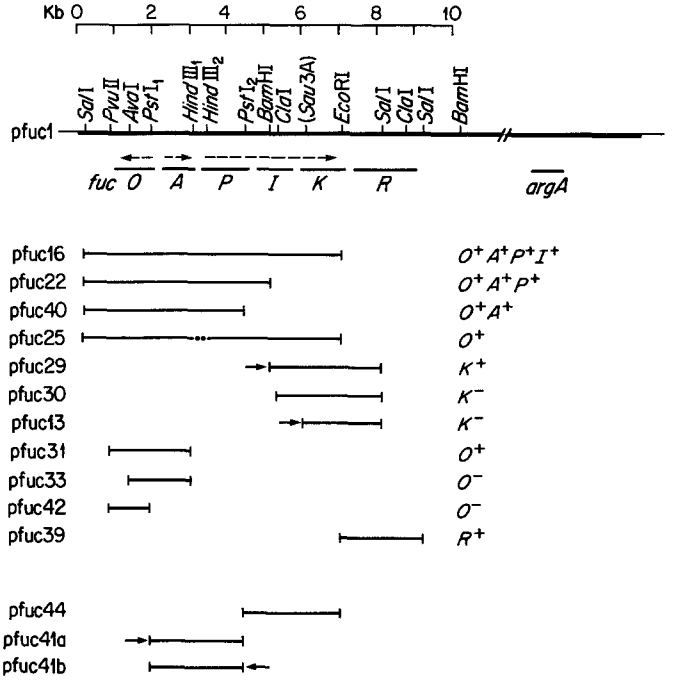

Fig. 2. Restriction map of the *fuc* region of the *Escherichia coli* chromosome. The *horizontal solid lines* represent the inserts of chromosomal DNA in the vector. *Dots* represent deleted DNA. *Arrows* show the location of the promoter and the direction of transcription

ECL357), was used for further restriction mapping and subcloning.

A restriction map of plasmid pfuc1 was constructed (Fig. 2). Subclones were examined for their ability to complement appropriate mutations in the *fuc* genes, e.g., *fucP* (strain ECL721), *fucI* (strain ECL722), *fucK* (strain ECL723), *fucA* (strain ECL476), *fucO* (strain ECL326) and *fucR* (strain ECL477). The results showed that pfuc1 carried the entire *fuc* region of about 8 kb with the *argA* gene approximately 9 kb away on the clockwise side.

Location of the fucP, fucI, *and* fucK *genes*

Plasmid pfuc16, constructed from pfuc1 by deleting a 14 kb fragment of the insert to the right of the *Eco*RI site, was able to complement the mutation in *fucO, fucA, fucP,* or *fucI* but not in *fucK*. Plasmid pfuc22, with a deletion to the right of the *Bam*HI site of the pfuc16 insert, complemented the mutation in *fucO, fucA,* or *fucP* but not in *fucI*. Plasmid pfuc40, with a deletion to the right of the *Pst*I$_2$ site of the pfuc16 insert, complemented the mutation in *fucO* or *fucA* but not in *fucP*. Plasmid pfuc25, constructed from pfuc16 by deleting the 0.4 kb fragment between the *Hin*dIII$_1$ and *Hin*dIII$_2$ sites, lost the ability to complement the mutation in *fucP* or *fucA*. Thus *fucP* (and also a portion of *fucA*) was located within the *Hin*dIII$_1$–*Bam*HI region.

Plasmid pfuc29 was constructed by inserting the 3 kb fragment between the *Bam*HI and *Sal*I sites of pfuc1 into the vector pBR322 doubly digested with *Bam*Hi and *Sal*I. The resulting product possessed a Tc promoter transcribing clockwise into the insert. This plasmid was able to complement the mutation in *fucK* but not in *fucI*. Strain ECL608, a *fucK* mutant derived from a parent with wild-type *fuc* regulon, was also complemented by the plasmid.

Plasmid pfuc13 contained the 2 kb fragment between the *Bam*HI (*Sau*3A) and *Sal*I sites on the right side of the pfuc29 insert and provided an external Tc promoter transcribing clockwise into the insert. (The *Bam*HI site was created by *Sau*3A partial digestion followed by ligation to the *Bam*HI end of the vector.) This plasmid did not complement the *fucK* mutation.

Since *fucK* was complemented by neither pfuc16 with the insert terminated on the right by the *Eco*RI site nor pfuc13 with the insert terminated on the left by the *Bam*HI (*Sau*3A) site, these two sites should be within the *fucK* gene. Since *fucI* was complemented by neither pfuc22 with the insert terminated to the right by the *Bam*HI site nor the pfuc29 with the insert terminated to the left by the *Bam*HI site, this *Bam*HI site should be inside the *fucI* gene.

The complementation patterns of the six plasmids described above thus indicate a clockwise gene order of *P-I-K*.

Location of fucA *and* fucO *genes*

Plasmid pfuc31, containing a 2.1 kb fragment located at the left end of the *fuc* region, complemented the mutation in *fucO* but not in *fucA*. Plasmid pfuc33, which is missing the left end (from the *Pvu*II site to the *Ava*I site spanning about 550 bp) of the pfuc31 insert, did not complement the *fucO* mutation. Plasmid pfuc42, which is missing the right end (from the *Pst*I$_1$ site to the *Hin*dIII$_1$ site spanning about 1.1 kb) of the pfuc31 insert, was also unable to complement the *fucO* mutation. Thus the *Ava*I and *Pst*I$_1$ sites are within the *fucO* gene. Insofar as pfuc22 complemented the mutation in *fucO*, *fucA* and *fucP*, pfuc25 (deleted in the *Hin*dIII$_1$–*Hin*dIII$_2$ segment of pfuc16) complemented the mutation in *fucO* but not in *fucA* or *fucP*, and pfuc40 complemented the mutation in *fucO* or *fucA* but not in *fucP*, it follows that the *fucA* gene is located between the *fucP* and *fucO* genes.

Isolation of fucR *mutants and location of* fucR *gene*

Because the inducer of the fucose enzymes is L-fuculose 1-phosphate, the product of the fuculose kinase, and because the fucose regulon is under positive control, it was necessary to distinguish the mutations in *fucP, fucI* or *fucK* genes that prevent inducer formation from the mutations in *fucR* that impair the synthesis of the positive regulator. This could be accomplished by testing with rhamnose. Mutants in a *fuc* structural gene remained inducible by rhamnose in the remaining *fuc* structural genes and retained the ability to grow anaerobically on rhamnose provided that *fucO* was intact. In contrast, mutants in *fucR* were not inducible in any of the *fuc* structural genes and grew poorly on rhamnose anaerobically. Of 18 independent pleiotropic fucose-negative mutants derived from wild-type strain ECL1 following mutagenesis with ethyl methanesulfonate (T. Chakrabarti, unpublished data), 5 were found by these criteria to be *fucR* mutations. The plasmid pfuc39, which did not complement the mutation in *fucO, fucA, fucP, fucI* or *fucK*, corrected the pleiotropic defects of these 5 mutants. Thus *fucR* is located at the right end of the *fuc* region and encodes a *trans*-activating product.

Additional evidence for the positive role of the *fucR* gene product was provided by other complementation tests. The introduction of plasmid pfuc16, containing *fucO, fucA,* and *fucPIK'* (truncated operon with incomplete *fucK*), into wild-type cells reduced their ability to ferment fucose (pale on MacConkey-fucose-Ap agar). This impairment suggested that the *fuc* promoters on the high copy number plasmid were "titrating" the activator protein encoded by the single copy of *fucR* on the chromosome, thereby limiting *fucK* expression. This interpretation was supported by two further experiments. First, the titration effect was cancelled by a chromosomal *pcnB* mutation which reduced the copy number of the plasmid. Second, the effect was attenuated by deleting the *fucP* promoter region of plasmid pfuc16 (to give plasmid pfuc25).

Direction of transcription of fucPIK

Plasmid pfuc16 complemented mutations in *fucP* and *fucI*, but plasmid pfuc25 did not complement the *fucP* mutation and only weakly complemented the *fucI* mutation (pink instead of red colonies on MacConkey-fucose-Ap agar). Thus, the promoter of *fucP* was apparently located within the *Hin*dIII$_1$–*Hin*dIII$_2$ fragment missing in pfuc25. The weak expression of *fucI* in the absence of the *fucP* promoter suggested that normally transcription from *fucP* continued through *fucI* and that in the absence of the *fucP* promoter either a readthrough occurred from *fucA* or a second transcription start site exists for *fucI*.

To find out whether *fucI* has a promoter of its own, plasmid pfuc44 was constructed by inserting the *Pst*I$_2$–*Eco*RI fragment that contains intact *fucI* and the 3′ terminal sequence of *fucP* into the pBR322 vector doubly digested

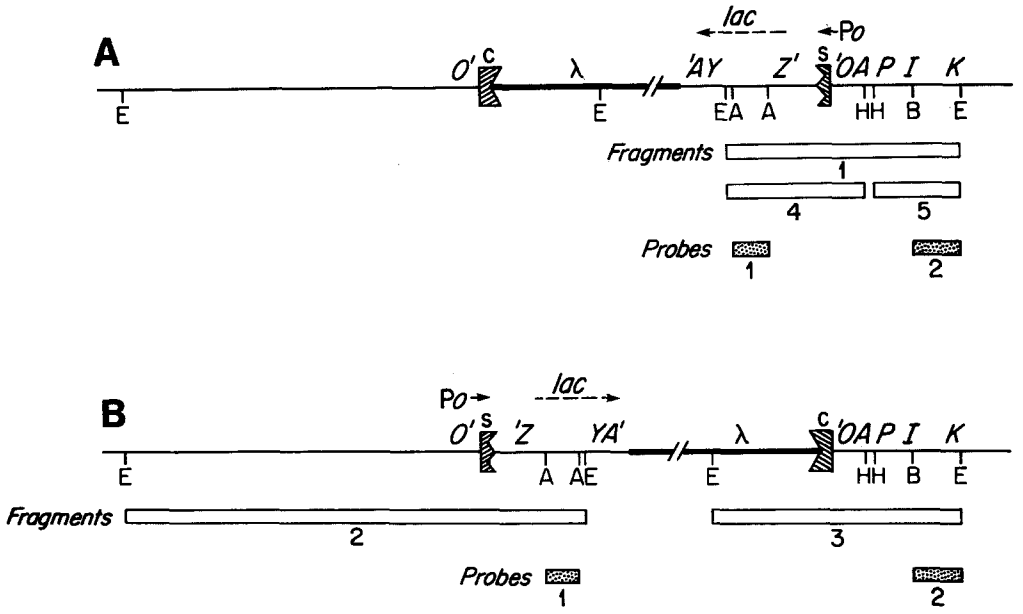

Fig. 3A, B. Strategy for determination of the direction of transcription of *fucO* by Southern blot hybridization. The *heavy horizontal solid line* represents lambda DNA and the *light horizontal line* represents *Escherichia coli* DNA. The segments s and c are the ends of the Mu phage. P*o* represents the promoter of *fucO*. *Arrows* indicate the direction of transcription. Restriction sites shown are only those used in this experiment: H, *Hin*dIII; E, *Eco*RI; B, *Bam*HI, A, *Ava*I. *Open bars* represent the DNA fragments expected to hybridize with the probes. *Stippled bars* represent the DNA fragments used as probes: probe 1, *Ava*I–*Ava*I segment of the *lacZ* gene; probe 2, *Bam*HI–*Eco*RI segment of the *fuc* region containing portions of *fucK* and *fucI*

by *Pst*I and *Eco*RI. A *fucI* mutant that had received this plasmid gave pink colonies on fucose-MacConkey-Ap agar and small colonies on minimal fucose-Ap agar. The activity of fucose isomerase in the transformant, however, was not inducible by fucose. This would indicate that the transcription was initiated from a weak exogenous promoter in the vector and that *fucI* did not have its own promoter.

Plasmid pfuc29, with a promoter of the Tc^r gene on the vector transcribing into the *fucK* insert, complemented *fucK* (normal growth of the mutant on minimal fucose-Ap medium). No complementation occurred when the Tc^r promoter was deleted (plasmid pfuc30). Thus, *fucK* lacks its own promoter.

The lack of evidence for *fucI* and *fucK* having their own promoters would suggest that the *fucP*, *fucI* and *fucK* genes constitute an operon transcribed clockwise. This conclusion is consistent with the observations that a Tn*5* insertion in *fucP* prevented the expression of *fucI* and *fucK*, and a Tn*5* insertion in *fucI* prevented the expression of *fucK* (Chakrabarti et al. 1984; T. Chakrabarti, unpublished).

Direction of transcription of fucA

Plasmids pfuc41a and pfuc41b were constructed by inserting the *Pst*I$_1$–*Pst*I$_2$ fragment of 2.5 kb, containing intact *fucA* and the 5′ terminal sequence of *fucP*, into the unique *Pst*I site in the polylinker region of vector pUC19. In pfuc41a, the insert was oriented with its *Pst*I$_1$ site proximal and its *Hin*dIII$_1$ site distal to the promoter of *lacZ* in the vector; in pfuc41b the insert was in the opposite orientation. Both plasmids complemented the *fucA* mutation in strain ECL476. However, the mutant cells harboring pfuc41a gave larger colonies on fucose minimal agar than those harboring pfuc41b. This would suggest that in both plasmids *fucA* was transcribed from its native promoter in the direction of *Pst*I$_1$–> *Hin*dIII$_1$ (i.e., the direction of *fucA* transcription was clockwise) and that in plasmid pfuc41a the gene was transcribed in addition from the *lacZ* promoter.

The mutant *crp-201* allele was found to render the *fuc*$^+$ cell phenotypically fucose negative by failing specifically to activate the *fucA*$^+$ promoter properly (Y. Zhu and E. Lin, to be published). When tested in strain ECL475, bearing *crp-201* and *fucA*::Tn*5*, only plasmid pfuc41a (*lacZ* promoter–> *Pst*I$_1$–> *Hin*dIII$_1$) allowed growth on fucose, confirming the clockwise direction of *fucA* transcription from its native promoter.

Direction of transcription of fucO

Southern blot hybridization was used to determine the direction of transcription of *fucO*. Figure 3 shows the two possible orientations of the *lacZ* fusion in the *fucO* gene in strains ECL326 and ECL327. Two probes were used to detect the DNA fragments expected in each case. Probe 1 was the *Ava*I–*Ava*I segment of the *lacZ* gene excised from plasmid pMLB1031. Probe 2 was the *Bam*HI–*Eco*RI segment of the *fuc* region containing portions of *fucK* and *fucI*.

There is only one *Eco*RI site in the *fuc* region and only one *Eco*RI site in the *lacZ* gene. The distance from the fusion joint of *fucO-lacZ* to the *Eco*RI site in the *lacZ* gene is deduced to be about 4 kb based on previous published data (Silhavy et al. 1984). If the direction of transcription of *fucO* were counterclockwise, the distance from the *Eco*RI site in *lacZ* to the *Eco*RI site in the *fuc* region should be about 10 kb (fragment 1 in Fig. 3A) and this same fragment should hybridize to both probes 1 and 2. If the transcription were clockwise, probes 1 and 2 should hybridize with two different fragments (fragments 2 and 3 in Fig. 3B). As can be seen in Fig. 4, when the *Eco*RI-digested chromosomal DNA of strains ECL326 and

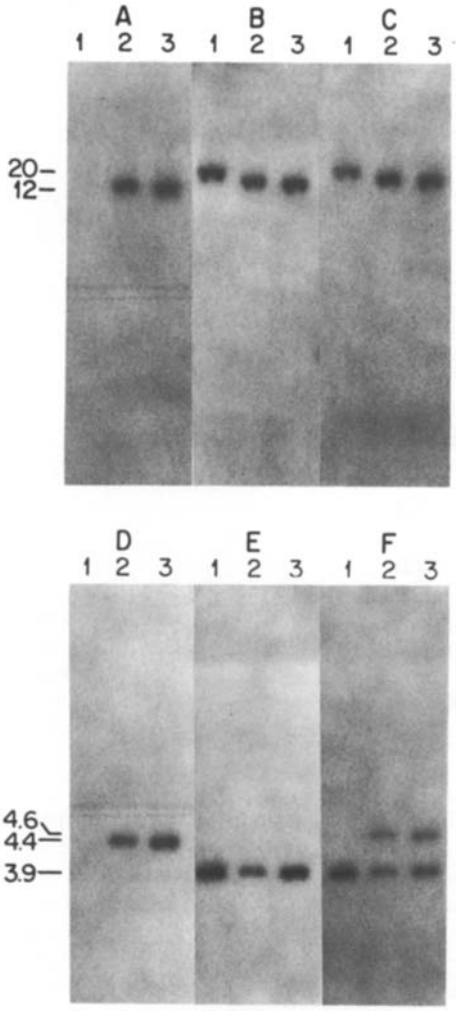

Fig. 4A–F. Southern blot analysis for determination of the direction of transcription of the *fucO* gene and the location of the fusion joint of the *fucO-lacZ* fusion strains ECL326 and ECL327. Chromosomal DNA was digested with *Eco*RI (**A–C**) or doubly digested with *Eco*RI and *Hin*dIII (**D–F**). The digested DNA was run on a 1% agarose gel, transfered to a nylon filter and hybridized with ^{32}P-dATP labeled probe 1 (**A, D**). (For probes, see Fig. 3.) After autoradiography, the filter was treated to wash off the probe, and used to rehybridize with probe 2 (**B, E**). After autoradiography, the filter was used to hybridize with probe 1 again (**C, F**). Lanes: 1, DNA from strain ECL116; 2, DNA from strain ECL326; 3, DNA from strain ECL327. Numbers on the left indicate the size of the hybridized chromosomal fragment in kb

ECL327 containing independent *fucO-lacZ*::lambda pl(209) fusions was hybridized with probe 1 (Fig. 4A, lanes 2 and 3), probe 2 (Fig. 4B, lanes 2 and 3), or both (Fig. 4C, lanes 2 and 3), the same band of about 12 kb was revealed in each case. This is approximately the size we expected for fragment 1. The results therefore are consistent with counterclockwise direction of *fucO* transcription.

Clockwise transcription of *fucO* was excluded by establishing the location of the chromosomal *Eco*RI site to the left of the *Eco*RI site in *fucK* in a parallel analysis of the *Eco*RI-digested chromosomal DNA of strain ECL116 (*fucO*$^+$ *Δlac*) from which the *fucO-lacZ* strain was derived. When hybridized with probe 1, no band was revealed as expected (Fig. 4A, lane 1). When hybridized with probe 2, a fragment of about 20 kb was seen (Fig. 4B, C, lanes 1). Since the distance from *fucO* to the *Eco*RI site on the right (in *fucK*) is about 5–6 kb, the *Eco*RI site on the left of *fucO* should be about 14–15 kb. Consequently, if the direction of transcription of *fucO* were clockwise (Fig. 3B), a 19 kb fragment (fragment 2) should hybridize with probe 1 and an 11 kb fragment (fragment 3) should hybridize with probe 2.

To locate the fusion joint of *fucO-lacZ*, a second Southern blot experiment was carried out, taking advantage of the nearby *Hin*dIII$_1$ site in *fucA*. Chromosomal DNA of fusion and parent strains doubly digested with *Hin*dIII and *Eco*RI hybridized with probes 1 and 2 separately and together. When hybridized with probe 1, no band was observed with strain ECL116; a band of 4.6 kb corresponding to fragment 4 in Fig. 3A was seen with strain ECL326 (Fig. 4D, lane 2) and a band of 4.4 kb was seen with strain ECL327 (Fig. 4D, lane 3). When hybridized with probe 2, a 3.9 kb band corresponding to fragment 5 in Fig. 3A was observed in each case, as expected from the restriction map (Fig. 4E, lanes 1–3). Consistent banding patterns were revealed when the DNA preparations were hybridized with both probes together (Fig. 4F, lanes 1–3). Since the distance from the fusion joint of *fucO-lacZ* to the *Eco*RI site in the *lacZ* gene is about 4 kb, the fusion joint of *fuco-lacZ* in strain ECL326 should be approximately 0.6 kb to the left of the *Hin*dIII$_1$ site and the corresponding distance for strain ECL327 should be approximately 0.4 kb. Thus in both strains the fusion joint was probably close to the promoter of *fucO*.

Discussion

Serial aerobic selection of mutants for progressively more rapid growth rates on propanediol as the sole source of carbon and energy resulted in the constitutive synthesis of the *fucO*-encoded oxidoreductase that remained catalytically active despite the presence of molecular oxygen (Sridhara et al. 1969; Cocks et al. 1974; Boronat et al. 1981a). In the initial-stage mutants, only the basal activity of the oxidoreductase increased, while the basal levels of the other *fuc* structural genes remained normal. At a critical step, when the expression of *fucO* became fully constitutive, *fucA* also became constitutively expressed. The expression of *fucP, fucI* and *fucK,* however, became noninducible. This pleiotropic change was observed during the selection of ten different clones on propanediol. On the basis of the shifting patterns of gene expression in the course of selection, it was suggested that the *fuc* structural genes were organized in at least three operons: *fucO, fucA,* and *fucPIK* (Hacking and Lin 1976, 1977). This suggestion was confirmed by the present study. Moreover, the divergent transcription of *fucO* and *fucA* and their simultaneous constitutive expression during the course of selection on propanediol raised the possibility of an overlap in the regulatory regions of the two operons. Such a possibility was reinforced by the observation that the *fucO-lacZ* fusions reduced the expression of *fucA* up to 50% (Chen et al. 1983), suggesting that the fusion disturbed an upstream controlling element of *fucA*.

More difficult to explain is the noninducibility of *fucPIK* that was associated with the double constitutivity of *fucO* and *fucA*. It might be that the final mutation altered the specificity of the *fucR*-encoded activator with different consequences on the expression of the three operons. It is also

conceivable that the regulation of the expression of the *fuc* operons involves a loop structure held together at the two promoter regions by subunits of the regulatory protein and that the pleiotropic effect of the final mutation results from a change in the complex structure. Experiments to explore these models are in progress.

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References

Bachmann BJ (1983) Linkage map of *Escherichia coli* (Edition 7). Microbiol Rev 47:180–230

Backman K, Chen Y-M, Magasanik B (1981) Physical and genetic characterization of the *glnA-glnG* region of the *Escherichia coli* chromosome. Proc Natl Acad Sci USA 78:3743–3747

Bartkus JM, Mortlock RP (1986) Isolation of a mutation resulting in constitutive synthesis of L-fucose catabolic enzymes. J Bacteriol 165:710–714

Boronat A, Aguilar J (1979) Rhamnose-induced propanediol oxidoreductase in *Escherichia coli*: purification, properties, and comparison with the fucose-induced enzyme. J Bacteriol 140:320–326

Boronat A, Aguilar J (1981a) Experimental evolution of propanediol oxidoreductase in *Escherichia coli*. Biochim Biophys Acta 672:98–107

Boronat A, Aguilar J (1981b) Metabolism of L-fucose and L-rhamnose in *Escherichia coli*: differences in induction of propanediol oxidoreductase. J Bacteriol 147:557–566

Chakrabarti T, Chen Y-M, Lin ECC (1984) Clustering of genes for L-fucose dissimilation by *Escherichia coli*. J Bacteriol 157:984–986

Chen Y-M, Lin ECC (1984a) Post-transcriptional control of L-1,2-propanediol oxidoreductase in the L-fucose pathway of *Escherichia coli* K-12. J Bacteriol 157:341–344

Chen Y-M, Lin ECC (1984b) Dual control of a common L-1,2-propanediol oxidoreductase by L-fucose an L-rhamnose in *Escherichia coli*. Bacteriol 157:828–832

Chen Y-M, Lin ECC, Ros J, Aguilar J (1983) Use of operon fusions to examine the regulation of the L-1,2-propanediol oxidoreductase gene of the fucose system in *Escherichia coli* K12. J Gen Microbiol 129:3355–3362

Chen Y-M, Chakrabarti T, Lin ECC (1984) Constitutive activation of L-fucose genes by an unlinked mutation in *Escherichia coli*. J Bacteriol 159:725–729

Chen Y-M, Zhu Y, Lin ECC (1987a) NAD-linked aldehyde dehydrogenase for aerobic utilization of L-fucose and L-rhamnose by *Escherichia coli*. J Bacteriol 169:3289–3294

Chen Y-M, Tobin JF, Zhu Y, Schleif RF, Lin ECC (1987b) Cross-induction of the L-fucose system by L-rhamnose in *Escherichia coli*. J Bacteriol 169:3712–3719

Cocks T, Aguilar J, Lin ECC (1974) Evolution of L-1,2-propanediol catabolism in *Escherichia coli* by recruitment of enzymes for L-fucose and L-lactate metabolism. J Bacteriol 118:83–88

Hacking AJ, Lin ECC (1976) Disruption of the fucose pathway as a consequence of genetic adaptation to propanediol as a carbon source in *Escherichia coli*. J Bacteriol 126:1166–1172

Hacking AJ, Lin ECC (1977) Regulatory changes in the fucose system associated with the evolution of a catabolic pathway for propanediol in *Escherichia coli*. J Bacteriol 130:832–838

Lin ECC (1976) Glycerol dissimilation and its regulation in bacteria. Annu Rev Microbiol 30:535–578

Lin ECC (1987) Dissimilatory pathways for sugars, polyols and carboxylates. In: Neidhardt FC, Ingraham JL, Low KB, Magasanik B, Schaechter M, Umbarger HE (eds) *Escherichia coli* and *Salmonella typhimurium:* cellular and molecular biology. Am Soc Microbiol, Washington, pp 244–284

Lin ECC, Wu TT (1984) Functional divergence of the L-fucose system in mutants of *Escherichia coli*. In: Mortlock RP (ed) Microorganisms as model systems for studying evolution. Plenum, New York, pp 134–165

Lopilato J, Garwin JL, Emr SD, Silhavy TJ, Beckwith JR (1984) D-ribose metabolism in *Escherichia coli* K-12: genetics, regulation and transport. J Bacteriol 158:665–673

Lopilato J, Bortner S, Beckwith J (1986) Mutations in a new chromosomal gene of *E. coli, pcnB*, reduce plasmid copy number of pBR322 and its derivatives. Mol Gen Genet 205:285–290

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, New York

Mekalanos JJ (1983) Duplication and amplification of toxin genes in *Vibrio cholerae*. Cell 35:253–263

Power J (1967) The L-rhamnose genetic system in *Escherichia coli* K-12. Genetics 55:557–568

Silhavy TJ, Berman ML, Enquist LW (1984) Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Skjold AC, Ezekiel DH (1982a) Analysis of lambda insertions in the fucose utilization region of *Escherichia coli* K-12: use of *fuc* and *argA* transducing bacteriophages to partially order the fucose utilization genes. J Bacteriol 152:120–125

Skjold AC, Ezekiel DH (1982b) Regulation of D-arabinose utilization in *Escherichia coli* K-12. J Bacteriol 152:521–523

Sridhara S, Wu TT (1969) Purification and properties of lactaldehyde dehydrogenase in *Escherichia coli*. J Biol Chem 244:5233–5238

Sridhara S, Wu TT, Chused TM, Lin ECC (1969) Ferrous-activated nicotinamide adenine dinucleotide-linked dehydrogenase from a mutant of *Escherichia coli* capable of growth on 1,2-propanediol. J Bacteriol 98:87–95

Sutcliffe JG (1979) Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp Quant Biol 43:77–90

Tanaka S, Lerner SA, Lin ECC (1967) Replacement of a phosphoenolpyruvate-dependent phosphotransferase by nicotinamide adenine dinucleotide-linked dehydrogenase for the utilization of mannitol. J Bacteriol 93:642–648

Yanisch-Perron C, Vieira J, Messing J (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33:103–109

Zagalak B, Frey PA, Karabatsos GL, Abeles RH (1966) The stereochemistry of the conversion of D and L 1,2-propanediols to propionaldehyde. J Biol Chem 241:3028–3035

Zhu Y, Lin ECC (1986) An evolvant of *Escherichia coli* that employs the L-fucose pathway also for growth on L-galactose and D-arabinose. J Mol Evol 23:259–266

Zhu Y, Lin ECC (1987) Loss of aldehyde dehydrogenase in an *Escherichia coli* mutant selected for growth on the rare sugar L-galactose. J Bacteriol 169:785–789

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