JOURNAL OF BACTERIOLOGY, May 1988, p. 2352–2358
0021-9193/88/052352-07$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 170, No. 5

A Mutant *crp* Allele That Differentially Activates the Operons of the *fuc* Regulon in *Escherichia coli*

YAN ZHU AND E. C. C. LIN*

Department of Microbiology and Molecular Genetics, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115

Received 10 November 1987/Accepted 14 February 1988

L-Fucose is used by *Escherichia coli* through an inducible pathway mediated by a *fucP*-encoded permease, a *fucI*-encoded isomerase, a *fucK*-encoded kinase, and a *fucA*-encoded aldolase. The aldolase catalyzes the formation of dihydroxyacetone phosphate and L-lactaldehyde. Anaerobically, lactaldehyde is converted by a *fucO*-encoded oxidoreductase to L-1,2-propanediol, which is excreted. The *fuc* genes belong to a regulon comprising four linked operons: *fucO*, *fucA*, *fucPIK*, and *fucR*. The positive regulator encoded by *fucR* responds to fuculose 1-phosphate as the effector. Mutants serially selected for aerobic growth on propanediol became constitutive in *fucO* and *fucA* [*fucO*(Con) *fucA*(Con)], but noninducible in *fucPIK* [*fucPIK*(Non)]. An external suppressor mutation that restored growth on fucose caused constitutive expression of *fucPIK*. Results from this study indicate that this suppressor mutation occurred in *crp*, which encodes the cyclic AMP-binding (or receptor) protein. When the suppressor allele (*crp-201*) was transduced into wild-type strains, the recipient became fucose negative and fucose sensitive (with glycerol as the carbon and energy source) because of impaired expression of *fucA*. The *fucPIK* operon became hyperinducible. The growth rate on maltose was significantly reduced, but growth on L-rhamnose, D-galactose, L-arabinose, glycerol, or glycerol 3-phosphate was close to normal. Lysogenization of *fuc*$^+$ *crp-201* cells by a λ bacteriophage bearing *crp*$^+$ restored normal growth ability on fucose. In contrast, lysogenization of [*fuc*O(Con) *fucA*(Con) *fucPIK*(Non) *crp-201*] cells by the same phage retarded their growth on fucose.

Escherichia coli can grow on L-fucose as the sole source of carbon and energy (Fig. 1). The dissimilatory pathway is mediated sequentially by a permease (22), an isomerase (21), a kinase (24), and an aldolase (20). The aldolase catalyzes the formation of dihydroxyacetone phosphate and L-lactaldehyde. Aerobically, lactaldehyde is converted by aldehyde dehydrogenase to L-lactate, which is further oxidized to pyruvate (14, 39). Anaerobically, lactaldehyde is converted by an oxidoreductase to L-1,2-propanediol, which is excreted as a terminal product (14).

With the exception of aldehyde dehydrogenase, which is specified by the *ald* gene at min 31 (11), all of the enzymes involved in fucose utilization are coded by a cluster of genes at min 60.2 (8, 37, 38): *fucP*, encoding the permease; *fucI*, encoding the isomerase; *fucK*, encoding the kinase; *fucA*, encoding the aldolase; *fucO*, encoding propanediol oxidoreductase; and *fucR*, encoding a positive regulatory protein. The *fuc* genes are organized into four operons arranged in clockwise order: *fucO* (transcribed counterclockwise), *fucA* (transcribed clockwise), *fucPIK* (transcribed clockwise), and *fucR* (12). Fuculose 1-phosphate is the true inducer (4).

Propanediol is unable to induce an enzymically active *fucO* product under aerobic conditions and thus to be salvaged by conversion to lactate via lactaldehyde. Mutants that grew aerobically on propanediol as the sole source of carbon and energy, however, were readily selected. In a series of selections from 10 different clones, all the mutants eventually expressed *fucO* constitutively [*fucO*(Con)]. When this occurred, *fucA* also became constitutive [*fucA*(Con)]. These mutants, however, were fucose negative because *fucPIK* was no longer inducible [*fucPIK*(Non)]. The mutations responsible for the propanediol positivity and fucose negativity all occurred at the *fuc* locus (14, 22, 23, 40; for reviews, see references 28 and 29). Two kinds of suppressor mutations were found to restore the growth ability on fucose without abolishing the growth ability on propanediol; one mapped at *fuc*, but the other did not. Both suppressions caused constitutive expression of *fucPIK*. The *fuc*-linked suppressor mutation was believed to be in either *fucR* or the promoter region of *fucPIK* (9). The subject of this study is the nature of the unlinked suppressor.

MATERIALS AND METHODS

Chemicals. L-Fucose and D-arabinose were obtained from Sigma Chemical Co., St. Louis, Mo. Vitamin-free casein acid hydrolysate was from ICN Nutritional Biochemicals, Cleveland, Ohio. MacConkey agar base (without lactose) was from Difco Laboratories, Detroit, Mich. L-[1-^{14}C]fucose (59 mCi/mmol) was from New England Nuclear Corp., Boston, Mass. L-Lactaldehyde, L-fuculose 1-phosphate, and fuculose isomerase were provided by Y.-M. Chen (9). All other chemicals were commercial products of reagent grade.

Bacterial and bacteriophage strains. The genotypes and phenotypes of the bacterial and phage strains used are given in Table 1. Unless otherwise specified, standard techniques were used for strain construction and genetic mapping by conjugation or transduction (32). Conjugational mapping was facilitated by Hfr strains constructed by the method of Chumley et al. (13). A population of strain ECL702 with Tn*10* randomly inserted in the chromosome was prepared with λNK55 (5). About 10,000 Tc^r colonies were pooled for the preparation of a P1 *vir* lysate. Transductants of strain ECL701 treated with this lysate were selected on fucose-tetracycline agar. Strain ECL706, in which the Tn*10* was about 20% linked to the *crp* locus, was thus obtained. Strain ECL367 was a transductant of strain ECL116 with strain ECL706 as the donor of *crp-201 zdh-4*::Tn*10*. The transductant was selected for Tc^r. Inheritance of *crp-201* by a *fuc*$^+$

* Corresponding author.

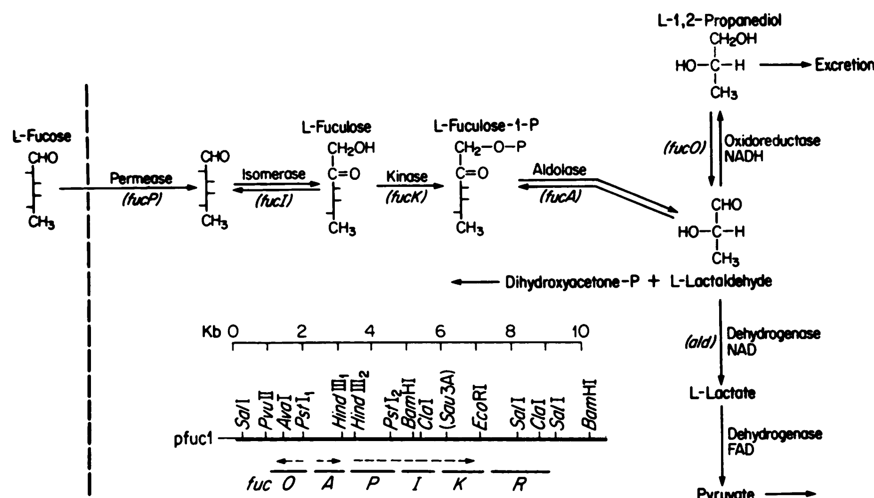

FIG. 1. The dissimilatory pathway for L-fucose and the genetic map of the *fuc* regulon.

recipient was scored by the fucose sensitivity (fucose inhibition of growth on glycerol) caused by the lowered expression of *fucA*$^+$ (see Results). By a similar procedure, strain ECL602 was isolated as a Tc^r Fuc^s transductant of strain ECL1. Strain ECL605 was isolated from strain ECL602 as a derivative with the Tn*10* excised (6). Strain ECL616 was a transductant of strain ECL1 that received *rpsL* (Sm^r) as the selected marker and Δ*crp-39* as the unselected marker from strain CA8439. Strain ECL720 was a λY1079 lysogen of strain ECL367. Strains ECL725 and ECL726 were, respectively, λY1079 and λZ1 lysogens of strain ECL701. Strain ECL727 was a Tc^r Fuc$^+$ transductant of strain ECL116 with strain ECL706 as the donor of *zhd-4*::Tn*10*. Strains ECL728 and ECL729 were, respectively, λY1079 and λZ1 lysogens of strain ECL702. Strain ECL731 was a transductant of strain ECL116 that received *rpsL* as the selected marker and Δ*crp-39* as the unselected marker from strain CA8439.

Lysogenization with λY1079 (*crp*$^+$) and λZ1 (*crp-201*). Bacterial strains to be lysogenized were grown in Luria-Bertani (LB) medium, and a drop of the culture was spread

TABLE 1. *E. coli* K-12 and phage strains

Bacterial strain or phage	Genotype or phenotype	Source or reference
Strains (derivations)		
CA8439 (I-7019)	Δ*crp-39 rpsL* Δ*cya thi aroB*(λ)	J. R. Beckwith (34)
SY777	F$^-$ *zhf*::Tn*10 malT*(Am) *lacY14 rpsL trp*(Am)	M. Syvanen
ECL1	HfrC *phoA8 relA1 tonA22* T2^r (λ)	27
ECL3 (ECL1)	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) (Fuc$^-$)	40
ECL56 (ECL3)	*crp-201 fucO*(Con) *fucA*(Con) *fucPIK*(Non) (Fuc$^+$)	22
ECL101	F$^-$ *argE gal his leu rpsL thr xyl*	L. Gorini
ECL116	F$^-$ *endA hsdR* Δ(*argF-lac*)*U169 thi*	3
ECL338 (ECL116)	*argA*::Tn*10*	Y.-M. Chen
ECL367 (ECL116)	*crp-201 zhd-4*::Tn*10* (Fuc^s)	This study
ECL602 (ECL1)	*crp-201 zhd-4*::Tn*10* (Fuc^s)	This study
ECL605 (ECL602)	*crp-201* (Fuc^s)	This study
ECL616 (ECL1)	Δ*crp-39 rpsL*	This study
ECL701 (ECL116)	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) (Fuc$^-$)	This study
ECL702 (ECL701)	*crp-201 fucO*(Con) *fucA*(Con) *fucPIK*(Non) (Fuc$^+$)	This study
ECL706 (ECL701)	*crp-201 zhd-4*::Tn*10 fucO*(Con) *fucA*(Con) *fucPIK*(Non) (Fuc$^+$)	This study
ECL720 (ECL367)	*crp-201 zhd-4*::Tn*10* λY1079 (Fuc^r)	This study
ECL725 (ECL701)	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) λY1079	This study
ECL726 (ECL701)	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) λZ1	This study
ECL727 (ECL116)	*zhd-4*::Tn*10*	This study
ECL728 (ECL702)	*crp-201 fucO*(Con) *fucA*(Con) *fucPIK*(Non) λY1079	This study
ECL729 (ECL702)	*crp-201 fucO*(Con) *fucA*(Con) *fucPIK*(Non) λZ1	This study
ECL730 (ECL367)	*crp-201 zhd-4*::Tn*10* λZ1 (Fuc^s)	This study
ECL731 (ECL116)	Δ*crp-39 rpsL*	This study
Phage		
λ *c*I	*c*I *h*80 Δ(*int*)9	J. Beckwith
λ *vir*		J. Beckwith
λNK55	*b*221 *c*I857 *c*III167::Tn*10 O*am29	M. Syvanen
λY1079	λ Bam λ 1° *sr*I λ *c*Its857 *int crp*$^+$	17, 19
λZ1	λ Bam λ 1° *sr*I λ *c*Its857 *int crp-201*	This study

on a tryptone-broth agar (32). A diluted stock of λY1079 or λZ1 was spotted on the lawn of cells and incubated overnight at 30°C. The lysogens were twice purified as single colonies on LB agar seeded with λ *c*I phage and verified by their resistance to λ *c*I and sensitivity to λ *vir*.

Preparation of λZ1 (*crp-201*). Growth of strain ECL720 [*crp-201*/λY1079(*crp*$^+$)] in LB medium was shifted at mid-exponential phase (about 100 Klett units, no. 42 filter) from 30 to 42°C. After 2.5 h, when extensive cell lysis was observed, the culture was centrifuged to remove the debris. Cells of the fucose-sensitive strain ECL367 (*crp-201*) exposed to this lysate formed two kinds of lysogens: the fucose-positive lysogen [*crp-201*/λY1079(*crp*$^+$)] and the fucose-sensitive lysogen [*crp-201*/λZ1(*crp*-201)]. A stock of the *crp-201* phage, λ Z1, was prepared by thermoinduction of the fucose-sensitive lysogen, strain ECL730.

Growth of cells and preparation of extracts. For testing growth rates on solid media, a diluted cell suspension (grown in LB medium) was spread on a sector of minimal agar to give 20 to 50 colonies. After incubation at 30°C for the desired time, diameters of 10 random colonies were measured to determine the average size. For enzyme assays, aerobic growth of cells was carried out in a 500-ml flask containing 100 ml of mineral medium (42) supplemented with 0.5% casein acid hydrolysate and 0.2% of the appropriate carbon and energy source, and anaerobic growth was carried out in a 150-ml tightly capped bottle (10). The media for anaerobic cultures were supplemented additionally with 0.1% casein amino acids and 20 mM pyruvate. Both aerobic and anaerobic cultures were grown at 37°C and harvested during the exponential phase by centrifugation. The collected cells were washed and suspended in 50 mM potassium phosphate (pH 7.0) for the preparation of an extract (43).

Transport and enzyme assays. Fucose permease activity was determined by the initial rate of cellular uptake of L-[1-^{14}C]fucose (22). Fucose isomerase activity was determined by the rate of fuculose formation by the cysteine carbazole method (15). Fuculose kinase was determined by the rate of L-[6-^{3}H]fuculose 1-phosphate formation (9). The assay of fuculose 1-phosphate aldolase activity was dependent on the formation of dihydroxyacetone phosphate (20). Propanediol oxidoreductase activity was measured by the rate of lactaldehyde-dependent oxidation of NADH (10). Fucose permease activity was expressed in nanomoles of substrate uptake per minute per milligram (dry weight) of cells at 30°C. Enzyme activities were expressed in nanomoles of product formed per minute per milligram of protein at 25°C. Protein concentration was determined with bovine serum albumin as the standard (31).

RESULTS

Stepwise transduction of the mutant *fuc* locus and the suppressor into a wild-type F$^-$ host. The propanediol-positive and fucose-negative strain ECL3 [*fucO*(Con) *fucA*(Con) *fucPIK*(Non)] was selected from strain ECL1, a λ lysogen of strain HfrC (40). Strain ECL56 was selected from strain ECL3 on fucose. In this strain, an unlinked suppressor (eventually designated as *crp-201* on the basis of the data presented below) allowed the constitutive expression of *fucPIK*(Non) (9, 14, 22). To facilitate genetic analysis, which required the lysogenization of the test strains by specially constructed λ phages, the [*fucO*(Con) *fucA*(Con) *fucPIK*(Non)] locus and the suppressor were sequentially transferred to an F$^-$ nonlysogen. First, the *fuc* locus of strain ECL3 was transduced to ECL116 by selection for growth on propanediol. Acquisition of [*fucO*(Con) *fucA*(Con) *fucPIK*(Non)] by the transductant, strain ECL701, was confirmed by the constitutive synthesis of propanediol oxidoreductase and fuculose 1-phosphate aldolase and the noninducibility of fucose isomerase (Table 2). The suppressor was then transduced from strain ECL56 into strain ECL701 to give strain ECL702. This was accomplished by selecting for growth on D-arabinose, a structural analog of fucose that can be utilized only when the proteins in the fucose pathway are present (26). Acquisition by strain ECL702 of the suppressor was likewise confirmed by enzyme analysis (Table 2; data not shown). Although the suppressor in the F$^-$ strain ECL702 allowed expression of *fucPIK*, expression of *fucA*(Con) and *fucO*(Con) was slightly lowered (Table 2). This is true also in the Hfr background (9, 22).

Proper expression of *fuc*$^+$ prevented by the suppressor. The suppressor, which allowed constitutive expression of *fuc PIK*(Non) in strain ECL702, surprisingly interfered with the expression of the *fuc*$^+$ regulon. This unexpected result was revealed by a transduction experiment in which strain ECL702 [*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp-201*] served as the recipient and strain ECL338 (*fuc*$^+$ *argA*::Tn*10*) served as the donor. Transductants were selected for *argA*::Tn*10*

TABLE 2. Activities of fucose enzymes in strains with different *crp* and *fuc* alleles

Strain	Relevant genotype or phenotype	Presence of fucose in growth medium	Relative activity (U)[a]			
			Fucose permease	Fucose isomerase	Fuculose 1-phosphate aldolase	Propanediol oxidoreductase[b]
ECL116	*fuc*$^+$ *crp*$^+$	−	2	3	<3	<2
		+	100	100	100	100
ECL701	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp*$^+$	−	3	1	180	510
		+	3	7	180	590
ECL702	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp-201*	−	70	48	37	110
		+	100	66	66	370
ECL367	*fuc*$^+$ *crp-201* Fuc^s	−	18	21	<3	<2
		+	160	160	15	60
ECL731	*fuc*$^+$ Δ*crp-39* Fuc$^-$	+	2	4	<3	<2

[a] The enzyme activities were normalized with those of the induced levels of strain ECL116. These standard specific activities (in units) are as follows: fucose permease, 33; fucose isomerase, 1,200; fuculose 1-phosphate aldolase, 41.

[b] Because the enzyme protein synthesized aerobically by *fuc*$^+$ cells was not fully active catalytically (see reference 10 for a review) the propanediol oxidoreductase activities of the *fuc*$^+$ strains ECL116, ECL367, and ECL731 were determined anaerobically and normalized with that of the induced level of ECL116 (specific activity, 1,700). The activities of *fucO*(Con) *fucA*(Con) *fucPIK*(Non) strains of ECL701 and ECL702 were determined with the cells grown aerobically and normalized with that of the aerobic induced level of ECL116 (specific activity, 150).

(about 50% linked to *fuc*$^+$). When the Tc^r transductants were analyzed, close to half (46%) appeared to have retained their original [*fucO*(Con) *fucA*(Con) *fucPIK*(Non)] locus on the basis of their remaining both fucose and propanediol positive. Close to half (42%) of the transductants, e.g., ECL367, appeared to have inherited the *fuc*$^+$ locus, since they lost the ability to grow on propanediol. However, most of the propanediol-negative transductants (88%) became fucose negative, despite the fact that both the donor and the recipient strains were fucose positive. Moreover, growth of these fucose-negative strains was actually inhibited by fucose when tested with glycerol as the carbon and energy source. A minority of transductants (12%) showed phenotypes indicative of crossovers within the *fuc* loci (Table 3). The results indicated that expression of the acquired *fuc*$^+$ was hindered by the resident suppressor. This interpretation was supported by a reciprocal transduction experiment in which the suppressor was introduced from strain ECL706 (*crp-201 zhd-4*::Tn*10*) into the Fuc$^+$ strains ECL1 and ECL116 using a linked Tn*10* as the selected marker. Again, despite the fact that both the donor and the recipient strains were fucose positive, significant fractions of Tc^r transductants (about 20%) lost the ability to grow on fucose and became fucose sensitive.

Impairment of *fucA*$^+$ expression by the suppressor. Since growth of mutants lacking fuculose 1-phosphate aldolase activity can be inhibited by fucose (37), an enzymic analysis was carried out to determine whether the fucose sensitivity of the transductant ECL367 (*fuc*$^+$ *crp-201*) was caused by that deficiency. Table 2 shows that the suppressor strongly impaired aldolase induction in strain ECL367. The induction of *fucO* was also reduced.

Results from additional experiments not presented in the table showed that this effect was even more dramatic in an Hfr background. In all transductants of strain ECL1 receiving the suppressor by selection of the linked *zhd-4*::Tn*10*, the aldolase activity was reduced more than 90% and the oxidoreductase activity was reduced more than 25%. We have not determined which element in the genetic background was critical for the degree of impairment of the expression of *fucA* and *fucO* by the suppressor.

The suppressor maps at *crp*. Preliminary mapping by conjugation of the Tn*10* linked to the suppressor indicated that the mutation was between *xyl* (80 min) and *argE* (70 min) on the chromosome (2). Transductions were then carried out to refine the mapping. In a cross with strain SY777 (*fuc*$^+$ and bearing a Tn*10* at min 76) as the donor and strain ECL605 (*fuc*$^+$ *crp-201*) as the recipient, 1% of Tc^r transductants lost the suppressor and became fucose positive (Table 3). In a cross with strain ECL101 (*fuc*$^+$ and bearing *rpsL* at min 73) as the donor and strain ECL605 as the recipient, 52% of Sm^r transductants lost the suppressor and became fucose positive.

The placement of the suppressor in the region of min 73 to 76 raised the possibility that this mutation occurred in *crp*, which is at min 74 and encodes the cyclic AMP (cAMP)-binding (or receptor) protein (CRP). This possibility was tested by introducing a copy of *crp*$^+$ into the fucose-negative strain ECL367 (*fuc*$^+$ *crp-201*) by the phage λY1079 (17). This phage bears the complete *crp*$^+$ gene in its 3.5-kilobase insert. Because of the *int* mutation in the phage, it integrates into and excises from the chromosome only by homologous recombination within the insert. In 10 lysogens tested, among which was strain ECL720, all were restored in their ability to grow on fucose. These results made it likely that the suppressor was a missense allele (*crp-201*) of the *crp* gene. Since the lysogens were expected to bear both the *crp*$^+$ and the *crp-201* allele, the former appears to be dominant over the latter in the *fuc*$^+$ background.

Genetic evidence for *crp* merodiploidy in a λY1079 lysogen of strain ECL367. The presence of both the *crp*$^+$ and the *crp-201* alleles in strain ECL720, a λY1079 lysogen of strain ECL367, was demonstrated by analyzing the phage progeny of strain ECL720. The heat-induced phage particles were used to infect strain ECL367. Among 16 resulting lysogens analyzed, 6 were fucose positive (expected to be *fuc*$^+$ *crp-201*/λ *crp*$^+$) and 10 were fucose negative (expected to be *fuc*$^+$ *crp-201*/λ *crp-201*). Strain ECL730, one of the fucose-negative lysogens, was in turn heat induced. The lysate produced only fucose-negative lysogens with strain ECL367, thus confirming that the phage crop contained homogeneous λZ1 (*crp-201*).

Dominance tests of *crp*$^+$ and *crp-201* on fucose utilization by strains with different *fuc* backgrounds. The growth rate on fucose was used to test the relative importance of the two *crp* alleles in *fuc*$^+$ and [*fucO*(Con) *fucA*(Con) *fucPIK*(Non)] backgrounds. In the *fuc*$^+$ background, *crp*$^+$ was dominant over *crp-201*, but in the [*fucO*(Con) *fucA*(Con) *fucPIK*(Non)] background, the two *crp* alleles were essentially codominant (Table 4). Control experiments showed that growth on rhamnose was relatively indifferent to the *crp* background.

Dependence of *fuc*$^+$ expression on *crp*$^+$. In contrast to the effect of the missense *crp-201* allele, a deletion in *crp* (Δ*crp-39*) virtually prevented the expression of all the *fuc* operons in the female strain ECL731 and the Hfr strain ECL616 (Table 2; data not shown).

Specificity of the *crp-201* mutation on sugar utilization. Three isogenic *fuc*$^+$ strains, ECL116 (*crp*$^+$), ECL367 (*crp-201*), and ECL720 (*crp-201*/*crp*$^+$) were compared for their growth on various sugars as the sole source of carbon and

TABLE 3. Transduction of the suppressor mutation and its fucose phenotype in wild-type strains

Donor	Recipient	Marker selected	Markers scored	% Cotransduction of unselected marker (no. cotransduced/total no. of colonies)
ECL338 (*argA*::Tn*10*)	ECL702 [*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp-201*]	Tc^r	Fuc$^+$ Prd$^+$	46 (23/50)
			Fuc$^-$ Prd$^-$	42 (21/50)
			Fuc$^+$ Prd$^-$	6 (3/50)
			Fuc$^-$ Prd$^+$	6 (3/50)
ECL706 [*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp-201 zhd-4*::Tn*10*]	ECL1 (*fuc*$^+$)	Tc^r	Fuc$^-$	16 (22/138)
ECL706	ECL116 (*fuc*$^+$)	Tc^r	Fuc$^-$	23 (11/48)
SY777 (*crp*$^+$ *zhf*::Tn*10*)	ECL605 (*fuc*$^+$ *crp-201*)	Tc^r	Fuc$^+$	1 (2/170)
ECL101 (*crp*$^+$ *rpsL*)	ECL605 (*fuc*$^+$ *crp-201*)	Sm^r	Fuc$^+$	52 (50/96)

TABLE 4. Growth on fucose and rhamnose as a function of *crp* alleles

Strain	Relevant genotype	Doubling time (h) on[a]:	
		Fucose	Rhamnose
ECL727	*fuc*$^+$ *crp*$^+$	3.5	6.5
ECL367	*fuc*$^+$ *crp-201*	>18.0[b]	7.5
ECL720	*fuc*$^+$ *crp-201*/λ *crp*$^+$	5.0	8.0
ECL701	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp*$^+$	>40.0	4.0
ECL726	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp*$^+$/λ *crp-201*	14.0	4.5
ECL725	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp*$^+$/λ *crp*$^+$	>40.0	3.0
ECL702	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp-201*	3.0	5.5
ECL728	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp-201*/λ *crp*$^+$	8.0	3.5
ECL729	*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp-201*/λ *crp-201*	4.0	4.5

[a] The minimal medium contained fucose or rhamnose at 0.2%, and the cultures were agitated on a rotary shaker at 30°C.
[b] Fucose-positive revertants occurred after 18 h of incubation.

energy (Fig. 2). The most striking defect of strain ECL367 was its failure to grow on fucose. Growth on maltose was significantly impaired, but no remarkable effects were observed with glucose, rhamnose, L-arabinose, galactose, xylose, glycerol, or glycerol 3-phosphate. The merodiploid strain ECL720 unexpectedly gave colonies larger than those of the wild-type strain ECL116 on all the carbon sources tested except glucose and fucose. In a control experiment, strains ECL731 (F$^-$ Δ*crp-39*) and ECL616 (HfrΔ*crp-39*) grew on glucose but not on fucose, rhamnose, maltose, L-arabinose, or galactose (data not shown).

DISCUSSION

Catabolite-repressible operons differ in their dependence on the cAMP-CRP concentration. In *E. coli*, higher cAMP levels are required for expression of the *ara* (encoding the L-arabinose pathway) and the *tnaA* operon (encoding tryptophanase) than for expression of the *lac* operon (30, 33). In vitro binding studies indicated the affinities for cAMP-CRP in the order of *lac* > *malT* > *gal* (25). Even in the absence of cAMP (mutants deleted in adenyl cyclase), the *lac* operon can be transcribed at levels 2 to 3% that of wild-type cells (7). In *Salmonella typhimurium*, progressively higher levels of cAMP were required for the utilization of succinate, citrate, glycerol, and ribose (1). Variations in the dependence on the cAMP-CRP complex were also observed among operons belonging to the same regulon. For instance, in an *E. coli* mutant lacking the specific repressor, glucose repressed *glpK* (encoding glycerol kinase) about 20-fold, *glpT* (encoding glycerol 3-phosphate permease) about 4-fold, and *glpD* (encoding aerobic glycerol 3-phosphate dehydrogenase) about 2-fold. The glucose repression was largely relieved by external cAMP (18). In the maltose system, the *lamB*, *malK*, and *malEFG* operons encoding the transport system are more dependent for their expression on the cAMP-CRP system than the *malPQ* operon encoding amylomaltase and maltodextrin phosphorylase (36). Results from our study suggest that the CRP-protein binding sites of the *fuc* operons also differ considerably in their affinity for the activator.

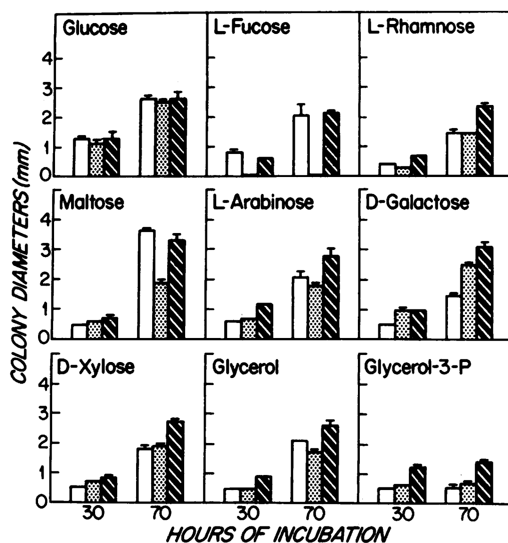

FIG. 2. Growth of *crp*$^+$, *crp-201*, and *crp-201*/λ *crp*$^+$ strains on different carbon sources. Cells of each strain were diluted and plated on a section of a minimal agar plate supplemented with 0.2% of the carbon source indicated. After 30 and 70 h of incubation at 30°C, the diameters of 10 colonies were measured. □, Strain ECL116 (*fuc*$^+$ *crp*$^+$); ▩, strain ECL367 (*fuc*$^+$ *crp-201*); ▧, strain ECL730 (*fuc*$^+$ *crp-201*/λ*crp*$^+$). Glycerol-3-P, Glycerol-3-phosphate.

Mutations in *crp* can affect the expression of catabolite-repressible operons in highly specific ways. A *crp* mutation selectively impaired the expression of *uidA*, encoding β-glucuronidase (41). One mutant *crp* product actually allowed a level of *lac* expression exceeding that observed with the *crp*$^+$ product (16, 17). Mutations in *crp* that enabled the activator protein to interact with cGMP in addition to cAMP were also observed (35).

The data from this study indicate that a mutation in *crp* can affect the expression of the *fuc* operons differentially. It should be borne in mind, however, that the identification of the suppressor as a mutant allele of *crp* is largely based on complementation tests with a 3.5-kilobase insert containing *crp*$^+$. It is remotely possible that an unknown gene very close to *crp* was involved in this complementation. Thus the designation of the *crp-201* mutation should be regarded as tentative. Nonetheless, several features of this mutation might be underscored. First, although the inducibility of *fucA* and *fucO* was impaired, the inducibility of *fucPIK* was enhanced (possibly because of fuculose 1-phosphate accumulation). Second, among the different catabolite pathways tested, only fucose utilization was virtually blocked. The symptom might have been aggravated by fuculose 1-phosphate accumulation resulting from overexpression of *fucPIK* and underexpression of *fucA*. The addition of 5 mM cAMP or cGMP to the growth medium did not significantly change the induction pattern (results not shown). The mutation also reduced the growth rate on maltose, although this effect was

not severe. Third, in the [*fucO*(Con) *fucA*(Con) *fucPIK*(Non)] strain, the *crp-201* mutation allowed expression of *fucPIK* but lowered the constitutive expression of *fucA* and *fucO* (compare strains ECL701 and ECL702 in Table 2). The coupled changes in the expression of *fucA* and *fucO* in strains with the wild-type and mutant *fuc* loci support the hypothesis that the promoters of these two divergently transcribed operons overlap (12). Fourth, the crp^+ allele seems more dominant over the *crp-201* allele in the fuc^+ background (the merodiploid showed an essentially normal growth rate on fucose) than in the [*fucO*(Con) *fucA*(Con) *fucPIK*(Non)] background (the *crp-201*-dependent growth on fucose was not severely curtailed by crp^+).

The mechanistic basis for suppression of *fucPIK*(Non) by *crp-201* remains unclear. At first we suspected that during selection for growth on propanediol a change occurred in *fucR*. The altered *fucR* would constitutively activate *fucO* and *fucA* but fail to activate *fucPIK*. The suppressor *crp-201* would then compensate for the deficiency of the altered *fucR* so that *fucPIK*(Non) could be transcribed. This *fucR*-mutation model, however, failed to accommodate certain additional observations. Deletions in strain ECL56 [*fucO*(Con) *fucA*(Con) *fucPIK*(Non) *crp-201*] from *argA* extending into *fucPIK*—and therefore excising *fucR*—did not affect the constitutive expression of *fucA* and *fucO* (Y. Zhu, unpublished data). Furthermore, *fucPIK*(Non) remained defective in its expression even when a multicopy plasmid bearing $fucR^+$ was introduced into strain ECL3 (Y.-M. Chen, personal communication).

An alternative model involving the participation of the wild-type promoter region of *fucO-fucA* in the expression of *fucPIK* can be proposed (E. C. C. Lin, Y.-M. Chen, and Y. Zhu, FEMS Microbiol. Lett., in press). For instance, a DNA loop structure with the promoter regions of *fucPIK* and *fucO-fucA* held together by the *fucR*-encoded protein may be required for transcription initiation. Mutations that render the expression of *fucO* (and *fucA*) constitutive prevent the formation of this loop structure which is necessary for *fucPIK* expression. The *crp-201*-encoded protein permitted *fucPIK*(Non) to be expressed in the linear form. Work is in progress to test the model.

ACKNOWLEDGMENTS

We thank Y.-M. Chen for helpful discussions and Alison Ulrich for editorial assistance.

This work was supported by grant DMB8314312 from the National Science Foundation and Public Health Service grant 5-R01-GM11983 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Alper, M. D., and B. N. Ames.** 1978. Transport of antibiotics and metabolite analogs by systems under cyclic AMP control: positive selection of *Salmonella typhimurium cya* or *crp* mutants. J. Bacteriol. **133**:149–157.
2. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
3. **Backman, K., Y.-M. Chen, and B. Magasanik.** 1981. Physical and genetic characterization of the *glnA-glnG* region of the *Escherichia coli* chromosome. Proc. Natl. Acad. Sci. USA **78**: 3743–3747.
4. **Bartkus, J. M., and R. P. Mortlock.** 1986. Isolation of a mutation resulting in constitutive synthesis of L-fucose catabolic enzymes. J. Bacteriol. **165**:710–714.
5. **Berman, M. L., L. W. Enquist, and T. J. Silhavy.** 1981. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Bochner, B., H.-C. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selections for loss of tetracycline resistance. J. Bacteriol. **143**:926–933.
7. **Brickman, E., L. Soll, and J. Beckwith.** 1973. Genetic characterization of mutations which affect catabolite-sensitive operons in *Escherichia coli*, including deletions of the gene for adenyl cyclase. J. Bacteriol. **116**:582–587.
8. **Chakrabarti, T., Y.-M. Chen, and E. C. C. Lin.** 1984. Clustering of genes for L-fucose dissimilation by *Escherichia coli*. J. Bacteriol. **157**:984–986.
9. **Chen, Y.-M., T. Chakrabarti, and E. C. C. Lin.** 1984. Constitutive activation of L-fucose genes by an unlinked mutation in *Escherichia coli*. J. Bacteriol. **159**:725–729.
10. **Chen, Y.-M., E. C. C. Lin, J. Ros, and J. Aguilar.** 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.
11. **Chen, Y.-M., Y. Zhu, and E. C. C. Lin.** 1987. NAD-linked aldehyde dehydrogenase for aerobic utilization of L-fucose and L-rhamnose by *Escherichia coli*. J. Bacteriol. **169**:3289–3294.
12. **Chen, Y.-M., Y. Zhu, and E. C. C. Lin.** 1987. The organization of the *fuc* regulon specifying L-fucose dissimilation in *Escherichia coli* as determined by gene cloning. Mol. Gen. Genet. **210**:331–337.
13. **Chumley, F. G., R. Menzel, and J. R. Roth.** 1979. Hfr formation directed by Tn*10*. Genetics **91**:639–655.
14. **Cocks, G. T., J. Aguilar, and E. C. C. Lin.** 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.
15. **Dische, Z., and E. Borenfreund.** 1951. A new spectrophotometric method for the detection and determination of keto sugars and trioses. J. Biol. Chem. **192**:583–587.
16. **Ebright, R. H., P. Cossart, B. Gicquel-Sanzey, and J. Beckwith.** 1984. Molecular basis of DNA sequence recognition by the catabolite gene activator protein: detailed inferences from three mutations that alter DNA sequence specificity. Proc. Natl. Acad. Sci. USA **81**:7274–7278.
17. **Ebright, R. H., P. Cossart, B. Gicquel-Sanzey, and J. Beckwith.** 1984. Mutations that alter the DNA sequence specificity of the catabolite gene activator protein of *E. coli*. Nature (London) **311**:232–235.
18. **Freedberg, W. B., and E. C. C. Lin.** 1973. Three kinds of controls affecting the expression of the *glp* regulon in *Escherichia coli*. J. Bacteriol. **115**:816–823.
19. **Garges, S., and S. Adhya.** 1985. Sites of allosteric shift in the structure of the cyclic AMP receptor protein. Cell **41**:745–751.
20. **Ghalambor, M. A., and E. C. Heath.** 1962. The metabolism of L-fucose. II. The enzymatic cleavage of L-fuculose-1-phosphate. J. Biol. Chem. **237**:2427–2433.
21. **Green, M., and S. S. Cohen.** 1956. The enzymatic conversion of L-fucose to L-fuculose. J. Biol. Chem. **219**:557–568.
22. **Hacking, A. J., and E. C. C. Lin.** 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.
23. **Hacking, A. J., and E. C. C. Lin.** 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.
24. **Heath, E. C., and M. A. Ghalambor.** 1962. The metabolism of L-fucose. I. The purification and properties of L-fuculose kinase. J. Biol. Chem. **237**:2423–2426.
25. **Kolb, A., A. Spassky, C. Chapon, B. Blazy, and H. Buc.** 1983. On the different binding affinities of CRP at the *lac*, *gal* and *mal*T promoter regions. Nucleic Acids Res. **11**:7833–7852.
26. **LeBlanc, D. J., and R. P. Mortlock.** 1971. Metabolism of D-arabinose: a new pathway in *Escherichia coli*. J. Bacteriol. **106**:90–96.
27. **Lin, E. C. C.** 1976. Glycerol dissimilation and its regulation in bacteria. Annu. Rev. Microbiol. **30**:535–578.
28. **Lin, E. C. C.** 1987. Dissimilatory pathways for sugars, polyols, and carboxylates, p. 244–284. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*:

cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
29. **Lin, E. C. C., and T. T. Wu.** 1984. Functional divergence of the L-fucose system in mutants of *Escherichia coli*, p. 135–164. *In* R. P. Mortlock (ed.), Microorganisms as model systems for studying evolution. Plenum Publishing Corp., New York.
30. **Lis, J. T., and R. Schleif.** 1973. Different cyclic AMP requirements for induction of the arabinose and lactose operons of *Escherichia coli*. J. Mol. Biol. **79:**149–162.
31. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
32. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
33. **Piovant, M., and C. Lazdunski.** 1975. Different cyclic adenosine 3′,5′-monophosphate requirements for induction of beta-galactosidase and tryptophanase. Effect of osmotic pressure on intracellular cyclic adenosine 3′,5′-monophosphate concentrations. Biochemistry **14:**1821–1825.
34. **Sabourin, D., and J. Beckwith.** 1975. Deletion of the *Escherichia coli crp* gene. J. Bacteriol. **122:**338–340.
35. **Sanders, R., and D. McGeoch.** 1973. A mutant transcription factor that is activated by 3′:5′-cyclic guanosine monophosphate. Proc. Natl. Acad. Sci. USA **70:**1017–1021.
36. **Schwartz, M.** 1987. The maltose regulon, p. 1482–1502. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
37. **Skjold, A. C., and D. H. Ezekiel.** 1982. Analysis of lambda insertions in the fucose utilization region of *Escherichia coli* K-12: use of λ *fuc* and λ *argA* transducing bacteriophages to partially order the fucose utilization genes. J. Bacteriol. **152:** 120–125.
38. **Skjold, A. C., and D. H. Ezekiel.** 1982. Regulation of D-arabinose utilization in *Escherichia coli* K-12. J. Bacteriol. **152:**521–523.
39. **Sridhara, S., and T. T. Wu.** 1969. Purification and properties of lactaldehyde dehydrogenase in *Escherichia coli*. J. Biol. Chem. **244:**5233–5238.
40. **Sridhara, S., T. T. Wu, T. M. Chused, and E. C. C. Lin.** 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.
41. **Stoeber, F., A. Lagarde, G. Nemoz, G. Novel, M. Novel, R. Portalier, J. Pouyssegur, and J. Robert-Baudouy.** 1974. Le métabolisme des hexuronides et des hexuronates chez *Escherichia coli* K 12: aspects physiologiques et génétiques de sa régulation. Biochimie **56:**199–213.
42. **Tanaka, S., S. A. Lerner, and E. C. C. Lin.** 1967. Replacement of a phophoenolpyruvate-dependent phosphotransferase by nicotinamide adenine dinucleotide-linked dehydrogenase for the utilization of mannitol. J. Bacteriol. **93:**642–648.
43. **Zhu, Y., and E. C. C. Lin.** 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.