Mutations Affecting Gluconate Catabolism in *Escherichia coli*. Genetic Mapping of the Locus for the Thermosensitive Gluconokinase

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An *Escherichia coli* strain unable to use gluconate was isolated by spontaneous curing of λ cI857 *s7 xis6 b515 b519*, λ cI857 *s7* $\Delta(A-att)$ *dargI valS* lysogens. Two lesions, linked to *asd* and *pyrB* markers, respectively, were necessary to produce this phenotype. The *asd*-linked mutation *gnt-17*, of regulatory type, seems to affect the expression of the major system of gluconate utilization (min 75) as well as that of 6-phosphogluconate dehydratase (gene *edd*, min 41), the first enzyme of the Entner–Doudoroff pathway. A closely linked suppressor of *gnt-17* causes constitutivity of these activities; this suppressor resembles *gntR*, which is also in the *asd* region. Hence, it is possible that *gnt-17* is a super-repressing allele of *gntR*, rather than a positive controlling element. Lesion *gnt-17* alone does not prevent the utilization of gluconate; for this, the mutation *gnt-18* at 96.9 min is also necessary. This mutation abolishes the thermosensitive gluconokinase activity and thus eliminates the subsidiary ability to catabolize gluconate. Accordingly, *gnt-18* seems to be allelic with *gntV*, the locus postulated as being in the *pyrB* region specifying the thermosensitive gluconokinase.

INTRODUCTION

The initial steps of gluconate metabolism in *Escherichia coli* are its entry into the cell and its subsequent phosphorylation to 6-phosphogluconate (Cohen, 1951). 6-Phosphogluconate is catabolized primarily by the Entner–Doudoroff pathway, but also by the pentose phosphate pathway (Eisenberg & Dobrogosz, 1967; Fig. 1). The two enzymes of the Entner–Doudoroff pathway, 6-phosphogluconate dehydratase (EC 4.2.1.12) and 6-phospho-2-dehydrogluconate aldolase (EC 4.1.2.14), convert 6-phosphogluconate to pyruvate and glyceraldehyde 3-phosphate. The dehydratase as well as the gluconate uptake and gluconokinase activity is induced when the cells grow on gluconate (Cohen, 1951; Fraenkel & Levisohn, 1967; Nagel de Zwaig *et al.*, 1973; Pouysségur *et al.*, 1974); the aldolase is partially constitutive and possibly induced by its substrate (Fradkin & Fraenkel, 1971).

The induction in normal *E. coli* strains of two transport activities and two gluconokinases has been reported (Hung *et al.*, 1970). In both cases, the carriers and the enzymes displayed different K_m values for gluconate. Later genetic and physiological studies (Nagel de Zwaig *et al.*, 1973; Faik & Kornberg, 1973; Zwaig *et al.*, 1973) indicated that presumptive structural genes for high-affinity transport (*gntTa* or *usgA*; here denoted as *gntT*, see Terminology), low-affinity transport (*gntTb*; here denoted as *gntU*), gluconate phosphorylation (*gntK*) as well as a regulatory gene *gntR*, were located at the *bioH-malA-glpRD-asd* region of the chromosome, about min 75 of the map; moreover, it was shown that, while *gntT* maps between *bioH* and *malA* markers, *gntU*, *gntK* and *gntR* map in the *asd* region (Nagel de Zwaig *et al.*, 1973). Apparently the product of the *gntR* gene negatively controls the expression of these activities as well as that of 6-phosphogluconate dehydratase, coded by the *edd* gene at min 41 (Peyru & Fraenkel, 1968).

Abbreviations: BTB, bromothymol blue; KU, Klett units.

Fig. 1. Catabolism of gluconate in *E. coli* and the location of relevant markers on its linkage map. Arrows indicate the probable sites for *gntT*, *gntK* and *gntU* (Nagel de Zwaig *et al.*, 1973). Location of *gntV* according to the present paper. For gluconate symbols, see Terminology. Other symbols according to Bachmann (1983).

The two reported gluconokinases were differentiated *in vitro* by their heat sensitivities. The thermoresistant one (activity stable for 3 h at 30 °C) mapped in the 75.5 min region (hence designated *gntKr*; here denoted *gntK*). The other, thermosensitive one, lost 75% or more of its activity in those conditions; moreover, a mutation abolishing its activity, probably by altering its structural gene designated *gntKs* (here denoted *gntV*), mapped at the *pyrB* region, min 96.5 (Zigheboim & Nagel de Zwaig, 1973; R. Nagel de Zwaig and co-workers, unpublished results).

It was suggested that the 75 min region governed the main gluconate utilization pathway. Phenotypes affected in its expression were designated GntM⁻; the residual growth in a GntM⁻ mutant (linkage to *malA*) was abolished by a second mutation (*gntS*) in the 95.3 min region (33% linkage to *fdp*) suggested as specifying the uptake system of a subsidiary system also involved in transport and phosphorylation of gluconate. Mutant phenotypes of *gntS* were designated GntS⁻ (Bächi & Kornberg, 1975).

Because *E. coli* *bioH*-*asd* deleted mutants do not grow in mineral medium with gluconate (Nagel de Zwaig *et al.*, 1973), the above findings suggested that in the absence of this region, the putative subsidiary system of gluconate utilization might not be expressed. In favour of this hypothesis, the isolation in mineral medium with gluconate of spontaneous fermenting pseudorevertants from mutant HfrG6ΔMD2 [*Δ(bioH*-*asd)*] was reported (Istúriz *et al.*, 1979). One representative pseudorevertant, mutant C177, constitutively expressed (*ΔgntR*) the dehydratase (*edd*) and formed, when grown in media with gluconate, both a high-affinity transport for this substrate and the thermosensitive gluconokinase. The isolation of a different *bioH*-*asd* deleted mutant (strain M119), able to grow on mineral medium with gluconate but still unable to ferment this sugar on indicator plates, has also been reported (Wecksler-Albo & Vitelli-Flores, 1979). Mutant M119, as strain C177 (above), inducibly expressed the activities of the subsidiary system and was constitutive for the dehydratase. To explain its phenotype, it was assumed that the activity of some genetic element of the gluconate system, not included in the deletion (for example, *gntU*) might favour the induction of the subsidiary system. It was additionally shown that strain M119 (on indicator plates with gluconate) gives spontaneously

fermenting colonies which were constitutive just for the activities inducibly expressed in their parent. The lesion responsible for this constitutivity as well as for that involved in the positive phenotype of strain C177 were 65% and 76%, respectively, linked to *pyrB*.

The present paper offers elaboration and support for the model of two gluconate utilization systems in *E. coli*.

METHODS

Most of the media and methods have been described (Nagel de Zwaig *et al.*, 1973). Gluconate bromthymol blue indicator plates (BTB-gluconate) contained, per litre: 2.5 g Bacto peptone, 1.5 g yeast extract, 10 g calcium carbonate, 50 mg bromthymol blue, 15 g agar and 10 g potassium gluconate. Sugars were added to mineral media at 0.4%.

Organisms. Bacterial strains used are listed in Table 1.

Terminology. The genetic markers (Table 1) are those listed by Bachmann (1983). In reference to the symbols concerned with the initial gluconate catabolism, we have tried, preserving the symbol *gnt*, to rationalize them according to Demerec *et al.* (1966). The gene previously denoted as *gntTa* (Zwaig *et al.*, 1973) or *usgA* (Faik & Kornberg, 1973) is written as *gntT*. The *gntTb* locus specifying the low-affinity transport for gluconate (Zwaig *et al.*, 1973) and the *gntKr* gene coding the thermoresistant gluconokinase are written as *gntU* and *gntK*, respectively. Together, the products of the above-mentioned three genes constitute the main system GntM of gluconate utilization suggested by Bächli & Kornberg (1975). The locus *gntKs*, postulated as specifying the thermosensitive gluconokinase (Zighebato & Nagel de Zwaig, 1973), is written as *gntV*. The product of this gene and that of *gntS* (the subsidiary gluconate uptake activity; Bächli & Kornberg, 1975) compose the subsidiary system GntS of gluconate utilization. Because GntM and GntS have been used to denote phenotypes concerned with sets of genes (above) and the same symbols with the lower-case *g* have been utilized as specific genotypic designations (Bächli & Kornberg, 1975; Bachmann, 1983), we suggest the use of GntI (instead of GntM) and GntII (instead of GntS) as symbols for phenotypes concerned with the main and subsidiary systems respectively. In this context, the symbol *gntM* (Bachmann, 1983) does not seem suitable to denote transport and phosphorylation of gluconate at min 75, since this region includes three genes (*gntT*, *gntK* and *gntU*) involved in these activities (Zwaig *et al.*, 1973).

Growth of bacteria. Cells were grown aerobically at 37 °C in volumes of 15 ml in 125 ml flasks fitted with side arms, on a gyratory water bath shaker (model G76, New Brunswick) at about 200 cycles min⁻¹. For anaerobiosis, cultures were inoculated into standing capped tubes with similar diameters to Klett tubes, filled to capacity with the respective medium and sealed. In each case, the growth was monitored by reading the optical density in a Klett colorimeter with a no. 42 filter.

To determine the effect of pyruvate on the expression of the initial activities of gluconate catabolism, 200 ml of late-exponential-phase cells growing on mineral medium with 0.4% fructose were centrifuged (3000 r.p.m., SS34 Sorvall) and resuspended in 20 ml mineral medium. Samples were inoculated into 700 ml volumes, contained in 4 litre flasks of the same medium with 0.4% gluconate, to give $6-7 \times 10^7$ cells ml⁻¹ (approximately 30 Klett units – KU). The cultures were grown aerobically at 37 °C to 60 KU and divided in two. Pyruvate (10 mM) was then added to one-half portions of each culture and the incubations were continued for a further generation. Samples of the cells thus grown were harvested for assays of both [U-¹⁴C]gluconate uptake and activity of gluconokinase (Fig. 2).

Enzyme assays. Gluconokinase and 6-phosphogluconate dehydratase were assayed by the procedure of Fraenkel & Horecker (1964). The gluconokinase heat inactivation is reported in each case as the percentage of the gluconokinase activity lost as determined in 0.2 ml volumes of cell extract incubated at 30 °C for 3 h and compared to that of the same extract without treatment. Activities are reported as nmol min⁻¹ (mg protein)⁻¹.

Assay of [U-¹⁴C]gluconate uptake. Washed cells were suspended in 0.1 M-Tris/HCl buffer (pH 7.5) at a concentration of 3×10^8 cells ml⁻¹. Approximately 10^7 cells were incubated with 2×10^{-5} M-[U-¹⁴C]gluconate (specific activity 5.6 mCi (0.21 GBq) mmol⁻¹; 300 µl final volume) in the presence of chloramphenicol (50 µg ml⁻¹ final concentration) at 30 °C for 1 min. The cells were collected on a membrane filter (0.45 µm pore size; Millipore) and washed with suction with 15 ml of the same buffer. The filter was dried in a scintillation vial to which 2 ml scintillation fluid (4 g PPO and 0.05 g PPOP per litre of toluene) was added. The concentration of [U-¹⁴C]gluconate, 2×10^{-5} M, is the approximate *K_m* value of the high-affinity transport system for gluconate (Nagel de Zwaig *et al.*, 1973). The specific rates of gluconate uptake are expressed as c.p.m. taken up by 10^7 cells min⁻¹. The radioactivity was counted in an automatic scintillation spectrometer (model 3385, Packard Tri-Carb.).

Phages and specialized transductions. The preparation of λ phage lysates and specialized transductions were done according to Kikuchi *et al.* (1975). A stock of P1Kc phage kept in our laboratory was used for generalized transductions; P1Kc lysates were prepared according to Miller (1972).

Selection of *E. coli* mutants affected in gluconate catabolism. To isolate *E. coli* mutants with lesions at the *pyrB* region which might be affected in gluconate catabolism, strain CA16 (AD9 lysogen carrying the helper *λ*c1857 *s7 xis6 b515 b519* at the *metA* region, min 91; F. Santos & T. Istúriz, unpublished results) was transduced to Arg⁺ with a λ lysate obtained from strain AD12yK5, a λ lysogen carrying both the helper prophage and an

Table 1. *Strains of E. coli*

Strain*	Sex	Genotype†	Relevant gluconate phenotype‡	Phenotypes on plates			Origin
				BTB† 48 h	Minerals§ 24 h 48 h		
AD9	HfrH	<i>argF</i> $\Delta(gal-att\lambda-bio)$ <i>thiA</i> $\Delta argI$ <i>str</i>		Y	Gnt ⁺		A. Kikuchi
AD12yK5	HfrH	$\Delta(proA-argF-lac)$ <i>thiA</i> $\Delta argI$ $\Delta c1857$ <i>s7</i> <i>xis6</i> <i>b515</i> <i>b519</i> <i>lac1857</i> <i>s7</i> $\Delta(A-att)$ <i>dargI</i> <i>valS</i>		Y	Gnt ⁺		A. Kikuchi
HfrG6	HfrG	<i>his</i>		Y	Gnt ⁺		M. Schwartz
HfrG6 Δ MD2	HfrG	<i>his</i> $\Delta(bioH-asd)$	GntI ⁻ GntII ⁻ Edd ^c	W	Gnt ⁻	Gnt ⁻	M. Schwartz
MT18	HfrH	<i>malA</i> <i>asd</i> <i>str</i>		Y	Gnt ⁺		M. Schwartz
PA505	F ⁻	<i>glpD</i> <i>asd</i> <i>str</i>		Y	Gnt ⁺		M. Schwartz
M49	HfrC	<i>gnd</i>		Y	Gnt ⁺		This laboratory
M85	HfrC	<i>fdp</i> <i>gntK</i>	GntK ⁻	Y	Gnt ⁺	Gnt ⁺	R. Nagel de Zwaig
M88	F ⁻	<i>gal</i> <i>trp</i> <i>his</i> <i>malA</i> <i>xyl</i> <i>gntV</i> <i>str</i>	GntV ⁻	Y	Gnt ⁺		R. Nagel de Zwaig
M90	F ⁻	<i>leu</i> <i>proA</i> <i>lacZY</i> <i>xyl</i> <i>fdp</i> <i>pyrB</i> <i>argI</i> <i>str</i>		Y	Gnt ⁺		This laboratory
M120	F ⁻	<i>leu</i> <i>his</i> <i>argG</i> $\Delta(bioH-asd)$ <i>metB</i> <i>pyrB</i>	GntI ⁻ GntII ^c Edd ^c	Y	Gnt ⁺		M. Weckler
CA16	HfrH	AD9 ($\Delta c1857$ <i>s7</i> <i>xis6</i> <i>b515</i> <i>b519</i>)		Y	Gnt ⁺		This study
CA17a	HfrH	<i>argF</i> $\Delta gal-att\lambda-bio$ <i>gnt-17</i> <i>thiA</i> <i>argI</i> ⁺ <i>gntV18</i> <i>str</i>	GntI ⁻ GntV ⁻ Edd ⁻	W	Gnt ⁻	Gnt ⁻	This study
TI42	HfrG	<i>his</i> <i>gnt-17</i>	GntI ⁻ Edd ⁻	W	Gnt ⁻	Gnt ⁺	This study
TI43	F ⁻	<i>leu</i> <i>his</i> <i>argG</i> $\Delta(bioH-asd)$ <i>metB</i> <i>gntV18</i>	GntI ⁻ GntV ⁻ Edd ^c	W	Gnt ⁻	Gnt ⁻	This study
TI44	F ⁻	<i>leu</i> <i>his</i> <i>argG</i> <i>metB</i> <i>gntV18</i>	GntV ⁻	Y	Gnt ⁺		This study
CA26	HfrH	$\Delta(gal-att\lambda-bio)$ <i>gnt-20</i> <i>gntV18</i> <i>thiA</i> <i>str</i>	GntI ^c GntV ⁻ Edd ^c	Y	Gnt ⁺	Gnt ⁺	This study
TI52	HfrG	<i>his</i> <i>gnt-19</i>	GntI ^c Edd ^c	Y	Gnt ⁺	Gnt ⁺	This study
TI75	F ⁻	<i>leu</i> <i>lacZY</i> <i>gnt-17</i> <i>xyl</i> <i>fdp</i> <i>pyrB</i> <i>str</i>	GntI ⁻ Edd ⁻	W	Gnt ⁻	Gnt ⁺	This study
TI103	F ⁻	<i>gal</i> <i>trp</i> <i>his</i> <i>xyl</i> <i>gnt-17</i> <i>gntV</i> <i>str</i>	GntI ⁻ GntV ⁻ Edd	W	Gnt ⁻	Gnt ⁻	This study

* All the strains except M90 (*E. coli* B derivative) are *E. coli* K12 derivatives.

† For gluconate symbols, see Methods; ° = constitutive.

‡ Y (yellow) and W (white) indicate fermenting and nonfermenting phenotypes respectively, on BTB gluconate plates, as tested by streaking fresh colonies and scoring after 48 h incubation.

§ Gnt⁺ and Gnt⁻ indicate growth and no growth, respectively, on mineral agar plates with gluconate, as tested by streaking fresh colonies and scoring 24 h and 48 h incubations.

|| M120 is a spontaneous pseudorevertant from strain M119 (see Introduction) able to ferment gluconate.

argI valS transducing prophage. Because of the *att* deletions present on both genomes, the transducing phage and the recipient strain, we expected that some *argI*⁺ transductants might have integrated the transducing prophage at the *argI-valS* region of the recipient chromosome. The subsequent spontaneous curing of such lysogens might produce survivors with lesions at this region which, in the case of those affecting gluconate metabolism, might be detected on indicator plates with gluconate. The transduction frequency was 2×10^{-6} . Groups of 70 of these transductants were mixed in tubes containing 2 ml LB broth, cultivated overnight at 30 °C and screened for cured cells by plating volumes of 0.2 ml, appropriately diluted, on LB plates at 42 °C. The spontaneous curing frequency (survival at 42 °C/survival at 30 °C), determined according to Shimada *et al.* (1972), was 1.6×10^{-5} . Of these colonies, 117 out of 120 were ArgI⁺; unexpectedly, three of them were neither able to grow on mineral agar plates with gluconate nor to ferment this sugar on indicator plates. The study of one of these mutants, designated strain CA17a, is reported here. Supporting the idea that the above three colonies arose by curing of λ lysogens with prophages inserted into (or near) gluconate genes, was the finding that two out of 200 independent Arg⁺ lysogens investigated displayed a gluconate phenotype similar to that of strain CA17a. These two lysogens, not considered in detail in this paper, gave (by spontaneous curing) survivors which kept the gluconate phenotype of the parents.

Chemicals. Sodium [U-¹⁴C]gluconate, specific activity 5.6 mCi (0.21 GBq) mmol⁻¹, was obtained from Amersham. D-Gluconic acid (potassium salt), 6-phosphogluconic acid (trisodium salt), lactic dehydrogenase, pyrimidine nucleotides and most other chemicals were purchased from Sigma. Culture media were obtained from Difco.

RESULTS AND DISCUSSION

Characteristics of mutant CA17a

This mutant grew normally at 37 °C on mineral medium supplemented with glucose, maltose, fructose or glycerol but not with gluconate; other sugars were not tested. Table 2 shows that it lacked both gluconokinase and 6-phosphogluconate dehydratase and was substantially affected in its inducible capacity to take up gluconate. Colonies of strain CA17a were white on BTB-gluconate plates; however, it was possible to detect on such plates yellow fermenting colonies which arose spontaneously and always showed constitutive activities of the gluconate system. One representative pseudorevertant, mutant CA26, was constitutive for a high-affinity transport, the thermoresistant gluconokinase and the dehydratase (Table 2). Since phenotypes like that shown by mutant CA26 arise from mutations in just the *gntR* gene (min 75.5; Zwaig *et al.*, 1973; Bächli & Kornberg, 1975), the location of the suppressor mutation (*gnt-20*) in mutant CA26 of the CA17a phenotype was directly investigated in the *malA-asd* region. When gluconate mutant HfrG6ΔMD2 was transduced to *asd*⁺ with phage P1 grown in strain CA26, all the transductants (56/56) resulted in yellow colonies on BTB-gluconate plates; eight independent transductants investigated were constitutive for gluconate activities. These results suggested that the *gnt-20* mutation might be allelic with *gntR*. One possibility was that the high-affinity uptake systems induced in mutant CA17a and derepressed in strain CA26 were different and differently regulated, e.g. that in mutant CA17a perhaps specified by *gntS* and that in mutant CA26 coded by *gntT*, as tested below.

Mapping of lesions affecting the gluconate system in mutant CA17a

Since mutations in the *pyrB* region (96.5 min) are not sufficient to prevent growth on gluconate (unpublished results), we thought that mutant CA17a might be affected both in this region (considering its origin as an ArgI⁺ transductant of an *argF argI* strain) and, additionally, in the *bioH-asd* region (75 min). To examine this possibility, mating experiments using this mutant as donor and strains PA505 and M90 as recipients were performed, and the Gnt⁻ phenotype (see Table 1) of the recombinants was investigated (Table 3). Among the PA505 recombinants, as scored after 24 h incubation, the Gnt⁻ phenotype was most closely linked (55%) to *asd*; but with a further 24 h incubation most Gnt⁻ recombinants grew sufficiently to be then scored as Gnt⁺ on mineral plates with gluconate [although they still appeared as mutants (white) on BTB-gluconate indicator plates]. In the case of M90 recombinants, those which were Gnt⁻ on mineral plates at 48 h always contained the *pyrB* allele from the donor (data not shown). Despite the effect that the operating restriction in K × B crosses could have in the recombination frequencies detected (Boyer, 1964), this result, together with that of strain

Table 2. *Activities of gluconate catabolism*

Cells were cultivated on mineral medium with the indicated carbon source at 0.4% and collected during the exponential phase, then grown on 1% casein hydrolysate with 0.4% of the indicated carbon source up to 200 KU. For units, see Methods. HfrG6 was used as control.

Strain	Carbon source	[U- ¹⁴ C]Gluconate uptake	Gluconokinase*	6-Phosphogluconate dehydratase
HfrG6	Fructose	25	4	2
	Gluconate	1925	472 (24%)	197
HfrG6ΔMD2	Fructose	12	ND	280
	Gluconate	18	6	310
M120	Fructose	373	67 (86%)	161
	Gluconate	416	127 (90%)	36
CA17a†	Fructose	41	2	3
	Gluconate	398	3	10
TI42	Fructose	48	7	5
	Gluconate	414	39 (83%)	4
TI43†	Fructose	350	ND	346
	Gluconate	405	ND	NI
TI44	Fructose	53	ND	ND
	Gluconate	1256	216 (9%)	99
CA26	Fructose	1018	275 (0%)	181
	Gluconate	792	123 (0%)	132
TI52	Fructose	1200	741 (0%)	343
	Gluconate	911	742 (0%)	400

ND, Not detected; NI, not investigated.

* Percentages in parentheses indicate gluconokinase lability (percentage lost after 30 °C preincubation; see Methods).

† Cultivated only on mineral medium with 0.4% fructose and then grown as indicated above.

Table 3. *Mapping of lesions in mutant CA17a by conjugation*

The matings were interrupted at 120 min.

Donor/recipient	Selected markers	No. scored	Unselected markers (%)		
			<i>pyrB</i> ⁺	Gnt ⁻ *	
				24 h	48 h
CA17a/PA505	<i>asd</i> ⁺ <i>str</i>	107		55.1	2.8
	<i>glpD</i> ⁺ <i>str</i>	78		29.4	0.0
CA17a/M90	<i>proA</i> ⁺ <i>str</i>	96	16.6	2.0	1.0
	<i>leu</i> ⁺ <i>str</i>	112	28.5	0.8	0.0
	<i>fdp</i> ⁺ <i>str</i>	92	NI	2.1	2.1
	<i>pyrB</i> ⁺ <i>str</i>	112		0.0	0.0

NI, Not investigated.

* Numbers indicate percentages of recombinants which did not grow on mineral plates with gluconate as tested by streaking and scoring after 24 h and 48 h incubation.

PA505, supported the idea that mutant CA17a carried two lesions affecting gluconate metabolism, one in the *glpD*–*asd* region and another in the *pyrB* region, with inheritance of both needed for complete incapacity to use gluconate.

To locate the lesions more precisely, several P1 transductions were performed using CA17a as donor. Among the *asd*⁺ transductants obtained when strains PA505 and HfrG6ΔMD2 were recipients, 73% (60/82) and 100% (62/62) respectively, gave white nonfermenting colonies on BTB-gluconate, but they grew (Gnt⁺) on gluconate mineral plates in 48 h. Furthermore, with

Table 4. Transductional analysis of lesions at *pyrB* affecting gluconate catabolism

Donor/recipient	Selected marker	No. scored	Unselected markers (%)							
			<i>fdp</i> ⁺	<i>pyrB</i> ⁺	<i>gnt-18</i>	<i>fdp</i> ⁺ <i>gnt-18</i>	<i>fdp</i> <i>gnt-18</i>	<i>pyrB</i> ⁺ <i>gnt-18</i>	<i>pyrB</i> <i>gnt-18</i>	
CA17a/TI75	<i>pyrB</i> ⁺	128	53.1		45.3	23.3	21.8			
	<i>fdp</i> ⁺	101		49.5	15.8			15.8	0.0	
TI103/TI75	<i>pyrB</i> ⁺	145	<i>fdp</i> ⁺	<i>pyrB</i> ⁺	<i>gntV</i>	<i>fdp</i> ⁺ <i>gntV</i>	<i>fdp</i> <i>gntV</i>	<i>pyrB</i> ⁺ <i>gntV</i>	<i>pyrB</i> <i>gntV</i>	
	<i>fdp</i> ⁺	127	48.9		44.8	21.3	23.4			
				40.1	17.3			15.7	1.5	

Table 5. Transductional analysis of lesions at *asd* affecting gluconate catabolism

The nonfermenting (*gnt-17*) and constitutive (*gnt-19*) phenotypes were identified on TTZ-gluconate plates as indicated in the text.

Donor/recipient	Selected marker	No. scored	Unselected markers (%)							
			<i>malA</i> ⁺	<i>asd</i> ⁺	<i>gnt-17</i>	<i>gnt-19</i>	<i>asd</i> ⁺ <i>gnt-17</i>	<i>asd</i> <i>gnt-17</i>	<i>malA</i> ⁺ <i>gnt-17</i>	<i>malA</i> <i>gnt-17</i>
TI42/MT18	<i>malA</i> ⁺	48		56.2	33.3		33.3	0.0		
	<i>asd</i> ⁺	48	62.5		79.1				47.9	31.2
TI42/M88*	<i>malA</i> ⁺	113			31.8					
TI52/M88†	<i>malA</i> ⁺	292			0.3	34.2				

* Transductants *gnt-17 gntV* were scored as Gnt⁻ in 48 h.

† Ten recombinants with the constitutive phenotype were confirmed by gluconokinase assays.

strain M88 (*malA gntV*) as recipient, 31% (28/89) of the *malA*⁺ transductants were Gnt⁻ on gluconate mineral plates in 48 h. Thus, in the presence of *gntV* in the *pyrB* region, a mutation (here designated *gnt-17*) linked to *asd* in CA17a caused complete incapacity to use gluconate. On the other hand, when a *gnt-17 pyrB* recombinant (Gnt⁻ 24 h, Gnt⁺ 48 h) from the conjugation CA17a × M90 (above) was used, 47% (42/90) of the *pyrB*⁺ transductants were Gnt⁻ in 48 h. The lesion involved, linked to *pyrB* in CA17a and causing complete incapacity to utilize gluconate in the presence of *gnt-17*, was designated *gnt-18*.

To determine more precisely the location of the *gnt-18* mutation, the same lysate of phage P1 grown on CA17a was used to transduce a *gnt-17 pyrB fdp* recombinant (strain TI75). The order *fdp-pyrB-gnt-18* (Table 4) revealed by such three-factor mapping, and the cotransduction frequencies shown by mutation *gnt-18* with both *fdp* and *pyrB* markers, allowed us to locate (Wu, 1966) the investigated lesion at about min 96.9, i.e. between *valS* and *leuX* (see Fig. 1). This region was reported by Kikuchi *et al.* (1975) as containing the integration site for the λ parent prophage in lysogens which originated the *argI-valS* transducing phage used here to transduce strain CA16 to *argI*⁺ (see Methods). Because this transducing phage (*A argI valS att int xis6 cIS R; A* gene and the *att* region partially deleted) carries about 0.4 min of the *E. coli* chromosome in its left arm which does not include the *pyrB* gene, it is possible to assume, as was expected, that its integration and subsequent excision in the *valS-leuX* region of the *E. coli* genome could have caused the *gnt-18* lesion.

Study of lesions affecting the gluconate system in mutant CA17a

One of the recombinants (*gnt-17 bioH*⁺ *malA*⁺ *glpD*⁺ *asd*⁺) from the P1(CA17a) × HfrG6ΔMD2 transduction, designated strain TI42, was chosen to characterize the *gnt-17* mutation of CA17a. This strain, like all the recombinants assayed from the above transduction, showed white nonfermenting colonies on BTB-gluconate plates but grew slowly on mineral plates with gluconate (Gnt⁻ 24 h, Gnt⁺ 48 h). The lesion at 75 min in strain TI42 was confirmed by transduction of strain M88 (*malA gntV*) to *malA*⁺; 31.8% of the transductants were Gnt⁻ in 48 h (Table 5). To investigate the *gnt-18* lesion of CA17a, strain M120 [*Δ(bioH-asd) gntS^c gntV^c*]

Table 6. Doubling times (min) of strains of *E. coli*

Strain	Carbon source*:			
	Glucose		Gluconate	
	Aerobiosis	Anaerobiosis	Aerobiosis	Anaerobiosis
HfrG6 (wild-type)	63	132	94	152
CA17a (<i>gnt-17 gnt-18</i>)	88	150	NG	NG
TI42 (<i>gnt-17</i>)	78	128	158	NG
TI43 [$\Delta(bioH-asd)$ <i>gnt-18</i>]	144	190	NG	NG
TI44 (<i>gnt-18</i>)	78	157	134	196
TI52 (<i>gnt-19</i>)	85	135	92	164

NG, No growth.

* For conditions, see Methods.

was first transduced to *pyrB*⁺ from CA17a and a *Gnt*⁻ (48 h) recombinant TI43 was then transduced to *asd*⁺ with phage P1 grown on strain HfrG6; strain TI44 was a *bioH*⁺ *malA*⁺ *asd*⁺ transductant carrying the *gnt-18* mutation and gave 'wild-type' (yellow) phenotype on BTB-gluconate indicator plates. The lesion at *pyrB* in strains TI43 and TI44 was confirmed by transduction of the former strain to *malA*⁺ *asd*⁺ from CA17a. All the transductants investigated (48/48) were *Gnt*⁻ in 48 h.

Strains TI42 and TI44 grew normally on mineral medium with glucose under both aerobic and anaerobic conditions. When cultivated in this medium with gluconate, although both strains grew aerobically, only the mutant TI44 grew also anaerobically, although with a generation time slightly longer than the HfrG6 control (Table 6). Additionally, the levels of [¹⁴C]gluconate uptake and gluconokinase activity of mutant TI42 were lower than those in mutant TI44; furthermore, mutant TI42 did not express 6-phosphogluconate dehydratase, even when grown on mineral medium with gluconate (Table 2). These results and the fact that the gluconokinases in TI42 and TI44 were the thermosensitive (83% heat inactivated) and the thermoresistant (9% heat inactivated) ones, respectively, suggested that the two strains were using different activities to catabolize gluconate. Indeed, when strain TI42 was transduced to *His*⁺ with phage P1 grown on strain M49 (*gnd edd*⁺), approximately 50% of the *His*⁺ transductants tested were *gnd* and *Gnt*⁻ in 48 h, showing that growth on gluconate in this strain (lacking 6-phosphogluconate dehydratase) depended on 6-phosphogluconate dehydrogenase (*gnd*⁺). Furthermore, the failure to grow on gluconate anaerobically when only 6-phosphogluconate dehydrogenase is available confirms an earlier report (Fraenkel & Vinopal, 1973).

We also investigated the effect of pyruvate on the expression of initial activities of gluconate catabolism in strains TI42 and TI44 growing on gluconate. The rationale of this approach was based on the report (Bächi & Kornberg, 1975) that the two initial systems for gluconate catabolism respond differently to the presence of pyruvate, or any carbohydrate catabolized via this metabolite, in the growth medium. Fig. 2 shows that pyruvate had a marked effect on gluconokinase and gluconate uptake in strain TI42 but not in strain TI44.

Mutant TI43, carrying the *gnt-18* mutation as well as having a *bioH-asd* deletion, lacked gluconokinase activity and remained as its parent strain M120 constitutive for both the subsidiary high-affinity transport for gluconate and the 6-phosphogluconate dehydratase (Table 2). Since the unique gluconokinase activity detected in strain M120 is the thermosensitive one, these results pointed to the *gnt-18* lesion as being allelic with *gntV*, the locus postulated to be at the *pyrB* region (Zigheboim & Nagel de Zwaig, 1973) specifying the thermosensitive gluconokinase activity. In support of this hypothesis, a three-factor mapping of *gntV* from strain TI103, a *Gnt*⁻ (48 h) recombinant (*gnt-17 gntV*) from the transduction P1(CA17a) × M88, gave the order *fdp-pyrB-gntV* (Table 4); moreover, the *gntV* marker was similar to *gnt-18* in its linkage to *fdp* and *pyrB*, respectively.

Fig. 2 Effect of pyruvate on the expression of initial activities of gluconate catabolism in strains TI42 (a) and TI44 (b). Gluconate uptake (●, ▲) and gluconokinase activity (○, △) in the absence (●, ○) or presence (▲, △) of pyruvate from cultures grown as indicated in Methods are shown. The arrows indicate when the pyruvate was added.

Two separate but closely linked regions of the *E. coli* map have so far been involved in the subsidiary ability of this bacterium to utilize gluconate (interestingly, two separate but closely linked regions, near 75 min, govern the main system also). The work of Bächli & Kornberg (1975) pointed to the 95.3 min region as containing the locus responsible for the subsidiary activity of gluconate uptake. The results presented in this communication reveal the 96.9 min region as being involved in the expression of another subsidiary activity: the thermosensitive gluconokinase.

Pseudorevertants from mutant TI42

Strain TI42 gave mainly white, nonfermenting colonies on BTB-gluconate plates, but as with the double mutant CA17a, it was always possible to detect a few yellow, fermenting colonies which arose spontaneously. Twenty independent colonies of this type were investigated, and all of them constitutively expressed the activities of gluconate metabolism, as shown in Table 2 for the representative pseudorevertant TI52. Furthermore, strain TI52, unlike its parent TI42, grew on gluconate mineral medium under anaerobic conditions. Under aerobiosis it showed a shorter period of latency (0.5 h vs 3 h, data not shown) and a shorter generation time (92 min vs 150 min) than strain TI42 (Table 6). The gluconate mutation in strain TI42 (*gnt-17*), as shown above, was in the 75.5 min region of the chromosome, and transductional three-factor crosses with respect to *malA* and *asd* markers gave the order *malA*-*asd*-*gnt-17* (Table 5).

Because a known locus for gluconate constitutivity, *gntR*, is also in the *asd* region (Zwaig *et al.*, 1973), a three-factor mapping was also done with TI52 as donor to find out whether the pseudoreversion mutation in TI52 (*gnt-19*) might be allelic with *gntR*. The recipient was strain MT18; by contrast with the mapping with TI42, only a small fraction of the transductants (1/318 and 1/491 among the *malA*⁺ and *asd*⁺ transductants, respectively) inherited the *gnt-17* marker (as tested on tetrazolium indicator plates with gluconate), indicating high linkage between the *gnt-17* marker and its suppressor as if this might indeed be *gntR*.

The same lysate was also used to transduce strain M88 (*malA gntV*). Scoring was done on tetrazolium gluconate plates (as well as on mineral plates), since *gntV* gives pink colonies, *gntR* white colonies and *gnt-17* red colonies. The 31.8% linkage of *gnt-17* and *malA* in TI42 was

replaced by 34.2% linkage with *gnt-19* in TI52, and *gnt-17* was recovered at a very low frequency (0.3%, Table 5). These results also fit with the very close linkage of *gnt-17* and *gnt-19*.

As shown above, the *gnt-17* mutation seems to affect the major determinants of gluconate catabolism, the GntI system: high-affinity uptake, thermostable gluconokinase and the expression of 6-phosphogluconate dehydratase (gene *edd*). Although it seemed reasonable to speculate that *gnt-17* might affect a positive control element, its suppression by a mutation (*gnt-19*) resembling *gntR* and the close linkage of *gnt-17* to *gntR* lead us to wonder instead whether it might be a super-repressing allele of *gntR*. Studies with merodiploids will help to answer this question.

It is not obvious how thermoinduction of *λ*I857 *argI valS* lysogens resulted in survivors affected at two separate loci. The possibility that the lysogen from which CA17a derived had prophages at those two positions has been supported by the preliminary study of one Gnt⁻ (48 h), nonfermenting *λ* lysogen (see Methods). This lysogen, strain TI65, and colonies cured from it lacked gluconate activities. One representative cured colony studied, strain TF2, carried lesions linked to *pyrB* and *asd* markers, respectively, just as for mutant CA17a reported here (F. Santos & T. Istúriz, unpublished). The question therefore arises as to whether physiological 'homology' in gluconate pathways for the 75.5 and 96.5 min regions might not also reflect nucleic acid homology, allowing the *argI* phage to integrate at both positions.

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