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Genetic Control of Isocitrate Lyase Activity in *Escherichia coli*

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Isocitrate lyase (EC 4.1.3.1) plays a necessary role in the growth of *Escherichia coli* on acetate as sole carbon source (1). Pyruvate and phosphoenolpyruvate both inhibit the activity and repress the synthesis of this enzyme (5). Mutants in which the synthesis of isocitrate lyase is no longer subject to control by these C_3-compounds, and which thus form isocitrate lyase constitutively, have been obtained as secondary mutants from parent organisms which lack the ability either to carboxylate phosphoenolpyruvate (9) or to form phosphoenolpyruvate from pyruvate (5). It is the purpose of this communication to report the location on the genome of *E. coli* K-12 of genes which specify the structure (*icl*) and constitutivity (*iclR*) of isocitrate lyase.

After treatment of a variety of strains of *E. coli* with ethylmethane sulfonate (7) and selection with penicillin (4) for inability to grow on acetate, four independently isolated mutants were affected in their ability to form isocitrate lyase; the genotypes of these organisms are listed in Table 1. By periodic interruptions of conjugation, it was established that all the *icl* alleles lay about 2 min from the *argHBCE* gene cluster, indistinguishably close to the *metA* marker and about 1 min from *pgi* (3); all *icl* mutants were thus mapped at 78 min on the linkage map of Taylor and Trotter (8). The genes affected were located more precisely, with reference to the *metBF*, *argHBCE*, *metA*, and *pgi* markers, by phage *P1*kc-mediated transduction. All *icl* mutants were cotransducible with well over 80% frequency with *metA* (Fig. 1), which is in good agreement with the findings of Vanderwinkel and de Vlieghere (10). The occurrence of icl^+ recombinants from genetic crosses among different icl^- mutants (which differed also in their *met* or *arg* alleles) showed that these mutants were not identical. Thus, of 886 arg^+ transductants obtained from the cross *icl-1* × *icl-2*, 10 were icl^+; of 941 arg^+ from *icl-1* × *icl-3*, 13 were icl^+; and of 833 arg^+ from *icl-2* × *icl-3*, 2 were icl^+. Thus, these results also support the order of these three *icl* mutations given in Fig. 1; *icl-4* has not yet been located to this degree of precision.

Similar procedures were used for the location of the marker which conferred constitutivity of isocitrate lyase synthesis on a variety of mutants. Such *iclR* mutants were obtained from pps^- parents and were recognized by their ability to grow on lactate, but neither on pyruvate nor on acetate plus pyruvate as carbon sources; that isocitrate lyase was formed constitutively was confirmed by enzymatic assay of sonic extracts of such *iclR* mutants (6).

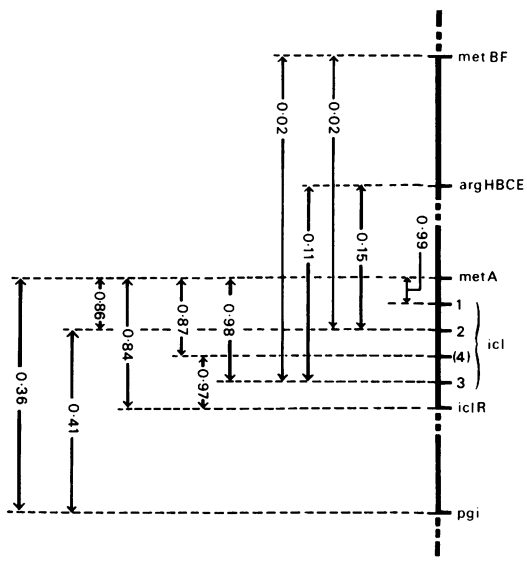

FIG. 1. *Genetic map of the icl region of the E. coli chromosome. The distances between markers are expressed as cotransduction frequencies.*

Like the *aceD* mutation, which confers constitutivity of glyoxylate cycle enzymes on *E. coli* (2), the *iclR* marker was closely linked both to *metA* and *icl*. To determine its position relative to *metA* and *icl*, the *iclR* and *metA* alleles were transduced from AT 2572-1-5^c (*iclR*, *pps*) into the recipient PA 505-1-5 (*metA*, *icl-4*, *pps*). Of 1,124 $metA^+$ transductants, 971 were also icl^+: of these latter, 939 had received both the *icl* and *iclR* markers, whereas 32 transductants formed

TABLE 1. *Characteristics of icl and iclR mutants*[a]

Strain	Derived from	Genetic markers	Response to streptomycin	Mating type
R4		*metBF*	S	Hfr
R4-5	R4	*metBF, icl-1*	S	Hfr
G6		*his, thy*	S	Hfr
G6-5	G6	*his, thy, icl-2*	S	Hfr
K8	AB 1911	*argHBCE, metBF*	R	F^-
K8-5a	[G6-5 × K8]	*arg, icl-2*	R	F^-
K8-5m	[G6-5 × K8]	*metBF, icl-2*	R	F^-
K8-5CB	K8	*argHBCE, metBF, icl-3*	R	F^-
AT 2572-1	AT 2572	*pps*	S	Hfr
AT 2572-1-5[c]	AT 2572-1	*pps, iclR*	S	Hfr
DF 11		*metA, pgi*	R	F^-
PA 505		*metA, argHBCE, pro, his*	R	F^-
PA 505-1	[AT 2572-1 × PA 505]	*metA, argHBCE, pps, pro*	R	F^-
PA 505-1-5	PA 505-1	*metA, argHBCE, pps, pro, icl-4*	R	F^-

[a] The symbols used indicate a requirement for *met* = methionine, *his* = histidine, *thy* = thymine, *arg* = arginine, *pro* = proline, or the absence of *icl* = isocitrate lyase, *iclR* = regulation of isocitrate lyase, *pps* = phosphoenolpyruvate synthase, *pgi* = phosphoglucose isomerase. R and S denote resistance and sensitivity to streptomycin; Hfr and F^- denote male (donor) and female (recipient) mating types.

isocitrate lyase inducibly. This phenomenon shows that the *metA* and *icl* markers can be cotransduced at high frequency, without simultaneously transducing *iclR*, and supports the order *metA . . . icl . . . iclR*.

Since the gene which specifies the second enzyme of the glyoxylate cycle, malate synthase A (*masA*), is also cotransducible with *metA* and *icl* at high frequency (the order being *metA . . . masA . . . icl*; 10), it is tempting to conclude that the structural and regulator genes of the glyoxylate cycle form an operon.

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