Autoregulation of *iclR*, the Gene Encoding the Repressor of the Glyoxylate Bypass Operon

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The *aceBAK* operon was partially induced by a multicopy plasmid which carried the promoter region of the gene which encodes its repressor, *iclR*. Gel shift and DNase I analyses demonstrated that IclR binds to its own promoter. Disruption of *iclR* increased the expression of an *iclR::lacZ* operon fusion. Although *aceBAK* and *iclR* are both regulated by IclR, *aceBAK* expression responds to the carbon source, while expression of *iclR* does not.

For *Escherichia coli*, adaptation to growth on acetate or fatty acids requires the induction of the glyoxylate bypass. This pathway, consisting of isocitrate lyase (*aceA*) and malate synthase (*aceB*), is essential for growth on these carbon sources because it prevents the quantitative loss of the entering carbon as CO_2 in the Krebs cycle (9, 11). Once induced, the flow of isocitrate through this pathway is controlled by the phosphorylation of isocitrate dehydrogenase (IDH), the Krebs cycle enzyme which competes with isocitrate lyase for isocitrate (1, 8, 15). This phosphorylation cycle is catalyzed by a bifunctional protein, IDH kinase/phosphatase (*aceK*) (12, 14).

The *aceBAK* operon is expressed from a single promoter during growth on acetate (2). Expression is regulated by a repressor protein encoded by *iclR* (10, 16, 19, 29). IclR binds to a site which overlaps the −35 region of the *aceBAK* promoter (2, 19). In this paper, we demonstrate that IclR also regulates its own expression.

***iclR* appears to compete with *aceBAK* for a common regulatory protein.** During a deletion analysis of a clone of the *iclR* gene, we obtained a surprising result: a truncated derivative of this gene activated expression of *aceBAK* under repressing conditions in an *iclR*⁺ background (Table 1). Further deletion analysis localized the region responsible for this effect to sequences between −45 and +3 relative to the start of translation. Activation of *aceBAK* expression was also observed in cells carrying the upstream region from *aceBAK* on a multicopy plasmid. These observations suggested that *iclR* and *aceBAK* were competing for a common repressor protein.

IclR binding to *iclR*. Examination of the region upstream of *iclR* revealed a site (−43 to −24 relative to the translational start site) which bears a strong resemblance to the binding site for IclR from *aceBAK* (Fig. 1). Gel shift analysis was used to test for IclR binding near this site. Purified IclR produced a single shifted band when added to a probe which contained the predicted IclR binding site of *iclR* (−152 to +3) (Fig. 2, lanes 2 and 3). DNA containing the IclR binding site from *aceBAK* prevented formation of this complex (Fig. 2, lanes 6 and 7).

The approximate location of the IclR binding site was determined by competition. An unlabeled fragment of *iclR* which included the predicted IclR binding site (−152 to +3) prevented the binding of IclR to the probe (Fig. 2, lanes 4 and 5). In contrast, an overlapping fragment of *iclR* which did not contain the predicted binding site (−152 to −45) failed to compete for binding (Fig. 2, lanes 8 and 9). Thus, sequences between −45 and +3 were required for effective competition.

The precise location of the IclR binding site on *iclR* was determined by footprint analysis with DNase I (Fig. 3). A single protected region was detected. This region corresponds to the proposed IclR binding site presented in Fig. 1. This site is within the region which was found to activate *aceBAK* expression when it was carried by a multicopy plasmid (see above).

Transcriptional start site for *iclR*. Primer extension analysis was used to determine whether the IclR binding site was near the transcriptional start site of *iclR*. Two major extension products were obtained (Fig. 4). It seems likely that the longer product resulted from the tendency of reverse transcriptase to add an extra nucleotide beyond the end of the RNA (6, 26). The location of the start site determined from the shorter product is shown in Fig. 4.

A match with the consensus sequence for σ^{70} promoters was found immediately upstream of the transcriptional start site. A sequence at −10 matched the consensus for −10 boxes at four of six positions, while sequences at −35 matched the −35 consensus at three of six positions. These regions were separated by 17 bp, which is consistent with the consensus separation of 17 ± 1 bp. The IclR binding site overlaps the −10 region of this possible promoter.

TABLE 1. Activation of *aceBAK* expression by multiple copies of the *iclR* promoter region

Insert[a]	Deletion endpoint[b] (nucleotide)	Amt of IDH phosphatase[c] (mU/mg)
None	NA	0.11 ± 0.04
iclR	+726	0.67 ± 0.15
	+377	0.64 ± 0.31
	+3	0.62 ± 0.08
	−45	0.16 ± 0.08
aceB	NA	0.85 ± 0.19

[a] The vector was pBR322. The plasmid carried either no insert or fragments of either *iclR* or *aceB*. The fragments of *iclR* included sequences between −1241 (relative to the translational start site) and the indicated endpoint. The fragment of *aceB* included sequences from −489 and +1213.

[b] Each position is the 3′ end of the fragment of *iclR* and is given relative to the translational start site. The structural gene includes 822 nt. NA, not applicable.

[c] Plasmids which carried the indicated inserts were introduced into strain SL1027 (*iclR*⁺). Cultures were grown on minimal glucose medium (repressing conditions) at 37°C with shaking. Mid-log-phase cultures were harvested and assayed for IDH phosphatase activity (13).

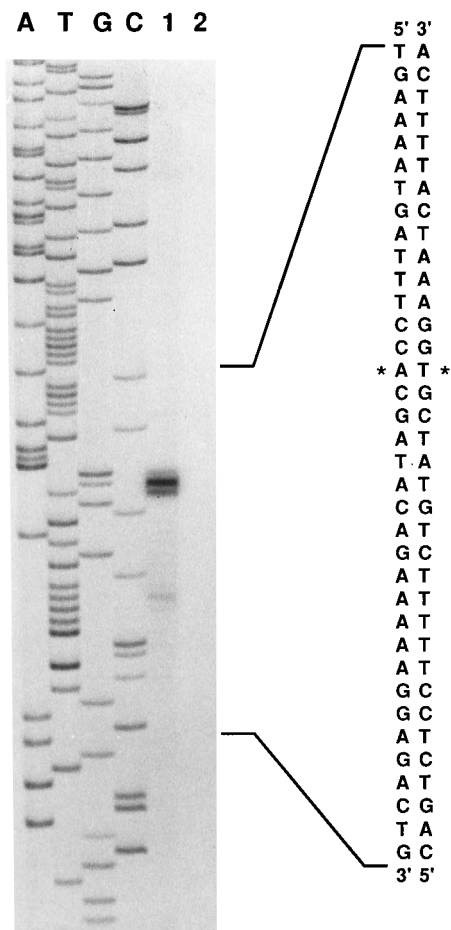* Corresponding author. Phone: (612) 625-4983. Fax: (612) 625-2163.

iclR

aceBAK

FIG. 1. Possible site for IclR binding upstream of *iclR*. The sequence of a region upstream of *iclR* (29) is compared with the binding site for IclR of *aceBAK*, which has been identified by footprint analysis (4). The region of *iclR* which was protected during DNase I footprint analysis (Fig. 3) is underlined. The transcriptional start site (Fig. 4) is indicated with an asterisk. Potential promoter elements are indicated ("−10" and "−35"). Arrows indicate the 3′ endpoints of the DNA fragments used for competition for Fig. 2.

IclR control of *iclR* expression. The observation that IclR appeared to bind to a site which overlaps its own promoter suggested that the gene encoding this protein is autogenously controlled. To test for autogenous repression, we created an *iclR::lacZ* operon fusion and inserted it at the *lac* locus of the chromosome. Disruption of *iclR* produced a substantial increase in expression of this fusion (Table 2), which is consistent with autogenous repression.

The repressive activity of IclR can be estimated by comparing levels of expression in the *iclR*[+] and *iclR* strains with a given carbon source. For example, disruption of *iclR* had a dramatic effect on IDH phosphatase expression during growth on glucose but had little effect on acetate (Table 2). Similar

FIG. 2. Binding of IclR near the *iclR* promoter. Binding of IclR to sequences upstream of *iclR* was tested by gel shift analysis. IclR was overexpressed and purified by a modification of the method described by Cortay et al. (4). The probe (1 ng) included sequences from −152 to +3 relative to the translational start site and was labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The binding reaction mixture included 10 mM Tris (pH 7.5), 1 mM EDTA, 50 mM NaCl, 5% glycerol, 1 mM dithiothreitol, and 2 μg of poly(dI-dC) in 30 μl. Following incubation for 30 min at 20°C, samples were applied to a 4.5% polyacrylamide gel. Lanes 1 to 3 included 0, 7, and 17 ng of IclR, respectively. All other lanes included 17 ng of IclR. Lanes 4 and 5, competition with 10 and 75 ng of unlabeled probe, respectively. Lanes 6 and 7, competition with 10 and 75 ng of sequences upstream of *aceBAK* (−118 to +9 relative to the start of transcription), respectively. Lanes 8 and 9, competition with 10 and 100 ng, respectively, of a fragment of *iclR* (−152 to −45) which does not contain the predicted IclR binding site. Lane 10, competition with 500 ng of salmon sperm DNA (ssDNA).

FIG. 3. Identification of the IclR binding site upstream of *iclR*. The IclR binding site was identified by DNase I footprint analysis. IclR was overexpressed and purified by a modification of the method described by Cortay et al. (4). DNase I footprint analysis of the IclR-DNA complex was carried out by a modification of the method described by Shih and Towle (27). The probe (−81 to +3 relative to the translational start site) was generated by PCR. The 5′ primer had been end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The binding reaction mixture included purified IclR, 25 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) (pH 7.5), 5 mM MgCl$_2$, 34 mM KCl, 2 μg of poly(dI-dC), and a probe (ca. 30,000 cpm) in 20 μl. Standards were generated by using the Maxam and Gilbert G and A+G reactions (17). Electrophoresis was performed with a 10% polyacrylamide gel which included 8 M urea. Lanes 1 through 6, results from reaction mixtures containing 0, 0.1, 0.3, 1, 2.5, and 0 μg of purified IclR, respectively. The sequence protected by IclR is boxed.

FIG. 4. Identification of the transcriptional start site of *iclR*. Primer extension analysis was used to identify the 5′ end of the *iclR* mRNA. RNA was isolated (28) from strain LLG1 harboring plasmid pKL5 (which carries *iclR* [29]). The primer spanned from +92 to +74 relative to the start of translation. Primer extension analysis was carried out by the method described by Domdey et al. (5). The same primer was used to generate the standards by using standard sequencing reactions (24, 25). Lane 1, 40 μg of RNA; lane 2, no RNA. Asterisks indicate the transcriptional start site of *iclR*.

TABLE 2. Effects of *iclR* on *iclR*::*lacZ* and *aceBAK* expression[a]

	Activity of:			
	β-Galactosidase[c]		IDH phosphatase	
iclR[b]	Glucose	Acetate	Glucose	Acetate
+	1,200	1,300	0.3	12
−	8,000	4,100	10	26

[a] Cultures were grown to mid-log phase on minimal media (20) containing the indicated carbon source. Assays for β-galactosidase (18) and IDH phosphatase (13) were performed as described previously. Results are expressed in Miller units for β-galactosidase and milliunits per milligram for IDH phosphatase. The standard errors were all less than 25%.

[b] An *iclR* disruption was generated by the insertion of a kanamycin resistance gene at the *Mlu*I site in vitro. This site is located at +84 within a coding region of 822 bp. The allele was transferred to the chromosome by recombination with Hfr-mediated conjugation to select for integrated plasmid (21). The strains are otherwise isogenic.

[c] An *iclR*::*lacZ* operon fusion was created by inserting the *Eco*RI-*Mlu*I fragment from *iclR* (29) between the *Eco*RI and *Bam*HI sites of plasmid pCL551 (3). This created a fusion which included 240 bp upstream of the translational start of *iclR* and 84 bp from the coding region while deleting 758 bp from the 3′ end of this gene. The fusion was transferred to the *lac* locus (22).

ence 7). We have found a binding site for IHF which is just upstream of the IclR binding site of *aceBAK* (23). This site greatly increases the sensitivity of *aceBAK* expression to the carbon source. IHF contributes to the induction of *aceBAK* by opposing repression by IclR during growth on acetate (inducing conditions) but not on glucose (repressing conditions). Expression of *iclR* may be relatively insensitive to the carbon source because it does not have a binding site of IHF. Consistent with this hypothesis, when the IHF site upstream of *aceBAK* was inactivated, the response of *aceBAK* expression to the carbon source closely resembled that of *iclR* expression.

results were observed for an *aceB*::*lacZ* operon fusion (not shown). These are the results expected since glucose is a repressing medium for *aceBAK* but growth on acetate yields induction. Surprisingly, the degrees of repression of *iclR* by IclR appear to be similar under inducing and repressing conditions. It appears that IclR responds strongly to growth conditions in its regulation of *aceBAK* but is far less affected when controlling its own expression.

The fact that autoregulation of *iclR* is relatively insensitive to carbon source is probably advantageous. Adaptation to acetate requires the induction of *aceBAK*, a process which results from the reduction in the repressor activity of IclR. Since IclR also represses its own expression, adaptation to acetate could produce a striking increase in the level of this repressor. Such an increase would be counterproductive, since it would oppose the induction of *aceBAK*.

Why does IclR control of *aceBAK* differ so markedly from its control of *iclR*? The available evidence suggests that integration host factor (IHF) may be largely responsible for this difference. IHF is a DNA-binding protein which participates in a variety of genetic processes in *E. coli* (for a review, see refer-

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