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Relative Expression of the Products of Glyoxylate Bypass Operon: Contributions of Transcription and Translation

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Although the genes of the *aceBAK* operon are expressed from the same promoter, the relative cellular levels of their products are approximately 0.3:1:0.003. Gene and operon fusions with *lacZ* were constructed to characterize this differential expression. The upshift in expression between *aceB* and *aceA* resulted from differences in translational efficiency. In contrast, inefficient translation and premature transcriptional termination contributed to the downshift in expression between *aceA* and *aceK*. Premature transcriptional termination occurred within *aceK* and appears to result from inefficient translation. Deletion of repetitive extragenic palindromic elements between *aceA* and *aceK* had little effect on the relative expression of these genes. Rather, the sequences responsible for inefficient expression of *aceK* lie within the *aceK* ribosome binding site.

The glyoxylate bypass of *Escherichia coli* is essential for growth on acetate (12, 13). The metabolic and regulatory enzymes of this pathway are encoded by the *aceBAK* operon. Isocitrate lyase and malate synthase are encoded by *aceA* and *aceB*, respectively, while isocitrate dehydrogenase (IDH) kinase/phosphatase is encoded by *aceK* (3, 5, 14, 18). During growth on acetate, this operon employs a single promoter upstream of *aceB*. Although these genes are expressed from the same promoter, the relative cellular levels of malate synthase, isocitrate lyase, and IDH kinase/phosphatase are ca. 0.3:1:0.003 (references 5, 6, and 15 and unpublished observations). Although the significance of the small upshift in expression observed between *aceB* and *aceA* is unclear, the striking downshift in expression exhibited by *aceK* undoubtedly reflects the fact that malate synthase and isocitrate lyase are metabolic enzymes while IDH kinase/phosphatase is a catalytic regulatory protein.

Differences in translational efficiency within the *aceBAK* operon. To determine whether transcriptional or translational effects contributed to differential expression in the *aceBAK* operon, we constructed a series of gene and operon fusions between these genes and *lacZ*, the gene encoding β-galactosidase. Gene fusions are dependent on both transcription and translation from upstream sequences. In contrast, the allele of *lacZ* used to construct operon fusions includes the sequences necessary for translational initiation and therefore reflects only transcriptional effects. Gene fusions with *aceB*, *aceA*, and the 5′ end of *aceK* expressed β-galactosidase activity at relative levels of 0.2:1:0.03 (Fig. 1), a pattern which is qualitatively similar to the levels of expression of the products of the native operon. In contrast, corresponding operon fusions were all expressed at similar levels. This result indicates that differential expression of the *aceBAK* operon's products results, in part, from differences in translational efficiency.

Transcriptional effects in the *aceBAK* operon. Although gene fusions at the 5′ end of *aceK* exhibited a striking downshift in expression relative to the gene fusion with *aceA*, the extent of this downshift (ca. 30-fold) was much less than that observed between isocitrate lyase and IDH kinase/phosphatase (ca. 300-fold). However, fusions inserted in the middle or at the 3′ end of *aceK* revealed a further downshift in expression of ca. fivefold (Fig. 1). In contrast to the results obtained with the 5′ *aceK* fusions, polarity within *aceK* was exhibited by both the gene and the operon fusions. Because operon fusions are not directly dependent on the translation of the upstream gene, these observations are consistent with premature transcriptional termination within *aceK*.

Identification of the determinants responsible for inefficient translation. Gene and operon fusions were inserted just downstream of the initiation codon of *aceK* (Fig. 1). These fusions exhibited the same translational effects as had fusions at position +37, indicating that the determinants responsible for inefficient translation are upstream of *aceK*.

The *aceA-aceK* intergenic region includes a cluster of repetitive extragenic palindromic (REP) elements (11, 20). REP sequences have been suggested to contribute to differential expression in operons either by interference with translation of downstream genes or by stabilization of mRNA sequences upstream of these elements. However, deletion of the REP elements from the *aceA-aceK* intergenic region was without effect on the expression of *aceK::lacZ* gene and operon fusions (Fig. 2). Furthermore, introduction of this deletion into the native operon had little effect on the expression of IDH kinase/phosphatase and yielded only a twofold decrease in the relative expression of isocitrate lyase (Table 1). This modest decrease in the expression of isocitrate lyase may suggest that the REP elements differentially stabilize the *aceA* region of the transcript, although this remains to be demonstrated. It is, however, clear that the REP elements are not primarily responsible for the downshift in expression between *aceA* and *aceK*.

Our deletion analysis had demonstrated that the determinants responsible for inefficient translation fell between positions −26 and +3 of *aceK*. This conclusion suggested that the sequences responsible for inefficient translation might correspond to the ribosome binding site. To test this possibility, we replaced the *aceK* ribosome binding site with the corresponding sequences from *aceA* (Fig. 2). This sub-

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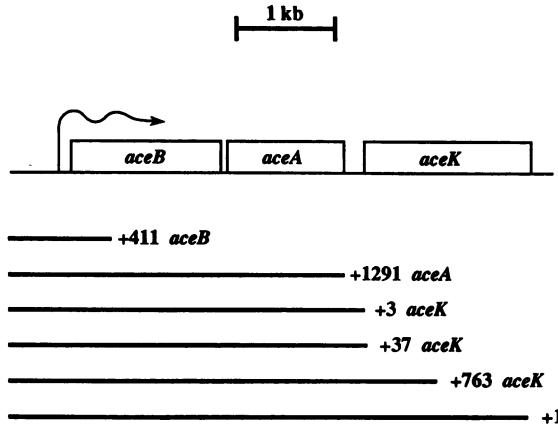

	Relative β-galactosidase Activity	
	gene fusions	operon fusions
+411 *aceB*	20	83
+1291 *aceA*	100	100
+3 *aceK*	2	55
+37 *aceK*	5	92
+763 *aceK*	1	17
+1686 *aceK*	1	22

FIG. 1. Gene and operon fusions with the glyoxylate bypass operon. Fusions between the genes of the *aceBAK* operon and *lacZ* were constructed in plasmid vectors, using the operon clone carried by plasmid pCL8 (5). The necessary deletions were generated by using available restriction sites, by BAL 31 digestion, or by introduction of an *Eco*RI site at +3 by site-directed mutagenesis. Operon fusions included translational stops at the 3′ ends of these deletions. After fusion to *lacZ*, clones were transferred to the chromosome of strain W4680 at the *lac* locus (22). The sequences included in each clone (horizontal lines) and the endpoints within the appropriate gene are indicated. Strains were grown to mid-log phase on minimal acetate medium (inducing conditions), and then β-galactosidase activity was assayed (19). Activity is expressed in relative units and is presented as a percentage of the value for the most active sample. For gene fusions, the most active sample contained 2,010 Miller units, while the most active operon fusion expressed 1,750 units. Results represent averages of three to six separate experiments. Standard errors of the means were less than ±10%.

stitution dramatically increased the expression of the *aceK*::*lacZ* fusion genes, indicating that the translational effects had been largely eliminated. Thus, inefficient translation of *aceK* appears to result from effects on translational initiation and is a consequence of an inefficient ribosome binding site.

Is premature transcriptional termination secondary to inefficient translation? Because transcription and translation are tightly coupled in *E. coli*, the premature transcriptional termination which appears to occur within *aceK* might be a secondary consequence of inefficient translational initiation. To test this possibility, we constructed operon fusions in which *aceK* employed the ribosome binding site from *aceA* (Fig. 2). The improved translation of the corresponding gene fusions (see above) correlated with the elimination of premature transcriptional termination, indicating that transcriptional termination had, in fact, resulted from inefficient translation.

Overview of the glyoxylate bypass operon. The modest increase in expression between *aceB* and *aceA* appears to result from a difference in translational efficiency, although the source of this difference has not been identified. The striking downshift in expression between *aceA* and *aceK* appears to result from two effects: inefficient translation and premature transcriptional termination. The disparity in the translation of *aceA* and *aceK* probably results from a difference in initiation efficiency, since the responsible sequences reside near the ribosome binding sites. Transcription and translation of *aceK* appear to be coupled: inefficient translation contributes to transcriptional termination within this gene. Together, these translational and transcriptional effects account for a 100-fold decrease in expression between *aceA* and *aceK*, a value very similar to that observed for the native operon (ca. 300-fold). Thus, these mechanisms appear to be primarily responsible for the downshift in expression between these genes.

Comparison with other operons. The mechanism responsible for the downshift in expression between *aceA* and *aceK* is unusual. For example, differential stabilization of regions of the primary transcript, which has been reported for the sigma operon of *E. coli* and the *rxcA* operon of *Rhodopseudomonas capsulata* (2, 4), contributes little to the downshift between *aceA* and *aceK*. Like *aceBAK*, inefficient translational initiation appears to contribute to the downshift between *uncE* and *uncF*. However, translation of *uncF* is inhibited by formation of a stem-loop structure which occludes the ribosome binding site (10). In contrast, deletion of the sequences at the 5′ end of *aceK* which had the potential for forming a stem-loop structure had no effect on the translation of *aceK* (Fig. 2). Transcriptional termination contributes to polarity in operons such as *rplKAJL-rpoBC* and the sigma operon (7, 16), as it does in *aceBAK*. However, terminations in these operons occur in intergenic regions rather than within structural genes and are not secondary consequences of inefficient translation.

Why is the ribosome binding site of *aceA* more efficient than that of *aceK*? This difference in efficiency was surprising, since the Shine-Dalgarno sequence (8) of *aceK* (GAGG) is quite similar to the corresponding sequence from *aceA* (GGAG), a gene which is translated much more efficiently. Furthermore, sequences between positions −1 and −26 which appear to be responsible for the differences in translational efficiency of *aceA* and *aceK* failed to reveal other *cis*-acting sequences which have been identified in highly expressed genes (24). It appears that this striking difference in translational efficiency results from the context of the Shine-Dalgarno sequence.

The observation that substitution of a more efficient ribosome binding site for the native sequences of *aceK* largely eliminated both the translational and the transcriptional effects argues that premature transcriptional termination within *aceK* was a secondary consequence of inefficient translation. This observation is consistent with evidence which indicates that transcription and translation are tightly coupled in *E. coli*. For example, it has been shown that premature transcriptional termination within *lacZ* can be experimentally induced by reducing the efficiency of translational initiation. Termination results probably because, in

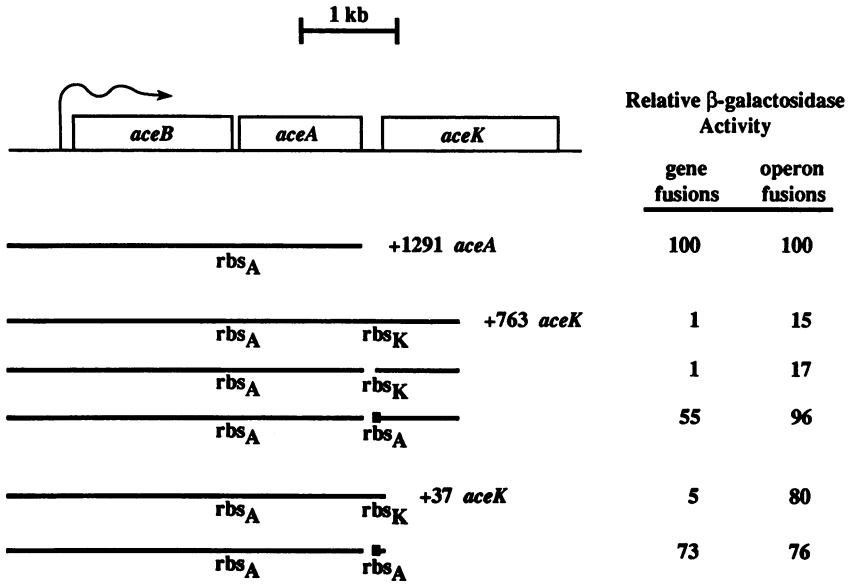

FIG. 2. Identification of determinants responsible for polarity. The transfer of clones to the chromosome and the assay of β-galactosidase activity were as described in the legend to Fig. 1. Horizontal lines indicate the sequences from the *aceBAK* operon which are present in the gene and operon fusions. Gaps in these lines indicate that these operons carry a deletion in the *aceA-aceK* intergenic region from positions −169 to −26 relative to the translational initiation codon of *aceK*, which does not remove any sequences from *aceA*. This deletion was created by oligonucleotide-directed mutagenesis (1) with a mutagenic oligonucleotide with the sequence

*Kpn*I
5′ CATCAGGAGCAGAGAGAATTGATATCGGTACCTTGTTGTTGCTTAGAACT 3′
*Eco*RV

where boldface type indicates sequences which hybridize with the native operon, and added restriction sites are indicated above and below the sequence. Boxes indicate clones that employ the ribosome binding site from *aceA* (rbs_A) for *aceK* rather than the normal ribosome binding site for this gene (rbs_K). The ribosome binding site of *aceK* was replaced by the corresponding sequences from *aceA* by using a synthetic DNA fragment with the sequence

5′ CGTAAACCACCACATAACTATGGAGCATCTGCATG 3′
3′ CATGGCATTTGGTGGTGTATTGATACCTCGTAGACGTACTTAA 5′

The derivative of the *aceBAK* operon used for this construction included a *Kpn*I site in the 17-bp sequence substituted for the region from −169 to −26 relative to *aceK* (see above) and an *Eco*RI site introduced at position +5 of *aceK* by site-directed mutagenesis. Activity is expressed in relative units and is presented as a percentage of the most active sample. For gene fusions, the most active sample contained 1,850 Miller units, while the most active operon fusion expressed 1,940 Miller units. The results represent averages of three or four separate experiments. Standard errors of the means were less than ±20%.

the absence of efficient translation, the transcriptional apparatus recognizes cryptic termination signals in the mRNA (23). It seems likely that *aceK* employs a similar mechanism, inefficient translational initiation allowing exposure of a transcriptional termination signal. However, in contrast to other cases, transcriptional termination resulting from inefficient translation appears to be a normal process in the *aceBAK* operon.

TABLE 1. Effect of deletion of REP sequences between *aceA* and *aceK* on operon polarity

Strain	Relevant genotype[a]	Expression (U/mg) of[b]: *aceA* (isocitrate lyase)	Expression (U/mg) of[b]: *aceK* (IDH phosphatase)
ST2010	Wild type	0.134	0.067
SL88	*aceBAΔK*	0.080	0.077

[a] ST2010 is wild type for the *aceBAK* operon (15). In strain SL88, the *aceBAK* operon carries a deletion in the *aceA-aceK* intergenic region from positions −169 to −26 relative to the translational initiation codon of *aceK*. This deletion was constructed in vitro (Fig. 2) and then transferred to the chromosome of ST2010 as described by Parker and Marinus (21). The identity of SL88 was confirmed by Southern blot analysis.

[b] Expression of *aceA* and *aceK* was measured by assay of isocitrate lyase (17) and IDH phosphatase (9) activities, respectively.

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