

## The *metD* D-Methionine Transporter Locus of *Escherichia coli* Is an ABC Transporter Gene Cluster

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**The *metD* D-methionine transporter locus of *Escherichia coli* was identified as the *abc-yaeE-yaeC* cluster (now renamed *metNIQ* genes). The *abc* open reading frame is preceded by tandem MET boxes bracketed by the –10 and –35 boxes of a promoter. The expression driven by this promoter is controlled by the MetJ repressor and the level of methionine.**

D-Methionine is an effective methionine source for *Escherichia coli* (5, 11, 14). The transport system reported to take up D-methionine in *E. coli* is encoded by the *metD* locus (11, 12). The system was found to be energized by ATP and regulated by the level of the internal methionine pool (10, 11, 13). The *metD* locus was mapped between the *fhuA* (previously called *tonA*) and the *proA* loci (12). The specific genes involved in D-methionine transport have not yet been reported.

We have identified the *abc-yaeE-yaeC* gene cluster (now renamed *metNIQ* genes) as a likely candidate for the *metD* locus in the *fhuA-proA* region. The *abc* gene was previously found in a search for ABC transporter ATP-binding domains (1). The PROSITE program (6) indicated that the Abc protein harbors an ATP- and GTP-binding site motif A (P-loop) (24) and an ABC transporter family signature (4). Gene *yaeE* encodes a putative membrane protein with a high sequence similarity to several bacterial amino acid transporters and contains a binding protein-dependent transport system inner membrane component signature (6, 25). The *abc* and *yaeE* open reading frames (ORFs) overlap by 8 nucleotides. The *yaeC* ORF is located 39 nucleotides downstream of the *yaeE* stop codon and was found to possess a probable signal sequence as well as a prokaryotic membrane lipoprotein lipid attachment site (6, 8), suggesting that it could be a periplasmic amino acid-binding protein.

A consensus MET box (5'-AGACGTCT-3'), the binding site of the MetJ repressor (2, 20, 22), was identified upstream of *abc* (28). There is a 62.5% consensus MET box next to the 100% box (Fig. 1). Recently, a conformational model-based prediction also identified the MetJ-binding site upstream of *abc* (15). This suggested that the *abc-yaeE-yaeC* cluster might be part of the MET regulon.

Using a neural network promoter prediction algorithm (<http://searchlauncher.bcm.tmc.edu/seq-search/gene-search.html>) (21), a very likely  $\sigma$ 70 promoter was predicted upstream

of *abc* (positions 2690 to 2726, reverse strand of the sequence registered under GenBank accession no. AE000129 [3]). Its spacer region between the –35 and –10 boxes is almost completely made up of the tandem MET boxes (Fig. 1). Even the –10 box of the putative promoter is part of a third, 50% MET box.

**Uptake of D-methionine.** To determine whether the *abc-yaeE-yaeC* putative ABC transporter gene cluster was involved in the ability of D-methionine to satisfy a methionine requirement, we deleted the cluster. The genomic region of the wild-type *E. coli* K-12 strain MG1655 corresponding to positions 90 to 2643 of the sequence with GenBank accession no. AE000129 was replaced with the kanamycin resistance cassette from pUC4K (Pharmacia) by using ET recombination (18), resulting in strain MK1958. The deletion was transduced into the methionine auxotroph strain MTD23 ( $\Delta metE \Delta methH$ ) (27) by using P1vir (17) with selection for kanamycin resistance, resulting in strain MK1962.

*E. coli* strains unable to synthesize L-methionine are known to grow in the presence of D-methionine (5, 11, 14). It was found that unlike the parental strain, strain MK1962 was unable to grow on M9 minimal plates (23) containing 0.2% glucose and 10  $\mu$ g of D-methionine/ml (Sigma).

Plasmids expressing the individual *abc*, *yaeE*, and *yaeC* ORFs were constructed on the basis of the pBAD18 and pBAD33 arabinose-inducible expression vectors (7). A plasmid expressing the *abc-yaeE* gene cluster was also generated. The expression from the pBAD-based plasmids was induced by the addition of 0.05% arabinose to the medium. Complementation studies on M9 glucose minimal plates showed that the expression of the three individual genes one at a time or two at a time in any combination did not enable MK1962 to grow on D-methionine. The ability to grow in the presence of 10  $\mu$ g of D-methionine/ml was restored only by the expression of all three genes, showing that all are necessary for the function of the transport system.

**Uptake of  $\alpha$ -methyl methionine.** The growth of strain MG1655 on M9 glucose minimal medium is severely inhibited by  $\alpha$ -methyl methionine, a toxic methionine analog. The analog is thought to be transported by the system encoded by the *metD* locus (11). Unlike strain MG1655, strain MK1958, harboring a deletion of the *abc-yaeE-yaeC* cluster, was resistant to

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FIG. 1. Structure of the predicted promoter upstream of *abc*. The  $-35$  and  $-10$  boxes are indicated in boldface type, and the tandem 100% and 62.5% MET boxes are shown as boxed sequences. The predicted transcriptional start nucleotide is underlined; stars indicate homology.

250  $\mu\text{g}$  of  $\alpha$ -methyl methionine/ml (Sigma). Complementation studies on M9 glucose minimal plates showed that the sensitivity to the analog was restored only by the expression of all three genes.

**Uptake of L-methionine.** There was no apparent difference in the growths of strains MK1962 and MTD23 in liquid M9 glucose minimal medium supplemented with L-methionine at concentrations ranging from 3.3 to 100  $\mu\text{g}/\text{ml}$ . It has been reported that there are at least two uptake systems for L-methionine in *E. coli*, a high-affinity system and a low-affinity system (9, 12). Therefore, it could not be ruled out that the Abc-YaeE-YaeC system transports L-methionine.

It has been hypothesized that the MmuP S-methylmethionine permease could also transport L-methionine (27). The *abc-yaeE-yaeC* deletion was transduced into MTD234 ( $\Delta\text{metE} \Delta\text{metH} \Delta\text{mmuP}$ ) (27), resulting in strain MK2053. The growth of MK2053 was indistinguishable from that of MTD23, MTD234, and MK1962 in liquid M9 glucose minimal medium supplemented with L-methionine as described above. However, because of the potential existence of another system(s) transporting L-methionine, we cannot exclude the possibility that the Abc-YaeE-YaeC system is one of the L-methionine transporters. The search for systems transporting L-methionine is under way and should be facilitated by the deletion of the *abc-yaeE-yaeC* cluster.

**Regulation of transcription of the *metD* locus.** To test whether the sequence shown in Fig. 1 is a promoter under the control of the MetJ repressor, it was cloned into the *EcoRI* and *BamHI* sites of the pRS415  $\beta$ -galactosidase-based promoter-probe vector (26), resulting in pROMET1. The expression of  $\beta$ -galactosidase from pROMET1 was assayed in the *E. coli* strain JM109 (29) harboring pBAD33 (7) or pMJ33, a pBAD33-derived, pROMET1-compatible plasmid expressing the *metJ* gene under the control of its native promoter. The strains were grown in liquid M9 minimal medium containing 0.2% glucose and 10  $\mu\text{g}$  of thiamine-HCl per ml, with or without 100  $\mu\text{g}$  of L-methionine per ml. The  $\beta$ -galactosidase specific activities of the cultures were determined by using the *o*-nitrophenyl- $\beta$ -D-galactopyranoside substrate (Sigma) (16). The data shown in Table 1 are the averages of three measurements. Assays with *E. coli* strain TN1, a *metJ* mutant derivative of JM109 (19), failed because of the very slow growth of the strains.

TABLE 1.  $\beta$ -Galactosidase specific activities in the JM109 host strain in liquid M9 glucose minimal cultures

Plasmids	Sp act (Miller units) with:	
	No added L-methionine	100 $\mu\text{g}$ of L-methionine/ml
pRS415, pBAD33	19.3	19.0
pROMET1, pBAD33	26,643	8,476
pROMET1, pMJ33	13,332	2,145

The results show that the segment behaves as a promoter. Its expression decreased about threefold upon the addition of L-methionine to the medium and about twofold when the *metJ* gene was present in multicopy. When both L-methionine was added and *metJ* was present in multicopy, the expression decreased about 12-fold. This suggests that the promoter is repressed by the MetJ repressor. The expression of the *abc-yaeE-yaeC* cluster is probably similarly regulated.

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