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

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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-yaE-yaEC gene 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 metD proA region. The expression driven by this promoter is controlled by the MetJ repressor and part of the MET regulon.

The metD D-methionine transporter locus of Escherichia coli was identified as the abc-yaE-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 part of the MET regulon.

Uptake of D-methionine. To determine whether the abc-yaE-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 MT2 (ΔmetE ΔmetH) (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 μg of D-methionine/ml (Sigma).

Plasmids expressing the individual abc, yaE, and yaC ORFs were constructed on the basis of the pBAD18 and pBAD33 arabinose-inducible expression vectors (7). A plasmid expressing the abc-yaE 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 μg 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 α-methyl methionine. The growth of strain MG1655 on M9 glucose minimal medium is severely inhibited by α-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-yaE-yaEC cluster, was resistant to
250 μg of o-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 growth of strains MK1962 and MTD23 in liquid M9 glucose minimal medium supplemented with l-methionine at concentrations ranging from 3.3 to 100 μg/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 Abs-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 (ΔmetH ΔmmuP) (27), resulting in strain MK2053. The growth of MK2053 was indistinguishable from that of MTD234, MTD23, 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 Abs-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 β-galactosidase-based promoter-probe vector (26), resulting in pROMET1. The expression of β-galactosidase from pROMET1 was assayed in the *E. coli* strain JM109 (29) harboring pBAD33 (7) or pMJ33, a pBAD33-derivative, pROMET1-compatible plasmid expressing the gene under the control of its native promoter. The strains were grown in liquid M9 minimal medium containing 0.2% glucose and 10 μg of thiamine-HCl per ml, with or without 100 μg of l-methionine per ml. The β-galactosidase specific activities of the cultures were determined by using the o-nitrophenyl-β-D-galactopyranoside substrate (Sigma) (16). The data shown in Table 1 are the averages of three measurements. Assays with *E. coli* strain TN1, a *metD* mutant derivative of JM109 (19), failed because of the very slow growth of the strains.

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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**REFERENCES**


**TABLE 1.** β-Galactosidase specific activities in the JM109 host strain in liquid M9 glucose minimal cultures

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<tr>
<th>Plasmids</th>
<th>Sp act (Miller units) with:</th>
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<tr>
<td></td>
<td>No added l-methionine</td>
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<tr>
<td>pRS415, pBAD33</td>
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