JOURNAL OF BACTERIOLOGY, June 1991, p. 3601–3604
0021-9193/91/113601-04$02.00/0

Vol. 173, No. 11

The Tryptophan-Specific Permease Gene, *mtr*, Is Differentially Regulated by the Tryptophan and Tyrosine Repressors in *Escherichia coli* K-12†

VIRGINIA M. HEATWOLE AND RONALD L. SOMERVILLE*
Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907

Received 7 January 1991/Accepted 1 April 1991

The regulation of transcription of the gene for the tryptophan-specific permease, *mtr*, was evaluated in several genetically marked *Escherichia coli* strains through the use of a single-copy *lacZ* reporter system. The expression of *mtr* was repressed 97-fold by tryptophan via the Trp repressor and induced 10-fold by phenylalanine or tyrosine via the Tyr repressor. By primer extension analysis two distinct *mtr* transcripts and their corresponding promoters were identified. One transcript was induced by the Tyr repressor. The tryptophan-dependent interaction of Trp repressor with an operator target within the *mtr* promoter was demonstrated by means of a restriction endonuclease protection assay.

Tryptophan is transported in *Escherichia coli* by a general aromatic amino acid permease, encoded by *aroP* (4), and by two specific transport systems. The first of these is a high-affinity permease encoded by *mtr* (10; see also the citation of C. Yanofsky in reference 16). The other is a low-affinity permease encoded by a gene of the *tna* operon (7). The gene for the high-affinity tryptophan-specific permease, *mtr*, has been cloned and sequenced (9). Probable operators for the tyrosine and tryptophan repressor proteins were distinguished within the DNA upstream of the *mtr* gene (9). An earlier study of tryptophan-specific uptake showed that this process was enhanced by phenylalanine, provided that functional tyrosine repressor was present (21). This report identifies the 5′ ends of *mtr* mRNA and characterizes the roles that the tyrosine and tryptophan repressors play in the regulation of *mtr* transcription.

Construction of an *mtr-lacZ* transcription-translation fusion on a λ phage. A 756-bp *Eco*RI-*Nla*IV DNA fragment containing 690 bp upstream of the *mtr* structural gene and the first 22 codons of *mtr* were cloned into the *Eco*RI-*Sma*I sites of M13mp18/p (1). The DNA corresponding to this transcription-translation fusion of *mtr* to *lacZ*α was excised from this construct, M13#17, and inserted into pMLB1034 (3) by using *Eco*RI-*Bam*HI. This resulted in an in-frame fusion of the first 22 codons of *mtr* to full-length *lacZ*. The plasmid bearing this *mtr-lacZ* fusion, pSLW20, was transformed into CSH26(λRZ11). Lac$^+$ recombinant λ phage bearing the *mtr-lacZ* fusion were isolated from this plasmid-bearing lysogen as described previously (1). The phage, designated λSLW20, were then inserted in single-copy form into appropriate *E. coli* hosts (Table 1) according to published procedures (15, 23).

Effects of the tyrosine and tryptophan repressors on transcription from the *mtr* promoter. Strains lysogenic for λSLW20 lacking either the tyrosine or tryptophan repressor or both repressors were used in β-galactosidase assays to evaluate the role of these regulatory proteins in *mtr* expression. The results are summarized in Table 2.

β-Galactosidase levels in wild-type cells (SP1312 in Table 2) grown in minimal medium and minimal medium supplemented with different aromatic amino acids indicated that expression from the *mtr* promoter was induced 8-fold by phenylalanine and 7-fold by tyrosine and repressed 26-fold by tryptophan, relative to the levels observed in minimal medium. Stimulation of β-galactosidase production by phenylalanine was not additive with respect to stimulation by tyrosine. In terms of their effects on expression of β-galactosidase from the *mtr* promoter, phenylalanine and tyrosine appeared to be interchangeable. When phenylalanine or tyrosine was present in addition to tryptophan, there was only a sixfold repression of β-galactosidase.

In a *tyrR* background (SP1313 in Table 2) phenylalanine- and tyrosine-mediated induction of β-galactosidase was abolished. The level of expression in minimal medium was only twofold different from that of the *tyrR*$^+$ control. The magnitude of tryptophan-mediated repression in strains lacking tyrosine repressor was unchanged in comparison with that in the wild-type control strain.

In a *trpR* background (SP1322 in Table 2), expression of β-galactosidase in minimal medium was fivefold higher than in the wild-type background. Phenylalanine- and tyrosine-mediated induction in the *trpR* strain was only fourfold above the levels seen in minimal medium. No tryptophan repression was observed when tryptophan alone was present. However when tryptophan and phenylalanine were both present, tryptophan appeared to reduce the phenylalanine-mediated induction from fourfold to twofold. This lower level of induction is probably attributable to the ability of tryptophan to compete with phenylalanine for uptake through the AroP system (21), thus reducing the entry of external phenylalanine. When both repressor proteins were absent (SP1323 in Table 2) no regulation of β-galactosidase expression in response to any of the aromatic amino acids was observed.

The net effect of each regulatory protein on β-galactosidase expression may be inferred by comparing the *trpR tyrR* (SP1323 in Table 2) double mutant with the appropriate single-mutant strains. Tyrosine repressor, in concert with phenylalanine or tyrosine, induced β-galactosidase expression 10-fold. Tryptophan repressor, in a tryptophan-dependent manner, repressed β-galactosidase expression 97-fold.

* Corresponding author.
† Journal paper no. 12858 from the Purdue University Agricultural Experimental Station.

TABLE 1. *E. coli* strains, phage, and plasmids

Strain, phage, or plasmid	Relevant genotype or description	Reference or source
Strains		
CSH26	*ara* Δ(*lac-pro*) *thi*	15
SP564-1	*thi* Δ(*lac-pro*) Val^r Δ(*tyrR*)	D. Johnson
SP1258	*trpR*::Ω*kan* Δ(*argF lac*)*205 rep-71 his-871 relA1 rpsL181 gal-3* F′ pOX38::Tn*10*-11(mini-Tet)	B. Hagewood
SP1312	As W3110, but *zah-735*::Tn*10*Δ (*argF-lac*)*U169*	This work
SP1313	As SP1312, but Δ(*tyrR*)	This work
SP1322	As SP1312, but *trpR*::Ω*kan*	This work
SP1323	As SP1313, but *trpR*::Ω*kan*	This work
W3110	Wild type	2
Plasmids		
pMLB1034	Promoterless *lacZ*, Amp^r	3
pSLW13	*mtr*$^+$	9
pSLW20	*mtr-lacZ* transcription-translation fusion	This work
Phage		
M13mp18/p	M13mp18 but promoterless *lacZ*α	1
M13#17	*mtr-lacZ*α transcription-translation fusion	This work
λRZ11	As λ*plac5* (*c*I857 *S*am7) but promoterless *lacZ*	23
λSLW20	*mtr-lacZ* transcriptional-translational fusion	This work

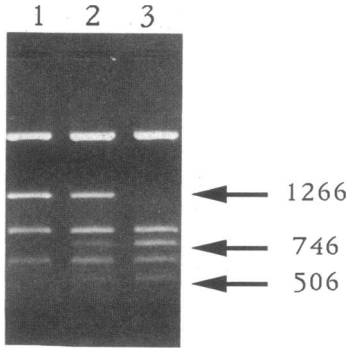

FIG. 1. Protection of three *Rsa*I restriction endonuclease sites within the *mtr* promoter by tryptophan repressor. A 6,350-bp plasmid, pSLW13 (9), that contains the *mtr* promoter region was used as the target DNA molecule. Purified tryptophan repressor and 0.5 mM tryptophan were incubated for 20 min at 37°C with 0.18 pmol of plasmid in *Rsa*I restriction buffer (50 mM NaCl, 6 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 6 mM mercaptoethanol; total volume, 20 μl). Then the plasmid was subjected to cleavage with *Rsa*I restriction endonuclease (14 U) for 45 min at 37°C. The products were electrophoretically separated on a 1% agarose gel. Lane 1, 38 pmol of purified tryptophan repressor; lane 2, 19 pmol; lane 3, 9.5 pmol. Controls containing either 76 pmol of repressor but no tryptophan or 0.5 mM tryptophan but no repressor gave results identical to lane 3 (data not shown). Arrows mark the 1,266-bp DNA fragment resulting from protection of the operator and DNA fragments of approximately 506 and 746 bp resulting from *Rsa*I cleavage within the operator.

In the wild-type background both induction and repression contributed to the level of β-galactosidase expression.

Tryptophan holorepressor binds to an operator upstream of *mtr*. Within the predicted tryptophan repressor operator site lie three *Rsa*I restriction endonuclease cleavage sites (see Fig. 3). Purified tryptophan repressor was found to prevent cleavage of the *mtr* DNA at all three *Rsa*I sites in a tryptophan-dependent manner (Fig. 1). The *Nsi*I site adjacent to the presumptive Trp repressor operator (see Fig. 3) remained susceptible to cleavage under conditions where *Rsa*I protection occurred (data not shown). The tryptophan repressor concentration required to protect approximately one-half of the operator sites from *Rsa*I cleavage, as observed by appearance of the 746- and 506-bp fragments, is shown in lane 2 of Fig. 1. From the concentrations of repressor and operator present, the in vitro dissociation constant is estimated to be 9.4×10^{-7} M under the conditions of this assay. This value, which is larger than previous estimates for related systems (8), may reflect the specific assay conditions and the quality of protein used. However, the data show that tryptophan repressor interaction with the DNA requires tryptophan and is operator specific.

Identification of the 5′ ends of *mtr* mRNA by primer extension analysis. RNA was isolated by the procedure of Salser et al. (17) from strain SP1312 grown either in minimal medium or phenylalanine (1 mM)-supplemented medium. RNA in the form of an ethanol slurry was stored at −20°C. Samples were withdrawn, centrifuged, and resuspended for primer extension analysis. The results are shown in Fig. 2. When RNA from cells grown in minimal medium was used as a template in extension reactions, three transcripts, P1, P2, and P3, were detected. When RNA from cells grown under conditions known to induce the expression of *mtr* (i.e., in the presence of phenylalanine) was examined, the relative abundance of transcript P3 was greatly increased while the amounts of P1 and P2 decreased slightly. It is not known whether the P2 transcript originates from a promoter distinct from P1 or is the result of inefficient elongation of the

TABLE 2. β-Galactosidase levels in strains lysogenic for λSLW20

Strain	Relevant genotype	β-Galactosidase level[a] in cells grown in:					
		Min[b]	Min+F	Min+Y	Min+W	Min+FY	Min+FW[c]
SP1312		187	1,470	1,290	7.2	1,730	30.0
SP1313	*tyrR*	91.0	168	104	3.6	217	4.0
SP1322	*trpR*	993	3,790	4,020	1,040	1,800	1,590
SP1323	*tyrR trpR*	348	390	405	348	441	414

[a] Results of β-galactosidase assays, performed on permeabilized cells grown to mid-log phase, are reported in Miller's units (15). Each value is the result of at least three independent assays. The standard deviation is no greater than 12%.

[b] Minimal medium (Min) contained 0.2% glucose, vitamin B_1 (1 mg/liter), biotin (0.1 mg/liter), and salt mix E of Vogel and Bonner (20). Aromatic amino acids were added at a concentration of 1 mM.

[c] Assays done in Min+YW and Min+FYW gave values similar to those of Min+FW for all four strains (data not shown).

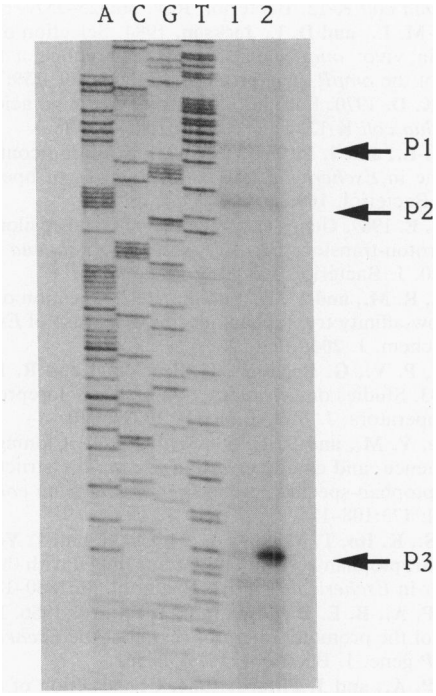

FIG. 2. Mapping the 5′ end of *mtr* mRNA by primer extension analysis. RNA was isolated from a wild-type strain, SP1312, grown to early log phase in either minimal medium or minimal medium supplemented with phenylalanine (1 mM). In each case, a 30-residue oligonucleotide (GCAGCGACGGTGACGTTTGGGTGGTGGTTA) was annealed to 40 μg of RNA and extended with avian myeloblastosis virus reverse transcriptase (United States Biochemical; 9 U) in the presence of dCTP, dGTP, dTTP, and [α-^{32}P]dATP as described by Curtis (6) except that the reactions were carried out at 42°C for 40 min. The extension products were separated on a 5% sequencing gel next to a dideoxy sequencing ladder of the strand complementary to the mRNA, generated with the same oligodeoxynucleotide primer. Lane 1, 40 μg of RNA from cells grown in minimal medium; lane 2, 40 μg of RNA from cells grown in minimal medium with phenylalanine (1 mM). Control reactions containing RNA but no oligodeoxynucleotide primer gave no observable cDNA products (data not shown). Three cDNA products, marked with arrows P1, P2, and P3, were detected (see text).

P1 transcript cDNA by reverse transcriptase. It should be noted that P1 RNA has a potential GC-rich stem-loop structure close to its 5′ end that may cause reverse transcriptase to prematurely terminate. For the present it will be assumed that the P1 and P2 cDNA products identified by primer extension represent a single set of transcripts that originate from the P1 promoter. It is judged that P3 originates from a separate promoter, because the relative amount of P3 mRNA was greatly elevated in phenylalanine-grown cells whereas the amount of P1 mRNA remained approximately the same. The locations of the 5′ ends of P1, P2, and P3, as well as possible −10 and −35 sequences for P1 and P3, are shown in Fig. 3.

The primer extension results, in combination with the β-galactosidase assays (Table 2), suggest that the increase of transcript P3 under inducing conditions reflects stimulation by the tyrosine repressor of transcriptional events at the *mtr* promoter. In phenylalanine-grown cells, P3 is the major transcript observed. The mechanism by which tyrosine repressor leads to this specific activation is not yet understood.

Although the amino acid sequence of tyrosine repressor has regions of identity to several proteins that activate σ^{54}-dependent promoters (19), σ^{54} is not required for the expression of *mtr* (data not shown). Furthermore, the presence of −10 and −35 hexamers for P3 that are characteristic of σ^{70} promoters (14) strongly suggests that the P3 transcript originates from a σ^{70} promoter. P1 was not induced under our conditions.

Expression from the *mtr* promoter is significantly repressed by tryptophan repressor and tryptophan. It is not known whether tryptophan repressor affects expression from the P1 promoter. In the case of the P3 promoter, the tryptophan repressor operator overlaps the −10 hexamer. One model for tryptophan repressor-mediated repression at this promoter involves straightforward physical exclusion of RNA polymerase. However, because multiple proteins are interacting at the *mtr* promoter, other models for tryptophan repressor-mediated repression cannot be ruled out.

Concluding comments. Sequence analysis previously suggested the existence of a tryptophan repressor operator site within the *mtr* promoter region (9). We have demonstrated that tryptophan repressor binds to the proposed operator

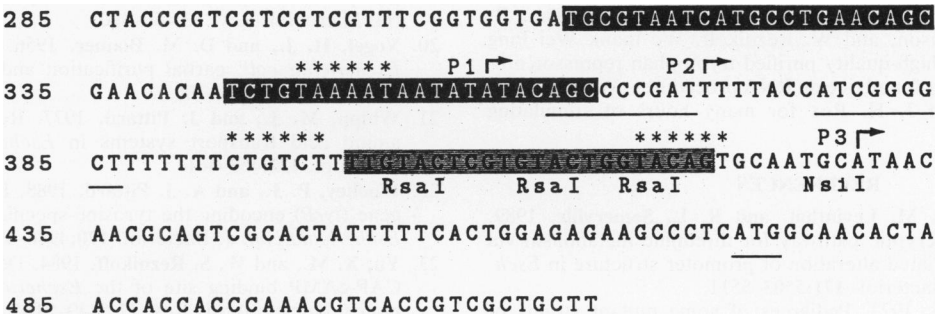

FIG. 3. Nucleotide sequence of the promoter region of *mtr*. The putative tyrosine repressor operators (black boxes) and the tryptophan repressor operator (gray box) as well as the previously identified initiation codon for *mtr* (9) are indicated. The nucleotide sequence coordinates are those of the previously published *mtr* sequence (9). The three *Rsa*I sites within the tryptophan repressor operator are indicated. The *Nsi*I site that remains cleavable in the presence of tryptophan repressor is also shown. The positions of the flanking *Rsa*I sites are 514 bp upstream and 752 bp downstream from the symmetry axis of the operator. The arrows indicate the 5′ ends of the cDNA products inferred by primer extension analysis. Arrow P3 indicates the transcript that appears to be induced by the tyrosine repressor. The GC-rich palindromic region near the 5′ end of the P1 mRNA transcript is underlined. The probable −10 and −35 hexamers for the P3 promoter and the probable −10 hexamer for the P1 promoter are indicated by asterisks. No obvious consensus −35 hexamer can be identified for the P1 promoter.

segment of DNA in a tryptophan-dependent manner. This result brings to four the number of *E. coli* operons known to be specifically and strongly repressed by the tryptophan repressor.

Tyrosine repressor induces the expression of *mtr* in a phenylalanine- or tyrosine-dependent manner. Depending upon whether tyrosine or phenylalanine is provided, tyrosine repressor either represses or induces transcription of *tyrP*, the gene for the tyrosine-specific permease (12). However, *mtr* is the first example of a gene in which the role of tyrosine repressor in transcription is limited solely to activation. It is not known whether tyrosine repressor utilizes one or both of the proposed operators to exert its effect. Further analysis will be required to elucidate this point. Sarsero and Pittard (18) also found that *mtr* is repressed by the tryptophan repressor via tryptophan and is induced by the tyrosine repressor via phenylalanine or tyrosine. These workers also detected an *mtr* promoter corresponding to the P3 promoter reported here (18).

In its regulation, the *mtr* gene bears certain similarities to *tyrP*. The expression of the *mtr* gene, like that of *tyrP*, involves at least two categories of transcripts, only the shorter of which is strongly regulated by the tyrosine repressor. In the case of *mtr*, the transcriptional start points are separated by 72 nucleotides, whereas the two major categories of *tyrP* transcripts differed in length by 140 nucleotides (11, 22). The structural similarities between the protein products of these two genes have been noted and discussed previously (9).

Tryptophan-mediated repression of *mtr* conforms to a pattern of regulation already established for two other aromatic amino acid permeases, *aroP* and *tyrP*. In each case a permease gene is repressed by the aromatic amino acid that the corresponding gene product transports into the cell (5, 12, 13, 21). In addition, either phenylalanine or tyrosine is able to competitively inhibit the uptake of tryptophan through the AroP system (21). These amino acids also repress the expression of *aroP* (5). Thus, it is likely to be advantageous to the cell to induce the expression of the tryptophan-specific permease, encoded by *mtr*, when phenylalanine or tyrosine is present in abundance.

This research was supported by Public Health Service grant GM22131 and by David Ross grant XR G901561 from the Purdue Research Foundation.

For their generous contributions of bacterial strains, viruses, and plasmids we thank B. Aronson, B. Bachmann, M. Berman, B. Hagewood, D. Johnson, and W. Reznikoff. We thank Wei Ping Yang for supplying high-quality purified tryptophan repressor protein and Duane Culler for advice on RNA procedures. We also thank B. D. Aronson and J. H. Rex for many hours of stimulating discussions.

REFERENCES

1. **Aronson, B. D., M. Levinthal, and R. L. Somerville.** 1989. Activation of a cryptic pathway for threonine metabolism via specific IS*3*-mediated alteration of promoter structure in *Escherichia coli*. J. Bacteriol. **171**:5503–5511.
2. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
3. **Berman, M. L., and D. E. Jackson.** 1984. Selection of *lac* gene fusions in vivo: *ompR-lacZ* fusions that define a functional domain of the *ompR* gene product. J. Bacteriol. **159**:750–756.
4. **Brown, K. D.** 1970. Formation of aromatic amino acid pools in *Escherichia coli* K-12. J. Bacteriol. **104**:177–188.
5. **Chye, M.-L., and J. Pittard.** 1987. Transcription control of the *aroP* gene in *Escherichia coli* K-12: analysis of operator mutants. J. Bacteriol. **169**:386–393.
6. **Curtis, S. E.** 1987. Genes encoding the beta and epsilon subunits of the proton-translocating ATPase from *Anabaena* sp. strain PCC 7120. J. Bacteriol. **169**:80–86.
7. **Edwards, R. M., and M. D. Yudkin.** 1982. Location of the gene for the low-affinity tryptophan-specific permease of *Escherichia coli*. Biochem. J. **204**:617–619.
8. **Haydock, P. V., G. Bogosian, K. Brechling, and R. L. Somerville.** 1983. Studies on the interaction of Trp holorepressor with several operators. J. Mol. Biol. **170**:1019–1030.
9. **Heatwole, V. M., and R. L. Somerville.** 1991. Cloning, nucleotide sequence, and characterization of *mtr*, the structural gene for a tryptophan-specific permease of *Escherichia coli* K-12. J. Bacteriol. **173**:108–115.
10. **Hiraga, S., K. Ito, T. Matsuyama, H. Ozaki, and T. Yura.** 1968. 5-Methyltryptophan-resistant mutations linked with the arginine G marker in *Escherichia coli*. J. Bacteriol. **96**:1880–1881.
11. **Kasian, P. A., B. E. Davidson, and J. Pittard.** 1986. Molecular analysis of the promoter operator region of the *Escherichia coli* K-12 *tyrP* gene. J. Bacteriol. **167**:556–561.
12. **Kasian, P. A., and J. Pittard.** 1984. Construction of a *tyrP-lac* operon fusion strain and its use in the isolation and analysis of mutants derepressed for *tyrP* expression. J. Bacteriol. **160**:175–183.
13. **Kuhn, J., and R. L. Somerville.** 1974. Uptake and utilization of aromatic D-amino acids in *Escherichia coli* K-12. Biochim. Biophys. Acta **322**:298–312.
14. **McClure, R. W.** 1985. Mechanism and control of transcription initiation in prokaryotes. Annu. Rev. Biochem. **54**:171–204.
15. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Oxender, D. L.** 1975. Genetic approaches to the study of transport systems, p. 214–231. *In* H. N. Christensen (ed.), Biological transport, 2nd ed. W. A. Benjamin, New York.
17. **Salser, W., R. F. Gesteland, and A. Bolle.** 1967. *In vitro* synthesis of bacteriophage lysozyme. Nature (London) **215**: 588–591.
18. **Sarsero, J. P., and J. Pittard.** 1990. Expression of the *mtr* gene of *Escherichia coli* K-12 is regulated by both TyrR and TrpR. Proc. Aust. Biochem. Soc., abstr. SP28.
19. **Stock, B. S., A. J. Ninfa, and A. M. Stock.** 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53**:450–490.
20. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
21. **Whipp, M. J., and J. Pittard.** 1977. Regulation of aromatic amino acid transport systems in *Escherichia coli* K-12. J. Bacteriol. **132**:453–461.
22. **Wookey, P. J., and A. J. Pittard.** 1988. DNA sequence of the gene (*tyrP*) encoding the tyrosine-specific transport system of *Escherichia coli*. J. Bacteriol. **170**:4946–4949.
23. **Yu, X. M., and W. S. Reznikoff.** 1984. Deletion analysis of the CAP-cAMP binding site of the *Escherichia coli* lactose promoter. Nucleic Acids Res. **12**:5449–5464.