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Regulation of methionine synthesis in *Escherichia coli*: Effect of the MetR protein on the expression of the *metE* and *metR* genes

(gene regulation/*met* regulon)

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ABSTRACT **A plasmid (pRSE562) containing the *metE* and *metR* genes of *Escherichia coli* was used to study the expression of these genes and the role of the MetR protein in regulating *metE* expression. DNA sequence analysis of the 236-base-pair region separating these genes showed the presence of seven putative *met* boxes. When this plasmid was used to transform either wild-type *E. coli*, *metE* mutant, or *metR* mutant, MetE enzyme activity increased 5- to 7-fold over wild-type levels. The *metR* gene was subcloned from pRSE562, and this plasmid, pMRIII, relieved the methionine auxotrophy of a *metR* mutant after transformation. The *metR* gene was also cloned into a vector containing the λP_L promoter, and the MetR protein was overexpressed and purified to near homogeneity. This protein, when added to an *in vitro* DNA-dependent protein synthesis system in which the MetE and/or MetR proteins were synthesized, caused a large increase in the expression of the *metE* gene but a decrease in the expression of the *metR* gene. The *in vitro* expression of both genes was inhibited by the MetJ protein and *S*-adenosylmethionine in the presence or absence of MetR protein. These results provide evidence that the product of the *metR* gene is a trans-activator of the expression of the *metE* gene and that the expression of the *metR* gene is under autogenous regulation and is repressed by the MetJ protein.**

The genes involved in the methionine biosynthetic pathway in *Escherichia coli* are part of a regulon (*met* regulon) that is controlled by the level of methionine in the medium (1, 2). Considerable knowledge has been obtained at the molecular level on the mechanism of this regulation. Studies on the expression of several of the *met* genes have shown that a repressor protein, the product of the *metJ* gene, can bind to a specific region on the DNA (*met* box) that is present in all *met* genes thus far examined (3–7). This interaction of the MetJ protein with the *met* operator region is markedly enhanced by the presence of *S*-adenosylmethionine (AdoMet), a primary metabolic product of methionine (5, 8, 9).

The *metE* gene has received special attention because it is regulated by both methionine and vitamin B_{12} (2, 10–14). This gene, which codes for 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (5-methylhydropteroyltri-L-glutamate:L-homocysteine *S*-methyltransferase, non-B_{12} methyltransferase, EC 2.1.1.14), has been cloned and expressed both *in vivo* and *in vitro* (15–17). In previous *in vitro* experiments no regulation of *metE* expression was observed, but a detailed study was not undertaken at that time (16). Recently, Urbanowski *et al.* (17) reported the presence of another *met* regulatory locus (*metR*) in both *Salmonella typhimurium* and *E. coli*. These genetic data suggested that *metR* codes for a trans-activator protein for the expression of the *metE* gene and, to a lesser extent, the *metH* gene that codes for the B_{12}-dependent methyltransferase (5-methyltetrahydrofolate:L-homocysteine *S*-methyltransferase, EC 2.1.1.13). These studies also showed that the *metR* gene was closely linked to the *metE* gene. The *S. typhimurium* *metR* gene has been cloned, and its nucleotide sequence has been determined (18). The *metR* gene product, as deduced from the DNA sequence, is a protein of 31 kDa. Previously Chu *et al.* (16), investigating the plasmid-directed *in vitro* synthesis of the MetE protein (92 kDa), observed the synthesis of a 34-kDa protein (based on gel migration) that in all probability was the *metR* gene product. We determined the nucleotide sequence of the *E. coli* *metE–metR* intergenic region.§ In the present study, we purified the MetR protein to near homogeneity and here we describe *in vivo* and *in vitro* experiments on expression and regulation of the *metE* and *metR* genes in *E. coli*, which show that the MetR protein is a trans-activator of *metE* expression.

MATERIALS AND METHODS

E. coli strain B was obtained from H. Ennis of the Roche Institute, and *E. coli* strain RRI/pRK248clts was supplied by R. Crowl (Department of Molecular Genetics, Hoffmann–La Roche). *E. coli* strain 2276 (*metE, thi*) was obtained from A. L. Taylor (University of Colorado, Denver), and *E. coli* strain GS244 (*metR, pheA905, thi*) was isolated as described elsewhere (17). *E. coli* strain JM108 (*recA1, gyrA96, thi, hsdl7, supE44, relA1, Δlac-proAB*) was obtained from H. R. Kaback of the Roche Institute. AdoMet was obtained from Sigma and purified by ion-exchange chromatography on Dowex 1 HCO_3^- (19). The MetJ protein was purified as described (4). Pteroyl-γ-glutamyl-γ-glutamic acid (pteroyltriglutamate) was provided by L. Ellenbogen (Lederle Laboratories). DL-tetrahydropteroyltriglutamate was prepared by reduction of pteroyltriglutamate with $NaBH_4$ (20), and ^{14}C-labeled DL-5-methyltetrahydropteroyltriglutamate was synthesized as described (21). The N-terminal amino acid analysis was performed by M. Miedel and Y. Pan of Hoffmann–La Roche.

***In Vivo* Analysis of MetE Activity.** *E. coli* strains B, 2276, and GS244 were grown at 37°C to an OD_{600} value between 0.5–0.7 unit in M9 salts medium (31) containing 0.4% glucose and either 3.3×10^{-5} M L-methionine (a nonrepressing concentration required for the growth of strains 2276 and GS244) or 10^{-2} M L-methionine (repressing concentration). The cells were harvested, broken by sonication, and centrifuged at 16,000 × *g* for 10 min. The supernatant was assayed for MetE enzyme activity, as described by Whitfield *et al.*

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Abbreviations: AdoMet, *S*-adenosylmethionine; S-30, supernatant from centrifugation of crude cell lysate at 30,000 × g for 30 min.

§The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04155).

(22). Protein concentrations were determined using the method of Lowry *et al.* (23).

Construction of Plasmids pRSE562, pMRIII, and pMMetR. Plasmid pRSE562 (Fig. 1) was constructed by digesting plasmid pJ19 (16) (which contains the *metE* and *metR* genes) with *Bam*HI and *Eco*RI. A 5.6-kilobase (kb) fragment containing both genes was isolated and inserted into the *Bam*HI and *Eco*RI sites of pBR322. DNA from pRSE562 was further digested with restriction enzymes *Pvu* I and *Pvu* II to yield a fragment of ≈3 kb that contained the *metR* gene, the intergenic sequence between the *metR* and *metE* genes, and ≈475 base pairs (bp) of the *metE* coding region. This fragment was cloned into the *Ssp* I and *Pvu* I sites of pBR322 to yield plasmid pMRIII.

Plasmid pRC25 was obtained from R. Crowl and carries a 250-bp *Bgl* II–*Hae* III DNA-fragment insert containing the bacteriophage λP_L promoter. This vector is identical to pRC23 (24) except for the change of an A → C 12 bases upstream from an *Eco*RI restriction site located at the 3′ end of the ribosome binding site; this base change creates a new *Hpa* I restriction site. This vector was digested sequentially with *Hpa* I and *Bam*HI and purified by electrophoresis from an agarose gel. Plasmid pRSE562 was restricted with *Eco*RI and *Bam*HI to liberate the 5.6-kb insert containing the *metE* and *metR* genes, and this fragment was subsequently digested with *Sma* I after gel isolation (16). The resulting 3.1-kb *Bam*HI–*Sma* I fragment, which carries the *metR* gene, was purified from an agarose gel and cloned into the modified pRC25 vector to yield pMMetR. This plasmid was used to transform *E. coli* strain RRI/pRK248cIts. pRK248cIts is a low-copy-number plasmid that contains a gene encoding a temperature-sensitive (42°C) λcIAt2 repressor (24).

Plasmid DNA was purified from cleared lysates by two successive cesium chloride/ethidium bromide equilibrium centrifugations and was used to transform *E. coli* strains B, 2276, and GS244. DNA sequencing was carried out by the procedures of Maxam and Gilbert (25), Sanger (26), and Chen and Seeburg (27).

Growth of *E. coli* RRI/pMMetR. Six liters of *E. coli* strain RRI/pRK248cIts that had been transformed with plasmid pMMetR were grown at 30°C in LB broth. This culture was then used to inoculate 200 liters of LB medium, and the cells were grown at 30°C with vigorous aeration (L. Bowski, Fermentation Unit, Hoffmann–La Roche). When the culture reached mid-logarithmic stage of growth, the temperature was raised to 42°C. After 2 hr at this temperature, the cells (≈1 kg) were recovered by centrifugation and frozen at −70°C.

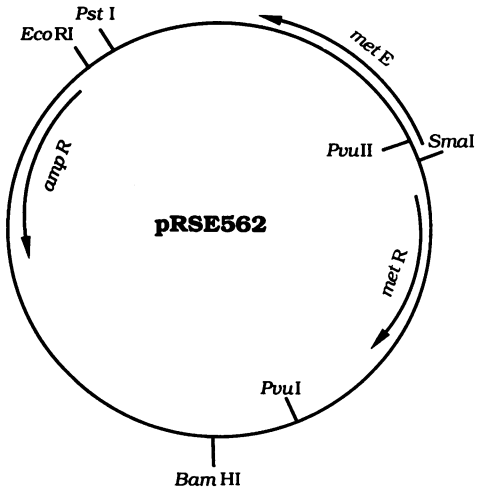

FIG. 1. Schematic diagram of pRSE562. Position of the *metE* and *metR* genes is shown with various restriction sites. Arrows indicate the direction of transcription.

Purification of MetR Protein. The purification of the MetR protein was followed by observing the enrichment of a protein band migrating at 34 kDa after gel electrophoresis. In a typical purification, 50 g of *E. coli* RRI/pRK248cIts transformed with pMMetR was suspended in 200 ml of a buffer containing 10 mM Tris acetate (pH 8.2), 14 mM $Mg(OAc)_2$, 60 mM KOAc, and 1 mM dithiothreitol and sonicated for 12 min at 0°C (Heat Systems-Ultrasonic, Farmingdale, NY). The suspension (crude lysate) was centrifuged for 30 min at 30,000 × *g*, and the supernatant (220 ml; 30 mg/ml) was removed (S-30 extract) and brought to 25% $(NH_4)_2SO_4$ saturation. After centrifugation the precipitate was dissolved in 8 ml of buffer A [50 mM Tris acetate, pH 7.4/1 mM $Mg(OAc)_2$/1 mM dithiothreitol/100 mM KCl] and then dialyzed overnight at 4°C against buffer A [$(NH_4)_2SO_4$ precipitate]. The dialyzed fraction (8 ml; 22 mg/ml) was loaded onto a DEAE-Sepharose column (3 × 5 cm) previously equilibrated with buffer A, and the column was washed with 2 column volumes of buffer A. The bulk of the MetR protein was not retained by the column and appeared in the wash fractions (DEAE fraction). The peak fractions were combined (13 ml; 3.4 mg/ml) and diluted 2-fold to adjust the buffer concentration to 25 mM Tris acetate, pH 7.4/0.5 mM $Mg(OAc)_2$/1 mM dithiothreitol/50 mM KCl (buffer B). Thirteen milliliters was loaded onto a CM-52 (Whatman) column (1 × 4 cm) previously equilibrated with buffer B, and the protein was eluted from the column with a linear gradient (40 ml) from 50 mM KCl to 350 mM KCl in buffer B. Fractions of 0.7 ml were collected, and fractions 35–45 were pooled and concentrated (1.1 mg) by centrifugal ultrafiltration (Centricon 10, Amicon). This preparation (CM-cellulose fraction) was used in the *in vitro* studies described below.

DNA-Directed *in Vitro* Protein Synthesis. An S-30 extract was prepared as described by Zubay *et al.* (28) from *E. coli* strain JM108 and used for the DNA-directed *in vitro* protein-synthesizing system. Each reaction mixture (35 μl) contained S-30 (180 μg of protein), 15 μCi of [^{35}S]methionine (1100 Ci/mmol; 1 Ci = 37 GBq), either 1.0 μg of pRSE562 or 1.2 μg of pMRIII as DNA templates, and other additions as noted in the text. The incubations were performed for 60 min at 37°C, and aliquots were removed and applied to $NaDodSO_4$/10% polyacrylamide gels to resolve the protein products that were detected by autoradiography.

RESULTS

Nucleotide Sequence of the *metE–metR* Intergenic Region. To gain more information on the regulation of the expression of the closely linked *metE* and *metR* genes in *E. coli*, the intergenic DNA sequence was determined (Fig. 2). The *metE* and *metR* genes are transcribed in opposite directions with 236 bases separating the translation initiator codons (underlined). This sequence differs from that reported for *S. typhimurium*, where 248 bases separate the two coding regions (18). The first four amino acids of the MetE protein from *E. coli* and *S. typhimurium* are identical (18, 29), and the present results show that 16 of the first 18 amino acids of the MetR protein in both organisms are also identical (18). Seven potential regulatory *met* boxes (3–7) (that contain at least 50% identity with the *met* box consensus sequence, 5′-AGACGTCT-3′) are overlined in the sequence shown in Fig. 2. Three such 8-base regions are located in tandem about 50 bases upstream of the *metE* coding sequence, and 4 other contiguous 8-base regions are present about 60 bases upstream of the *metR* coding region. In addition, an area of dyad symmetry similar to that found in *S. typhimurium* (18) is present in the *E. coli metE–metR* intergenic region (Fig. 2). A single base change (A → G) in the *E. coli* sequence generates a *Sma* I restriction site (vertical arrow).

***In Vivo* Levels of MetE.** Plasmids pRSE562 (containing the *metR* and *metE* genes) and pMRIII (containing the *metR*

FIG. 2. DNA sequence of the intergenic region of *metE* and *metR* of *E. coli*; the coding strand of *metE* is shown. Seven potential regulatory *met* boxes (at least 50% homologous to the consensus sequence 5'-AGACGTCT-3') are overlined, and the initiation codons for the MetE and MetR proteins are underlined; × and ● indicate regions of dyad symmetry, and the vertical arrow marks the *Sma* I restriction site.

gene) were transformed into wild-type, *metE* mutant, and *metR* mutant strains of *E. coli*, and the non-B_{12} methyltransferase (MetE protein) activity in these cells was determined (Table 1). Transformation of the three strains of bacteria with plasmid pRSE562 caused a 5- to 7-fold increase in MetE activity compared to wild-type *E. coli* B. Note that both *E. coli* 2276 and *E. coli* GS244 are phenotypically methionine auxotrophs—evident by the lack of basal MetE activity in both organisms (Table 1). As expected, after transformation with pRSE562 both mutants grew in the absence of methionine (data not shown). As also shown in Table 1, when methionine (10^{-2} M) was added to the growth medium, >90% repression of *metE* expression was seen in wild-type non-transformed *E. coli*, but only a partial repression was seen in all three strains transformed with pRSE562 in which MetE is overexpressed.

When pMRIII was used to transform *E. coli* B, there was a small increase in MetE activity as compared with wild type and a 64% repression by methionine. No MetE activity occurred in *E. coli* 2276 transformed with this plasmid, and this transformant did not not grow in the absence of methionine. This finding confirms that this organism is a *metE* and not a *metR* mutant. When GS244 was transformed with pMRIII, wild-type MetE levels were restored, and the organism could grow in the absence of methionine. In addition, 10^{-2} M methionine repressed MetE activity by 83% in this transformant.

PAGE analysis of the extracts used in Table 1 agreed with the observed changes in MetE enzyme activity. For example, although a band corresponding to MetE was seen in extracts of wild-type *E. coli* B grown in low methionine, this band was absent in extracts of wild-type cells grown in high methionine or extracts from GS244 strain. However, a prominent MetE band could be seen in extracts of GS244 that had been transformed with either plasmid pRSE562 or pMRIII (data not shown). These results together with those shown in Table 1 indicate that the changes in MetE activity seen after transformation correlate with increased *de novo* synthesis of the MetE protein.

Table 1. MetE enzyme activity in various *E. coli* strains transformed with plasmids containing the *metE* and *metR* genes

E. coli strain	MetE enzyme, specific activity: Low methionine	MetE enzyme, specific activity: High methionine	Repression, %
No plasmid			
B	137	14	93
2276	0		
GS244	0		
Plasmid pRSE562			
B	662	508	23
2276	984	723	26
GS244	864	296	66
Plasmid pMRIII			
B	177	63	64
2276	0		
GS244	136	23	83

Cells were grown in either 3.3×10^{-5} M (low) or 10^{-2} M (high) methionine, and cell-free extracts were prepared and assayed for MetE activity. Specific activity is defined as nmol of methionine synthesized per mg of protein per 15 min. Plasmid pRSE562 contains the *metR* and *metE* genes, and plasmid pMRIII contains the *metR* gene. *E. coli* B is a wild-type strain, whereas strains 2276 and GS244 lack *metE* and *metR*, respectively.

These *in vivo* results confirm previous studies (17) and are consistent with the view that the *metR* gene product is a transactivator for *metE* expression. As shown below, we can now demonstrate this directly by showing an effect of purified MetR protein on the *in vitro* expression of the *metE* gene.

Purification of MetR Protein. The MetR protein was purified to near homogeneity from extracts of *E. coli* RRI/pRK248cIts that had been transformed with a plasmid (pM-MetR) containing the *metR* gene under control of λP_L promoter (see *Materials and Methods*). Fig. 3 shows, by gel analysis, the proteins present in the fractions at various stages of purification. Crude lysates from these cells show the presence of a protein band migrating at ≈34 kDa, corresponding to the MetR protein (lane 1). This protein band was not present in either untransformed *E. coli* RRI/pRK248cIts or in transformed cells that had not been induced by heating to 42°C (data not shown). Although the bulk of the MetR protein was insoluble, due presumably to the formation of inclusion bodies, some MetR protein is present in the S-30 extract (lane

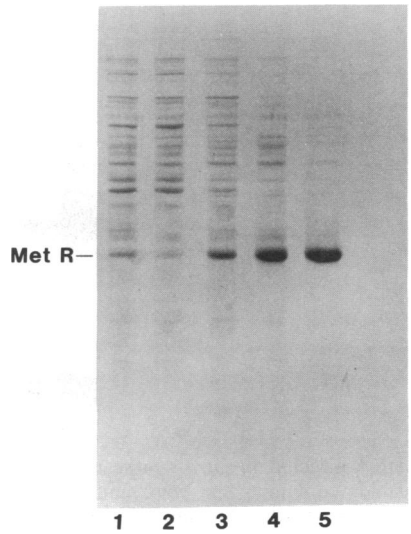

FIG. 3. $NaDodSO_4$/PAGE of MetR protein-containing fractions at different stages of purification. The MetR protein was purified as described in the text. Lanes: 1, crude lysate; 2, S-30 extract; 3, $(NH_4)_2SO_4$ precipitate; 4, DEAE-Sepharose fraction; 5, CM-cellulose fraction. Five micrograms of protein was used in each lane.

2). The protein in this soluble fraction was further purified by $(NH_4)_2SO_4$ fractionation (lane 3), followed by DEAE-Sepharose (lane 4) and CM-52 chromatography (lane 5). The MetR protein in the final fractions was estimated to be >90% pure. Identity of the protein migrating at 34 kDa was confirmed as the MetR protein by N-terminal amino acid analysis. The first 15 amino acids of the protein and those deduced from the DNA sequence were found to be identical (data not shown).

***In Vitro* Expression of *metE* and *metR* Genes.** An S-30 DNA-directed *in vitro* protein-synthesizing system employing [^{35}S]methionine (see *Materials and Methods*) was used to study expression of the *metE* and *metR* genes with plasmids pRSE562 and pMRIII used as templates. Fig. 4, lanes 1–4, summarizes the results with the plasmid containing *metE* and *metR* genes; this plasmid directs the synthesis of the MetR protein, whereas there is poor synthesis of the MetE protein (lane 1). Expression of both genes is inhibited when the MetJ protein and AdoMet are added to the incubations (lane 2). The availability of purified MetR protein (see above) now made it possible to test its effect on *metE* gene expression. Lane 3 shows that the addition of MetR protein (2 μg) to the *in vitro* incubation markedly stimulated the expression of the *metE* gene while causing a small but reproducible inhibition of the synthesis of the MetR protein. Even in the presence of the MetR protein, the MetJ protein and AdoMet still inhibited *in vitro* synthesis of both the MetE and MetR proteins (lane 4). MetR protein and/or MetJ protein and AdoMet had no effect on β-lactamase synthesis (directed by the plasmid by *amp*^{r} gene) in these experiments. Lanes 5–8 show the results when the *metR* plasmid was used as template. This plasmid does not contain the *amp*^{r} gene (β-lactamase) or *metE* gene, so the only *in vitro* product observed is the MetR protein (lane 5). In the presence of the MetJ protein and AdoMet, MetR synthesis is significantly repressed (lane 6), although the effect of the MetJ protein was not as pronounced as seen when the plasmid containing the *metR* and *metE* genes was used (compare lanes 1 and 2 versus lanes 5 and 6). The addition of MetR protein to incubations containing the *metR* gene again showed a small inhibition of MetR synthesis (lane 7), and the presence of MetR protein did not alter the inhibition of MetR synthesis by MetJ protein and AdoMet (lane 8).

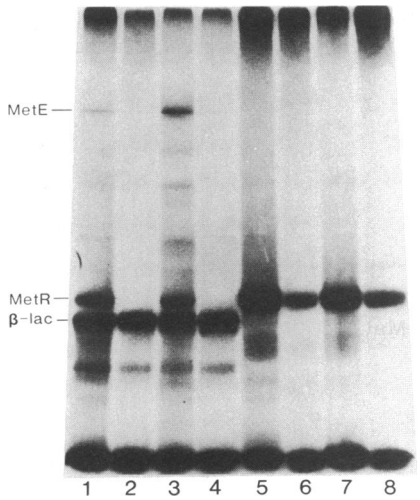

FIG. 4. DNA-directed *in vitro* synthesis of MetE and MetR proteins. Plasmids containing the *metE* and/or *metR* genes were incubated in an S-30 extract containing [^{35}S]methionine as described. The MetR protein (2 μg), the MetJ protein (0.4 μg), and AdoMet (5×10^{-5} M) were added as indicated. Aliquots were removed, subjected to NaDodSO$_4$/PAGE, and autoradiographed. Lanes: 1–4, plasmid pRSE562; 5–8, plasmid pMRIII; 1 and 5, no addition; 2 and 6, MetJ protein and AdoMet; 3 and 7, MetR protein; 4 and 8, MetJ protein, AdoMet, and MetR protein.

DISCUSSION

Present results confirm previous studies on the presence of an additional *met* regulatory locus, *metR* (17, 29, 30) and provide direct evidence that purified MetR protein can stimulate synthesis of the MetE protein in an *in vitro* protein synthesis system. As in *S. typhimurium* the *metR* gene in *E. coli* is closely linked to the *metE* gene. The intergenic space is 236 bases in *E. coli* compared with 248 bases in *S. typhimurium*. A minor difference in the *E. coli* sequence is the presence of a *Sma* I site that is missing in *S. typhimurium*. The *metR* and *metE* genes are transcribed in opposite directions, and the gene products are similar in size in both organisms. Seven potential *met* boxes (3–7) that could be sites for repression by the MetJ protein are present in the *E. coli metE–metR* intergenic region.

Regulation of the synthesis of the MetE protein is thought to be mediated by the MetR protein that acts as a transactivator for *metE* expression (17). This was first demonstrated in an *E. coli* mutant lacking MetE activity. The methionine requirement for growth was relieved when the cells were transformed with a plasmid containing the *metR* gene (17), and the transformant possessed MetE activity. It was also found that when these cells were grown in the presence of 4×10^{-4} M methionine, the synthesis of both MetE–Lac and MetR–Lac fusion proteins was repressed and that this repression depended upon the presence of the MetJ protein (17, 30).

Our *in vivo* results on MetE activity after transformation of wild-type and *metE* and *metR* mutant *E. coli* strains with plasmids containing both *metE* and *metR* genes corroborate these earlier studies (17), although some differences were seen. As example, although transformation of all three strains with the plasmid containing the *metR* and *metE* genes resulted in a large increase in MetE activity (as compared to wild-type cells) only partial repression of MetE activity occurred in the presence of methionine. Perhaps the incomplete repression by methionine in cells producing large amounts of MetE protein was due to limiting amounts of the MetJ repressor protein or of another unknown protein required for MetJ and AdoMet activity.

Gel analysis showed no overproduction of the MetR protein from any of these plasmids with the exception of a high-expression vector in which the *metR* gene was under control of the λP_L promoter (see Fig. 3). This fact suggests that autoregulation of the synthesis of the MetR protein may occur when the *metR* gene is expressed from its own promoter, as indicated in a previous study (30) and in the *in vitro* experiments described here.

Although the previous genetic experiments, as well as the *in vivo* results described in the present work, indicated that the *metR* gene codes for a positive effector of *metE* expression, direct evidence was lacking. To prove this relationship, we isolated the MetR protein and demonstrated an effect of the purified protein on *metE* gene expression *in vitro*. Although the high-expression vector we used, in which MetR synthesis was directed by the λP_L promoter, overproduced the MetR protein *in vivo*, our initial attempts to purify an active protein were unsuccessful. In these early small-scale experiments practically all the MetR protein was insoluble and appeared to be in inclusion bodies. Although the protein could be solubilized with urea, the protein was not active in our *in vitro* protein synthesis system. However, when cells were grown on a larger scale (see *Materials and Methods*), a significant portion of the synthesized MetR protein remained soluble. It was then possible to develop a rapid and simple purification involving $(NH_4)_2SO_4$ fractionation of a 30,000 ×

g supernatant, negative adsorption on DEAE-Sepharose, and a purification by CM-cellulose chromatography. The MetR protein thus obtained appeared >90% pure, and although the yield was low (1–2 mg of pure protein from 50 g of cells) due to the large amounts of insoluble MetR protein, sufficient purified protein was isolated to test in the *in vitro* protein synthesis system. The *in vitro* studies confirmed what was predicted from *in vivo* experiments. When the plasmid containing both *metE* and *metR* genes was used as template, synthesis of MetE protein was markedly stimulated by the MetR protein, whereas synthesis of the MetR protein was decreased when the MetR protein was added to the incubation, suggesting that synthesis of the MetR protein is autoregulated. Addition of the MetJ protein and AdoMet to the *in vitro* incubations caused almost complete repression of synthesis of both MetR and MetE proteins. The observation that the MetJ protein and AdoMet repressed expression of the *metE* gene even in the presence of exogenously added MetR protein suggests that synthesis of the MetE protein can be regulated in a dual manner. Thus, MetJ protein and AdoMet not only repress synthesis of the MetR protein, which is required for the expression of the *metE* gene (17), but the *in vitro* results suggest that the MetJ protein and AdoMet also directly repress expression of the *metE* gene. The latter mechanism ensures that synthesis of the MetE protein is repressed by methionine, even in the presence of the MetR protein. These results also suggest that the putative *met* boxes located upstream of the *metE* coding sequence are functional (Fig. 2). In this regard the *in vivo* expression of the *metE* gene from a plasmid that contains the *metE* coding region and only 73 bases upstream of the initiation codon was repressed by methionine in a *metR* strain (data not shown).

Although synthesis of the MetE protein is greatly stimulated by the MetR protein, *in vitro* synthesis of the MetE protein, relative to the MetR protein, still appears low (Fig. 4). As noted above, the MetE protein accounts for as much as 5% of the soluble *E. coli* protein (22). Preliminary data indicate that there may be a specific inhibitor of *metE* gene expression in the S-30 extracts because expression of the *metE* gene was increased when the S-30 extract was replaced with a partially defined protein synthesis system (data not shown). Further *in vitro* studies are required to elucidate details of the molecular mechanism by which the MetR protein regulates expression of *metE* gene.

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