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Heat Shock RNA Polymerase ($E\sigma^{32}$) Is Involved in the Transcription of *mlc* and Crucial for Induction of the Mlc Regulon by Glucose in *Escherichia coli**

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Mlc is a global regulator of carbohydrate metabolism. Recent studies have revealed that Mlc is depressed by protein-protein interaction with enzyme $IICB^{Glc}$, a glucose-specific permease, which is encoded by *ptsG*. The *mlc* gene has been previously known to be transcribed by two promoters, P1(+1) and P2(+13), and have a binding site of its own gene product at +16. However, the mechanism of transcriptional regulation of the gene has not yet been established. *In vitro* transcription assays of the *mlc* gene showed that P2 promoter could be recognized by RNA polymerase containing the heat shock sigma factor σ^{32} ($E\sigma^{32}$) as well as $E\sigma^{70}$, while P1 promoter is only recognized by $E\sigma^{70}$. The cyclic AMP receptor protein and cyclic AMP complex (CRP·cAMP) increased expression from P2 but showed negative effect on transcription from P1 by $E\sigma^{70}$, although it had little effect on transcription from P2 by $E\sigma^{32}$ *in vitro*. Purified Mlc repressed transcription from both promoters, but with different degrees of inhibition. *In vivo* transcription assays using wild type and *mlc* strains indicated that the level of *mlc* expression was modulated less than 2-fold by glucose in the medium with concerted action of CRP·cAMP and Mlc. A dramatic increase in *mlc* expression was observed upon heat shock or in cells overexpressing σ^{32}, confirming that $E\sigma^{32}$ is involved in the expression of *mlc*. Induction of *ptsG* P1 and *pts* P0 transcription by glucose was also dependent on $E\sigma^{32}$. These results indicate that $E\sigma^{32}$ plays an important role in balancing the relative concentration of Mlc and $EIICB^{Glc}$ in response to availability of glucose in order to maintain inducibility of the Mlc regulon at high growth temperature.

When Mlc is overproduced on a multicopy plasmid in *Escherichia coli* grown in the presence of glucose, it causes reduction of acetate accumulation and *E. coli* makes large colonies (1). Mlc has been proposed to be a new global regulator of carbohydrate metabolism (2–5). It has been reported that Mlc regulates *manXYZ* encoding enzyme II of the mannose PTS (4), *malT* encoding the activator of maltose operon, and *mlc* itself negatively (2). Moreover, *ptsG* encoding enzyme IICB of the glucose PTS ($EIICB^{Glc}$) and the *pts* operon encoding general PTS proteins also proved to be repressed by Mlc (3, 5–8). The Mlc regulon is also under the positive control of the CRP[1]·cAMP complex. It has been discovered that repression of the Mlc regulon is relieved in cells grown in the media containing glucose or other PTS sugars (7). It has been shown that the unphosphorylated $EIICB^{Glc}$ can sequester Mlc from its binding sites by direct protein-protein interaction to induce expression of the Mlc regulon in response to glucose (9–11).

The *mlc* gene encoding a 44-kDa Mlc protein is located around 35 min of chromosomal locus (1). This gene was also identified as the same allele of the *dgsA* gene (4, 12, 13). Decker *et al.* (2) have shown that *mlc* transcription starts from two promoters called upstream "+1" and downstream "+13" and there exists one Mlc-binding site centered at +16. In addition, a highly conserved CRP-binding site is present within the *mlc* promoter. However, the detailed mechanisms of transcriptional regulation of *mlc* have not yet been reported.

The majority of the *E. coli* promoters are recognized by the RNA polymerase containing the housekeeping sigma factor, σ^{70} (14, 15). Several genes that are necessary to respond to various environmental or nutritional changes require specific recognition by RNA polymerase associated with the alternative sigma factors, σ^{32} (16), σ^{E} (17), σ^{54} (18), or σ^{S} (19). The heat shock response in *E. coli* is mediated by $E\sigma^{32}$ (20) and it is known that expression of at least 26 genes is induced by heat shock in *E. coli* (21). Many essential genes in *E. coli* have multiple promoters including one recognized by $E\sigma^{32}$ in order to respond to various environmental conditions (22–24). It has been shown that the *pts* P0 promoter is recognized by $E\sigma^{32}$ as well as $E\sigma^{70}$ (25) as is expected for a system as central to carbohydrate metabolism as the PTS. In this work, we studied the transcriptional regulation of the *mlc* gene *in vitro* as well as *in vivo* and the role of $E\sigma^{32}$ in maintaining glucose-dependent induction of the Mlc regulon at high growth temperature.

EXPERIMENTAL PROCEDURES

Materials—Cyclic AMP was obtained from Sigma, RNA polymerase saturated with σ^{70}, nucleotide triphosphates, [γ-^{32}P]ATP, and [α-^{32}P]UTP were purchased from Amersham Pharmacia Biotech. The cycle sequencing kit was from Epicentre Technologies (Madison, WI).

Bacterial Strains—MC4100 (*araD139* Δ*argF-lacU169rpsL150 thiA relA1 flb5301 deoC1 ptsF25 rbsR*) was used as a wild type strain in this study. The *mlc* strain SR505 is the same as KD413 (MC4100, *mlc*::Tn*10*Tet). To construct the *rpoH*-deleted mutant, SR701 (SR702, Δ*rpoH*), Δ*rpoH* allele of KY1603 (26) was transferred to SR702

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[1] The abbreviations used are: CRP, cyclic AMP receptor protein; PTS, phosphoenolpyruvate: carbohydrate phosphotransferase system.

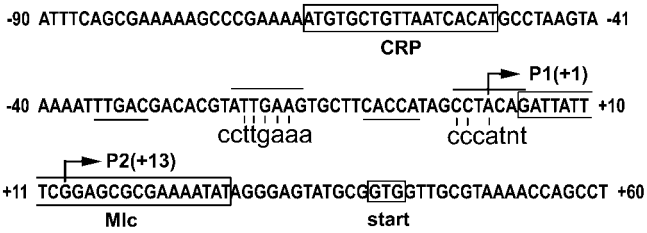

FIG. 1. **Nucleotide sequence of the promoter region of *mlc*.** The transcription start points of P1(+1) and P2(+13) are shown with *arrows*. The −10 and −35 region of the P1 promoter is *underlined* and that of P2 is indicated with a *line over* the sequence. The known consensus −10 and −35 sequence recognized by Eσ^{32} is shown in *lowercase* (20). The binding site of CRP and Mlc is indicated as *box*. The start codon of *mlc*, GTG, is also indicated. All of the numbering of this sequence is based on the transcription start point of the P1 promoter.

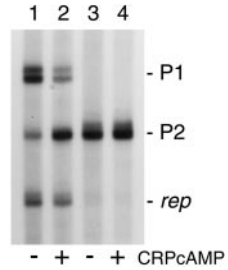

FIG. 2. **The effect of CRP·cAMP on *mlc* transcription *in vitro*.** The supercoiled DNA template, pMX, was used for the *in vitro* transcription by Eσ^{70} (*lanes 1* and *2*) and Eσ^{32} (*lanes 3* and *4*) in the absence (*lanes 1* and *3*) and presence (*lanes 2* and *4*) of the CRP·cAMP complex. The transcripts from the plasmid origin of replication (106/107 nucleotides) are marked as *rep*. The 132-nucleotide transcript from P1 and the 120-nucleotide transcript from P2 are indicated.

(MC4100, *suhX1* (6)) using P1 transduction. To study the effect of σ^{32} on *mlc* expression, a plasmid pKV10 in which the *rpoH* gene is under control of *tac* promoter was introduced into MC4100.

Plasmid Construction and Preparation—Basic cloning protocols used were described in Sambrook and Russell (27). Polymerase chain reaction cloning of the *mlc* promoter was carried out using primers that have unique restriction sites in their sequences. The clone was verified by DNA sequencing. The supercoiled plasmid pMX, which contains the *mlc* promoter region, was made by inserting the DNA segment from base pair −272 to +105 (all of these numberings are based on the transcription start site of the P1 promoter of *mlc* in Fig. 1) between the *Eco*RI and the *Pst*I sites in front of the *rpoC* terminator in plasmid pSA600 (28). Supercoiled DNA was prepared by Concert kit (Life Technologies, Inc.) in RNase-free condition for *in vitro* transcription assay.

Primer Extension—Cells were grown aerobically at 30 °C in tryptone broth (1% Bacto-tryptone, 0.8% NaCl) either in the presence or absence of 0.2% glucose. At A_{600} = 0.5, growth temperature was shifted to 42 °C and incubation was continued for the designated time and total *E. coli* RNA was purified using Trizol reagent (Life Technologies, Inc.) to study the heat shock effects on *mlc* and *ptsG* transcription. Purified RNA was resuspended in sterile distilled water. To study *mlc* transcription, ^{32}P-labeled primer MLC7 (5′-ATTTTAGTGATACTGGCAGGAGCCAGTTGC-3′), which is complementary to +162 to +192, was co-precipitated with 40 μg of total cell RNA. The primer PG1 (5′-AATTGAGAGTGCTCCTGAGTATGGGTGC-3′, complementary to +74 to +102) and the primer P11 (6) were co-precipitated with 30 μg of total RNA to study *ptsG* and *pts* transcription, respectively. The pellet was resuspended in 20 μl of 250 mM KCl, 2 mM Tris-HCl, pH 7.9, and 0.2 mM EDTA. Primer extension reactions were done as described by Ryu and Garges (29).

Purification of RNA Polymerase Holoenzyme Containing σ^{32}—E. coli MC4100 cells harboring the pKV10 plasmid were grown on 2 × LB (1% Bacto-tryptone, 0.5% yeast extract, 2% NaCl) in a 5-liter fermenter at 30 °C in the presence of 1 mM isopropyl-1-thio-β-D-galactopyranoside. The cells were harvested 10 min after a temperature shift from 30 to 42 °C at A_{600} = 2.5 in late exponential growth phase. RNA polymerase was purified by fast protein liquid chromatography according to Harger *et al*. (30) with some modifications as described by Sukhodolets *et al*. (31). Crude RNA polymerase was obtained from 10 g of wet cell paste by Polymin P precipitation and the drained pellet was solubilized with TGED (10 mM Tris, pH 7.9, 5% (v/v) glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol) containing 0.2 M NaCl (TGED^N). This enzyme solution was loaded on a single-stranded DNA-agarose column (Amersham Pharmacia Biotech) pre-equilibrated with TGED^N. After the column was washed with 3 column volumes of TGED^N, RNA polymerase was eluted with a linear salt gradient (from TGED^N to TGED containing 1.0 M NaCl) at a flow rate of 2 ml/min. The elution of RNA polymerase was monitored by the presence of ββ′ on a sodium dodecyl sulfate-polyacrylamide gel. The fractions containing RNA polymerase (from 0.3 to 0.5 M NaCl gradient) were loaded onto a 5/5 Mono-Q column (Amersham Pharmacia Biotech), and RNA polymerase containing σ^{32} was eluted with a linear gradient of NaCl (0.3 M to 0.5 M in TGED) at 1 ml/min. The yield for Eσ^{32} was 1.0∼1.5 mg.

In Vitro Transcription Assay—Reactions were done as described by Ryu and Garges (29) in a 25-μl volume containing the following: 20 mM Tris acetate, pH 8.0, 3 mM magnesium acetate, 200 mM potassium glutamate, 1 mM dithiothreitol, 1 mM ATP, 0.2 mM GTP, 0.2 mM CTP, 0.02 mM UTP, 10 μCi of [α-^{32}P]UTP (800 Ci/mmol), 2 nM supercoiled DNA template, 20 nM RNA polymerase saturated with σ^{70} or σ^{32}, 100 μg/ml bovine serum albumin, and 5% glycerol. Additional regulators such as 40 nM CRP, 100 μM cAMP, or 0–1.5 μM Mlc were added to the reaction as needed. All components except nucleotides were incubated at 37 °C for 10 min. Transcriptions were started by the addition of nucleotides and terminated after 10 min by the addition of 25 μl of formamide loading buffer (80% formamide, 89 mM Tris base, 89 mM boric acid, 2 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol). RNA was resolved by electrophoresis on an 8 M urea, 6% polyacrylamide gel. The amounts of transcripts were measured using a phosphoimage analyzer, BAS2500 (Fuji Photo Film Co.).

RESULTS

The mlc Promoter Is Recognized by Both Eσ^{70} and Eσ^{32} in Vitro and Its Activity Is Modulated by CRP·cAMP and Mlc—As shown in Fig. 1, there is a CRP-binding site centered at −58.5 and one Mlc-binding site centered at +16 within the *mlc* promoter region (2). The sequence of the P2 promoter region has a partial homology with a known consensus sequence (CTTGAAA, 11∼16 base pairs, CCCATNT) of the promoters recognized by Eσ^{32} (20). To investigate how the two promoters of the *mlc* gene are regulated, we performed *in vitro* transcription assays with supercoiled DNA template, purified proteins, and either Eσ^{70} or Eσ^{32}. When Eσ^{70} was used, transcripts were made from both the P1 and P2 promoters (Fig. 2, *lanes 1* and *2*). However, Eσ^{32} could initiate transcription only from P2, as expected from the sequence of the promoter region of *mlc* shown in Fig. 1. No transcription from P1 by Eσ^{32} was detected (Fig. 2, *lanes 3* and *4*). The specificity of Eσ^{32} used for the *in vitro* transcription assay was confirmed by the lack of "*rep*" transcript that is transcribed only by Eσ^{70}. Interestingly, CRP·cAMP showed a negative effect on P1 transcription by Eσ^{70}, while it exerted a positive effect on P2 transcription directed by Eσ^{70}. However, CRP·cAMP had little effect on Eσ^{32}-directed transcription from P2.

The purified Mlc could inhibit transcription from both promoters but the degree of repression was dependent on the kind of σ factor and the promoter (Fig. 3). Transcription from the P2 promoter by Eσ^{70} was most sensitive to the repression by Mlc, and half-repression of Eσ^{70}-directed P2 expression by Mlc was obtained at the level of 11.5 and 6.5 nM of the protein in the presence and absence of CRP·cAMP, respectively (Fig. 3*A*). However, about 100-fold more Mlc (0.7 and 0.6 μM of Mlc in the presence and absence of CRP·cAMP, respectively) was required in order to repress the P1 transcription (Fig. 3*B*). For half-repression of the Eσ^{32}-directed P2 promoter activity, 20 and 12.5 nM Mlc were required in the presence and absence of CRP·cAMP, respectively (Fig. 3*C*). Repression of P1 transcription and induction of Eσ^{70}-directed P2 transcription by CRP·cAMP shown in Fig. 2 were also observed in the presence of Mlc (compare *lanes 1-4* with *lanes 5-8* in Fig. 3, *A* and *B*).

Transcription of mlc Was Modulated Less Than 2-fold by Glucose—We studied further the regulation of *mlc* transcription *in vivo* by primer extension assay. The wild type strain

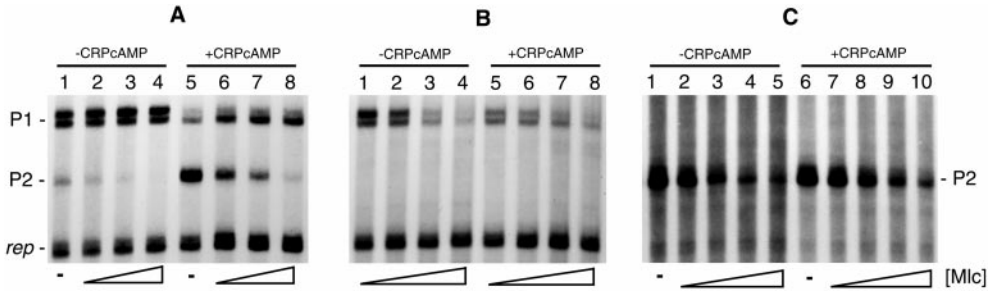

FIG. 3. **The autoregulatory effect of Mlc on its own gene *in vitro*.** *In vitro* transcription was carried out using pMX as a DNA template. Forty nM of CRP·cAMP was added to the reactions as marked in the *top* of the figures. *A,* Eσ^{70}-directed transcription at low level of Mlc. The amount of Mlc used in each lane is following: *lanes 1–4* and *lanes 5–8* contains 0, 6.3, 12.5, and 25 nM Mlc in the reaction, respectively. *B,* Eσ^{70}-directed transcription at high level of Mlc. 0.25, 0.5, 1.0, and 1.5 μM Mlc was added to the *lanes 1–4* and *5–8*, respectively. *C,* Eσ^{32}-directed transcription in the presence of Mlc. *Lanes 1–5* and *6–10* contain 0, 12.5, 25, 50, and 100 nM Mlc, respectively.

MC4100 and its isogenic mutant strains were grown at 30 °C in the presence or absence of glucose then total RNA was extracted as described under "Experimental Procedures." Transcription from both P1 and P2 promoter was detected and the level of expression from two promoters was similar when the wild type cells were grown in the absence of glucose (Fig. 4A, *lane 2*). However, when cells were grown in the presence of glucose, transcription from P1 was increased slightly while that from P2 was not detectable (Fig. 4A, *lane 1*). These results establish that P1 and P2 promoter activity was dependent on CRP·cAMP *in vivo* in accordance with the *in vitro* transcription assay results as described above and that the overall expression level of *mlc* was changed less than 2-fold by glucose.

We analyzed the *mlc* expression in SR505 (MC4100, *mlc*::Tn*10*) to test the autoregulatory effect of Mlc *in vivo*. The level of transcription could be analyzed in SR505 because Tn*10* was inserted in the C-terminal region of the *mlc* gene. Transcription from both promoters was increased clearly when the *mlc::* strain was grown in the absence of glucose (Fig. 4*A*, compare *lanes 2* and *4*), suggesting that Mlc acts as a repressor of the *mlc* promoter. A strong stimulatory effect of CRP·cAMP on *mlc* P2 promoter activity could also be seen when we compared the P2 promoter activities in SR505 grown in the presence and absence of glucose (Fig. 4*A*, *lanes 3* and *4*).

The P2 Promoter of mlc Is a Bona Fide Heat Shock Promoter—In vitro transcription assay showed that the P2 promoter of *mlc* was recognized by Eσ^{32} as well as Eσ^{70}. We analyzed expression of *mlc* in the Δ*rpoH* strain using primer extension assay. SR701 strain (MC4100, *suhX1*, Δ*rpoH*) can grow at 30 °C because it expresses a high level of GroELS due to an IS*1* element inserted upstream of the *groE* gene (6, 26). Overexpression of GroELS did not affect *mlc* expression (data not shown). As shown on Fig. 4*A* (*lanes 5* and *6*), the expression level of *mlc* was decreased but the overall expression pattern was not changed in the Δ*rpoH* cell compared with wild type. However, P2 transcription was increased and its activity was not much affected by glucose in cells overexpressing σ^{32} (Fig. 4*A*, *lanes 7* and *8*). It is also interesting to note that even the activity of P1 that is not recognized by Eσ^{32} *in vitro* was increased significantly when cells overexpressing Eσ^{32} was grown in the presence of glucose (Fig. 4*A*, *lane 7*).

We also examined changes in the *mlc* expression upon heat shock by primer extension analysis. Growth temperature of a wild type strain, MC4100, was shifted from 30 to 42 °C, and cells were further incubated for the designated time as described under "Experimental Procedures." As shown on Fig. 4*B*, temperature upshift caused dramatic changes in the *mlc* expression. The *mlc* expression reached the maximum level 5 min after heat shock (Fig. 4*B*, *lanes 3* and *4*), when the intracellular concentration of Eσ^{32} is known to be at the highest level (20, 32). When cells were grown in the presence of glucose

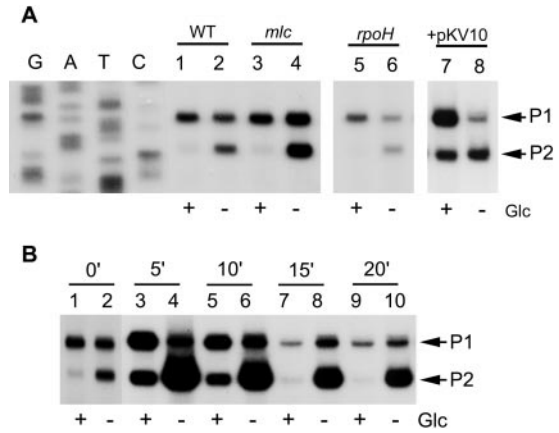

FIG. 4. ***In vivo* transcription analysis and heat shock response of *mlc*.** Primer extension analysis using 40 μg of total RNA per reaction was carried out as described under "Experimental Procedures." The start site of each promoter was marked with the name of each promoter. Sequence ladder was generated using the same end-labeled primer used for primer extension assay. (A) Wild type strain, MC4100 (*lanes 1* and *2*), MC4100 *mlc*::Tn*10* (*lanes 3* and *4*), Δ*rpoH* strain (SR701, *lanes 5* and *6*), and MC4100 harboring pKV10 in which *rpoH* gene is under control of *tac* promoter (*lanes 7* and *8*) were grown at 30 °C in the presence or absence of glucose. In the case of MC4100 carrying pKV10, isopropyl-1-thio-β-D-galactopyranoside was added to overexpress σ^{32}. *B,* changes in *mlc* transcription in response to heat shock were monitored using primer extension analysis. Duration of heat shock was indicated on *top*.

(Fig. 4*B*, *odd lanes*), expression of both P1 and P2 was increased transiently after heat shock as in the cells overexpressing σ^{32} (Fig. 4*A*). The increased level of *mlc* expression from both P1 and P2 upon heat shock was decreased sharply after 15 min at 42 °C in the presence of glucose. Activation of *mlc* by heat shock was not dependent on the presence of glucose. The cells grown in the absence of glucose showed relatively little change in the P1 transcription but a high level of activation in the P2 transcription in response to heat shock (Fig. 4*B*, *even lanes*). However, heat shock effect was not observed in the Δ*rpoH* strain (data not shown), confirming that activation of *mlc* transcription by heat shock was dependent on Eσ^{32}. These results show that Eσ^{32} can recognize the P2 promoter *in vivo* and Eσ^{32}-directed transcription from P2 becomes more prominent when cells were grown at a high temperature or overexpressing σ^{32}.

Transcription of ptsG Is Increased by Heat Shock and Glucose Induction of ptsG Requires Eσ^{32}—How does the highly increased level of *mlc* expression in heat-shocked cells affect glucose induction of Mlc regulon? To answer this question, we analyzed changes in the *ptsG* transcription upon heat shock. It has been known that transcription of the *ptsG* gene in *E. coli* encoding the major membrane-bound glucose transporter, EI-ICB^{Glc}, is initiated from a major promoter, P1, and a minor

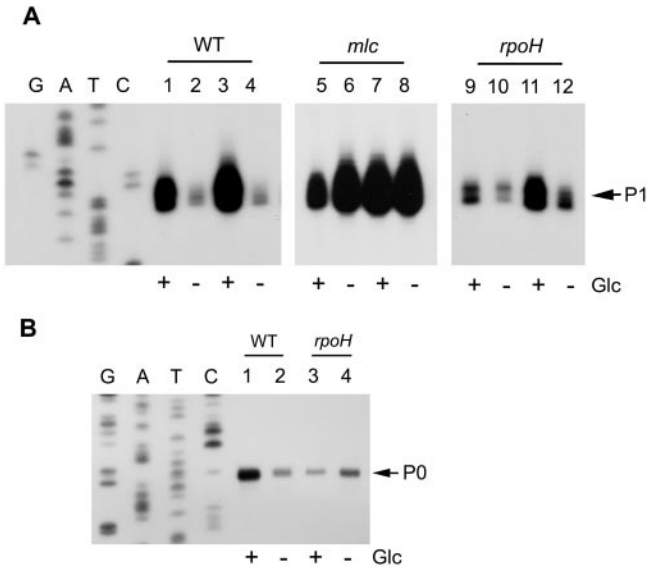

FIG. 5. **Effects of heat shock RNA polymerase and Mlc on *ptsG* P1 and *pts* P0 transcription analyzed by primer extension analysis.** *A,* heat shock effects on *ptsG* P1 transcription. Total RNA was extracted from cells grown at 30 °C to A_{600} = 0.5 in the presence or absence of glucose (*lanes 1, 2, 5, 6, 9,* and *10*) or cells held at 42 °C for 5 min before RNA extraction (*lanes 3, 4, 7, 8, 11,* and *12*). *B,* effects of heat shock RNA polymerase on glucose induction of *pts* P0 transcription. The *pts* P0 transcription was not induced by glucose in *rpoH* strain.

promoter, P2, and that both P1 and P2 transcription is regulated negatively by Mlc (3, 5). There were no changes in P2 transcription of *ptsG* by heat shock (data not shown). But P1 transcription of *ptsG* was increased 5 min after heat shock when wild type cells were grown in the presence of glucose (Fig. 5*A*, compare *lanes 1* and *3*) despite that *mlc* expression was also increased at this condition as shown in Fig. 4*B*. However, *ptsG* P1 transcription was not changed by heat shock when cells were grown in the absence of glucose. Activation of *ptsG* P1 transcription by heat shock was also seen in the Δ*mlc* strain (Fig. 5*A*, *lane 7*), suggesting the possibility that Mlc was not involved in the activation of *ptsG* P1 by heat shock. In that expression of both $EIICB^{Glc}$ and its negative regulator Mlc was increased by heat shock, it seems that there is another mechanism(s) to maintain glucose induction of the Mlc regulon under the heat shock condition.

As shown on Fig. 5*A*, induction of *ptsG* P1 transcription by glucose was reduced significantly in the Δ*rpoH* strain. From these results, together with our previous data (9), it could be assumed that the Mlc regulon could not be induced by glucose because not enough $EIICB^{Glc}$ was available to sequester Mlc in the Δ*rpoH* strain. As expected, *pts* P0, one of several genes known to be under Mlc control (6–8), was not induced in the Δ*rpoH* strain (Fig. 5*B*). These results support the view that the expression of $EIICB^{Glc}$ and Mlc is balanced in response to glucose uptake even when the cells were heat-shocked and that $E\sigma^{32}$ plays an important role in this regulation. However, it should be noted that heat shock still increased P1 transcription of *ptsG* in the Δ*rpoH* strain albeit to a lower degree when cells were grown in the presence of glucose (Fig. 5*A*).

DISCUSSION

It has been suggested that the *mlc* gene has two promoters, P1 and P2, which are separated by 12 bases and autoregulated by its product (2). Here, we report that the transcription of the *mlc* gene is regulated in a highly sophisticated manner and that heat shock σ factor, σ^{32}, is involved in its transcription.

It is known that the expression level of several genes encoding transcriptional repressors such as *galS* (33), *nagC* (34), *purR* (35), and *trpR* (36) of *E. coli* is low and that their expression level is not modulated much in various growth conditions. It seems likely that both CRP·cAMP and Mlc work together in *E. coli* to maintain the level of Mlc optimum in response to availability of glucose. All genes known to be regulated negatively by Mlc, such as *manXYZ*, *malT*, *ptsG,* and *pts*, are also regulated positively by CRP·cAMP (2–8). *In vitro* transcription assay with $E\sigma^{70}$ and CRP·cAMP showed two opposite effects on each promoter of *mlc*, that is the positive effect on P2 and the negative effect on P1 (Fig. 2). This can be explained based on the fact that the CRP-binding site of P2 centered at −71.5 to the transcription start site is more compatible for a functional CRP site (37) compared with that of P1 centered at −58.5 to the transcription start site. However, $E\sigma^{32}$-directed P2 transcription was insensitive to CRP·cAMP. The P2 promoter of *mlc* should be a good model system to assess the effect of σ factor on transcription activation by CRP·cAMP because it has been known that CRP·cAMP activates transcription by direct protein-protein interaction with the α-subunit of RNA polymerase (38).

Repression of the P2 transcription when cells were grown in the presence of glucose implies that the action of CRP·cAMP is dominant over the self-repression by Mlc in the regulation of the *mlc* P2 promoter. The low binding affinity of Mlc to its own promoter that is 10 times weaker than that to *ptsG* or *pts* P0 promoters (9) seems to be a major reason for the low influence of Mlc on regulation of its own gene. *In vitro* transcription assay revealed that each promoter of *mlc* has a different sensitivity to Mlc (Fig. 3). When cells were grown in the absence of glucose, a similar level of expression from both P1 and P2 was observed even though the P2 transcription is more sensitive to Mlc repression probably because the intracellular concentration of Mlc is limiting in *E. coli* (3). The condition seems to be similar to the *in vitro* transcription condition where both CRP·cAMP and a small amount of Mlc were present as shown in *lane 6* of Fig. 3*A*. However, P1 was as active as P2 when the *mlc* strain was grown in the absence of glucose. These results imply that the concentration of intracellular CRP·cAMP is lower than that of CRP·cAMP used for *in vitro* transcription reactions (40 nM) (39). When Mlc was induced and the concentration of CRP·cAMP was lowered by the addition of glucose in the growth medium, the P1 promoter was activated slightly while the P2 promoter was repressed because the P2 promoter is active only in the presence of CRP·cAMP. This situation is similar to the *in vitro* transcription condition where neither CRP·cAMP nor Mlc were present (*lane 1* of Fig. 3*A*). Therefore, the addition of glucose in the growth medium resulted in the reduction of *mlc* expression by about half. These results also agree with the previous report by Decker *et al*. (2) that expression of *mlc* is reduced by half when cells were grown in the presence of glucose by measuring the β-galactosidase activity of the *mlc-lacZ* fusion. Level of Mlc expression can vary precisely in response to the available sugars but the variation range is less than 2-fold in that the availability of unphosphorylated $EIICB^{Glc}$ may be more critical than the intracellular level of Mlc for induction of the Mlc regulon by glucose as shown in our previous report (9).

$E\sigma^{32}$ is involved in the transcription of the *mlc* gene. *In vitro* transcription assay with $E\sigma^{32}$ showed that $E\sigma^{32}$ could recognize the P2 promoter of the *mlc* gene. Transcription of P2 was increased when σ^{32} was overexpressed (Fig. 4*A*). Moreover, *mlc* expression was increased upon heat shock. It is known that the intracellular concentration of σ^{32} in *E. coli* increases from 15–20-fold within 5 min then declines to a new steady-state level severalfold higher than the preshift level in response to tem-

perature shift from 30 to 42 °C (20, 32). Transcription from P2 recognized by Eσ^{32} was induced transiently to an extraordinary level upon heat shock when cells were grown in the absence of glucose (Fig. 4*B*, *lane 4*). The level of *mlc* transcription was changed parallel to the changes in intracellular concentration of σ^{32}. In addition, P2 transcription was activated upon heat shock even when cells were grown in the presence of glucose. These results imply that the major RNA polymerase which activated the P2 transcription upon heat shock was Eσ^{32} because Eσ^{32} was less sensitive to Mlc than Eσ^{70} and the P2 transcription by Eσ^{32} was not dependent on CRP·cAMP as revealed by the *in vitro* transcription assay (Fig. 3). It is not clear why the P1 transcription was reduced in the Δ*rpoH* strain and activated by heat shock or when cells overexpressing σ^{32} were grown in the presence of glucose even though P1 promoter was not recognized by Eσ^{32} *in vitro*. Because heat shock should exert pleiotropic effects by regulating transcription of various genes (21), further study on the mechanism of heat shock is needed for a better understanding of these phenomena.

To investigate whether the increased level of *mlc* expression resulting from heat shock can influence Mlc-dependent gene expression, we analyzed changes in *ptsG* expression by heat shock. The P1 expression of *ptsG* was increased significantly by heat shock only when cells were grown in the presence of glucose regardless of the presence of Mlc (Fig. 5*A*). These results suggest that activation of *ptsG* P1 by heat shock was not mediated by Mlc or CRP·cAMP even though Mlc repression might be dominant over activation of *ptsG* P1 by heat shock. We have reported that the unphosphorylated form of $EIICB^{Glc}$ sequesters Mlc from its target promoters upon glucose uptake by direct protein-protein interaction (9). Therefore, glucose is required to maximize the level of dephosphorylated $EIICB^{Glc}$ necessary to sequester Mlc that is increased by heat shock. However, contrary to the case of *mlc* P2 transcription in which Eσ^{32} plays a major role in its regulation, it is likely that additional factors independent of Eσ^{32} are involved in regulation of the *ptsG* expression because *ptsG* P1 expression was increased partially upon heat shock even in the Δ*rpoH* strain grown in the presence of glucose. It means that two separate mechanisms involving Eσ^{32}-dependent and Eσ^{32}-indendent activation of *ptsG* P1 may work additively for full activation of *ptsG* P1 by glucose when cells were heat-shocked. The importance of Eσ^{32} in glucose induction of the Mlc regulon was manifested by the fact that glucose induction of *ptsG* could not be observed in the Δ*rpoH* strain. The inability of glucose to activate *ptsG* expression resulted in an insensitivity of the *pts* P0 promoter to glucose in Δ*rpoH* strain. We are trying to elucidate the mechanism of activation of *ptsG* transcription by heat shock in the absence of Eσ^{32} in order to understand the general role of Eσ^{32} in regulation of genes involving carbohydrate metabolism.

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Heat Shock RNA Polymerase ($E\varsigma^{32}$) Is Involved in the Transcription of *mlc* and Crucial for Induction of the Mlc Regulon by Glucose in *Escherichia coli*

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