Biochemical and Biophysical Research Communications 370 (2008) 609–612

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Up-regulation of the cellular level of *Escherichia coli* PTS components by stabilizing reduced transcripts of the genes in response to the low oxygen level

Dongwoo Shin [b], Namwook Cho [a], You-Jin Kim [c], Yeong-Jae Seok [c], Sangryeol Ryu [a,*]

[a] Department of Food and Animal Biotechnology, School of Agricultural Biotechnology, Center for Agricultural Biomaterials, and Research Institute for Agriculture and Life Sciences, Seoul National University, Shilim Dong, Seoul 151-921, Republic of Korea
[b] Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon 440-746, Republic of Korea
[c] Department of Biological Sciences, Seoul National University, Seoul 151-921, Republic of Korea

ARTICLE INFO

Article history:
Received 13 March 2008
Available online 8 April 2008

Keywords:
pts
ArcA
mRNA stability

ABSTRACT

When *Escherichia coli* cells were grown with limited levels of oxygen, the glucose-induced transcription of *ptsG* was decreased whereas deletion of the *arcA* gene partially restored it, which was consistent with the previous report that the ArcA protein represses *ptsG* transcription. However, under this circumstance, we found that the level of $EIICB^{Glc}$ protein encoded by the *ptsG* gene was rather increased. This paradoxical phenomenon can be explained by the delayed turnover of *ptsG* mRNA in cells anaerobically grown in the presence of glucose. Finally, our data showed that anaerobic expression of the *ptsHIcrr* operon is also enhanced by increasing the longevity of the reduced mRNA levels.

In addition to the phosphorylation-coupled transport of various sugars, the bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS) [1] exerts multiple roles such as chemoreception [2], catabolite repression [3,4], carbohydrate transport and metabolism [5,6], carbon storage [7], and the coordination of carbon and nitrogen metabolism as well as sugar uptake [8,9]. For this reason, synthesis of the PTS components should be regulated in a highly sophisticated way.

It was reported that expression of the *ptsG* gene and the *ptsHIcrr* operon encoding the enzyme $IICB^{Glc}$ ($EIICB^{Glc}$) protein and the general PTS proteins, respectively, is increased to a few fold during cells' growth on glucose or other PTS substrates, which is mediated by two antagonistic regulatory proteins, transcriptional activator CRP (i.e. CRP·cAMP complex) and repressor Mlc [10–14]. We and other research groups demonstrated that unphosphorylated form of $EIICB^{Glc}$ can relieve the Mlc repression on these genes by sequestering Mlc through direct protein–protein interaction when *Escherichia coli* cells are grown in the presence of glucose [15–17]. Previously, we also found that glucose-induced expression of *ptsG* is enhanced under heat shock condition and proposed that the elevated $EIICB^{Glc}$ level may counteract the highly increased *mlc* expression upon the same stress, whereby normal glucose metabolism is maintained at a high growth temperature [18]. In addition to the regulations at the transcriptional level, expression of *ptsG* is regulated post-transcriptionally by modulation of the mRNA stability in response to glycolytic flux [19]. It has been reported that accumulation of glucose-6-phosphate induces expression of the SgrS small RNA and subsequently possible base paring with 5′-UTR of the *ptsG* mRNA accelerates RNase E-dependent degradation of the *ptsG* transcript [19,20]. These findings suggest that expression of the PTS components is regulated in a highly complex and dynamic manner in response to various growth environments.

In a recent study, we have established that the ArcA protein, a response regulator of the ArcB/ArcA two-component system, binds to regulatory regions of the *ptsG* gene and the *ptsHIcrr* operon and especially represses *ptsG* transcription [21]. As the ArcB/ArcA system controls transcription of numerous genes in *E. coli* cells experiencing oxygen-limitation [22–24], we now address how the levels of PTS proteins are regulated by the availability of oxygen.

Materials and methods

Bacterial strains and growth conditions. All *E. coli* strains used in this study are MC4100 (*araD139 ΔargF-lacU169 rpsL150 thiA relA1 flb5301 deoC1 ptsF25 rbsR*) derivatives. To construct the *arcA* mutant strain, SR509, the *arcA*::Km region of PC35 [25] was transferred to MC4100 by P1 transduction. All strains were grown in Luria–Bertani (LB) medium in the presence of 1% glucose at 37 °C.

Primer extension analyses. Cells were grown aerobically at 37 °C overnight in LB. Overnight cultures were diluted 1:100 into fresh LB supplemented with 1% glucose. Total RNA was isolated from *E. coli* cells grown to mid-exponential phase using Trizol reagent (Invitrogen). To study *ptsG* and *ptsHIcrr* transcription, 50,000 cpm of ^{32}P-labeled primer PG1 (5′-AATTGAGAGTGCTCCTGAGTATGGGTGC-3′) and primer P11 (5′-GCCAGTTTTTAACAGACGCGACGCACGAAG-3′) was coprecipitated with 30 μg of total cell RNA. The pellet was suspended in 20 μl of 250 mM KCl, 2 mM Tris–HCl pH 7.9, and 0.2 mM EDTA. Primer extension reactions were performed as described by Ryu and Garges [26].

* Corresponding author. Fax: +82 2 873 5095.
E-mail address: sangryu@snu.ac.kr (S. Ryu).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.03.145

Determination of mRNA half-life. Cells were grown aerobically or anaerobically in the presence of glucose to mid-logarithmic growth phase. After rifampicin (200 μg/ml) was added to prevent the initiation of transcription, total RNA was extracted from cells at the designated times. The stability of mRNA was measured by primer extension analysis as described above using 30 μg of total RNA. The amounts of transcripts were quantified using BAS 2500 (Fuji Photo Film Co., Japan) phosphoimage analyzer. The half-life of *ptsG* and *ptsHlcrr* mRNA was estimated based on the plot of quantified transcripts.

Western blot analyses. To compare the protein level of $EIIA^{Glc}$ and $EIICB^{Glc}$ between aerobic and anaerobic growth conditions, cells extracts prepared from aliquots (standardized by OD_{600}) of *E. coli* MC4100 cells grown at both conditions were resolved in 12% SDS–polyacrylamide gels. Proteins were transferred to nitrocellulose membrane, followed by Western blot using polyclonal antibodies against purified $EIIA^{Glc}$ and the enzyme IIB domain of $EIICB^{Glc}$.

Results

The level of $EIICB^{Glc}$ protein is increased even under the reduced ptsG mRNA expression when E. coli cells grow in anaerobic condition

Two promoters, P1 and P2, have been identified for transcription of the *ptsG* gene [12]. Even though both P1 and P2 promoters are activated in *E. coli* cells grown with glucose, ~90% of the *ptsG* mRNA is transcribed from a major promoter P1 [12]. Previously, we have demonstrated that the ArcA protein, a response regulator of the ArcB/ArcA two-component system, represses transcription initiation from the *ptsG* P1 promoter [21]. As the ArcB/ArcA regulatory system switches on/off transcription of many genes in anaerobically grown *E. coli* cells [22–24], we initially investigated how the levels of oxygen affect transcription of the *ptsG* gene. The wild-type and *arcA*-deleted mutant strains were grown to the mid-exponential phase in Luria–Bertani (LB) supplemented with 1% glucose under aerobic or anaerobic condition, and total RNA was isolated from these strains. To examine transcription from the *ptsG* P1 promoter, a primer extension analyses was conducted on the isolated RNA, which revealed that the glucose-induced mRNA expression from the *ptsG* P1 promoter was significantly decreased in wild-type *E. coli* cells grown anaerobically (Fig. 1A). The *ptsG* P1 transcription was partially restored in the *arcA* mutant strain grown anaerobically, whereas we barely found difference of the mRNA levels of *ptsG* P1 when these strains were grown aerobically (Fig. 1A). This result suggests that the activated ArcA protein (i.e. phosphorylated ArcA) under anaerobic growth condition [27] could repress transcription of *ptsG* P1 promoter [21]. In addition, the partial recovery of *ptsG* transcription in *arcA* mutant could be explained by the notion that the FNR protein is also implicated in down-regulation of *ptsG* transcription during anaerobiosis [28].

Our findings above appeared to be contradictory to a report that anaerobic growth conditions favor a higher expression of the PTS [29]. To investigate the $EIICB^{Glc}$ protein levels, cell extracts were prepared from wild-type cells grown in LB + 1% glucose under aerobic or anaerobic condition. Western blot analyses using polyclonal antibody against purified IIB domain of the $EIICB^{Glc}$ protein showed that in anaerobically grown cells, the $EIICB^{Glc}$ protein level was higher than that in cells grown with oxygen (Fig. 1B), which was a condition that the amount of *ptsG* P1 transcript was lower than the other (Fig. 1A). These data suggest that the expression of *ptsG* might be regulated at the post-transcriptional level in response to the availability of oxygen.

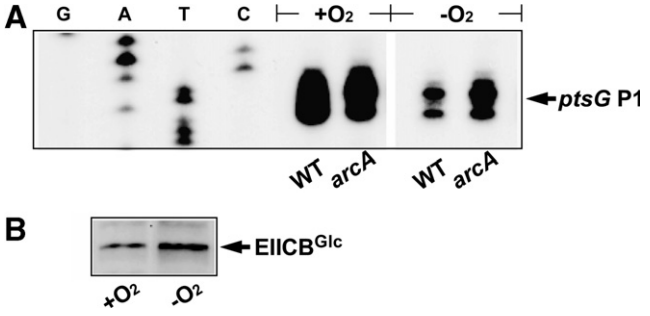

Fig. 1. The level of $EIICB^{Glc}$ protein is increased even when *ptsG* transcription is repressed by the ArcA protein in cells grown anaerobically. (A) A primer extension analyses conducted using the isolated RNA from the wild-type MC4100 strain and the *arcA* mutant strain grown with in the presence or absence of oxygen. (B) The level of $EIICB^{Glc}$ protein was determined in wild-type cells grown in the presence or absence of oxygen. All strains were grown in LB supplemented with 1% glucose to induce *ptsG* expression.

Slow ptsG mRNA turnover is responsible for the increased level of $EIICB^{Glc}$ protein under anaerobic growth condition

It has been demonstrated that expression of the $EIICB^{Glc}$ protein is decreased even when transcription initiation of the *ptsG* gene normally occurs, where the *ptsG* mRNA is being rapidly degraded in response to glycolytic flux [19]. Therefore, we hypothesized that the low levels of oxygen might delay the turnover of *ptsG* mRNA, which in turn causes to increase expression of the $EIICB^{Glc}$ protein. To test this idea, we compared the stability of *ptsG* P1 mRNA between cells grown under aerobic and anaerobic condition. The wild-type strain was grown either aerobically or anaerobically to the mid-exponential phase in LB supplemented with 1% glucose and then rifampicin (200 μg/ml) was added to prevent *de novo* synthesis of RNA. A primer extension analyses using total RNA isolated from the rifampicin-treated cells indicated that *ptsG* P1 mRNA was likely to be stabilized in anaerobic growth condition despite the fact that the level of transcription was lowered (Fig. 2A). To calculate the half-life of *ptsG* P1 mRNA, the amounts of transcripts were quantified using phosphoimage analyzer, which revealed that the half-life of *ptsG* P1 mRNA in aerobically grown cells was 4.5 min while it was increased to 8.4 min in anaerobic culture (Fig. 2B). Thus, these experiments suggest that the enhanced longevity of *ptsG* mRNA increases the $EIICB^{Glc}$ protein levels, despite the

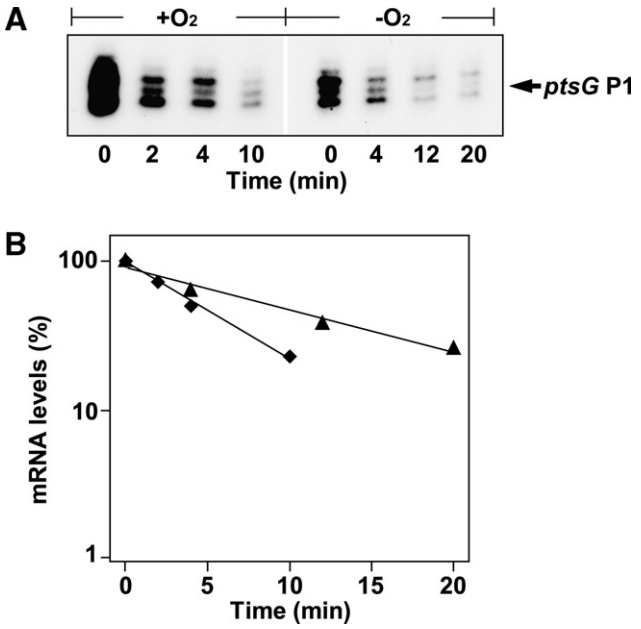

Fig. 2. The turnover of *ptsG* mRNA is delayed in cells grown in the absence of oxygen. (A) The half-life of *ptsG* P1 mRNA was measured using primer extension. Right after addition of rifampicin to the wild-type (MC4100) strain grown in the presence or absence of oxygen, total RNA was isolated at the indicated times. Cells were grown in LB supplemented with 1% glucose to induce *ptsG* expression. (B) The levels of mRNA in (A) were quantified using phosphoimager. The half-life of *ptsG* P1 mRNA in cells grown with (a line with squares) or without (a line with triangles) oxygen was 4.5 min and 8.4 min, respectively.

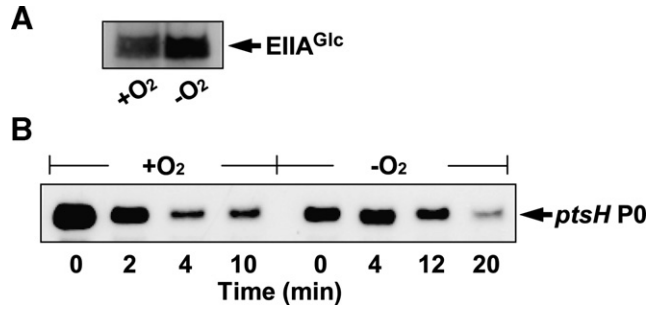

Fig. 3. The delayed degradation of *ptsHIcrr* mRNA may also cause to increase the level of $EIIA^{Glc}$ protein during anaerobic growth of cells. (A) The level of $EIIA^{Glc}$ protein was determined using Western blot in cells grown in the presence or absence of oxygen. (B) A primer extension analyses was carried out to measure the stability of *ptsHIcrr* P0 mRNA as described in Fig. 2. The half-life of *ptsHIcrr* P0 mRNA in cells grown with or without oxygen was 4.4 min and 11 min, respectively (data not shown). Cells were grown in LB supplemented with 1% glucose to induce expression of the *ptsHIcrr* operon.

decreased promoter activity of the *ptsG* gene in *E. coli* cells grown anaerobically.

Expression of the ptsHIcrr operon is also up-regulated in a similar manner to ptsG expression upon oxygen limitation

As followed further by the previous report that the anaerobiosis increases PTS expression [29], we investigated how the level of another PTS component is regulated by oxygen levels. A Western blot analyses using polyclonal antibody against purified $EIIA^{Glc}$ revealed that the level of $EIIA^{Glc}$ protein encoded in the *pts* operon was also increased ~2-fold when cells were grown anaerobically (Fig. 3A). We next examined glucose-induced transcription from the P0 promoter of the *pts* operon using primer extension assay. As observed in *ptsG* P1 transcription (Fig. 1A), the level of *pts* P0 mRNA was reduced in the wild-type strain grown anaerobically (Fig. 3B), which was consistent with the previous findings that both the ArcA and the Fnr proteins are involved in negative regulation of *crr* transcription [24,28], yet the P0 mRNA turnover was delayed (Fig. 3B): the half-life of mRNA was 4.4 min and 11 min in the presence and absence of oxygen, respectively (data not shown). In sum, these results suggest that the expression level of PTS proteins encoded by the two independent genetic loci, *ptsG* and *ptsHIcrr*, is controlled by a common mechanism (i.e. ArcA- and Fnr-mediated repression on transcription and modulation of mRNA stability post-transcription) in response to the availability of oxygen.

Discussion

For concomitant uptake and phosphorylation of glucose, *E. coli* uses a signal-transduction pathway mediated by the PTS which is composed of the $EIICB^{Glc}$ protein encoded by the *ptsG* gene and HPr, EI, and $EIIA^{Glc}$ proteins expressed from the *ptsHIcrr* operon [1]. It has been well established that the optimum levels of PTS proteins are maintained by elaborate control mechanisms at both the transcriptional and post-transcriptional levels according to cells' physiological demands.

Here, we have shown that the low levels of oxygen increased cellular concentrations of PTS proteins such as $EIICB^{Glc}$ and $EIIA^{Glc}$, which seems to be due to the changed stability of *ptsG* and *ptsHIcrr* mRNAs regardless of the reduced transcription initiation of these genes. Recent studies have demonstrated that the accumulation of phospho-glucose by mutations blocking a glycolytic pathway induces expression of the SgrS sRNA, which in turn promotes the RNase E-dependent degradation of the *ptsG* mRNA by possible base pairing between these two RNA molecules [19,20]. Consequently, this mechanism leads to a decrease in the $EIICB^{Glc}$ protein levels without affecting transcription initiation of the *ptsG* gene [19]. Thus, one might imagine that anaerobic growth of *E. coli* could decrease *srgS* expression to stabilize the *ptsG* mRNA further. However, the finding that the accumulation of phospho-glucose does not affect expression of the $EIIA^{Glc}$ protein [19] suggests that there might be rather a common mechanism explaining stabilization of both the *ptsG* and the *ptsHIcrr* mRNAs by oxygen limitation. Contrary to this gene-specific control of mRNA stability, a previous study showed that slow anaerobic growth of *E. coli* results in retarded turnover of RNA such as: (i) prolonged half-life of *ompA* and *bla* mRNA; and (ii) retarded processing of 9S RNA and suggested that intracellular activity of RNase E is down-regulated as the availability of oxygen becomes limited [30]. In this sense, it is possible that the PTS mRNA could be also generally stabilized during anaerobic growth of *E. coli* cells.

What is the biological relevance of our findings? Under anaerobic growth conditions, pathways generating cellular energy from sugar substrates work less efficiently, even in facultative anaerobes such as *E. coli*. For this reason, cells may need to convert their metabolic routes toward an energy-saving mode while maintaining the glycolytic metabolic pathways to an appropriate level to produce limited amount of cellular energy through respiration using less efficient electron acceptors (such as nitrate, fumarate, etc.) or fermentation. Under these circumstances, cells could avoid wasting the energy consumed for *de novo* synthesis of mRNA to minimize transcription initiation of the PTS genes by employing two regulatory proteins, ArcA and Fnr. Finally, maintenance of the increased cellular levels of PTS proteins by extending the mRNA longevity would enable *E. coli* cells to enhance glucose uptake compensating for inefficient energy conversion during anaerobic growth.

Acknowledgments

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-005-J04702) and the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (No. R01-2005-000-10038-0). N. Cho and Y.-J. Kim were recipients of a graduate fellowship provided by the Ministry of Education through the Brain Korea 21 Project. We thank Dr. Sankar Adhya for critical reading of the manuscript.

References

[1] P.W. Postma, J.W. Lengeler, G.R. Jacobson, Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria, Microbiol. Rev. 57 (1993) 543–594.
[2] R. Lux, K. Jahreis, K. Bettenbrock, J.S. Parkinson, J.W. Lengeler, Coupling the phosphotransferase system and the methyl-accepting chemotaxis protein-dependent chemotaxis signaling pathways of *Escherichia coli*, Proc. Natl. Acad. Sci. USA 92 (1995) 11583–11587.
[3] Y.H. Park, B.R. Lee, Y.J. Seok, A. Peterkofsky, *In vitro* reconstitution of catabolite repression in *Escherichia coli*, J. Biol. Chem. 281 (2006) 6448–6454.
[4] J. Stulke, W. Hillen, Carbon catabolite repression in bacteria, Curr. Opin. Microbiol. 2 (1999) 195–201.
[5] J.H. Hurley, H.R. Faber, D. Worthylake, N.D. Meadow, S. Roseman, D.W. Pettigrew, S.J. Remington, Structure of the regulatory complex of *Escherichia coli* III^{Glc} with glycerol kinase, Science 259 (1993) 673–677.
[6] Y.J. Seok, J. Sun, H.R. Kaback, A. Peterkofsky, Topology of allosteric regulation of lactose permease, Proc. Natl. Acad. Sci. USA 94 (1997) 13515–13519.
[7] Y.J. Seok, M. Sondej, P. Badawi, M.S. Lewis, M.C. Briggs, H. Jaffe, A. Peterkofsky, High affinity binding and allosteric regulation of *Escherichia coli* glycogen phosphorylase by the histidine phosphocarrier protein, HPr, J. Biol. Chem. 272 (1997) 26511–26521.
[8] C.R. Lee, B.M. Koo, S.H. Cho, Y.J. Kim, M.J. Yoon, A. Peterkofsky, Y.J. Seok, Requirement of the dephospho-form of enzyme IIA^{Ntr} for derepression of *Escherichia coli* K-12 *ilvBN* expression, Mol. Microbiol. 58 (2005) 334–344.
[9] B.S. Powell, D.L. Court, T. Inada, Y. Nakamura, V. Michotey, X. Cui, A. Reizer, M.H. Jr Saier, J. Reizer, Novel proteins of the phosphotransferase system encoded within the *rpoN* operon of *Escherichia coli*. Enzyme IIA^{Ntr} affects growth on organic nitrogen and the conditional lethality of an erats mutant, J. Biol. Chem. 270 (1995) 4822–4839.

[10] S.Y. Kim, T.W. Nam, D. Shin, B.M. Koo, Y.J. Seok, S. Ryu, Purification of Mlc and analysis of its effects on the *pts* expression in *Escherichia coli*, J. Biol. Chem. 274 (1999) 25398–25402.
[11] K. Kimata, T. Inada, H. Tagami, H. Aiba, A global repressor (Mlc) is involved in glucose induction of the *ptsG* gene encoding major glucose transporter in *Escherichia coli*, Mol. Microbiol. 29 (1998) 1509–1519.
[12] J. Plumbridge, Expression of *ptsG*, the gene for the major glucose PTS transporter in *Escherichia coli*, is repressed by Mlc and induced by growth on glucose, Mol. Microbiol. 29 (1998) 053–1063.
[13] J. Plumbridge, Expression of the phosphotransferase system both mediates and is mediated by Mlc regulation in *Escherichia coli*, Mol. Microbiol. 33 (1999) 260–273.
[14] Y. Tanaka, K. Kimata, T. Inada, H. Tagami, H. Aiba, Negative regulation of the *pts* operon by Mlc: mechanism underlying glucose induction in *Escherichia coli*, Genes Cells 4 (1999) 391–399.
[15] S.J. Lee, W. Boos, J.P. Bouche, J. Plumbridge, Signal transduction between a membrane-bound transporter, PtsG, and a soluble transcription factor, Mlc, of *Escherichia coli*, EMBO J. 19 (2000) 5353–5361.
[16] T.W. Nam, S.H. Cho, D. Shin, J.H. Kim, Y.J. Jeong, J.H. Lee, J.H. Roe, A. Peterkofsky, S.O. Kang, S. Ryu, Y.J. Seok, The *Escherichia coli* glucose transporter enzyme $IICB^{Glc}$ recruits the global repressor Mlc, EMBO J. 20 (2001) 491–498.
[17] Y. Tanaka, K. Kimata, H. Aiba, A novel regulatory role of glucose transporter of *Escherichia coli*: membrane sequestration of a global repressor Mlc, EMBO J. 19 (2000) 5344–5352.
[18] D. Shin, S. Lim, Y.J. Seok, S. Ryu, 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*, J. Biol. Chem. 276 (2001) 25871–25875.
[19] K. Kimata, Y. Tanaka, T. Inada, H. Aiba, Expression of the glucose transporter gene, *ptsG*, is regulated at the mRNA degradation step in response to glycolytic flux in *Escherichia coli*, EMBO J. 20 (2001) 3587–3595.
[20] C.K. Vanderpool, S. Gottesman, Involvement of a novel transcriptional activator and small RNA in post-transcriptional regulation of the glucose phosphoenolpyruvate phosphotransferase system, Mol. Microbiol. 54 (2004) 1076–1089.
[21] J.Y. Jeong, Y.J. Kim, N. Cho, D. Shin, T.W. Nam, S. Ryu, Y.J. Seok, Expression of *ptsG* encoding the major glucose transporter is regulated by ArcA in *Escherichia coli*, J. Biol. Chem. 279 (2004) 38513–38518.
[22] C.E. Bauer, S. Elsen, T.H. Bird, Mechanisms for redox control of gene expression, Annu. Rev. Microbiol. 53 (1999) 495–523.
[23] R.P. Gunsalus, S.J. Park, Aerobic-anaerobic gene regulation in *Escherichia coli*: control by the ArcAB and Fnr regulons, Res. Microbiol. 145 (1994) 437–450.
[24] K.A. Salmon, S.P. Hung, N.R. Steffen, R. Krupp, P. Baldi, G.W. Hatfield, R.P. Gunsalus, Global gene expression profiling in *Escherichia coli* K12: effects of oxygen availability and ArcA, J. Biol. Chem. 280 (2005) 15084–15096.
[25] P.A. Cotter, R.P. Gunsalus, Contribution of the *fnr* and *arcA* gene products in coordinate regulation of cytochrome o and d oxidase (*cyoABCDE* and *cydAB*) genes in *Escherichia coli*, FEMS Microbiol. Lett. 70 (1992) 31–36.
[26] S. Ryu, S. Garges, Promoter switch in the *Escherichia coli pts* operon, J. Biol. Chem. 269 (1994) 4767–4772.
[27] D. Georgellis, O. Kwon, E.C. Lin EC, Quinones as the redox signal for the *arc* two-component system of bacteria, Science 292 (2001) 2314–2316.
[28] K. Salmon, S.P. Hung, K. Mekjian, P. Baldi, G.W. Hatfield, R.P. Gunsalus, Global gene expression profiling in *Escherichia coli* K12. The effects of oxygen availability and FNR, J. Biol. Chem. 278 (2003) 29837–29855.
[29] J. Lengeler, H. Steinberger, Analysis of regulatory mechanisms controlling the activity of the hexitol transport systems in *Escherichia coli* K12, Mol. Gen. Genet. 167 (1978) 75–82.
[30] D. Georgellis, T. Barlow, S. Arvidson, A. von Gabain, Retarded RNA turnover in *Escherichia coli*: a means of maintaining gene expression during anaerobiosis, Mol. Microbiol. 9 (1993) 375–381.