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Dynamic Gene Expression Profiling of *Escherichia coli* in Carbon Source Transition from Glucose to Acetate

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Abstract DNA microarray was used to study the transcription profiling of *Escherichia coli* adapting to acetate as a sole carbon source. Bacteria grown in glucose minimal media were used as a reference. The dynamic expression levels of 3,497 genes were monitored at seven time points during this adaptation. Among the central metabolic genes, the glycolytic and glucose phosphotransferase genes were repressed as the bacteria entered stationary phase, whereas the glyoxylate pathway, TCA cycle, and gluconeogenic genes were induced. Distinct induction or repression patterns were recognized among different pathway genes. For example, the repression of glycolytic genes and the induction of gluconeogenic ones started immediately after glucose was depleted. On the other hand, the regulation of the pentose phosphate pathway genes and glyoxylate genes gradually responded to the glucose depletion or was more related to growth in acetate. When the whole genome was considered, many of the CRP, FadR, and Cra regulons were immediately responsive to the glucose depletion, whereas the σ^s, Lrp, and IHF regulons were gradually responsive to the glucose depletion. The expression profiling also provided differential regulations between isoenzymes; for example, malic enzymes A (*sfcA*) and B (*maeB*). The expression profiles of three genes were confirmed with RT-PCR.

Key words: DNA microarray, gene expression profiling, carbon source, glucose, acetate, *Escherichia coli*

Recent advent of the DNA microarray technology has provided a new paradigm in measuring and understanding gene expressions. It allows a thorough analysis of gene expression patterns at the genomic level and opens a research field, transcriptomics. An enormous amount of gene expression data has rapidly been accumulated and become powerful information for understanding the physio-logical state of the cell and its regulatory networks.

Genome-wide gene expression profiling with the DNA microarray has been used for understanding the metabolic responses of microbial cells to various conditions [5, 7, 9, 12, 16, 24]. Gene expression profiling of *Escherichia coli* by using acetate or glucose as a sole carbon and energy source was one of them [15, 18]. In the absence of glucose, *E. coli* activates adenylate cyclase (Cya), which synthesizes cAMP. The cAMP binds to the cAMP receptor protein (CRP), and then induces the expression of a large set of genes, which is known as catabolite derepression [8, 27]. Acetate is transported into the cell and converted to acetyl-CoA, which is further metabolized through the glyoxylate shunt and the TCA cycle. The induction and regulation of acetate-metabolizing genes have been studied extensively [4]. The global gene expression profile, comparing glucose- and acetate-grown *E. coli*, not only proved many of these known features, but also showed many previously unknown results, such as induction of malic enzymes, *ppsA*, and the glycolate pathway, and repression of glycolytic and glucose phosphotransferase genes in acetate [17].

One of the interesting results from the expression profiling of the acetate-grown *E. coli* was its correlation with the metabolic fluxes [17]. The qualitative correlation between fluxes and expression profiles helped identify the gene coding malic enzyme B [18]. Scientists have further tried to look for a theoretical ground between transcriptomic and fluxomic data using elementary flux modes [3, 22]. These research activities are expected to extend the scope of metabolic engineering [10, 19]. The expression profiling was also used to unravel the relationship between transcription factors and their regulons. These relationships will guide us to the complete understanding of cellular

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regulatory networks in response to physiological and environmental signals [2, 11, 13, 14].

A more important metabolic feature to be studied by *E. coli* transcriptomics is the dynamic regulation of the genes, since the availability of carbon sources varies dynamically in the natural environment. Therefore, the adaptation from one condition to another is critical for their survival. The gene expression profiling of long-term adaptation of *E. coli* in various conditions has been reported [21]. In this experiment, short-term gene expression regulation of *E. coli* was monitored, when its primary carbon source was changed from glucose to acetate. The expression profiles were monitored at seven time points with a cDNA-type microarray.

MATERIALS AND METHODS

Strain and Culture Conditions

To monitor the dynamic regulations of the transcripts under nutritional change from glucose to acetate, *E. coli* MC4100 (F^- *araD139 (argF-lac) U169 rpsL150 relA1 flb5301 deoC1 ptsF25 rbsR*) was cultured in M9 minimal medium containing glucose (0.5%, w/v) as a sole carbon source. The arginine was added to the medium to 125 mg/l. After two overnight cultures in the test tube, the bacterial cells were inoculated to M9 medium with glucose (0.15%, w/v) in the flask to OD_{550} of 0.1. At 3 h after inoculation, when glucose was almost depleted, sodium acetate (0.15%, w/v) at pH 7.0 was added to the medium. The *E. coli* strain was sampled at seven time points (2, 3, 4, 6.5, 8.5, 10.5, and 12.5 h) after the inoculation. The sampled cultures were quickly chilled in an ethanol/dry ice bath, and the cells were harvested by centrifugation for RNA purification.

Detection of Metabolites

Glucose concentration in the medium was monitored by a Glucose (Trinder) kit (Sigma, St. Louis, MO, U.S.A.), and acetate and pyruvate concentrations were measured by HPLC with an acid column (Aminex HP-87H, Bio-Rad, Hercules, CA, U.S.A.).

RNA Purification and Labeling

Total RNA was extracted from roughly 1×10^9 cells at each time point using the hot-phenol method. Briefly, cell pellet was resuspended in TE buffer (pH 8.0) with 1 mg/ml lysozyme. After 1/10 volume of 10% SDS and 1/10 volume of 1 M sodium acetate (pH 5.2) were added, the sample was placed on the water bath at 64°C for 1–2 min. Equal volume of phenol was added, and the sample was incubated at 64°C for 6 min with occasional mixing. After centrifugation at 14,000 rpm for 10 min, the aqueous phase was transferred to a new tube. RNA was purified using Qiagen RNeasy Kit according to the manufacturer's protocol.

RNA sample was labeled with Cy-3 dCTP during reverse transcription. The reverse transcription was performed by 200 U Superscript RNase H^- Reverse Transcriptase (Gibco BRL), random hexamers (Gibco BRL), 0.5 mM each of dATP, dTTP, and dGTP, 0.2 mM dCTP, and 0.1 mM Cy-3 dCTP (Amersharm Pharmacia, Piscataway, NJ, U.S.A.). After reverse transcription, the RNA was degraded by incubating at 65°C for 40 min after adding 2 µl of 0.5 M EDTA (pH 8.0) and 5 µl of 1 N NaOH. The Cy-3 dCTP-labeled cDNA was mixed with Cy-5 dCTP-labeled cDNA, which was originated from *E. coli* grown in the glucose (0.5%, w/v) M9 minimal medium. After dilution with 120 ml TE buffer (pH 8.0), the cDNA mixture was concentrated to 1–2 µl using the Micron-50 (Millipore, Bedford, MA, U.S.A.).

Hybridization and Scanning

The concentrated Cy-3 and Cy-5 cDNA were resuspended in 10 µl of hybridization solution, containing 50% formamide, 3× SSC, 1% SDS, 5× Denhardt's solution, 0.1 mg/ml salmon sperm DNA, and 0.05 mg/ml yeast total RNA. The labeled cDNA in hybridization solution was denatured at 95°C for 2 min and cooled for 5 min at room temperature. The hybridization solution was then placed on the DNA microarray slide, which was manufactured in our laboratory as previously reported [25]. The hybridiza-tion was continued for 14–20 h at 42°C in the water bath. The slide was washed in 2× SSC and 0.1% SDS for 5 min at room temperature, and then 0.2× SSC for 5 min prior to scanning.

After drying by centrifugation, the hybridized slide was scanned by the Affymetrix GMS-418 Scanner (Santa Clara, CA, U.S.A.). The two images with the wavelengths of Cy-3 and Cy-5 dyes were individually analyzed by a microarray image analysis software, Imagene (Biodiscovery, Santa Monica, CA, U.S.A.). The median intensities of each spot calculated by the program were obtained for further analysis.

Statistical Analysis

The correlation between Cy-3 and Cy-5 intensity is nonlinear and slide-dependent. Thus, the expression data from each slide need an internal normalization. We used a rank-invariant method to select genes that were nondifferentially expressed in each slide [25]. This method was based on the assumption that if a gene were regulated, its intensity rank in one channel, Cy-5, should be significantly different one from the other. Therefore, the genes of which the ranks in both dyes are similar can be considered as housekeeping genes and used to determine the normalization curve. After the selection of the rank-invariant genes, the LOWESS (locally weighted polynormial regression) method in S-Plus (Insightful Corp.) was used to fit the data. After normalization, the logarithmic residuals were calculated as

$\log(Cy5_i/f(Cy3_i))$, where $Cy3_i$ and $Cy5_i$ represent Cy-3 and Cy-5 intensities on spot i, and f(x) is the normalization function between the two channels. Then, the expression levels of the genes were computed using a previously developed statistical method [25]. This method considered the slide-to-slide and culture-to-culture variations, when the gene expression levels were determined using a Markov Chain Monte Carlo (MCMC) simulation [25].

In this experiment, the hybridization was duplicated with the RNA samples at each time point to minimize errors incorporated during the experiment, and the duplicated gene expressions that differed more than 50% were flagged. The genes having the intensities less than a threshold were also flagged, and the genes flagged more than 2 time points were filtered out. With this method, the expression levels of 3,497 genes were monitored at least six time points and further analyzed.

For hierarchical cluster analysis and self-organizing map (SOM), the log-transformed expression ratios were imported into the CLUSTER program [6]. The samples were clustered by the average linkage method with Pearson coefficients. The clustered tree was drawn by the Treeview program [6].

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Semiquantitative RT-PCR was used to confirm DNA microarray gene expression data. Total RNA was converted to cDNA using Superscript II by the manufacturer's recommendation (Invitrogen). Remaining RNA was digested with RNase H, and the first-strand cDNA was used directly as a template for PCR. The following primers were used in RT-PCRs: *aceB*-F (5'-ATTACATGGACACAGA CTGG-3'), *aceB*-R (5'-TTCATCCATCTGGAACAC-3'), *gapA*-F (5'-TCTGACATCGAGATCGTTGC-3'), *gapA*-R (5'-ATCAGACCTTCGATGATGC-3'), *pgpB*-F (5'-TTGC TTGTCATGCCAGTAGC-3'), *pgpB*-R (5'-TCTTTCTCG CAGTGTGAACG-3'). The total number of PCR cycles ranged between 20 and 25, and its annealing temperatures ranged between 56 and 58°C. The PCR products were examined by agarose gel electrophoresis, and the amounts of PCR products were calculated based on the areas of the bands in the gel using AlphaEaseFC software (Alpha Innotech Corp).

RESULTS AND DISCUSSION

Design of Experiment

E. coli was inoculated into glucose (0.15%, w/v) minimal medium. The growth of *E. coli* in glucose ceased at 4 h after inoculation, and entered a lag phase, and 3 h later, *E. coli* restarted growing with acetate as a carbon source. To promote the growth in acetate, a nontoxic concentration (0.15%, w/v) of sodium acetate (pH 7.0) was added to the medium at 3 h after inoculation. The growth curve and glucose, acetate, and pyruvate concentrations in the medium are illustrated in Fig. 1.

The *E. coli* strain was sampled at seven time points (2, 3, 4, 6.5, 8.5, 10.5, and 12.5 h) from the culture, and total RNAs were extracted from the samples and labeled with Cy-3 dCTP during reverse transcription. These samples were mixed with the RNA purified from the strain growing in the 0.5% glucose minimal medium and labeled with Cy-5 dCTP to measure the relative amount of gene expression using the DNA microarray. After hybridization, the gene expression levels were computed using the statistical methods previously reported [25]. The microarray experiment was duplicated with the same RNA. If the duplicated gene expressions differ more than 50%, the genes were filtered out. Otherwise, the data were averaged at each time point.

Cluster Analysis of Microarray Data

We performed a cluster analysis to unravel the similarities of 3,497 gene expression patterns among the samples, using Pearson coefficient. Figure 2 shows that the global expression patterns were similar among lag-phase and acetate-grown samples (6.5, 8.5, 10.5 and 12.5 h), and also between glucose-growth samples (2 and 3 h). However, the expression pattern of the transition-state sample (4 h) was most distinct, and was slightly more similar to acetate-grown samples than glucose-grown ones (Fig. 2).

Expression Pattern of the Central Metabolic Genes

We first examined the responses of central metabolic genes to the carbon source transition. The overall pattern of the expression level changes of central metabolic genes agreed with our expectations based on the comparison data between glucose- and acetate-grown *E. coli* [18]. Phosphotransferase system genes (*ptsG*, *ptsHIcrr*) and glycolytic genes (*pgi*, *pfkA*, *pfkB*, *fba*, *gapA*, *epd*, *pgk*, *gpmA*, *eno*, *pykF*, *aceEF*, *ppc*) were downregulated after the cell

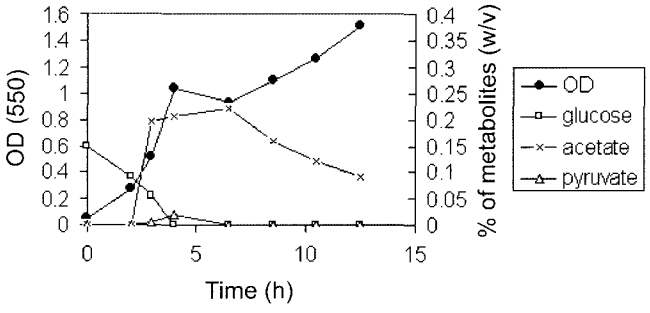

Fig. 1. *E. coli* growth curve and metabolite concentrations during the transition of carbon source from glucose to acetate.
The *E. coli* samples were taken at 2, 3, 4, 6.5, 8.5, 10.5, and 12.5 h after inoculation. Just before the sample was taken at 3 h, sodium acetate (0.15%, w/v) at pH 7.0 was added to the medium.

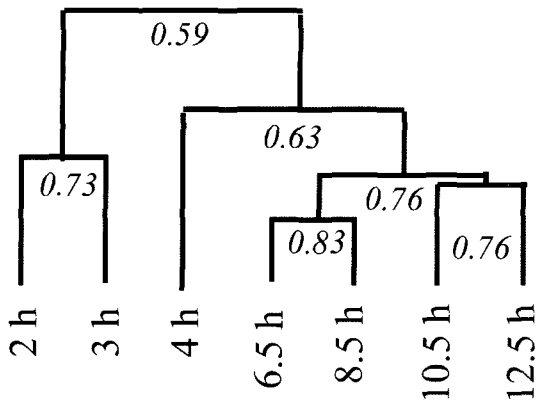

Fig. 2. The cluster analysis between the global gene expression patterns of *E. coli* sampled at seven time points.
The numbers in the trees are the Pearson coefficients, which represent similarities between the groups of samples. The closer the correlation coefficient is to 1, the similar the expression patterns.

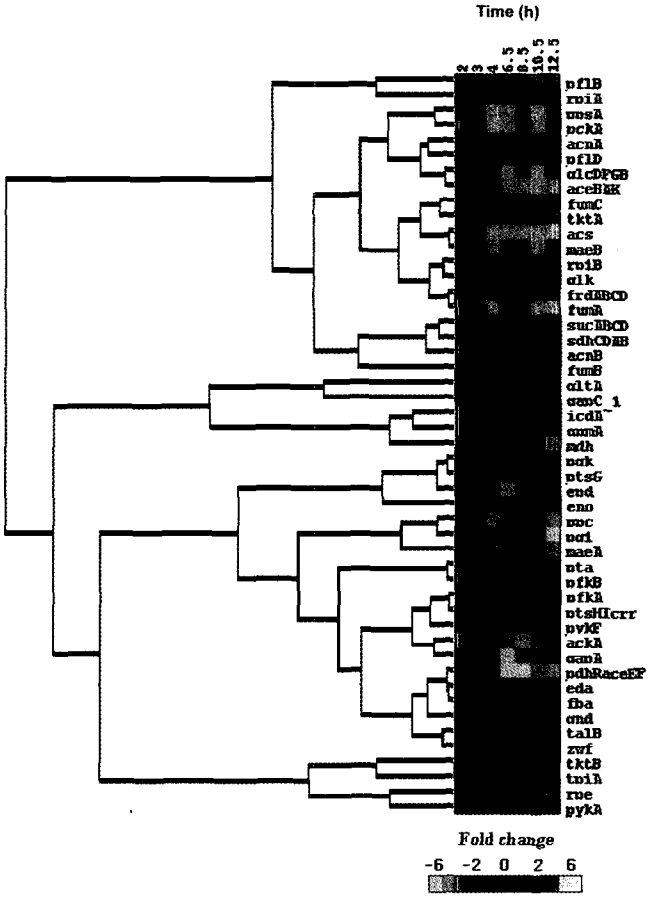

Fig. 3. The expression profiles of central metabolic genes.
The gene expression patterns were clustered using Spearman coefficient. The red grid represents an induced expression level, compared to a reference, whereas the green one represents a repressed expression level. The grey one indicates no measurable data for the time point. The expression levels of genes on the operon were averaged before the analysis.

entered the stationary phase (Fig. 3). Several pentose phosphate pathway genes were also downregulated (*zwf*, *gnd*, *talB*). Interestingly, the pattern of the downregulation of pentose phosphate genes were different from that of glycolytic and phosphotransferase system genes. Most of the glycolytic genes were significantly downregulated at 4 h, while pentose phosphate genes started to be repressed from 6.5 h. It should be noted that a differential gene expression pattern was observed among different pathway genes.

The glyoxylate, citric acid cycle, and gluconeogenic genes were all upregulated as glucose was depleted. The differential expression patterns between different pathway genes were also noted among these upregulated genes. The gluconeogenic genes (*ppsA*, *pckA*, *maeB*, *acs*) were highly upregulated at 4 h, while the expression of the glyoxylate shunt pathway genes (*aceBAK*, *glcDFGB*) were gradually induced and maximally expressed at 6.5 h. On the other hand, the citric acid cycle genes were generally upregulated throughout the experiment (*acnB*, *sucABCD*, *sdhCDAB*, *fumA*, *fumB*), suggesting that the regulation of citric acid cycle genes is more related to the low glucose concentration, but not to the availability of acetate.

One interesting result was the different regulation between malic enzymes A and B (*sfcA* and *maeB*). The NAD^+-dependent malic enzyme (*sfcA*) was downregulated together with glycolytic genes as glucose was depleted, whereas the $NADP^+$-dependent malic enzyme (*maeB*) was upregulated along with gluconeogenic genes. Although both malic enzymes carry out reversible reactions, NAD^+-dependent malic enzyme is possibly more active in converting pyruvate to malate, whereas $NADP^+$-dependent malic enzyme is for the reverse reaction. The differential regulations between isoenzymes were also recognized in pyruvate kinases (*pykA* and *pykF*). Although these enzymes compensate for each other when one of them is deleted, their major functions under ordinary conditions may be distinct, considering regulation of their gene expression.

The expression level changes of central metabolic genes suggest that there are at least two distinct regulations of genes during the transition of carbon source from glucose to acetate; 1) immediate response to the glucose depletion, and 2) gradual response. The differential regulations must result from distinct transcription factors involved. Indeed, most of the immediate responding genes were CRP, FadR, or Cra (catabolite repressor and activator) regulons, whereas gradual responding genes were under the control of IclR, IHF, and/or σ^s. In the central metabolic genes, the differential regulations were observed among the genes involved in different pathways.

Upregulated Genes

To select the significantly regulated genes from 3,497 genes, the average gene expression levels between 6.5 h and 12.5 h were compared with those of glucose-grown

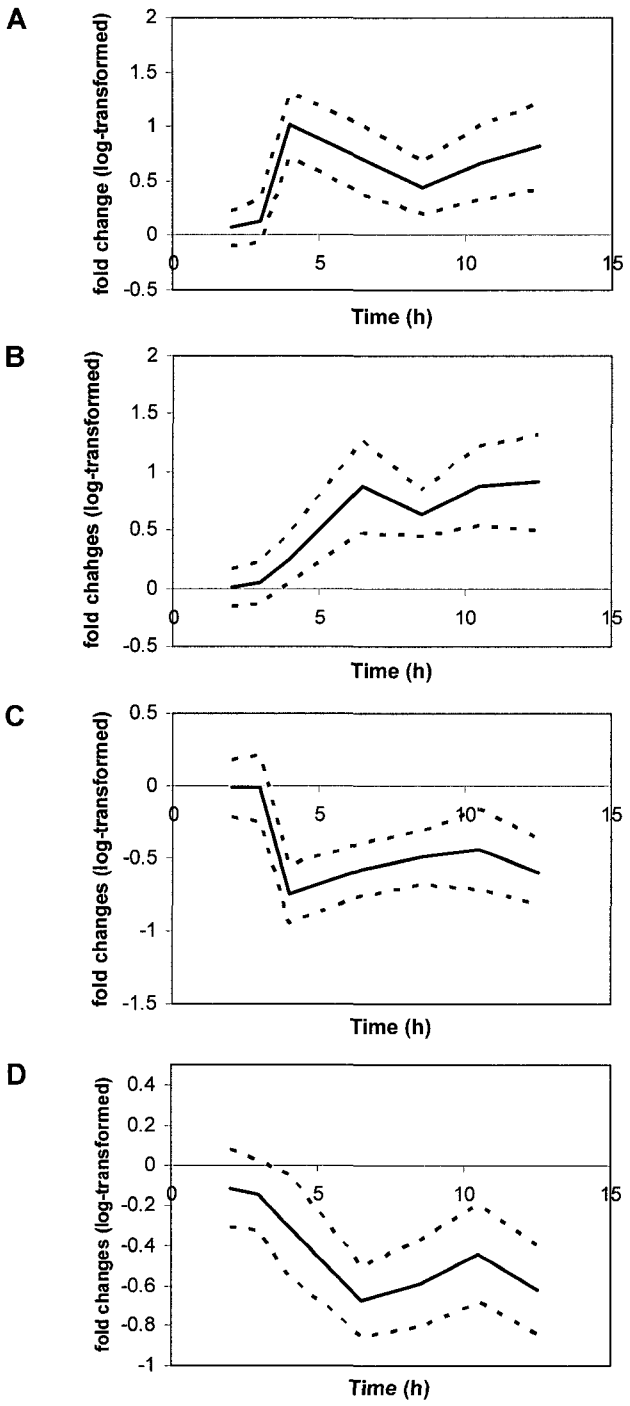

Fig. 4. The average expression patterns of the gene groups listed in Table 2.

The solid line represents the average expression level changes of the genes belonged to each group, and the dashed lines represent standard deviations of the expression levels. The y-axis is $\log_{10}$-transformed fold change.

samples (2 and 3 h). A total of 692 genes showed the gene expression levels being different by more than 2-fold in the two stages. With the 692 genes, the gene expression patterns were classified into 20 different groups using the self-organizing map (SOM) program. A few gene groups showed expression patterns similar to those of central metabolic genes.

Group I genes showed an upregulation pattern similar to the gluconeogenic genes, which immediately responded to the glucose depletion (Fig. 4a and Group a in Table 1). Indeed, some gluconeogenic genes (*ppsA*, *maeB*, *pckA*, *acs*) were included in this group. Many genes in this group are known to be regulated by CRP, Cra, or FadR. Examples are fatty acid degradation genes [*fadAB*, *fadH*, *fadL*, *fadIJ* (*b2341-b2342*), and *fadD*], which are FadR regulons. Other genes in this group include various carbohydrate metabolic or transporting genes (*gatYZ*, *mglAB*, *dctA*, *rbsD*, and *fucAO*) as well as amino acid degradation genes (*astA*, *tnaA*, and *aspA*), which are known as CRP and/or Cra regulons [8].

The second upregulated gene group has a regulation pattern similar to the *aceBAK* and *glcBGFD* operons (Fig. 4b and Group b Table 1). These genes are regulons of σ^S and Lrp as well as IHF [1]. The σ^S regulons found in this group included *dps*, *poxB*, *csgA*, *gabT*, *astD*, *otsB*, and *csiE* [20, 26]. Many of them are also Lrp regulons [23]. The genes in this group are possibly more related to the stationary phase or acetate metabolism. About 50% of the genes in this group are functionally unidentified, implying that the bacterial physiology in this phase has been less intensely studied than that in the glucose-grown phase.

Downregulated Genes

Group III included the genes downregulated immediately after glucose depletion (Fig. 4c and Group c Table 1). Some of the glycolytic genes (*ppc* and *pgi*) are listed in this group. The group contained many biosynthesis genes, including threonine (*thrA*, *thrC*), glutamate (*gltB*, *gdhA*), aromatic amino acids (*aroA*, *aroG*), purine (*guaB*), and pyrimidine (*carA*). Peptide transport genes (*oppA* and *dppA*) and the RNA polymerase gene (*rpoA*) also showed immediate response to glucose depletion (Fig. 4c). Many of the genes encoding ribosomal proteins (*rpsA*, *rne*, *rpsQ*, *rplP*, *rpsC*, *rplV*, *rpsS*, *rplW*, *rplD*, *rplC*) were also included in the group.

Many other biosynthetic and ribosomal proteins were gradually downregulated as the bacteria entered the acetate-grown phase (Fig. 4d and Group d Table 1). The group of genes included pyruvate dehydrogenase (*pdhR-aceEF*), methionine biosynthetic genes (*metB*, *metE*, *metF*, *metJ*), isoprenoid biosynthetic genes (*ispA*, *ispE*), ATP biosynthetic genes (*atpCHE*), and fatty acid synthetic genes (*accBC*). Some of them (*metE*, *guaA*, *ilvE*, *ilvG*) are known as IHF regulons [1]. Many of the ribosomal proteins as well as some heat-shock proteins (*mopB*, *tig*) were also included in group IV. The downregulations of these genes reflect low biological activities of the bacteria in the acetate-grown phase. Many of the genes belonging to group IV were also functionally unknown.

Table 1. The genes whose expression patterns belonged to four groups (A–D) in Fig. 4.

	Genes*
Group I	*cstA, ybeL, phoH, dadAX, b1376, b1398, ydbC, aldA, b1449,* ***ppsA****, b1725, astA, fadD, gatYZ, mglBA, galS, argT, b2341-b2342, fadL,* ***maeB(b2463)****, b2537, b2659, fucAO, b3001,* ***pckA****, ugpB, dctA, mtlA, rbsD, fadAB,* ***acs****, aspA, yjfO, ytfJ, ytfQ*
Group II	*dps, poxB, ycaC, rmf, csgA, b1444, b1511, b1516, b1517, b1518, uxaB, b1674, astE, astD, b1750, b1783, otsB, b1955, b1967, b2080, b2097, yohC, csiE, b2584, gabDTP,* ***glcBGFD****, uxaC,* ***aceAK***
Group III	*thrA, thrC, carA, secA, ompT, aroG, b0905, aroA, rpsA, rne, oppA, gdhA, b1762, b1964, b1983, guaB, trmD, gltB, rpoA, prlA, rpsQ, rplP, rpsC, rplV, rpsS, rplW, rplD, rplC, dppA, b3830, yijP,* ***ppc****,* ***pgi***
Group IV	*guaA,* ***pdhR-aceEF****, panD, pyrH, yajC, ispA, tig, apt, ahpC, pyrD, prsA, ispE, pntA,* ***gapA****, b1832, b1839, b1840, wzzB(b2027), ugd, yefJ, yefI, yefH, yefE, glf, rfbADB, rplY, mqo, nrdB, b2834,* ***epd****, yqgC, b3071, rpsO, yhbC, yhbY, yrb-ABC, b3193, yrbE, yrbG, rpsI, rplM, yhcM, cafA, yhdE, accBC, rplB, rpsG, aroK, rpmB, gmk, atpCHE, ilvG_12, ilvE, rffE, rffG, hemD, metE, hslU, metJ, metB, metF, rplK, rplA, yjaD, lysC, mopB, rpsF, priB, rplI, ppa*

*Bold characters represent central metabolic genes.

Confirmation of Gene Expression Profiling with RT-PCR

The expression profiles of three genes, *aceB*, *gapA*, and *pgpB*, were confirmed with RT-PCR. According to the microarray data, the expression level of *pgpB* was not significantly changed during glucose-to-acetate transition, and the amounts of RT-PCR products of the *pgpB* gene verified the microarray data (Fig. 5A): When RT-PCR products with 25 cycles were examined, the expression levels changed less than 25% during the carbon source transition. Therefore, the *pgpB* gene was used as a reference for normalizing gene expression levels detected by RT-PCR.

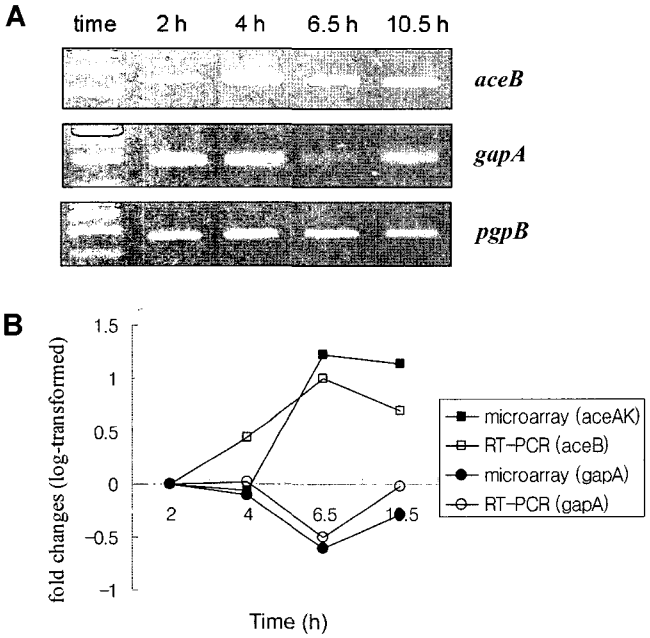

Fig. 5. A. RT-PCR products analyzed by agarose gel electrophoresis. The areas of the product bands were calculated by AlphaEaseFC software and considered as the gene expression levels. The expression levels of *aceB* and *gapA* were normalized by *pgpB* at the same hour sample and then normalized to 2 h data of its own gene. **B.** The normalized amounts of RT-PCR products were plotted with microarray results. The y-axis of the plot is $\log_{10}$-transformed fold change.

The gene expression of *aceB*, encoding the first enzyme of the glyoxylate pathway, was not detected in the microarray experiment, because of the low quality of the PCR probe on the DNA microarray. However, the expression levels of the gene could be estimated, based on those of the other genes on the same operon, *aceA* and *aceK*: These two genes were significantly upregulated as the microorganism adjusted to the acetate media. Assuming the genes on the same operon have similar gene expression patterns, the expression pattern of *aceB* measured by RT-PCR was comparable with those of *aceA* and *aceK* detected by DNA microarray. The minor difference was that *aceB* was upregulated 4-fold in 4-h samples by the RT-PCR experiment, whereas *aceA* and *aceK* were not upregulated in the DNA microarray experiment (Fig. 5). The gene expression of *gapA*, a glycolytic gene, was also confirmed by RT-PCR: The gene was significantly downregulated in the microarray analysis after glucose was depleted, and a similar pattern was also observed in the RT-PCR experiment (Fig. 5). In general, the RT-PCR result supported the pattern of gene expression levels measured by the DNA microarray experiment during the transition of carbon source.

Acknowledgments

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References

1. Arfin, S. M., A. D. Long, E. T. Ito, L. Tolleri, M. M. Riehle, E. S. Paegle, and G. W. Hatfield. 2000. Global gene

expression profiling in *Escherichia coli* K12. The effects of integration host factor. *J. Biol. Chem.* **275:** 29672–29684.
2. Balazsi, G., A. L. Barabasi, and Z. N. Oltvai. 2005. Topological units of environmental signal processing in the transcriptional regulatory network of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **102:** 7841–7846.
3. Cakir, T., B. Kirdar, and K. O. Ulgen. 2004. Metabolic pathway analysis of yeast strengthens the bridge between transcriptomics and metabolic networks. *Biotechnol. Bioeng.* **86:** 251–260.
4. Cozzone, A. J. 1998. Regulation of acetate metabolism by protein phosphorylation in enteric bacteria. *Annu. Rev. Microbiol.* **52:** 127–164.
5. DeRisi, J. L., V. R. Iyer, and P. O. Brown. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278:** 680–686.
6. Eisen, M. B., P. T. Spellman, P. O. Brown, and D. Botstein. 1998. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* **95:** 14863–14868.
7. Gonzalez, R., H. Tao, K. T. Shanmugam, S. W. York, and L. O. Ingram. 2002. Global gene expression differences associated with changes in glycolytic flux and growth rate in *Escherichia coli* during the fermentation of glucose and xylose. *Biotechnol. Prog.* **18**: 6–20.
8. Gosset, G., Z. Zhang, S. Nayyar, W. A. Cuevas, and M. H. Saier, Jr. 2004. Transcriptome analysis of Crp-dependent catabolite control of gene expression in *Escherichia coli*. *J. Bacteriol.* **186**: 3516–3524.
9. Hua, Q., C. Yang, T. Oshima, H. Mori, and K. Shimizu. 2004. Analysis of gene expression in *Escherichia coli* in response to changes of growth-limiting nutrient in chemostat cultures. *Appl. Environ. Microbiol.* **70**: 2354–2366.
10. Kang, M. J., S. H. Yoon, Y. M. Lee, S. H. Lee, J. E. Kim, K. H. Jung, Y. C. Shin, and S. W. Kim. 2005. Enhancement of lycopene production in *Escherichia coli* by optimization of the lycopene synthetic pathway. *J. Microbiol. Biotechnol.* **15**: 880–886.
11. Kao, K. C., Y. L. Yang, R. Boscolo, C. Sabatti, V. Roychowdhury, and J. C. Liao. 2004. Transcriptome-based determination of multiple transcription regulator activities in *Escherichia coli* by using network component analysis. *Proc. Natl. Acad. Sci. USA* **101**: 641–646.
12. Khodursky, A. B., B. J. Peter, N. R. Cozzarelli, D. Botstein, P. O. Brown, and C. Yanofsky. 2000. DNA microarray analysis of gene expression in response to physiological and genetic changes that affect tryptophan metabolism in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **97**: 12170–12175.
13. Lee, S. G., K. S. Hwang, and C. M. Kim. 2005. Dynamic behavior of regulatory elements in the hierarchical regulatory network of various carbon sources-grown *Escherichia coli*. *J. Microbiol. Biotechnol.* **15**: 551–559.
14. Liao, J. C., R. Boscolo, Y. L. Yang, L. M. Tran, C. Sabatti, and V. P. Roychowdhury. 2003. Network component analysis: Reconstruction of regulatory signals in biological systems. *Proc. Natl. Acad. Sci. USA* **100**: 15522–15527.
15. Liu, M., T. Durfee, J. E. Cabrera, K. Zhao, D. J. Jin, and F. R. Blattner. 2005. Global transcriptional programs reveal a carbon source foraging strategy by *Escherichia coli*. *J. Biol. Chem.* **280**: 15921–15927.
16. Oh, K. S., O. Kwon, Y. W. Oh, M. J. Shon, S. G. Jung, Y. K. Kim, M. G. Kim, S. K. Rhee, G. Gellissen, and H. A. Kang. 2004. Fabrication of a partial genome microarray of the methylotrophic yeast *Hansenula polymorpha*: Optimization and evaluation of transcript profiling. *J. Microbiol. Biotechnol.* **14**: 1239–1248.
17. Oh, M. K. and J. C. Liao. 2000. Gene expression profiling by DNA microarrays and metabolic fluxes in *Escherichia coli*. *Biotechnol. Prog.* **16**: 278–286.
18. Oh, M. K., L. Rohlin, K. C. Kao, and J. C. Liao. 2002. Global expression profiling of acetate-grown *Escherichia coli*. *J. Biol. Chem.* **277**: 13175–13183.
19. Park, S. J., J. I. Choi, and S. Y. Lee. 2005. Short-chain-length polyhydroxyalkanoates: Synthesis in metabolically engineered *Escherichia coli* and medical applications. *J. Microbiol. Biotechnol.* **15**: 206–215.
20. Patten, C. L., M. G. Kirchhof, M. R. Schertzberg, R. A. Morton, and H. E. Schellhorn. 2004. Microarray analysis of RpoS-mediated gene expression in *Escherichia coli* K-12. *Mol. Genet. Genomics* **272**: 580–591.
21. Polen, T., D. Rittmann, V. F. Wendisch, and H. Sahm. 2003. DNA microarray analyses of the long-term adaptive response of *Escherichia coli* to acetate and propionate. *Appl. Environ. Microbiol.* **69**: 1759–1774.
22. Stelling, J., S. Klamt, K. Bettenbrock, S. Schuster, and E. D. Gilles. 2002. Metabolic network structure determines key aspects of functionality and regulation. *Nature* **420:** 190–193.
23. Tani, T. H., A. Khodursky, R. M. Blumenthal, P. O. Brown, and R. G. Matthews. 2002. Adaptation to famine: A family of stationary-phase genes revealed by microarray analysis. *Proc. Natl. Acad. Sci. USA* **99**: 13471–13476.
24. Tao, H., C. Bausch, C. Richmond, F. R. Blattner, and T. Conway. 1999. Functional genomics: Expression analysis of *Escherichia coli* growing on minimal and rich media. *J. Bacteriol.* **181**: 6425–6440.
25. Tseng, G. C., M. K. Oh, L. Rohlin, J. C. Liao, and W. H. Wong. 2001. Issues in cDNA microarray analysis: Quality filtering, channel normalization, models of variations and assessment of gene effects. *Nucleic Acids Res.* **29**: 2549–2557.
26. Weber, H., T. Polen, J. Heuveling, V. F. Wendisch, and R. Hengge. 2005. Genome-wide analysis of the general stress response network in *Escherichia coli*: SigmaS-dependent genes, promoters, and sigma factor selectivity. *J. Bacteriol.* **187**: 1591–1603.
27. Zheng, D., C. Constantinidou, J. L. Hobman, and S. D. Minchin. 2004. Identification of the CRP regulon using *in vitro* and *in vivo* transcriptional profiling. *Nucleic Acids Res.* **32**: 5874–5893.