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# Analysis of Gene Expression in *Escherichia coli* in Response to Changes of Growth-Limiting Nutrient in Chemostat Cultures

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**Studies of steady-state metabolic fluxes in *Escherichia coli* grown in nutrient-limited chemostat cultures suggest remarkable flux alterations in response to changes of growth-limiting nutrient in the medium (Hua et al., J. Bacteriol. 185:7053–7067, 2003). To elucidate the physiological adaptation of cells to the nutrient condition through the flux change and understand the molecular mechanisms underlying the change in the flux, information on gene expression is of great importance. DNA microarray analysis was performed to investigate the global transcriptional responses of steady-state cells grown in chemostat cultures with limited glucose or ammonia while other environmental conditions and the growth rate were kept constant. In slow-growing cells (specific growth rate of 0.10 $h^{-1}$), 9.8% of a total of 4,071 genes investigated, especially those involved in amino acid metabolism, central carbon and energy metabolism, transport system and cell envelope, were observed to be differentially expressed between the two nutrient-limited cultures. One important characteristic of *E. coli* grown under nutrient limitation was its capacity to scavenge carbon or nitrogen from the medium through elevating the expression of the corresponding transport and assimilation genes. The number of differentially expressed genes in faster-growing cells (specific growth rate of 0.55 $h^{-1}$), however, decreased to below half of that in slow-growing cells, which could be explained by diverse transcriptional responses to the growth rate under different nutrient limitations. Independent of the growth rate, 92 genes were identified as being differentially expressed. Genes tightly related to the culture conditions were highlighted, some of which may be used to characterize nutrient-limited growth.**

Microorganisms are capable of varying their metabolic fluxes and sizes of intermediate pools over a wide range in response to changes in environmental conditions. These metabolic responses are usually studied in chemostat cultures, where growth conditions can be well controlled. By designing the composition of the feeding medium carefully, growth in chemostats may be limited by a single or multiple nutrients. Carbon limitation is the most widely used type and has been applied to investigate cell metabolism (9, 17, 18, 30, 37), metabolic differences among knockout mutants (11, 30, 38), metabolic reaction models (7), and so on. Growth in carbon-deficient medium may be regarded as limiting energy or catabolism. On the other hand, although energy generation rates are high in carbon-replete cultures, cell yields are usually low, resulting in anabolism limitation and overflow metabolism (9, 18, 23). Other nutrient-limited chemostat cultures include limitation of the nitrogen source, phosphorus, or sulfur, among which nitrogen limitation is the most frequently studied (9, 18, 30, 37). Growth, metabolism and energetics in chemostat cultures under carbon-limited and nitrogen-limited conditions have been investigated for *Saccharomyces cerevisiae*, *Bacillus subtilis*, and other microorganisms (5, 9, 13, 17, 18). For *Escherichia coli*, the influences of nutrient limitation on metabolic responses has been investigated in recent studies by using information on isotopomer distribution (11, 30). Metabolic changes and kinetic properties during the transition from glucose-excess to glucose-limited growth conditions have also been studied (37).

Recently, we developed the method of flux ratio analysis (30) to identify central reaction networks and obtained reliable metabolic fluxes in wild-type W3110 and some knockout mutants of *Escherichia coli* grown in either glucose-limited or ammonia-limited chemostats (16, 38). Steady-state flux information suggested remarkable metabolic alterations in these strains in response to changes of the limiting nutrient. It is known that *E. coli* growing in nutrient-limited chemostat cultures, as in natural conditions, is capable of adapting to these environments quickly and achieving suboptimal growth. To elucidate these physiological adaptations through the flux change and understand the molecular mechanisms underlying the change in the flux, it is therefore very important to investigate the transcriptional responses to different nutrient-limited growth conditions. On the other hand, although a number of genes responsible for cell adaptation to carbon- or nitrogen-limited conditions and many important results relating to its molecular mechanisms have been revealed (2, 12, 13, 19, 20, 28), the transcriptional responses of most genes in the *E. coli* genome are not yet clear. A DNA microarray is one of the most effective techniques to investigate global gene expression and has been used in many studies (15, 25–27, 34).

In this work we investigated gene expression in *E. coli* grown in glucose-limited or ammonia-limited chemostat cultures by DNA microarray analyses. Analyses of transcriptional responses to the change of the growth-limiting nutrient were emphasized, which may provide valuable information to eluci-

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date the change in metabolic flux and cell adaptation. Comparisons of gene expression between two nutrient-limited cultures were conducted for both slow- and fast-growing cells to study the influence of growth rate and identify the genes most important to nutrient-limited growth.

## MATERIALS AND METHODS

**Strains and culture conditions.** *E. coli* K-12 W3110 [$F^-$ $\lambda^-$ IN(*rmD-rmE*)1 *rph-1*] was used to investigate the difference in global gene expression under two nutrient-limited growth conditions. Cells were grown in glucose- or ammonia-limited chemostat cultures. The composition of the glucose-limited medium was (per liter): 5.0 g of glucose, 1.0 g of $NH_4Cl$, 2.7 g of $(NH_4)_2SO_4$, 6.8 g of $Na_2HPO_4$, 3.0 g of $KH_2PO_4$, 0.6 g of NaCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1.0 μg of thiamine HCl, 2.0 μl of polypropylene glycol 2000 as an antifoaming agent, and 10 ml of trace element solution (30). For the ammonia-limited culture, the concentrations of $NH_4Cl$ and $(NH_4)_2SO_4$ were reduced to 0.20 g and 0.24 g per liter, respectively, while all other components remained constant.

Chemostat cultures were operated at 37°C in a BMJ-02PI bioreactor (ABLE, Tokyo, Japan) with a working volume of 1 liter. The culture medium was continuously fed to the bioreactor at a dilution rate ($D$) of 0.10 ± 0.005 or 0.55 ± 0.025 $h^{-1}$, and the working volume was kept constant by withdrawing culture broth through a continuously operating pump. The pH of the culture was maintained at 7.0 by automatic addition of 2.0 M NaOH. An agitation speed of 450 rpm and constant airflow of 1.0 liter/min ensured dissolved oxygen concentrations above 60% saturation. The concentrations of oxygen and carbon dioxide exhausted from the bioreactor were monitored with an exhaust gas analyzer, Off-Gas Jr. DEX-2562 (ABLE). The steady-state condition was ascertained when the optical density of the biomass at 600 nm, the dissolved oxygen concentration, and the exhaust gas concentrations had remained constant for at least three volume changes. In steady-state cultures, almost all cells in the reactor could be considered to be growing at the same specific growth rate, which is equal to the dilution rate. Chemostat cultures were performed at least twice under each dilution rate-nutrient limitation combination.

**Determination of biomass and metabolite concentrations.** Cell growth was monitored by measuring the optical density at 600 nm. Cell dry weight was determined by collecting cell pellets from 100 to 200 ml of culture aliquots, washing them with distilled water, and drying them at 85°C until a constant weight was obtained. The concentrations of glucose and ammonium in the medium were determined with enzymatic test kits (Roche Molecular Biochemicals, Mannheim, Germany) on a microplate spectrofluorometer (SPECTRAmax GEMINI XS; Molecular Devices, Sunnyvale, Calif.), following the changes in NAD(P)H fluorescence at the 355- and 460-nm wavelength pair. The concentrations of other metabolites were determined as given elsewhere (16). At least three measurements were performed for each metabolite concentration.

**Isolation of total RNA.** Total RNA was prepared with a modified hot phenol method (1). Cells were first harvested by centrifugation at 11,000 × *g* for 3 min, resuspended in 0.5 ml of solution A (0.5% sodium dodecyl sulfate, 20 mM sodium acetate, 10 mM EDTA) and mixed by pipetting with 0.5 ml of acidic phenol (pH 5.5) preheated at 60°C. The mixture was incubated at 60°C for 5 min. After centrifugation at 13,000 × *g* for 3 min at room temperature, the supernatant was recovered and the phenol extraction process was repeated. A phenol-chloroform (1:1, pH 5.5) extraction was then performed, and the RNA was precipitated by the addition of 3 volumes of ethanol. The RNA pellet was dried and dissolved in a DNase solution (100 mM sodium acetate, 50 mM $MgSO_4$) containing 5 U of RNase-free DNase (Takara Shuzo Co., Ltd., Kyoto, Japan), and incubated at room temperature for 1 h. A second phenol-chloroform extraction and RNA precipitation were then performed. Purified total RNA was subjected to 1% agarose gel electrophoresis to check for degradation and whether the 23S and 16S rRNAs were recovered without contamination of genomic DNA.

**DNA microarrays, cDNA preparation, hybridization, and data analysis.** High-density custom-made DNA microarrays containing 4,071 independent genes cloned from the *E. coli* K-12 W3110 strain were prepared by the Takara Shuzo Company. In addition to genes of the *E. coli* genome, 24 spots containing the human β-actin gene were present on the slide as negative controls. The details of the preparation and accuracy checking of the DNA microarray were described elsewhere (26, 27).

The preparation of cDNA fluorescently labeled with indocarbocyanine (Cy3) and indodicarbocyanine (Cy5) and microarray hybridization were performed essentially according to the MGuide (http://cmgm.stanford.edu/pbrown/mguide/index.html) with some modifications. Fluorescently labeled cDNA probes were prepared by random priming methods with 5.3 nmol of random hexamer (Takara Shuzo). Reverse transcriptase reactions were performed twice with 50 U of avian myeloblastosis virus reverse transcriptase XL (Life Sciences, St. Petersburg, Fla.) and 4 nmol of either Cy3-dUTP or Cy5-dUTP (Amersham Pharmacia Biotech, Piscataway, N.J.) with 30 μg of total RNA from *E. coli* grown under each of the four dilution rate-nutrient limitation conditions. Labeled cDNA probes were purified with Centri-Sep columns (Princeton Separations, Adelphia, N.J.), phenol-chloroform extraction, and ethanol precipitation. After drying, the cDNA probe was dissolved in 9 μl of water. Both Cy3- and Cy5-labeled cDNA probes were then added to a final volume of 21 μl of hybridization buffer consisting of 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.2% sodium dodecyl sulfate, 5× Denhardt's solution, and 100 ng of salmon sperm DNA $\mu l^{-1}$ and denatured by heating at 98°C for 2 min.

The denatured cDNA probe was applied to the microarray, and hybridization was carried out at 65°C for 16 h. The slides were then washed consecutively with 2× SSC (60°C, 5 min), 0.2× SSC containing 0.1% sodium dodecyl sulfate (60°C, 5 min), and 0.2× SSC (room temperature, 5 min or more). The fluorescence intensities of the slides were scanned immediately after centrifugation (200 × *g*, room temperature, 2 min) with a GMS 418 array scanner (Genetic Microsystems, Woburn, Mass.) and recorded to 16-bit image files. The signal intensity of each spot in the microarray was quantified with IMAGENE software (BioDiscovery, Marina del Rey, Calif.).

For each growth-nutrient condition, two steady-state cell samples from two separate cultures were taken for analysis. Differences in gene expression between ammonia-limited growth and glucose-limited growth at the same growth rate were studied. For each comparison, two hybridization samples were prepared from two independent cell sample pairs (with reversal of dyes), and DNA microarrays were duplicated for each cDNA hybridization, generating four transcriptional data per gene. The signal intensity of each gene was first corrected by subtracting the local background value. Then, genes were classified into three groups by comparison with the intensity of the negative control spots. Group I consisted of genes for which both Cy3 and Cy5 signal intensities were greater than the mean + 1 standard deviation (SD) of the negative controls. Group II consisted of genes for which either Cy3 or Cy5 signal intensity was greater than the mean + 1 SD of the negative controls. Group III consisted of genes for which both Cy3 and Cy5 signal intensities were lower than the mean + 1 SD of the negative controls. Expression differences (ratios of the fluorescence intensities of Cy3 and Cy5) of all group I genes were then normalized by defining the mean of ratios of all genes as 1.0. Group II genes with sufficiently high Cy3 or Cy5 signal intensities (usually exceeded the high-intensity cutoff) were considered. Group III genes were ignored due to extremely low expressions under both growth conditions.

To ensure that the observed transcript alterations were really caused by the change of the growth-limiting nutrient, we also assessed the random fluctuation inherent in our microarray system. Cy5- and Cy3-labeled cDNA probes were simultaneously synthesized from the same template RNA, and their intensities were compared after hybridization. Only a few spots were found to have reproducible twofold changes in intensity due to artificial errors and systematic biases. When transcription in ammonia-limited growth was compared with that in glucose-limited growth, significantly more genes with twofold or greater transcriptional changes were observed, suggesting the transcriptional responses to nutrient-limited cultures could be well investigated by this microarray system.

Significant alteration in transcription was then recognized if one of the following criteria was satisfied: (i) for genes whose four measurements were all group I data, expression ratios should be reproducibly >2.0 or <0.5 and in good consistency ($P < 0.05$, paired Student's $t$ test); (ii) for genes whose four measurements were all group II data, sufficiently high Cy3 or Cy5 intensities should be observed for all measurements; (iii) for genes with both group I and group II data, reproducible twofold or more expression changes should be observed for all group I data and sufficiently high Cy3 or Cy5 intensities should be observed for all group II data.

## RESULTS

**Chemostat cultures of *E. coli* under nutrient-limited conditions.** When *E. coli* strain W3110 was cultivated at a $D$ value of 0.10 $h^{-1}$ with either limited glucose or limited ammonia supply, steady-state cultures could be achieved after six to eight volume changes, which was further ascertained by the measurement of residual substrate concentrations at regular intervals. The residual nutrient concentrations were about 23 μmol/

TABLE 1. Parameters for glucose- and ammonia-limited chemostat cultures at different growth rates[a]

| Limitation | Dilution rate ($h^{-1}$) | $Y_{X/C}$ (g $mol^{-1}$) | Residual glucose (mmol $liter^{-1}$)[b] | Residual ammonium (mmol $liter^{-1}$)[b] | Specific carbon uptake/formation rate (mmol of carbon $g^{-1}$ $h^{-1}$) | | | | Carbon recovery (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | From glucose | To $CO_2$ | To acetate | To other by-products | |
| Glucose | 0.10 | 72 ± 5.4 | 23 ± 4.0* | 38 ± 3.4 | 8.4 ± 0.6 | 4.2 ± 0.4 | 0 | 0 | 99 ± 7 |
| Ammonia | 0.10 | 41 ± 3.6 | 8.3 ± 0.6 | 50 ± 5.0* | 17.4 ± 1.8 | 7.2 ± 0.9 | 3.2 ± 0.3 | <0.5 | 93 ± 9 |
| Glucose | 0.55 | 86 ± 5.4 | 27 ± 5.0* | 40 ± 4.0 | 38.4 ± 1.8 | 16.6 ± 1.8 | 0 | 0 | 104 ± 8 |
| Ammonia | 0.55 | 58 ± 5.4 | 14.6 ± 0.7 | 50 ± 6.0* | 51.6 ± 3.6 | 17.0 ± 2.0 | 5.1 ± 0.3 | <0.5 | 95 ± 6 |

[a] $Y_{X/C}$, cell yield on glucose. Other by-products included pyruvate, ethanol, α-ketoglutarate, fumarate, and extracellular polysaccharide (16).
[b] Units are micromoles per liter if marked (*).

liter for glucose and 50 μmol/liter for ammonia when cells were grown at the low growth rate. When the dilution rate of the chemostat culture increased to 0.55 $h^{-1}$, 20 to 25 volume changes after the initiation of feeding were necessary to ensure steady-state cultures. In this case of high growth rates, there were about 27 μmol of residual glucose and 50 μmol of residual ammonia per liter in the medium of glucose-limited and ammonia-limited cultures, respectively. Cell yields on glucose in cultures deficient in glucose were more than 1.5-fold higher than those in cultures deficient in ammonia under both dilution rate conditions. Several metabolic by-products were excreted from the cells in the case of ammonia deficiency, where the primary by-product was acetate and others included pyruvate, α-ketoglutarate, ethanol, fumarate, and some extracellular polysaccharide. In chemostat cultures with glucose limitation, however, no other metabolic by-products except $CO_2$ were excreted into the medium at detectable levels (Table 1).

**Transcriptome analyses of cells in different nutrient-limited cultures with DNA microarrays.** For each growth rate condition, the number of genes that could be classified in groups I and II after one hybridization exceeded 3,300 (more than 80% of the total genes on the slide). By combining four individual hybridizations, it was found that 400 genes (9.8% of the total genes analyzed) were differentially expressed in slow-growing cells adapting to glucose- or ammonia-deprived environments. The number of genes with significant transcriptional changes was, however, decreased markedly with an increase in the growth rate. The number of differentially expressed genes under two nutrient limitations and the corresponding percentages in their functional categories are summarized in Table 2. For genes with known functions, it was observed that ammonia limitation might upregulate genes involved in amino acid metabolism and transport system to high transcription levels, while a deficiency of glucose in chemostat cultures might greatly induce genes involved in central carbon and energy metabolisms, transport systems, and the cell envelope.

**Significant upregulation of genes in amino acid metabolism and nitrogen metabolism by ammonia limitation.** At the low growth rate, expressions of most of the 138 genes encoding enzymes of amino acid metabolism were induced in ammonia-limited cells. Among these upregulated genes, the transcript levels of 32 were significantly elevated in response to nitrogen deficiency. It is known that there are two routes of ammonia assimilation in enterobacteria. One involves the synthesis of glutamine and glutamate by glutamine synthetase (GS, product of the *glnA* gene) and glutamate synthase (GOGAT, product of the *gltB* and *gltD* genes). Another route is direct glutamate synthesis from α-ketoglutarate and ammonia by NADP-linked glutamate dehydrogenase, the product of the *gdhA* gene. In cells growing under ammonia limitation, unlike the slight in-

TABLE 2. Genes differentially expressed between ammonia-limited growth and glucose-limited growth

| Functional category of genes analyzed | No. of genes | Low growth rate (0.10 $h^{-1}$) | | | High growth rate (0.55 $h^{-1}$) | | | Both growth rates | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. higher on N-limited | No. higher on C-limited | %[a] | No. higher on N-limited | No. higher on C-limited | % | No. higher on N-limited | No. higher on C-limited | % |
| Total | 4,071 | 235 | 165 | 9.8 | 58 | 108 | 4.1 | 42 | 50 | 2.3 |
| Amino acid metabolism | 138 | 32 | 4 | 26 | 11 | 0 | 8.0 | 11 | 0 | 8.0 |
| Biosynthesis of cofactors, etc. | 128 | 9 | 2 | 8.6 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| Cell envelope | 202 | 8 | 37 | 22 | 1 | 10 | 5.4 | 0 | 2 | 1.0 |
| Cellular processes | 124 | 7 | 7 | 11 | 1 | 1 | 1.6 | 0 | 0 | 0.0 |
| Central intermediary metabolism | 153 | 12 | 11 | 15 | 1 | 9 | 6.5 | 1 | 6 | 4.6 |
| Energy metabolism | 362 | 18 | 21 | 11 | 2 | 34 | 9.9 | 0 | 14 | 3.9 |
| Fatty acid/phospholipid metabolism | 60 | 5 | 4 | 15 | 1 | 3 | 6.7 | 1 | 2 | 5.0 |
| Nucleotide metabolism | 120 | 10 | 2 | 10 | 2 | 4 | 5.0 | 2 | 0 | 1.7 |
| Regulatory function | 104 | 7 | 2 | 8.7 | 2 | 1 | 2.9 | 2 | 1 | 2.9 |
| Replication | 91 | 3 | 0 | 3.3 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| Transport/binding protein | 373 | 28 | 26 | 15 | 14 | 27 | 11 | 11 | 16 | 7.2 |
| Translation | 152 | 6 | 1 | 4.6 | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| Transcription | 52 | 5 | 0 | 9.6 | 0 | 2 | 3.8 | 0 | 0 | 0.0 |
| Other | 185 | 7 | 3 | 5.4 | 3 | 4 | 3.8 | 0 | 2 | 1.0 |
| Hypothetical | 1,827 | 78 | 45 | 6.7 | 20 | 13 | 1.8 | 14 | 7 | 1.1 |

[a] Percentage of differentially expressed genes in the functional category.

TABLE 3. Amino acid metabolism genes differentially expressed between ammonia-limited and glucose-limited chemostat cultures[a]

| Identification | Gene | Logarithmic ratio[b] (N-limited/C-limited) at a *D* of: | | Description[c] |
|---|---|---|---|---|
| | | 0.10 $h^{-1}$ | 0.55 $h^{-1}$ | |
| JW0001 | *thrA* | 1.58 | | ThrA bifunctional enzyme (EC 2.7.2.4, EC 1.1.1.3) |
| JW0029 | *dapB* | 2.19 | | Dihydrodipicolinate reductase (EC 1.3.1.26) |
| JW0076 | *ilvI* | 1.89 | | Acetolactate synthase (EC 4.1.3.18) III large subunit |
| JW0077 | *ilvH* | 1.22 | | Acetolactate synthase (EC 4.1.3.18) III small subunit |
| JW0161 | *dapD* | 1.46 | | Succinyldiaminopimelate transaminase (EC 2.6.1.17) |
| JW0162 | *glnD* | 2.09 | 1.18 | $P_{II}$ uridylyl transferase (EC 2.7.7.59) |
| JW0266 | *argF* | 1.65 | | Omithine carbamoyltransferase (EC 2.1.3.3) chain F |
| JW0660 | *asnB* | 5.08 | 2.13 | Asparagine synthase (glutamine-hydrolyzing) (EC 6.3.5.4) |
| JW0812 | *ybiK* | 1.96 | | Hypothetical L-asparaginase |
| JW0890 | *serC* | 2.11 | | Phosphoserine transaminase (EC 2.6.1.52) |
| JW0891 | *aroA* | 1.63 | | 3-Phosphoshikimate 1-carboxyvinyltransferase (EC 2.5.1.19) |
| JW1253 | *trpB* | 1.94 | | Tryptophan synthase beta chain (EC 4.2.1.20) |
| JW1254 | *trpC* | 1.88 | | Anthranilate isomerase |
| JW1255 | *trpD* | 1.98 | | Anthranilate synthase (EC 4.1.3.27) component II |
| JW1256 | *trpE* | >4.15 | | Anthranilate synthase (EC 4.1.3.27) component I |
| JW1733 | *astE* | 1.59 | 1.66 | Succinylglutamate desuccinylase |
| JW1734 | *astB* | 1.76 | 1.37 | Succinylarginine dihydrolase |
| JW1735 | *astD* | 2.16 | 1.27 | Succinylglutamic semialdehyde dehydrogenase |
| JW1736 | *astA* | 3.26 | 1.61 | Arginine succinyltransferase |
| JW1737 | *astC* | 3.02 | 2.04 | Succinylornithine aminotransferase (EC 2.6.1.—) |
| JW2001 | *hisG* | 1.47 | | ATP phosphoribosyltransferase |
| JW3044 | *oat* | 3.48 | 2.72 | Probable ornithine aminotransferase (EC 2.6.1.13) |
| JW3140 | *argG* | 1.26 | | Argininosuccinate synthase (EC 6.3.4.5) |
| JW3396 | *asd* | >2.35 | | Aspartate-semialdehyde dehydrogenase (EC 1.2.1.11) |
| JW3839 | *ntrC* | 1.65 | 1.67 | Nitrogen regulation protein NtrC ($NR_I$) |
| JW3840 | *ntrB* | 1.61 | 1.90 | Nitrogen regulation protein NtrB ($NR_{II}$) (EC 2.7.3.—) |
| JW3841 | *glnA* | 2.97 | 2.14 | Glutamate-ammonia ligase (EC 6.3.1.2) |
| JW3929 | *argE* | 1.34 | | Acetylornithine deacetylase (EC 3.5.1.16) |
| JW3930 | *argC* | 2.45 | | *N*-Acetyl-gamma-glutamylphosphate reductase (EC 1.2.1.38) |
| JW3931 | *argB* | 1.08 | | Acetylglutamate kinase (EC 2.7.2.8) |
| JW3932 | *argH* | 1.36 | | Argininosuccinate lyase (EC 4.3.2.1) |
| JW3984 | *lysC* | 3.37 | | Lysine-sensitive aspartokinase III (EC 2.7.2.4) |
| JW1290 | *ycjK* | −1.70 | | Putative glutamine synthetase (EC 6.3.1.2) |
| JW1393 | *paaK* | −2.55 | | Phenylacetate-coenzyme A ligase |
| JW2582 | *aroF* | −3.00 | | Tyrosine-sensitive 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase |
| JW3740 | *ilvG* | −1.71 | | Acetolactate synthase (EC 4.1.3.18) II large chain |

[a] The results obtained are representative of four independent hybridizations from total RNAs extracted from two independent chemostat cultures.
[b] The expression level was described by the relative log ratio ($\log_2$ ratio) of ammonia-limited growth/glucose-limited growth. When both group I and group II data existed, the expression level was described by more than or less than the mean of the ratio evaluated from only group I data. The details of group I and group II are described in Materials and Methods.
[c] Function was described according to SWISSPROT, Genobase, and GenBank.

crease in expression of the *gdhA* gene, the transcript level of the *glnA* gene was significantly elevated (7.8- and 4.4-fold increase at the low and high growth rates, respectively) (Table 3), indicating that the former route is primarily responsible for the assimilation of ammonia in this condition. The *glnA* gene is the first gene of the *glnA-ntrBC* operon, which involves another two genes, *ntrB* and *ntrC*, that encode the nitrogen-regulatory proteins NtrB and NtrC, respectively. When the nitrogen source in the medium is abundant, the expression of three genes is regulated by NtrC, resulting in relatively low intracellular concentrations of GS, NtrB, and NtrC. The extremely low residual ammonia concentration in ammonia-limited culture, however, results in a drop in the intracellular concentration of glutamine, which enables $P_{II}$ uridylyltransferase (product of the *glnD* gene) to uridylylate $P_{II}$ protein to form $P_{II}$-UMP and release NtrB from its association with $P_{II}$. NtrC is then phosphorylated by NtrB to form NtrC phosphate, which activates the initiation of transcription at the $\sigma^{54}$-dependent promoter *glnAp2* and results in high expression of the *glnA-ntrBC* operon (2, 20). The expression of the *glnD* gene was also induced markedly (two- to fivefold) in ammonia-limited cells. The resulting product of $P_{II}$ uridylylation ($P_{II}$-UMP) may then stimulate the deadenylylation (activation) of partly inactive GS (GS-AMP) by adenylyltransferase. Therefore, significantly elevated transcriptional and posttranscriptional levels of glutamine synthetase, together with high expression of the *gltB* and *gltD* genes, which encode glutamate synthase, provided high intracellular concentration of GS and GOGAT, capable of harvesting the remaining ammonia in the medium.

Among the genes involved in amino acid metabolism, the *asnB* gene, encoding glutamine-dependent asparagine synthetase, is one of the genes that showed the most significant increase in expression when culture conditions were changed from glucose limitation to ammonia limitation (Table 3). Although there is another ammonia-dependent asparagine synthetase encoded by *asnA* in prokaryotes, this enzyme is not preferred for asparagine synthesis under nitrogen limitation due to insufficient intracellular ammonia ($K_m$ for ammonia,

about 0.3 mM). Indeed, no change in expression of the *asnA* gene in response to ammonia limitation was observed in our experiments, which may be due to the repression of *asnA* expression by the elevated NtrC concentration (28). Most of the other genes with large increases in transcript levels were genes involved in the synthesis of amino acids, including *thrA* and *asd*, involved in the common biosynthetic pathway of aspartate family amino acids; *lysC*, *dapB*, and *dapD*, involved in lysine synthesis; *hisG*, which encodes the first-step enzyme in histidine synthesis; and two genes of the *ilvIH* operon, encoding acetolactate synthase, which catalyzes first enzymatic step of valine and isoleucine synthesis. Besides these, the expression of the *trpEDCAB* operon genes (encoding the enzymes of tryptophan synthesis) and the *argECBH* operon genes (encoding the enzymes of arginine synthesis) were also elevated in slow-growing ammonia-deprived cells.

In contrast to most of the upregulated genes involved in amino acid biosynthesis, no significant expression was found for genes involved in the degradation of amino acids except for the genes of the *astCADBE* operon (2-fold to 10-fold increase in ammonia-deprived cells) (Table 3). Genes of the *astCADBE* operon encode the enzymes of the arginine succinyltransferase pathway, the dominant arginine catabolic pathway in *E. coli*. Previous studies suggested that some enzymes of the arginine succinyltransferase pathway are nitrogen regulated, and the activities of these enzymes increased greatly when arginine or aspartate was used as the nitrogen source (29, 31). This was also confirmed by our results for gene expression under ammonia limitation.

**Expression of genes involved in central carbon and energy metabolism.** Of the 515 genes involved in central carbon and energy metabolism, differentially expressed genes were equally distributed between glucose-limited and ammonia-limited cells when the growth rate was low. With an increase in the growth rate, the number of genes with elevated expression in ammonia-deprived cells decreased significantly, whereas an increase in number was observed for genes that were differentially expressed when glucose was deficient. A lack of ammonia in the medium generally induced genes of the Embden-Meyerhof-Parnas and pentose phosphate pathways. Besides these, all of five genes in two glycogen operons (*glgBX* and *glgCAP*) were also expressed at significantly higher levels (Table 4), corresponding to the large amount of glycogen storage (more than 10% of the cell's dry weight) when the cell grows slowly under ammonia limitation (16). Moreover, significant elevation of transcript levels of pyruvate dissimilation genes were also observed in ammonia-deprived cells. These included the *pflA* gene (encoding pyruvate formate-lyase I activating enzyme), the *ackA* gene (encoding acetate kinase), and the *adhE* gene (encoding alcohol dehydrogenase/acetaldehyde dehydrogenase). High transcript levels of these genes were probably responsible for the excretion of acetate, ethanol, and formate as overflow metabolites in cells growing under ammonia limitation (16).

Unlike the general upregulation of genes in the Embden-Meyerhof-Parnas and pentose phosphate pathways, expression of most of the genes of the tricarboxylic acid cycle as well as glyoxylate and dicarboxylate metabolism declined in ammonia-limited cells under both growth rates (Table 4). Among these downregulated genes, four genes of the *sdhCDAB* operon that encode succinate dehydrogenase; the *sucD* gene, encoding succinate-coenzyme A ligase α chain; the *fumA* and *fumC* genes, which encode fumarase; and *aceA*, encoding isocitrate lyase were expressed with significantly lower transcript levels. The expression of a considerable number of oxidative phosphorylation genes was upregulated in glucose-limited cells in response to the limitation of energy. Moreover, with the increase in growth rate, an increase was observed for the oxidative phosphorylation genes that had elevated transcript levels under glucose limitation, among which 8 of 13 genes of the *nuo* operon encoding NADH dehydrogenase I were differentially expressed in glucose-limited cells. The remarkable elevation in aerobic respiration suggested the further requirement of ATP synthesis by faster-growing cells to satisfy the rapid biosynthesis of cell macromolecules in carbon- and energy-limited conditions.

**Strong induction of transporter genes by two nutrient limitations.** A considerable number of genes involved in membrane transport were found to have differential expression in cells grown in the two different nutrient limitation conditions (Table 2). Ammonia deficiency in the medium significantly induced the transcript levels of a number of genes that encode transport proteins for nitrogen-containing metabolites. These included the *proP* gene (encoding proline/betaine transport protein), the *cycA* gene (encoding D-serine/D-alanine/glycine permease), the *gabP* gene (encoding γ-aminobutyrate permease), the *nupC* gene (encoding a nucleoside permease), and genes of the *potFGHI* operon (encoding the ATP-binding cassette [ABC] transport system for putrescine) (Table 5). Besides these genes, the functions of which are well annotated, expression of most of the genes involved in two hypothetical ABC transport systems, YddOPQRS and YliABCD, was also induced significantly by ammonia limitation, indicating the possibility of transport of certain nitrogen-containing metabolites by these systems. Cells grown under ammonia limitation also expressed the transporter gene *amtB* (encoding ammonium/methylammonium transport B protein [AmtB]) at markedly elevated transcript levels (more than 27-fold and 44-fold changes in slow- and fast-growing cells, respectively).

It was proposed that AmtB and its homologue proteins are responsible for the increase in diffusion rate of ammonia ($NH_3$) across the cytoplasmic membrane rather than actively transporting $NH_4^+$ or $NH_3$ (32, 33). Although it was reported that AmtB function is required when the external $NH_3$ concentration drops to 50 nM (about 10 μM external ammonium concentration at pH 7) or less (33), the result of the remarkable increase in expression of the *amtB* gene implied that its product may function earlier, such as when the residual ammonium concentration was about 50 μM, as in our ammonia-limited chemostats. Another significantly expressed gene was the *nac* gene, which, like the *amtB* gene, is a nitrogen-regulatory (Ntr) gene which is transcriptionally regulated by the nitrogen-regulatory proteins NtrB and NtrC (3, 21, 39). The *nac* gene encodes the nitrogen assimilation control protein NAC, which activates transcription from a number of $\sigma^{70}$-dependent promoters to transcribe operons whose products provide the cell with alternative nitrogen sources (21). A >100-fold increase in the transcript levels of this gene in both slow- and fast-growing cells suggests full induction of the nitrogen

TABLE 4. Genes involved in carbon and energy metabolism that were differentially expressed between ammonia-limited and glucose-limited chemostat cultures

| Category | Identification | Gene | Logarithmic ratio (N-limited/C-limited) at a *D* of: | | Description |
|---|---|---|---|---|---|
| | | | 0.10 $h^{-1}$ | 0.55 $h^{-1}$ | |
| Central intermediary metabolism | JW0007 | *talB* | 1.32 | | Transaldolase B (EC 2.2.1.2) |
| | JW1005 | *phoH* | 2.67 | | PhoH protein (phosphate starvation-inducible protein PsiH) |
| | JW1488 | *gadB* | 4.27 | | Glutamate decarboxylase (EC 4.1.1.15) beta |
| | JW2010 | *ugd* | >3.04 | | UDP-glucose 6-dehydrogenase (EC 1.1.1.22) |
| | JW2037 | *fcl* | 2.15 | | Fucose synthatase |
| | JW2038 | *gmd* | 2.48 | | GDP-mannose 4,6-dehydratase (EC 4.2.1.47) |
| | JW2198 | *mqo* | >2.68 | 2.19 | Malate:quinone oxidoreductase |
| | JW2636 | *gabD* | 1.72 | | Succinate-semialdehyde dehydrogenase ($NADP^+$) (EC 1.2.1.16) |
| | JW2637 | *gabT* | 2.07 | | 4-Aminobutyrate transaminase (EC 2.6.1.19) |
| | JW3179 | *gltB* | 1.91 | | Glutamate synthase (NADPH) large-chain precursor (EC 1.4.1.13) |
| | JW3180 | *gltD* | 1.84 | | Glutamate synthase (NADPH) small chain (EC 1.4.1.13) |
| | JW3485 | *gadA* | >4.50 | | Glutamate decarboxylase alpha (EC 4.1.1.15) (Gad-alpha) |
| | JW0987 | *agp* | −1.83 | <−1.71 | Glucose-1-phosphatase precursor (EC 3.1.3.10) (G1Pase) |
| | JW1179 | *dadX* | −1.24 | | Alanine racemase, catabolic precursor (EC 5.1.1.1) |
| | JW2447 | *maeB* | −1.74 | −1.77 | NADP-dependent malic enzyme |
| | JW2943 | *glcB* | | −1.73 | Malate synthase (EC 4.1.3.2) isoenzyme G |
| | JW2944 | *glcG* | | −1.81 | GlcG protein |
| | JW2947 | *glcC* | −1.84 | | Glc operon transcriptional activator |
| | JW3366 | *pckA* | −3.35 | −3.42 | Phosphoenolpyruvate carboxykinase (ATP) (EC 4.1.1.49) |
| | JW3686 | *tnaA* | <−6.09 | −5.39 | Tryptophanase (EC 4.1.99.1) (L-tryptophan indole-lyase) |
| | JW3772 | *aslB* | −1.38 | | Putative arylsulfatase regulatory protein |
| | JW3897 | *glpK* | −2.53 | −3.11 | Glycerol kinase (EC 2.7.1.30) |
| | JW3975 | *aceA* | −2.17 | −1.53 | Isocitrate lyase (EC 4.1.3.1) (isocitrase) (isocitratase) (ICU) |
| | JW3976 | *aceK* | −2.32 | | Isocitrate dehydrogenase ($NADP^+$) kinase/phosphatase precursor (EC 2.7.1.116) |
| | JW4092 | *cadA* | −1.39 | | Lysine decarboxylase (EC 4.1.1.18) |
| | JW4099 | *aspA* | | −1.54 | Aspartate ammonia-lyase (EC 4.3.1.1) |
| Energy metabolism | JW0120 | *gcd* | | 1.47 | Glucose dehydrogenase (pyrroloquinoline-quinone) (EC 1.1.99.17) |
| | JW0142 | *sfsA* | 2.15 | | Sugar fermentation stimulation protein |
| | JW0885 | *pflA* | 1.91 | | Pyruvate formate-lyase 1 activating enzyme (EC 1.97.1.4) |
| | JW1206 | *kdsA* | 1.11 | | 3-Deoxy-D-manno-octulosonic acid 8-phosphate synthetase |
| | JW1228 | *adhE* | 2.55 | | Alcohol dehydrogenase (EC 1.1.1.1) (ADH), acetaldehyde dehydrogenase (acetylating) (EC 1.2.1.10), pyruvate-formate-lyase deactivase |
| | JW1724 | *celD* | 1.47 | | Regulatory protein CelD |
| | JW1841 | *zwf* | 1.83 | | Glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49) |
| | JW2155 | *fruK* | 1.53 | | 1-Phosphofructokinase (EC 2.7.1.56) |
| | JW2293 | *ackA* | 1.28 | | Acetate kinase (EC 2.7.2.1) |
| | JW2750 | *eno* | 1.66 | | Enolase (EC 4.2.1.11) |
| | JW2892 | *fbaA* | 1.33 | | Fructose-bisphosphate aldolase (EC 4.1.2.13) |
| | JW2894 | *epd* | 1.80 | | Erythrose-4-phosphate dehydrogenase (homologous to *gapB*) |
| | JW2978 | *yqhD* | | 3.39 | Hypothetical oxidoreductase |
| | JW3065 | *exuR* | 1.35 | | Exu regulon regulator |
| | JW3391 | *glgP* | 2.13 | | Glycogen phosphorylase (EC 2.4.1.1) |
| | JW3392 | *glgA* | >1.68 | | Glycogen synthase (EC 2.4.1.21) |
| | JW3393 | *glgC* | 1.62 | | Glucose-1-phosphate adenylyltransferase (EC 2.7.7.27) |
| | JW3394 | *glgX* | 1.86 | | Glycogen operon protein GlgX (EC 3.2.1.—) |
| | JW3395 | *glgB* | 1.87 | | 1,4-Alpha-glucan branching enzyme (EC 2.4.1.18) |
| | JW3928 | *ppc* | 2.41 | | Phosphoenolpyruvate carboxylase (EC 4.1.1.31) (pepcase) |
| | JW0201 | *mltD* | | −1.48 | Membrane-bound lytic murein transglycosylase D |
| | JW0711 | *sdhC* | −1.42 | −2.60 | Succinate dehydrogenase cytochrome *b*-556 subunit |
| | JW0712 | *sdhD* | −1.57 | −1.95 | Succinate dehydrogenase (EC 1.3.99.1) 13k hydrophobic protein |
| | JW0713 | *sdhA* | −1.69 | −1.39 | Succinate dehydrogenase (EC 1.3.99.1) flavoprotein |
| | JW0714 | *sdhB* | −2.04 | −1.51 | Succinate dehydrogenase (EC 1.3.99.1) iron-sulfur protein |
| | JW0718 | *sucD* | −1.69 | −1.79 | Succinate-coenzyme A ligase (ADP-forming) (EC 6.2.1.5) alpha chain |
| | JW1268 | *acnA* | | −1.49 | Aconitate hydratase I (EC 4.2.1.3) |
| | JW1293 | *aldH* | | −1.23 | Aldehyde dehydrogenase homolog |
| | JW1412 | *aldA* | −2.88 | −2.54 | Aldehyde dehydrogenase ($NAD^+$) (EC 1.2.1.3) |
| | JW1603 | *fumC* | −2.36 | | Fumarate hydratase class II (EC 4.2.1.2) |
| | JW1604 | *fumA* | −1.77 | −1.34 | Fumarate hydratase class I (EC 4.2.1.2), aerobic |
| | JW1609 | *uidA* | <−1.29 | | Beta-glucuronidase (EC 3.2.1.31) |
| | JW1794 | *fadD* | −2.60 | −1.86 | Long-chain fatty-acid-coenzyme A ligase (EC 6.2.1.3) |
| | JW1912 | *amyA* | −1.72 | | Alpha-amylase (EC 3.2.1.1), cytoplasmic |
| | JW2075 | *gatD* | −5.29 | −2.45 | Galactitol-1-phosphate 5-dehydrogenase (EC 1.1.1.251) |
| | JW2078 | *gatA* | | −1.93 | Phosphotransferase system, galactitol-specific IIA component |
| | JW2081 | (*gatA*) | | −1.91 | Phosphotransferase system, galactitol-specific IIA component |
| | JW2082 | *gatZ* | | −1.83 | Putative tagatose 6-phosphate kinase (EC 2.7.1.143) |
| | JW2274 | *nuoK* | | −1.17 | NADH dehydrogenase I chain K (EC 1.6.5.3) |
| | JW2275 | *nuoJ* | | −1.14 | NADH dehydrogenase (ubiquinone) (EC 1.6.5.3) chain J |
| | JW2276 | *nuoI* | | −1.53 | NADH dehydrogenase I chain I (EC 1.6.5.3) |

*Continued on following page*

TABLE 4—*Continued*

| Category | Identification | Gene | Logarithmic ratio (N-limited/C-limited) at a *D* of: | | Description |
|---|---|---|---|---|---|
| | | | 0.10 $h^{-1}$ | 0.55 $h^{-1}$ | |
| | JW2279 | *nuoF* | | −1.33 | NADH dehydrogenase I chain F (EC 1.6.5.3) |
| | JW2280 | *nuoE* | | −1.44 | NADH dehydrogenase (ubiquinone) (EC 1.6.5.3) chain E |
| | JW2281 | *nuoD/C* | | −1.38 | NADH dehydrogenase I chain C/D (EC 1.6.5.3) |
| | JW2282 | *nuoB* | | −1.85 | NADH dehydrogenase (ubiquinone) (EC 1.6.5.3) chain B |
| | JW2283 | *nuoA* | | −1.99 | NADH dehydrogenase I chain A (EC 1.6.5.3) |
| | JW2338 | *yfcX* | −2.07 | −1.88 | Putative fatty oxidation complex alpha subunit (EC 4.2.1.17) |
| | JW2385 | *glk* | | −1.21 | Glucokinase (EC 2.7.1.2) |
| | JW2771 | *fucA* | −1.78 | | L-Fuculose phosphate aldolase (EC 4.1.2.17) |
| | JW2812 | *yqeF* | −1.90 | −1.80 | Probable acetyl-coenzyme A acetyltransferase |
| | JW3021 | *glgS* | | −1.39 | RpoS-dependent glycogen synthesis protein |
| | JW3205 | *mdh* | | −1.46 | Malate dehydrogenase (EC 1.1.1.37) |
| | JW3381 | *malT* | | −2.13 | MalT regulatory protein |
| | JW3543 | *malS* | −2.54 | | Alpha-amylase (EC 3.2.1.1) precursor, periplasmic |
| | JW3561 | *aldB* | −2.79 | | Aldehyde dehydrogenase (EC 1.2.1.—) |
| | JW3716 | *atpB* | | −1.19 | ATP synthase *a* chain (EC 3.6.1.34) (protein 6) |
| | JW3717 | *atpI* | | −1.57 | ATP synthase protein I |
| | JW3821 | *fadA* | −2.42 | −2.10 | 3-Ketoacyl-coenzyme A thiolase (EC 2.3.1.16) |
| | JW3822 | *fadB* | −2.28 | −2.23 | Fatty oxidation complex alpha subunit |
| | JW3934 | *udhA* | −2.01 | −2.16 | Soluble pyridine nucleotide transhydrogenase |
| | JW4112 | *frdD* | −1.38 | | Fumarate reductase (EC 1.3.99.1) 13k membrane anchor protein |

regulatory system in *E. coli* to cope with nitrogen starvation and optimize cell growth.

In contrast to the induction of transport systems of possible nitrogen sources under ammonia limitation, expression of a number of genes encoding membrane transporter proteins for various carbon sources was significantly induced in nitrogen-replete but carbon-limited chemostat cultures (Table 5). About half of these are genes involved in a wide range of high-affinity ABC transport systems that facilitate the uptake of maltose (all genes of the *malGFE* and *malK-lamB-malM* operons), galactose (three genes of the *mglBAC* operon), xylose (*xylF*), and ribose (*rbsB*). Other transporter genes induced by glucose limitation included those encoding the galactitol-specific phosphotransferase system (*gatB* and *gatC*), mannose-specific phosphotransferase system (*manXYZ*), and an ion-coupled transporter responsible for the aerobic transport of $C_4$ dicarboxylates (*dctA*).

Significantly high expression of ABC transporter genes may be under the influence of high internal cyclic AMP (cAMP) levels and the induction of endogenous sugar inducers (13, 35). Among the above-mentioned ABC transporter genes, the expression of the *malM* gene dropped markedly to below the intensity of the negative controls when ammonia was deficient. The function of this gene is, however, not yet clear. The *lamB* gene, located in the same operon, also showed a significant change in expression. This gene encodes the maltoporin LamB, which facilitates the diffusion of maltose and glucose across the outer membrane and acts as a general porin for small solutes. The transcript level of the *lamB* gene was more than 20-fold higher in glucose-deprived cells than in ammonia-deprived cells. Ammonia-limited growth was found to be not greatly impaired by the loss of LamB, possibly due to the small size of $NH_3$ and $NH_4^+$ ions (19) and the improved ammonia acquisition ability resulting from the overexpressed *amtB* gene. Although LamB and a general porin, OmpF, were considered the major players in outer membrane permeability under sugar, iron, or phosphate limitation (13, 19), the results of the insignificant expression changes in the *ompF* gene between glucose and ammonia limitation indicate that LamB might be the primary porin that contributes to glucose permeability through the outer membrane under glucose limitation.

**Other genes differentially expressed in response to nutrient limitation.** Other genes that were regulated significantly by nutrients include the *codA*, *guaD*, and *cfa* genes, involved in nucleotide and fatty acid metabolism, and the *glnK* and *cbl* genes, involved in regulatory function. All of these genes showed differential expression independent of the growth rate (Table 6). The *codA* gene, one gene of the *codBA* operon, encodes cytosine deaminase, one enzyme involved in pyrimidine catabolism. Since another gene of the *codBA* operon (*codB*, encoding cytosine permease) was also upregulated, the remarkable increase in the expression of this operon under ammonia limitation is in good agreement with the recent results that *codBA* expression is regulated by the nitrogen-regulatory system through the direct activation of nitrogen assimilation control protein NAC (22, 39).

The expression of the *guaD* gene (encoding guanine deaminase of the pyrine catabolic pathway) was induced most among the genes involved in nucleotide metabolism when ammonia in the medium became deficient (more than 20-fold and 4-fold increases at the low and high growth rates, respectively). Our results support the hypothesis that this pyrine catabolism gene from *E. coli* is also a nitrogen-regulated gene, though further verification is required. Indeed, the *gde* gene, which encodes guanine deaminase in *Bacillus subtilis*, was found to be expressed only during nitrogen-limited conditions, and the control of *gde* expression required regulatory proteins such as GlnA, GlnR, and TnrA, which are important for regulating the expression of nitrogen metabolism genes (24).

Differential expression was also observed for the *cfa* gene. This gene encodes cyclopropane fatty acid (CFA) synthase, which catalyzes the postsynthetic modification of the lipid bilayer (8). Although it is known that one of the two promoters of this gene is activated by RpoS and that acid conditions

TABLE 5. Transport genes differentially expressed between ammonia-limited and glucose-limited chemostat cultures[a]

| Identification | Gene | Logarithmic ratio (N-limited/C-limited) at a *D* of: | | Description |
|---|---|---|---|---|
| | | 0.10 $h^{-1}$ | 0.55 $h^{-1}$ | |
| JW0441 | *amtB* | 4.80 | 5.46 | Probable ammonium transporter |
| JW0734 | *pnuC* | 1.58 | | PnuC protein |
| JW0813 | *yliA* | 2.04 | | Hypothetical ABC transporter ATP-binding protein |
| JW0814 | *yliB* | 1.87 | | Hypothetical protein |
| JW0815 | *yliC* | 1.34 | | Putative transport system permease protein |
| JW0838 | *potF* | 2.59 | | Putrescine transport protein PotF |
| JW0839 | *potG* | 2.52 | | Putrescine transport protein PotG |
| JW0840 | *potH* | 1.53 | | Putrescine transport protein PotH |
| JW0874 | *lolA* | 1.50 | | Outer membrane lipoprotein carrier protein precursor (p20) |
| JW0953 | *yccA* | >1.40 | | Probable transport permease |
| JW1209 | *chaC* | 1.98 | | Cation transport protein ChaC |
| JW1478 | *yddO* | 2.51 | | Hypothetical ABC transporter ATP-binding protein |
| JW1479 | *yddP* | 2.94 | 2.61 | Hypothetical ABC transporter ATP-binding protein |
| JW1480 | *yddQ* | 2.37 | 2.39 | Putative transport protein |
| JW1481 | *yddR* | 2.46 | 2.48 | Hypothetical ABC transporter permease protein |
| JW1482 | *yddS* | 4.06 | 3.97 | Putative hemin-binding lipoprotein |
| JW1487 | *xasA* | 5.17 | | Extreme acid sensitivity protein |
| JW1521 | *ydeA* | 2.45 | | L-Arabinose and isopropyl-β-D-thiogalactopyranoside exporter protein |
| JW1967 | *nac* | >7.24 | >7.12 | Nitrogen assimilation control protein |
| JW2156 | *fruB* | 2.86 | | Phosphotransferase system, fructose-specific IIA/FPr component (EIIA-Fru) |
| JW2389 | *nupC* | 2.78 | 1.20 | Nucleoside permease |
| JW2638 | *gabP* | >3.25 | >2.27 | GabA permease (γ-aminobutyrate transport carrier) |
| JW2850 | *ygfO* | 1.76 | 1.66 | Hypothetical purine permease |
| JW3239 | *yhdZ* | | 2.43 | Hypothetical amino acid ABC transporter ATP-binding protein |
| JW3371 | *feoA* | 2.25 | | Iron(II) transport system protein FeoA |
| JW3789 | *corA* | 1.74 | | Magnesium transport protein CorA |
| JW3798 | *yigK* | 1.47 | | Amino acid exporter (homoserine, homoserine lactone) |
| JW4066 | *phnD* | | >1.14 | Phosphonate-binding periplasmic protein precursor |
| JW4072 | *proP* | 3.45 | 2.31 | Proline/betaine transport protein |
| JW4166 | *cycA* | 2.57 | 1.77 | D-Serine/D-alanine/glycine transporter |
| JW4168 | *ytfF* | | 1.61 | Probable cationic amino acid transporter |
| JW0542 | *nmpC* | −1.79 | | Outer membrane porin protein NmpC precursor |
| JW0665 | *nagE* | −1.28 | −1.22 | Phosphotransferase system, *N*-acetylglucosamine-specific IIABC component (EIIABC-Nag) |
| JW0746 | *modA* | −1.56 | | Molybdate-binding periplasmic protein precursor |
| JW1087 | *ptsG* | | −1.30 | Phosphotransferase system, glucose-specific IIBC component (EIIBC-glc) |
| JW1758 | *ydjE* | −1.73 | | Hypothetical metabolite transport protein |
| JW1806 | *manX* | | −2.50 | Phosphotransferase system enzyme II (EC 2.7.1.69), mannose-specific, factor III |
| JW1807 | *manY* | | −2.63 | Phosphotransferase system enzyme II (EC 2.7.1.69), mannose-specific, factor II-P |
| JW1808 | *manZ* | | −2.40 | Phosphotransferase system, mannose-specific IID component (EIID-Man) |
| JW1889 | *araF* | −1.80 | | L-Arabinose-binding protein precursor |
| JW1893 | *ftn* | | −1.40 | Ferritin |
| JW2074 | (*gatR*) | <−3.59 | −1.92 | Galactitol utilization operon repressor |
| JW2076 | *gatC* | −4.25 | −2.88 | Phosphotransferase system, galactitol-specific IIC component (EIIC-GAT) |
| JW2077 | *gatB* | −2.97 | −2.79 | Phosphotransferase system enzyme II, galactitol specific, protein B |
| JW2083 | *gatY* | | −2.04 | Tagatose-bisphosphate aldolase (EC 4.1.2.—) |
| JW2135 | *mglC* | <−3.52 | −1.58 | Galactoside transport system permease protein |
| JW2136 | *mglA* | <−4.32 | <−3.77 | Galactoside transport ATP-binding protein |
| JW2137 | *mglB* | −3.71 | −4.02 | D-Galactose-binding protein precursor |
| JW2234 | *glpT* | −1.39 | | Glycerol-3-phosphate transport protein. |
| JW2341 | *fadL* | | −2.20 | Long-chain fatty acid transport protein precursor |
| JW2672 | *srlA* | −1.66 | | Phosphotransferase system, glucitol/sorbitol-specific IIBC component (EIIBC-Gut) |
| JW2760 | *gudT* | −1.36 | | Probable glucarate transporter |
| JW3101 | *agaZ* | | −1.88 | Putative tagatose 6-phosphate kinase AgaZ (EC 2.7.1.—) |
| JW3496 | *dctA* | −2.20 | −2.32 | DctA protein |
| JW3538 | *xylF* | <−3.90 | <−1.77 | D-Xylose-binding periplasmic protein precursor |
| JW3540 | *xylH* | −1.47 | | Xylose transport permease |
| JW3632 | *yicJ* | −1.10 | | Hypothetical permease protein |
| JW3687 | *tnaB* | GII (↓) | | Low-affinity tryptophan permease |
| JW3727 | *rbsD* | | −1.25 | High-affinity ribose transport protein RbsD |
| JW3728 | *rbsA* | | −1.32 | Ribose transport protein RbsA (ATPase) |
| JW3730 | *rbsB* | −2.03 | −2.16 | D-Ribose-binding periplasmic protein precursor |
| JW3898 | *glpF* | | −1.23 | Glycerol diffusion facilitator |
| JW3992 | *malG* | −1.96 | −1.65 | Maltose transport protein MalG |
| JW3993 | *malF* | −3.32 | −2.44 | Inner membrane protein MalF |
| JW3994 | *malE* | <−5.30 | −4.28 | Maltose-binding protein precursor |
| JW3995 | *malK* | −2.19 | −2.20 | Maltose/maltodextrin transport ATP-binding protein MalK |
| JW3996 | *lamB* | −4.79 | <−4.53 | Maltoporin precursor (lambda receptor protein) |
| JW3997 | *malM* | GII (↓) | GII (↓) | Maltose operon periplasmic protein precursor |

[a] For genes whose four measurements were all group II data and expression in glucose-limited growth was sufficiently high for all measurements, the log ratio was substituted by GII, with (↓) indicating significant upregulation under glucose limitation. The details of group II are described in Materials and Methods.

TABLE 6. Operons with differential expression under different nutrient limitations at both growth rates[a]

| Category | Identification | Operon | Differentially expressed genes of the operon |
|---|---|---|---|
| Operons significantly upregulated by ammonia limitation | | | |
| Amino acid metabolism and nitrogen regulation | JW0162 | *glnD* | *glnD** |
| | JW0440-1 | *glnK-amtB* | ***glnK*** |
| | JW0660 | *asnB* | *asnB** |
| | JW1733-7 | *astCADBE* | *astC*, *astA*, *astD*, *astB*, *astE* |
| | JW1967 | *nac* | ***nac*** |
| | JW3044 | *oat* | *oat* |
| | JW3839-41 | *glnA-ntrBC* | *glnA*, *ntrB*, *ntrC* |
| Transport of nitrogen sources | JW0440-1 | *glnK-amtB* | ***amtB*** |
| | JW1478-83 | *ddpXABCDE* | *yddP*, *yddQ*, *yddR*, *yddS*, *ddpX* |
| | JW2389 | *nupC* | *nupC* |
| | JW2636-9 | *gabDTPC* | *gabP* |
| | JW2850 | *ygfO* | *ygfO** |
| | JW3236-9 | *yhdWXYZ* | **yhdW** |
| | JW4072 | *proP* | *proP** |
| | JW4165-6 | *fklB-cycA* | *cycA* |
| Other functions | JW0327-8 | *codBA* | *codA* |
| | JW0991-7 | *ycdGHIJKLM* | *ycdG*, *228#6*, *228#7*, *228#9*, *228#10* |
| | JW1588 | *ynfM* | *ynfM** |
| | JW1653 | *cfa* | *cfa** |
| | JW1772-3 | *yeaGH* | *yeaG*, *yeaH* |
| | JW1917 | *yedL* | ***yedL*** |
| | JW1966 | *cbl* | ***cbl*** |
| | JW2198 | *mqo* | *mqo** |
| | JW2851 | *guaD* | *guaD** |
| | JW2891 | *yggB* | *yggB** |
| | JW3477-8 | *hdeAB* | *hdeB** |
| | JW3479 | *hdeD* | *hdeD** |
| Operons significantly upregulated by glucose limitation | | | |
| Transport or uptake of carbon sources | JW0665 | *nagE* | *nagE* |
| | JW2074 | *(gatR)* | *(gatR)* |
| | JW2075-80 | *gatYZABCD* | *gatB*, *gatC*, *gatD* |
| | JW2135-7 | *mglBAC* | ***mglB***, ***mglA***, *mglC* |
| | JW3496 | *dctA* | *dctA* |
| | JW3538 | *xylF* | *xylF* |
| | JW3730 | *rbsB* | *rbsB* |
| | JW3992-4 | *malEFG* | ***malE***, *malF*, *malG* |
| | JW3995-7 | *malK-lamB-malM* | *malK*, ***lamB***, ***malM*** |
| Carbon and energy metabolism | JW0711-4 | *sdhCDAB* | *sdhC*, *sdhD*, *sdhA*, *sdhB* |
| | JW0715-8 | *sucABCD* | *sucD* |
| | JW0987 | *agp* | *agp* |
| | JW1412 | *aldA* | *aldA* |
| | JW1604 | *fumA* | *fumA* |
| | JW1794 | *fadD* | *fadD* |
| | JW2338 | *yfcX* | *yfcX* |
| | JW2447 | *maeB* | *maeB* |
| | JW2812 | *yqeF* | *yqeF* |
| | JW3366 | *pckA* | ***pckA*** |
| | JW3686-7 | *tnaAB* | ***tnaA*** |
| | JW3821-2 | *fadBA* | *fadB*, *fadA* |
| | JW3897-8 | *glpFK* | *glpK* |
| | JW3934 | *udhA* | *udhA* |
| | JW3975 | *aceA* | *aceA* |
| Other functions | JW0590 | *cstA* | ***cstA*** |
| | JW0864 | *cspD* | *cspD* |
| | JW1060-8 | *flgBCDEFGHIJ* | *flgB* |
| | JW1291 | *ycjL* | *ycjL* |
| | JW1418 | *ydcI* | *ydcI* |
| | JW1493 | *301#2* | *301#2* |
| | JW1914 | *yedE* | *yedE* |
| | JW1921 | *fliE* | *fliE* |
| | JW1958 | *yeeI* | *yeeI* |
| | JW2419 | *ucpA* | *ucpA* |
| | JW2521 | *yfhT* | *yfhT* |
| | JW3542 | *bax* | *bax* |
| | JW4028 | *yjcG* | ***yjcG*** |
| | JW4030 | *acs* | ***acs*** |

[a] Genes with a more than 10-fold expression difference between slow- and fast-growing cells are shown in boldface. Genes with hypothetical functions are underlined. Genes that were significantly induced by ammonia limitation under both growth conditions but were not identified or considered to be nitrogen regulated (39) are indicated with an asterisk.

increase *cfa* transcription (6, 8), there was only a slight expression difference for the *rpoS* gene and no pH change between the two nutrient-limited chemostat cultures, which may indicate other possibilities that induce the expression of the *cfa* gene. Further studies are therefore required to show whether the *cfa* gene is nitrogen regulated or whether a nitrogen assimilation-related activator is involved in the transcription of this gene.

The *glnK* gene, which encodes signal transduction protein GlnK, was a strict group II gene in this study, which agreed well with the result of Atkinson and Ninfa (3) that no GlnK was found in nitrogen-replete cells and a high concentration of GlnK was found in nitrogen-starved cells. The product of *glnK* is a paralogue of the signal transduction protein $P_{II}$ (product of *glnB*) and is known to play a role distinct from that of $P_{II}$ protein in nitrogen regulation (3, 4). It is supposed that most $P_{II}$ has been uridylylated to $P_{II}$-UMP before the accumulation of GlnK, and the high expression of the *glnK* gene in ammonia-limited cells may provide additional GlnK-UMP to activate the deadenylylation of GS-AMP. Although the presence of GlnK may also activate the phosphorylase activity of NtrB and adenylylate GS, its effect in regulating adenylyltransferase and controlling the level of NtrC-P appeared less potent than that of $P_{II}$ protein. Further study and quantification of the relative contents of GlnK and GlnK-UMP would help a lot in understanding the role of this protein.

The *cbl* gene, lying downstream of the *nac* gene and encoding a CysB-like regulator, Cbl, for sulfur utilization, is probably expressed from the *nac* promoter under nitrogen limitation (39). Absolutely elevated transcript levels of the *cbl* gene (85-fold and 13-fold increases in slow- and fast-growing cells, respectively) may imply an important role of the Cbl protein in nitrogen metabolism in addition to its role in sulfate utilization (36).

Although more than 40% of the genes in *E. coli* have unknown functions, our results suggested that only about 1.1% of them (21 genes) were differentially expressed at both growth rates in response to nutrient-limited growth (Table 6). In ammonia-limited cells, most genes in the *ycdGHIJKLM* (JW0991-7) and *yeaGH* (JW1772-3) operons were expressed at higher transcript levels than under glucose limitation, while the most significant expression changes (more than 16-fold increase) could be observed for the *yedL* and *yhdW* genes in ammonia-limited cells and the *yjcG* gene in glucose-limited cells. Further experiments are required to clarify the functions of these genes in cell growth under nutrient limitation.

## DISCUSSION

**Genes characterizing nutrient-limited growth of *E. coli*.** Chemostat cultures provide microorganisms with steady-state growth under well-controlled conditions, making them suitable for the study of genome-wide transcriptional responses to environmental changes such as nutrient limitation for *S. cerevisiae* (5) or *E. coli* in this study. Regardless of the growth rate, about 1.0% of *E. coli* genes (42 genes) were significantly upregulated by ammonia limitation, half of which were involved in amino acid metabolism and transport systems for nitrogen-containing compounds. On the other hand, glucose deficiency in the medium induced another 50 genes (mainly involved in central carbon and energy metabolism and the transport or uptake of carbon sources) at high transcript levels.

Comparing transcriptional profiles between steady-state cells grown under different nutrient limitations allows the investigation of genes involved in the nitrogen-regulatory system. It is known that the remarkably decreased intracellular glutamine level under nitrogen limitation triggers the initiation of the $\sigma^{54}$-dependent promoter of the *glnA-ntrBC* operon, resulting in elevated transcription of the three genes of this operon. The nitrogen-regulatory protein of NtrC then activates the transcription of a number of $\sigma^{54}$-dependent genes, including the *nac* gene. The nitrogen assimilation control protein NAC, the product of the *nac* gene, can induce a number of $\sigma^{70}$-dependent genes, which, together with the NtrC-activated genes, encode proteins responsible for scavenging, transport, and utilization of many nitrogen-containing compounds (2, 20, 21).

Although growth rate affected markedly the relative expression of genes between the two nutrient conditions, our results suggest that 42 genes involved in 26 operons may be tightly related to the physiological adaptation to nitrogen limitation independent of the growth rate (Table 6). Fifteen of the 26 operons were also identified as being nitrogen regulated by Zimmer et al. when the mRNA levels in an *ntrB*-overexpressing mutant were compared to those in a strain with an *ntrC* null allele (39). These include four genes involved in nitrogen regulation (*ntrB*, *ntrC*, *nac*, and *glnK*); nine genes involved in the transport of ammonia (*amtB*), nucleosides (*nupC*), γ-aminobutyric acid (*gabP*), D-serine/D-alanine/glycine (*cycA*), D-alanyl-D-alanine dipeptide (*yddP*, *yddQ*, *yddR*, and *yddS*) and amino acid (*yhdW*, hypothetical); 10 genes of nitrogen-containing compound metabolism or other functions (five genes of the *astCADBE* operon, *oat, glnA*, *codA*, *cbl*, and *ddpX*); and another eight hypothetical genes (*yedL*, *yeaGH*, and five genes in the *ycdGHIJKLM* operon). Except for the putrescine transport genes of the *potFGHI* operon (significantly expressed in slow-growing cells and upregulated in fast-growing cells), differential expression was not observed in our experiments for nine other operons that were determined to be under nitrogen regulation (*gltIJKL*, *glnHPQ*, *ompF*, *chaBC*, *oppABCDF*, *ydcSTUV*, *hisJQMP*, *argT*, and *dppABCDF*) (39). On the other hand, 11 additional genes that are significantly induced by ammonia limitation were identified in our chemostat cultures (Table 6), suggesting that these genes may play important roles in the adaptation to nitrogen deficiency. Moreover, among 42 significantly upregulated genes, the transcript levels of 6 genes (*glnK*, *nac*, *amtB*, *yhdW*, *yedL*, and *cbl*) were increased by more than 10-fold, which may then be used to indicate ammonia limitation in the medium.

Limitation of carbon source such as glucose obviously induced another group of genes (Table 6). The first noteworthy result is the ability of *E. coli* to scavenge carbon from glucose-deficient medium through elevating the expression of a number of transport genes, among which all genes of the *malEFG*, *malK-lamB-malM*, and *mglBAC* operons were expressed at significantly high levels. Since nitrogen limitation has less influence on the expression of these genes in glucose-replete cultures (12), our transcription results indicate the significant induction of these genes by glucose limitation, which is also consistent with the results with *lacZ* fusions (12) and two-

TABLE 7. Transcriptional responses to growth rate under different nutrient limitations[a]

| Category | Identification | Gene | Logarithmic expression ratio | | Difference in expression ratio [$\log_2(k_C) - \log_2(k_N)$] |
|---|---|---|---|---|---|
| | | | Glucose-limited growth [$\log_2(k_C)$] | Ammonia-limited growth [$\log_2(k_N)$] | |
| $k_C \approx k_N$ (similar transcriptional responses to growth rate) | JW3366 | *pckA* | −0.13 | 0.00 | −0.13 |
| | JW0713 | *sdhA* | 0.89 | 1.02 | −0.13 |
| | JW1480 | *yddQ* | −1.23 | −1.21 | −0.02 |
| | JW0712 | *sdhD* | 1.29 | 1.31 | −0.02 |
| | JW2850 | *ygfO* | −1.14 | −1.17 | 0.03 |
| | JW3730 | *rbsB* | −0.07 | −0.12 | 0.05 |
| | JW0752 | *ybhH* | −0.03 | −0.08 | 0.06 |
| | JW2521 | *yfhT* | 0.12 | 0.04 | 0.08 |
| | JW3996 | *lamB* | 1.70 | 1.57 | 0.14 |
| | JW3839 | *ntrC* | 0.12 | −0.09 | 0.21 |
| | | Others (*bax*, *crcB*, *ddpX*, *fadB*, *fadD*, *fumA*, *maeB*, *malF*, *malK*, *mqo*, *nagE*, *ntrB*, *pyrG*, *sdhB*, *slp*, *sucD*, *ybiK*, *ycjK*, *ydcI*, *yddS*, *yfbU*, *yfcX*, *yfcY*, *yieE*, *yqeF*) | | | |
| $k_C > 5\ k_N$ (higher expression ratio in glucose-limited growth) | JW0626 | *ybeD* | 1.37 | −1.55 | 2.93 |
| | JW2156 | *fruB* | 0.31 | −2.67 | 2.98 |
| | JW3516 | *yhjX* | −1.39 | −4.61 | 3.22 |
| | JW3480 | *yhiE* | −0.17 | −3.42 | 3.25 |
| | JW1966 | *cbl* | 0.87 | −2.38 | 3.25 |
| | JW0791 | *ybiM* | −1.16 | <−4.46[b] | >3.30 |
| | JW1256 | *trpE* | 2.00 | −1.70 | 3.70 |
| | JW1487 | *xasA* | −0.97 | −5.24 | 4.28 |
| | JW3478 | *hdeA* | 1.53 | −4.08 | 5.61 |
| | JW3477 | *hdeB* | 1.90 | −4.35 | 6.25 |
| | | Others (*adhE*, *argC*, *asd*, *hha*, *lysC*, *nadA*, *priB*, *rplI*, *sfsA*, *trpC*, *ugd*, *yafK*, *yaiB*, *ydfG*, *yeaH*, *yeeX*, *yhdN*, *ylcB*, *yrbA*) | | | |
| $k_C < 0.2\ k_N$ (higher expression ratio in ammonia-limited growth) | JW1061 | *flgC* | 0.72 | >7.17 | <−6.45 |
| | JW1065 | *flgG* | 0.55 | 6.42 | −5.87 |
| | JW1063 | *flgE* | −0.02 | 5.68 | −5.70 |
| | JW1064 | *flgF* | 0.44 | >5.75 | <−5.31 |
| | JW1066 | *flgH* | 0.18 | >5.09 | <−4.91 |
| | JW1906 | *fliZ* | 0.61 | 5.26 | −4.66 |
| | JW1062 | *flgD* | 0.34 | 4.92 | −4.59 |
| | JW1908 | *fliC* | 0.75 | 4.83 | −4.08 |
| | JW1909 | *fliD* | 1.19 | >5.18 | <−3.99 |
| | JW1924 | *fliH* | −0.41 | 3.54 | −3.95 |
| | | Others (*aldB*, *aroF*, *flaG*, *flgB*, *flgJ*, *flgK*, *flgL*, *flgM*, *flgN*, *flhA*, *flhB*, *fliA*, *fliF*, *fliG*, *fliI*, *fliL*, *fliM*, *fliN*, *fliO*, *fliS*, *fliT*, *gatD*, *tar*, *yecR*, *yhfT*, *yjcZ*, *ypjA*) | | | |

[a] Genes differentially expressed in slow-growing cells ($D = 0.10\ h^{-1}$) were investigated. Only those affected by growth rate with similar or a more than fivefold difference in expression ratio between the two nutrient-limited cultures are listed. Ten typical genes in each group are listed with expression data. Only gene names are given for other genes.

[b] When both group I and group II data existed for a gene, the expression level was described by more than (>) or less than (<) the mean of the ratio evaluated from group I data only. The details for group I and group II are described in Materials and Methods.

dimensional gel electrophoresis (37). The significance of highly expressed maltose regulon (*malEFG* and *malK-lamB-malM*) and galactose transport operon (*mglBCA*) genes to glucose-limited growth has also been demonstrated by Ferenci's group (10, 12–14, 19). It was found that the LamB porin (product of the *lamB* gene, which was induced by more than 20-fold) has glucose affinity, through which most glucose may diffuse across the outer membrane rather than through other porins such as OmpF and OmpC when *E. coli* grows on extremely low glucose. On the other hand, the ABC transporter of galactose (Mgl), which also has high affinity for glucose, showed more than a 10-fold increase in transcription under glucose limitation and is likely to play an important role in transporting periplasmic glucose into the cytoplasm in addition to the glucose-specific phosphotransferase system. Investigations suggested that high cAMP levels and elevated endogenous inducers of maltotriose and galactose are possible regulators that induce the high expression of the *mgl*/*lamB* high-affinity system in an environment of extremely low glucose concentration (12, 13). An upregulation in expression of two genes required for endogenous synthesis of galactose, *galE* and *galU* (10), was also confirmed by our transcriptional analysis. Similarly, a >10-fold increase in transcription of genes in glucose-deprived cells can be used as indicators for this nutrient-limited condition. These genes include five genes involved in carbon transport systems (*mglB*, *mglA*, *malE*, *lamB*, and *malM*) and five other genes involved in central carbon metabolism (*pckA* and *tnaA*) and other functions (*cstA*, *yjcG*, and *acs*). For *S. cerevisiae*, however, glucose-limited growth may be characterized by the transcription of many more genes (5).

**Effects of growth rate on gene expression in nutrient-limited growth.** Comparison of gene transcription indicates that nearly

10% of the total genes were differentially expressed in slow-growing cells in response to the change of the growth-limiting nutrient. The number of differentially expressed genes, however, decreased markedly when chemostat cultures were carried out at the high growth rate (Table 2). To investigate the influence of growth rate on gene expression, additional DNA microarray experiments were performed to compare transcriptional profiles between slow- and fast-growing cells grown under nutrient limitation, i.e., glucose-limited growth at $D = 0.55$ $h^{-1}$ (C0.5) versus that at $D = 0.10$ $h^{-1}$ (C0.1) and ammonia-limited growth at $D = 0.55$ $h^{-1}$ (N0.5) versus that at $D = 0.10$ $h^{-1}$ (N0.1). Similarly, for each growth rate, two independent steady-state RNA samples were prepared and DNA microarrays were duplicated for each hybridization, yielding four sets of data for each experiment.

Only genes that were differentially expressed at the low growth rate were investigated in this study. Based on the transcriptional responses to growth rate, these genes were roughly divided into two groups. For group A genes, the transcriptional response to the change in growth rate was similar under two nutrient limitations, i.e., $k_C \approx k_N$, where $k_C$ and $k_N$ indicate the average ratio of transcription in fast-growing cells to that in slow-growing cells grown under glucose or ammonia limitation, respectively. Therefore, for most group A genes, differential expression between the two nutrient-limited conditions could be maintained even if the growth rate varied markedly. Group B includes genes for which $k_C$ is very different from $k_N$, suggesting significant differences in transcriptional responses to the growth rate between the two nutrient-limited cultures. Group B genes can be further divided into two subgroups, i.e., genes whose expression ratios (logarithmic ratios) in glucose-limited growth were much higher than those in ammonia-limited growth ($k_C \gg k_N$) and vice versa ($k_C \ll k_N$).

Among 400 genes that were differentially expressed in slow-growing cells with either ammonia limitation or glucose limitation, the effects of growth rate on the transcription of 35 genes may be independent of the growth-limiting nutrient if the maximum relative difference permitted between $k_C$ and $k_N$ was 25% (Table 7). Moreover, the growth rate did not significantly affect the expression of most group A genes, particularly genes such as *pckA*, *yfhT*, *rbsB*, and *ybhH* ($k_C \approx k_N \approx 1$). For most of the genes considered, transcriptional responses to growth rate were also affected by the growth-limiting nutrient in the medium. A >5-fold difference in transcriptional response was observed for about one-sixth of the genes investigated when the growth-limiting nutrient was changed. It was also observed that the expression of most genes was much more sensitive to the growth rate in ammonia-limited cells than in glucose-limited cells.

For genes highly induced by ammonia limitation in slow-growing cells, high positive differences in transcriptional responses to the growth rate between the two nutrient-limited growth conditions $[\log_2(k_C) - \log_2(k_N)]$ generally resulted in the decreased induction or even repression by ammonia limitation in fast-growing cells, such as the *xasA* and *trpE* genes and genes around the *hdeAB* (histone-like protein-determined expression) operon (*hdeB*, *hdeA*, and *yhiE*) (Table 7). On the other hand, for genes highly repressed by ammonia limitation in slow-growing cells, a high negative value for $[\log_2(k_C) - \log_2(k_N)]$ generally resulted in decreased repression by ammonia limitation in fast-growing cells. Most genes in this group were flagellar genes (Table 7), which agrees well with the results that most flagellar genes that were significantly downregulated under ammonia limitation at the low growth rate were no longer differentially expressed with the increase in the growth rate (data not shown). It can then be concluded that *E. coli* is capable of regulating gene transcription in a wide variety of ways in response to a change in growth rate and growth-limiting nutrient.

Our results also indicate that an increase in the growth rate may reduce the transcriptional difference between the two nutrient-limited growth conditions. Indeed, although 74 new genes were differentially expressed at the high growth rate, 308 genes were no longer differentially expressed in fast-growing cells (Table 2), which also suggests that the effects of growth rate on gene expression in *E. coli* may be much greater than the effects of nutrient-limited growth.

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