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Functional Genomics: Expression Analysis of *Escherichia coli* Growing on Minimal and Rich Media

HAN TAO,[1] CHRISTOPH BAUSCH,[1] CRAIG RICHMOND,[2] FREDERICK R. BLATTNER,[2] AND TYRRELL CONWAY[1]*

Department of Microbiology, The Ohio State University, Columbus, Ohio 43210-1292,[1] and Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706[2]

Received 1 June 1999/Accepted 6 August 1999

DNA arrays of the entire set of *Escherichia coli* genes were used to measure the genomic expression patterns of cells growing in late logarithmic phase on minimal glucose medium and on Luria broth containing glucose. Ratios of the transcript levels for all 4,290 *E. coli* protein-encoding genes (cds) were obtained, and analysis of the expression ratio data indicated that the physiological state of the cells under the two growth conditions could be ascertained. The cells in the rich medium grew faster, and expression of the majority of the translation apparatus genes was significantly elevated under this growth condition, consistent with known patterns of growth rate-dependent regulation and increased rate of protein synthesis in rapidly growing cells. The cells grown on minimal medium showed significantly elevated expression of many genes involved in biosynthesis of building blocks, most notably the amino acid biosynthetic pathways. Nearly half of the known RpoS-dependent genes were expressed at significantly higher levels in minimal medium than in rich medium, and *rpoS* expression was similarly elevated. The role of RpoS regulation in these logarithmic phase cells was suggested by the functions of the RpoS dependent genes that were induced. The hallmark features of *E. coli* cells growing on glucose minimal medium appeared to be the formation and excretion of acetate, metabolism of the acetate, and protection of the cells from acid stress. A hypothesis invoking RpoS and UspA (universal stress protein, also significantly elevated in minimal glucose medium) as playing a role in coordinating these various aspects and consequences of glucose and acetate metabolism was generated. This experiment demonstrates that genomic expression assays can be applied in a meaningful way to the study of whole-bacterial-cell physiology for the generation of hypotheses and as a guide for more detailed studies of particular genes of interest.

The field of microbial physiology was launched in 1958 with the fundamental discovery that the macromolecule composition of the bacterial cell changes with the growth rate (58). Faster-growing cells contain proportionally more stable RNAs—rRNA and tRNA. The reason for this increased abundance of stable RNA is simple: in order to grow faster, bacteria must synthesize protein faster. The growth rate of the bacterial cell increases in proportion to the quality of the growth medium (although not necessarily in proportion to its exact composition), and this increase in growth rate is accomplished by an increase in the number of ribosomes and the concentrations of translation accessory factors (8). It is now understood that the seven *Escherichia coli* rRNA operons are under the control of growth rate-dependent promoters and that expression of the ribosomal proteins, translation factors, and the transcription apparatus are all tied to the cellular concentration of rRNA (8, 27, 35). The rate of transcription initiation of the growth rate-dependent *rrn* promoters is physiologically connected to the metabolic state of the cell by the concentration of nucleoside triphosphates—efficient transcription initiation from these promoters requires a high concentration of the initiating nucleotide (22). The presence of high-quality nutrients in the growth medium results in high intracellular nucleoside triphosphate concentrations; hence, this model unifies the idea that the quality of the growth medium dictates the growth rate of the cell.

Growth rate-dependent changes in cell composition are realized at the level of gene expression; for example, transcript levels corresponding to the protein components of the protein synthesis apparatus change in proportion to the growth rate as the rates of transcription or mRNA turnover are modulated (27, 35). Other changes in cellular physiology can be more subtle, such as redirection of intermediary metabolism in response to changes in growth medium composition or the flow of carbon and electrons that is coupled to ATP generation, although many of these adjustments in metabolism are accompanied by changes in the concentrations of metabolic enzymes and electron transport chain components (40, 41, 56, 63, 64). The expression of numerous other genes is affected by environmental stresses (9, 17, 26, 29, 48, 60, 69, 71). Almost all aspects of microbial physiology, including the myriad adjustments made by the cell in response to changes in the environment, have been cataloged by the scientific community in the form of the book *Escherichia coli and Salmonella: Cellular and Molecular Biology*. Since the publication of this compendium, the sequence of the *E. coli* genome has been completed and the way that we look at gene expression is forever changed (6). The genome sequence provides the tools necessary to take a global view of *E. coli* physiology.

Genomic expression assays provide an unprecedented ability not only to look at a single aspect of physiology but also to see how a particular gene, regulon, or modulon interacts with every other aspect of physiology. Genomewide methods have been developed for a number of uses, including drug discovery (43), measurement of gene copy number (50), discovery of disease-related genes in humans (18, 28), gene mapping (12), and gene expression: in humans (73), in yeast (13, 19, 31, 37, 65), and in *Arabadopsis* (59).

From the *E. coli* MG1655 genome sequence (6), 4,290 open

* Corresponding author. Present address: Department of Botany and Microbiology, 770 VanVleet Oval, University of Oklahoma, Norman, OK 73019-0245. Phone: (405) 325-1683. Fax: (405) 325-7619. E-mail: tconway@ou.edu.

reading frame (ORF)-specific primer pairs were designed for PCR amplification of all *E. coli* ORFs, and this set of 4,290 PCR-amplified, ORF-specific DNA fragments was used to develop DNA arrays for gene expression profiling (54). A similar set of ORF-specific DNA fragments was used to generate commercially available DNA macroarrays (12 by 24 cm) on nylon membranes (Sigma-GenoSys Biotechnologies, Inc., Woodland, Tex.). The advantage of the commercial arrays is that they can be used with equipment found in typical molecular biology laboratories. For these utilitarian investigations of bacterial physiology to be successful, it will be necessary to determine if DNA macroarrays can reveal differences in gene expression across the genome. Here we report on the expression profiles of *E. coli* under two very different growth conditions, and from the data we provide insights into growth rate-dependent gene expression, global regulation of biosynthetic regulons, and stress responses that appear to be involved in growth on minimal glucose medium.

MATERIALS AND METHODS

Growth conditions. *E. coli* MG1655 cultures were grown in 50-ml batch cultures in 250-ml Erlenmeyer flasks at 37°C with aeration by gyrotary shaking (300 rpm). The culture media used were M63 minimal medium (57) containing 0.2% glucose and a rich medium, Luria broth (39) containing 0.2% glucose. Growth was monitored spectrophotometrically at 600 nm on a Spectronic 601 (Milton Roy). Cells were harvested in late logarithmic growth phase (absorbance at 600 nm = 0.6) from cultures that had been inoculated at low density and had maintained a constant growth rate for at least 10 generations.

Handling of RNA. The ability to isolate pure, intact mRNA is critical to the success of genomic expression assays. Cells in growing cultures were pipetted directly into boiling lysis buffer. The lysed cells were extracted twice with phenol (pH 5.0) at 60°C and then with phenol-chloroform (66). The RNA was precipitated with isopropanol, redissolved in water, treated with DNase I, and applied to an RNeasy column. The purified RNA was redissolved in water and stored at −70°C in 2 volumes of ethanol.

Probe synthesis. Hybridization probes were generated by standard cDNA synthesis. The protocol supplied by the manufacturer of the DNA arrays was suitable for achieving >70% incorporation of the ^{33}P-labeled nucleotide. Since it is not possible to purify bacterial mRNA from total RNA (i.e., by purification of polyadenylated mRNA as in eukaryotes), the labeling protocol takes into account the presence of rRNA and tRNA, which constitute 85% of the total RNA. The C-terminal primer set (4,290 ORF-specific C-terminal primers [Sigma-GenoSys Biotechnologies, Inc.]) was used to generate the hybridization probe in a standard first-strand cDNA synthesis. Briefly, 1 μg of RNA was mixed with dATP, dGTP, and dTTP (final concentrations, 0.33 mM each), and cDNA-labeling primers (Sigma-GenoSys), in a volume of 25 μl of first-strand buffer, heated to 90°C for 2 min and cooled to 42°C in 20 min. Then 200 U of Superscript II, 10 U of RNase inhibitor, and 20 μCi of [α-^{32}P]dCTP (2,000 to 3,000 Ci/mmol) were added, bringing the total volume to 30 μl, and the cDNA synthesis reaction mixture was incubated at 42°C for 2 h. Unincorporated nucleotides were removed by gel filtration through a G-50 Sephadex column (57).

Hybridization. The DNA arrays (Panorama *E. coli* gene arrays) used in the hybridization experiments were produced by Sigma-GenoSys Biotechnologies, Inc. Each DNA array consists of a 12- by 24-cm positively charged nylon membrane on which 10 ng each of all 4,290 PCR-amplified ORF-specific DNA fragments are robotically printed in duplicate. The hybridization and washing steps were carried out as described by the manufacturer. Briefly, the blots were prehybridized in hybridization solution (5× SSPE [1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA, pH 7.7], 2% sodium dodecyl sulfate [SDS], 1× Denhardt's reagent, 100 μg of sheared salmon sperm DNA per ml) at 65°C for 1 h in a 30- by 3.5-cm roller bottle in a hybridization oven. The entire cDNA probe, generated as described above, was added to 3 ml of hybridization buffer, and the blot was hybridized with this solution for 15 h at 65°C. The blots were washed with buffer (0.5× SSPE, 0.2% SDS) three times for 5 min each at room temperature and three times for 30 min each at 65°C. The blots were then wrapped in clear plastic food wrap and exposed to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, Calif.) for 48 h. For each of the data sets used in this study, the same blot was consecutively hybridized, stripped, and rehybridized (this can be done up to four times). The blots were stripped at 100°C with 1% SDS in Tris-EDTA buffer as specified by the manufacturer.

Data analysis. The exposed PhosphorImager screens were scanned with a pixel size of 100 μm (10,000 dots/cm^2) on a STORM 840 PhosphorImager (Molecular Dynamics). The resulting TIFF image files were analyzed by determining the pixel density (intensity) for each spot in the array by using ImageQuant (version 5.0) software (Molecular Dynamics). A grid of individual ellipses corresponding to each of the DNA spots on the blots was laid down on the image to designate each spot to be quantified. Background was subtracted automatically by the software by using the local median background subtraction method. The intensities for each spot were exported from ImageQuant into a Microsoft Excel spreadsheet. Each ORF-specific spot was present in duplicate, and the intensities were averaged for analysis. Each averaged spot intensity was expressed as a percentage of the total of intensities of all the spots on the DNA array, which allowed direct comparison of the two conditions by normalizing with regard to the specific activity of the probes used. The correlation coefficients of the percent intensities determined individually for the duplicate spots on a single blot ranged from 0.986 to 0.999, and the standard deviations for the log ratios of intensities of the duplicate spots (determined as described below) ranged from 0.073 to 0.095 for four different hybridizations, thus providing a measure of reproducibility.

Two growth conditions were compared by determining the ratio of the corresponding averaged percent intensities of each pair of ORF-specific spots on the two blots. These ratios represent the relative transcript levels of each *E. coli* ORF under the two growth conditions. Ratios were calculated such that the log of the absolute value of the expression ratio was positive for percent intensities that were higher under the first condition and negative for percent intensities that were higher under the second condition. Also taken into account in the calculation were situations where the percent intensities for both conditions fell below a threshold value equal to the background, that is, when the gene was not expressed at detectable levels under either condition; in this case, the calculated log expression ratio was zero. A threshold value, equal to the background, was used to calculate ratios where a gene was not expressed at detectable levels under one of the growth conditions. A statistical analysis of the log expression ratios of all 4,290 genes in the minimal glucose versus gluconate experiment indicated a standard deviation from the mean (0.000) of 0.180. There is 95.5% confidence that any expression ratio is significant if the value of the log expression ratio is greater than 2 standard deviations (0.360) from the mean. Thus, a log expression ratio of 0.400 (2.5-fold) was considered to indicate significantly higher expression (99% confidence of each tail) in the analyses, and this value is shown graphically in Fig. 3 to 6. The experiment presented here, comparing the expression profile of cells grown on minimal versus rich medium, was repeated, and qualitatively similar data were obtained (data not shown). The blot-to-blot reproducibility of DNA macroarray hybridization data has been addressed in detail elsewhere (54).

Functional groups. Two schemes for functional grouping of genes have been applied to the expression data generated in these experiments. The first scheme assigns genes to groups in accordance with their cellular function, as described previously (6). The second scheme of functional assignments is that of Riley (55), version M54, submitted by Plunkett et al. (19a), as it appears on the *E. coli* K-12 MG1655 complete genome at the National Center for Biotechnology Information (43a).

Internet access to data. An Internet accessible version of the expression data and details of the protocols has been created (49a). The data can also be accessed from a database (19a).

Chemicals. SuperScript II, an RNase H$^-$ reverse transcriptase used for cDNA synthesis, was purchased from Gibco BRL (Bethesda, Md.). RNase inhibitor and DNase I were also purchased from Gibco BRL. PCR grade deoxyribonucleoside triphosphates were purchased from Roche Molecular Biochemicals (Indianapolis, Ind.). RNeasy columns were purchased from Qiagen, Inc. (Valencia, Calif.). [α-^{33}P]dCTP (2,000 to 3,000 Ci/mmol) was purchased from New England Nuclear (Wilmington, Del.). Biochemicals were purchased from Sigma (St. Louis, Mo.).

RESULTS AND DISCUSSION

The genomic expression profiles of *E. coli* MG1655 growing on rich and on minimal culture media (Fig. 1) were determined. The rich medium (Luria broth) contained amino acids as the nitrogen source, a number of other preformed building blocks of macromolecule synthesis (e.g., nucleosides and vitamins, etc., provided by tryptone and yeast extract), and also glucose as a carbon and energy source. The minimal medium contained glucose as the sole carbon and energy source and ammonia as the nitrogen source. In glucose minimal medium, the carbon backbone of the glucose molecule was rearranged through the biosynthetic pathways to generate each of the building blocks de novo. In addition to having fundamentally different metabolisms, the two cultures grew at significantly different rates: G = 25 min on the rich medium and G = 57 min on minimal glucose medium. As a control, data are provided for a culture growing on minimal gluconate medium (G = 60 min).

Whole-genome perspective. RNA isolated from the cultures in Fig. 1 were used to generate the probes used for hybridization of the DNA arrays shown in Fig. 2, and the data were

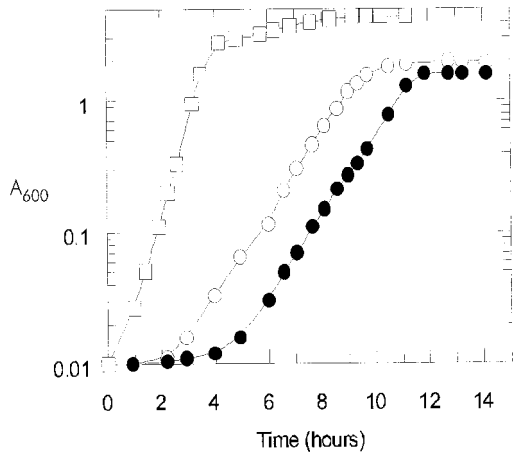

FIG. 1. Growth of *E. coli* MG1655 on Luria broth plus glucose (open squares), minimal glucose medium (open circles), and minimal gluconate medium (solid circles). Cells were harvested for genomic expression analysis at an absorbance at 600 nm (A_{600}) of 0.6.

quantified as described in Materials and Methods. Calculation of the log expression ratios of corresponding spots allowed pairwise comparisons of the relative transcript levels for each of the 4,290 *E. coli* protein-encoding genes under the different growth conditions. The log expression ratios indicate whether gene expression is higher under one condition or the other or remains unchanged. The results are summarized in Table 1 and presented in chart form in Fig. 3 to 6. It is important to keep in mind that in vivo transcript levels are dynamically balanced by the rates of transcription initiation and transcript turnover. Thus, the data presented here as expression ratios reflect the relative transcript levels for individual genes without providing any indication of the mechanism of regulation. Furthermore, some individual expression ratios may be in error, due to technical problems, including cross-hybridization, PCR failures, misapplied DNA spots on the arrays, or scatter in the data (see reference 54 for a more comprehensive review of the technical aspects of using *E. coli* DNA arrays). A few of the ratios are in conflict with published results, and it is possible that other ratios will not be validated in subsequent experiments. Thus, these data should not be taken as specific evidence for gene regulation and should be independently verified. Nevertheless, the general trends of the data are substantially clear and will be of value for generating experimental leads.

Expression levels of the majority of genes did not differ significantly (log ratio ≥ 0.4) between growth conditions. This was particularly true for the comparison of the cultures grown on minimal glucose versus minimal gluconate media; 80 genes (1.9%) were expressed at significantly higher levels on glucose, and 82 genes were expressed at significantly higher levels on gluconate (Table 1; Fig. 3). Thus, the overall similarity of these two growth conditions, being identical in basal medium composition, aeration, pH, and temperature and differing only in the nature of the carbon source, was reflected in their gene expression profiles. The comparison of genomic expression patterns of cells grown on minimal versus rich media was more revealing: 225 genes (5.2%) were expressed at significantly higher levels on minimal glucose, and 119 genes (2.8%) were expressed at significantly higher levels on rich medium (Fig. 3). A larger number of genes (3,496 versus 3,284 genes) had expression intensities above the background value on minimal glucose compared to rich medium (data not shown). By these measures, the cells growing on glucose minimal medium expressed more genes than did cells growing on rich medium. The nature of these differences in global gene expression was examined in detail, as described below.

Translation apparatus. The culture containing rich medium plus glucose grew more than twice as fast as did the cultures on minimal media (Fig. 1). It is known that faster-growing cells synthesize protein faster and contain more ribosomes (27, 35). There are 128 known genes encoding the enzymes, factors, and structural components that make up the translation apparatus. Of these 128 genes of the translation apparatus, 53 (41.4%) were expressed at significantly higher levels in the cells growing on rich medium and none of them were expressed at significantly higher levels on the minimal medium. Of the 53 translation genes that were expressed at higher levels on rich medium, 42 encoded ribosomal proteins. These data are charted in Fig. 4 and can be compared to the data for the cultures on minimal glucose versus gluconate medium, which had nearly identical growth rates and showed very few significant differences in expression of the translation genes. A comparison of the general pattern of expression of the translation genes (Fig. 4) to that of the entire *E. coli* gene set (Fig. 3) further illustrates the dramatic increase in production of the translation apparatus in the faster-growing cells.

(i) tRNA synthetase genes. There are 37 known genes encoding the tRNA synthetases and other enzymes involved in tRNA modification. While none of the expression ratios of the tRNA synthetase genes varied significantly, it is clear from the chart in Fig. 4 that the transcript levels for these genes followed the same general trend as the complete set of translation genes. This result is consistent with the notion that synthesis of the tRNA synthetases is coupled to the synthesis of other ribosomal components (27).

(ii) Translation factors. There are 17 known genes that encode factors involved in translation and ribosome modification, including the initiation and elongation factors, and 7 of these genes were expressed at significantly higher levels on rich medium (Fig. 4; Table 2). This result is generally consistent with the coupled synthesis of translation factors and ribosome components (27). The expression ratio of *infB* was significantly higher on rich medium. The regulation of *infB*, which is downstream of and cotranscribed with the transcription factor gene *nusA*, is complex and is thought to be the result of autoregulation of the extent of readthrough at upstream terminators by NusA (27). The expression ratio of *infB* was 1.8-fold higher than that of *nusA* (data not shown). The expression ratios of the translation elongation factor genes *tsf*, *tufB*, *tufA*, and *fusA* were all significantly higher, in that order, on rich medium, which is consistent with their coordinate regulation with the ribosomal protein genes (27). The growth rate-dependent regulation of *tsf*, *tufA*, and *fusA*, all of which are located in ribosomal protein operons, is the result of mRNA destabilization in slowly growing cells (27). Interestingly, regulation of *tufB* appears to be at least partially dependent upon Fis (68), and the *fis* gene had one of the highest expression ratios on rich medium, as described in more detail below. A fifth elongation factor encoded by *efp* has been shown to be essential in *E. coli* for protein synthesis and viability, although the details of *efp* regulation have not been published (2). The results of this study indicate that *efp* was expressed at a significantly higher level (log ratio = −0.425) in the faster-growing cells on rich medium, paralleling the expression of the other elongation factors.

(iii) Ribosomal proteins. Of the 55 genes encoding the ribosomal proteins, 42 were expressed at significantly higher

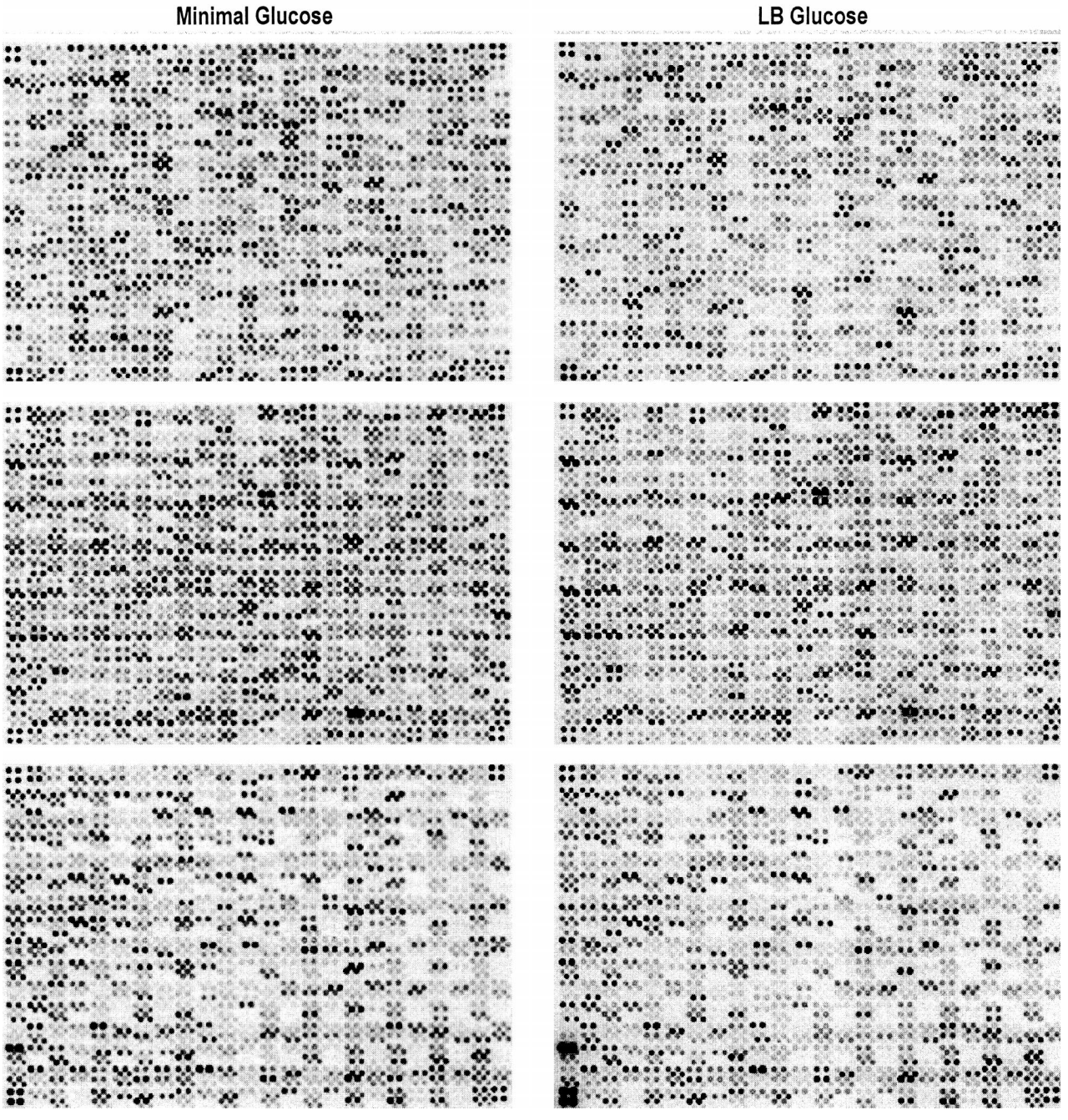

FIG. 2. DNA arrays of the entire set of *E. coli* genes hybridized with probes generated from RNA extracted from cells growing in late logarithmic phase on minimal glucose medium (left) and on Luria broth (LB) containing glucose (right).

levels in the more rapidly growing cells in rich medium (Fig. 4; Table 2). This result is consistent with the paradigm of growth rate-dependent regulation of ribosome number (35). Although the ribosomal S10 operon is at least partially regulated at the transcriptional level, it is generally accepted that regulation of the 21 ribosomal protein operons is not at the level of transcription initiation (23, 35). Rather, the regulation of ribosomal protein synthesis involves a combination of translational control and transcriptional control at the level of mRNA stability. In general, growth conditions which lead to a decreased rate of ribosome synthesis result in an excess of ribosomal proteins, with certain ones serving as autoregulators by binding to their transcript and decreasing the translation rate of the mRNA, thus leading to destabilization of the transcript (35). While not all of the ribosomal protein operons have been studied at this level of detail, the experiment presented here indicates that most of the operons are regulated in such a way that their transcript levels are higher in faster growing cells. Clearly, these data demonstrate that any regulatory mechanism that contributes to the dynamic control of a particular mRNA concentration, whether it be the rate of transcription or the rate of turnover, can be visualized in genomic expression

TABLE 1. Expression ratios of functional groups

Functional group	No. of genes[a]				
	Total	Minimal glucose vs Luria broth plus glucose		Minimal glucose vs minimal gluconate	
		Higher on minimal	Higher on LB	Higher on glucose	Higher on gluconate
Whole genome	4,290	225	119	80	82
Amino acid biosynthesis	97	22	0	3	0
Biosynthesis of cofactors, prosthetic groups, and carriers	106	9	1	0	2
Carbon compound catabolism	124	3	0	1	2
Cell processes	170	19	2	5	1
Cell structure	85	2	0	8	0
Central intermediary metabolism	149	15	1	4	5
DNA replication, repair, restriction/modification	105	1	0	1	1
Energy metabolism	136	14	5	4	3
Fatty acid and phospholipid metabolism	41	2	7	0	0
Hypothetical, unclassified, unknown	1,428	43	26	10	30
Nucleotide biosynthesis and metabolism	66	6	5	0	3
Phage, transposon, or plasmid	91	5	1	0	9
Putative cell structure	43	1	0	2	0
Putative enzymes	453	12	8	7	4
Putative factors	67	3	0	3	0
Putative membrane proteins	54	4	0	1	0
Putative regulatory proteins	167	11	0	4	1
Putative transport proteins	291	14	3	2	8
Regulatory function	208	14	3	6	3
Transcription, RNA processing, and degradation	28	0	1	0	1
Translation and posttranslational modification	128	0	53	6	1
Transport and binding proteins	254	24	2	13	8

[a] Number of genes showing significant (99% confidence) log expression ratios ($\geq \pm 0.400$).

assays. The global regulation and coordination of ribosome number and components of the translation apparatus was the most obvious result of this experiment.

Nitrogen metabolism. The minimal medium used in this study contained ammonia as the nitrogen source and the rich medium contained amino acids as the nitrogen source. In general, cells growing on minimal medium are limited for amino acids while cells growing on rich medium are limited for nucleotides (47, 52, 76). These differences were reflected in the transcript levels of the genes involved in nitrogen assimilation

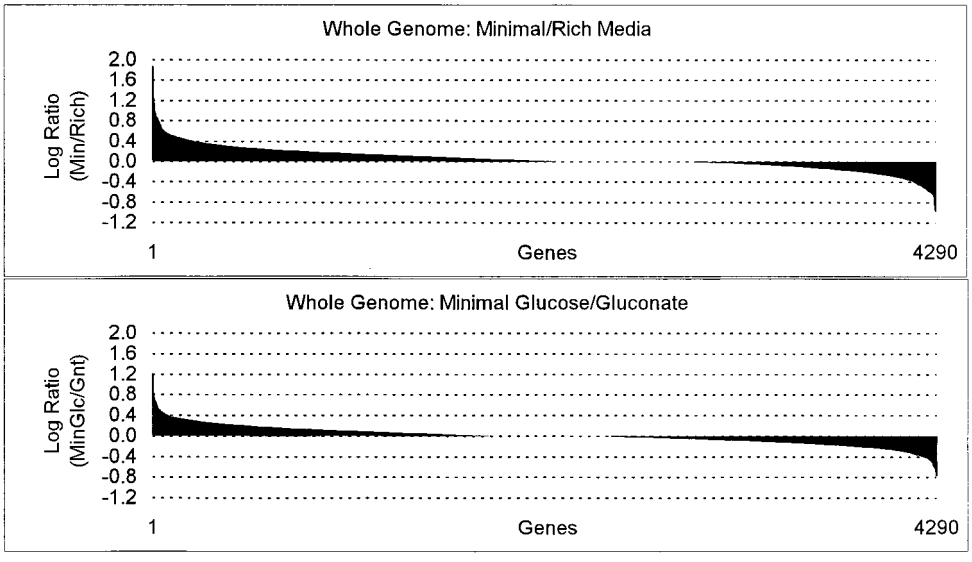

FIG. 3. The log expression ratios of all *E. coli* genes were plotted for minimal glucose versus Luria broth plus glucose (top) and for minimal glucose versus minimal gluconate (bottom). The entire data sets were sorted in Excel spreadsheets by the log expression ratio values, and a bar chart was generated by the software, with individual genes plotted on the *x* axis and the log expression ratios plotted on the *y* axis. Genes more highly expressed under the first condition are positive, and genes more highly expressed under the second condition are negative. The horizontal divisions (dashed lines) represent 99% confidence levels, such that any gene with a value extending beyond the first horizontal division in either direction is significantly expressed at a higher level under that condition.

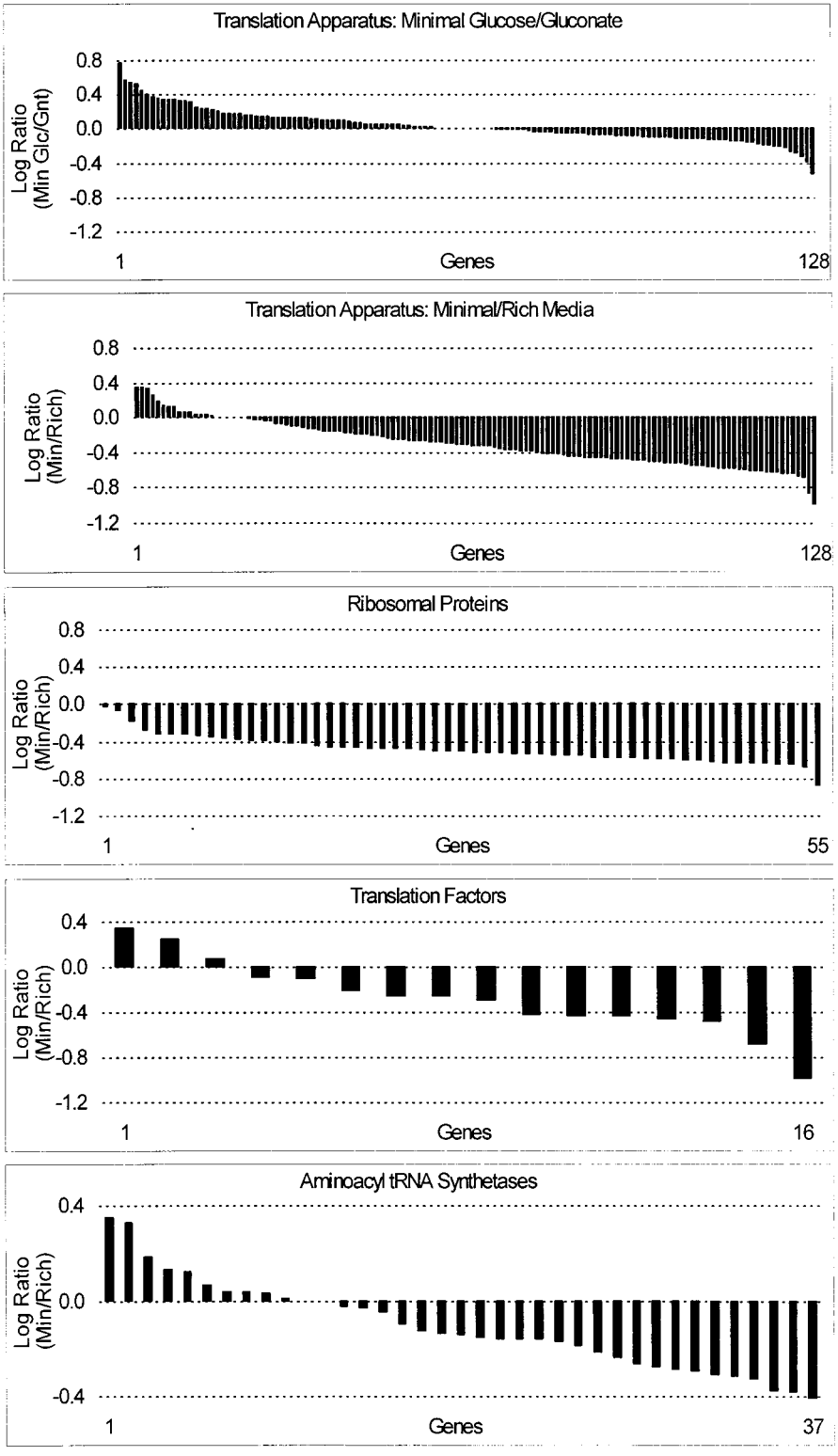

FIG. 4. Log expression ratios of the translation apparatus genes sorted by value. The set of all translation apparatus genes is shown in the top two panels for the minimal glucose versus minimal gluconate and minimal glucose versus Luria broth plus glucose experiments (see the legend to Fig. 3). The bottom three panels show the results of the minimal glucose versus Luria broth plus glucose experiment for functionally grouped subsets of the translation apparatus genes.

TABLE 2. Genes of the translation apparatus showing significant expression ratios

Gene	Gene product	Log ratio (minimal/rich medium)[a]
rplY	50S ribosomal subunit protein L25	−0.405
ileS	Isoleucine-tRNA synthetase	−0.414
rpsU	30S ribosomal subunit protein S21	−0.416
rpmH	50S ribosomal subunit protein L34	−0.419
efp	Elongation factor P	−0.425
fusA	GTP-binding protein chain elongation factor G	−0.435
tufA	Protein chain elongation factor Tu	−0.441
slyD	FKBP-type peptidylprolyl *cis-trans* isomerase	−0.450
rplL	50S ribosomal subunit protein L7/L12	−0.452
rpsO	30S ribosomal subunit protein S15	−0.461
rpmA	50S ribosomal subunit protein L27	−0.462
infB	Protein chain initiation factor 2	−0.463
rpmD	50S ribosomal subunit protein L30	−0.466
rpmE	50S ribosomal subunit protein L31	−0.471
rpsN	30S ribosomal subunit protein S14	−0.471
rplD	50S ribosomal subunit protein L4, regulates S10 operon	−0.475
prfB	Peptide chain release factor 2	−0.481
rpsD	30S ribosomal subunit protein S4	−0.483
rplF	50S ribosomal subunit protein L6	−0.494
rpsG	30S ribosomal subunit protein S7, initiates assembly	−0.499
ppiA	Peptidylprolyl *cis-trans* isomerase A	−0.501
rpsI	30S ribosomal subunit protein S9	−0.503
rpsK	30S ribosomal subunit protein S11	−0.504
rplK	50S ribosomal subunit protein L11	−0.514
rpmB	50S ribosomal subunit protein L28	−0.518
rpmC	50S ribosomal subunit protein L29	−0.522
rplA	50S ribosomal subunit protein L1, regulates L1 and L11	−0.529
rpsL	30S ribosomal subunit protein S12	−0.530
rpsB	30S ribosomal subunit protein S2	−0.540
rplS	50S ribosomal subunit protein L19	−0.548
rplN	50S ribosomal subunit protein L14	−0.553
rplC	50S ribosomal subunit protein L3	−0.555
rplP	50S ribosomal subunit protein L16	−0.570
rplU	50S ribosomal subunit protein L21	−0.575
rplQ	50S ribosomal subunit protein L17	−0.577
rplV	50S ribosomal subunit protein L22	−0.580
rpsT	30S ribosomal subunit protein S20	−0.587
rplE	50S ribosomal subunit protein L5	−0.587
rplR	50S ribosomal subunit protein L18	−0.596
rplM	50S ribosomal subunit protein L13	−0.603
rplI	50S ribosomal subunit protein L9	−0.607
prmA	Methylase for 50S ribosomal subunit protein L11	−0.615
rpsA	30S ribosomal subunit protein S1	−0.620
rplW	50S ribosomal subunit protein L23	−0.627
rpsS	30S ribosomal subunit protein S19	−0.634
rplB	50S ribosomal subunit protein L2	−0.636
rpsR	30S ribosomal subunit protein S18	−0.637
rpsJ	30S ribosomal subunit protein S10	−0.644
rpsE	30S ribosomal subunit protein S5	−0.646
rplJ	50S ribosomal subunit protein L10	−0.676
tufB	Protein chain elongation factor Tu	−0.688
rplX	50S ribosomal subunit protein L24	−0.875
tsf	Protein chain elongation factor Ts	−0.990

[a] Log expression ratios of measured transcript levels determined for the two cultures. The log expression ratio is positive for genes that were more highly expressed on minimal glucose medium and negative for genes that were more highly expressed on Luria broth plus glucose.

and biosynthesis of amino acids. The genes involved in assimilation of ammonia as the nitrogen source were expressed at significantly higher levels on minimal medium, including *gdhA*, which encodes glutamate dehydrogenase, and *gltD*, which encodes a subunit of glutamate synthase (Table 3). While it is known that *gdhA* is transcriptionally regulated by ammonia, next to nothing is known about the mechanism (53). The *gltBD* operon is subject to complex regulation by certain amino acids and in a concentration-dependent fashion by leucine-responsive protein (Lrp) (20); thus, the high induction ratio of *gltBD* on minimal medium (0.329 for *gltB*; 0.889 for *gltD*) can be explained by amino acid repression in rich medium and a high induction ratio of Lrp on minimal medium (see below). Conversely, *glnA*, which encodes glutamine synthase and is induced by nitrogen limitation (as indicated by a low ratio of intracellular glutamine to α-ketoglutarate), had the highest (although not significantly so) expression ratio (−0.316) of any of the amino acid biosynthetic genes in rich medium (52). In summary, the genes involved in ammonia assimilation were induced for growth on minimal medium where ammonia was the nitrogen source.

Biosynthesis of amino acids. The overall expression pattern of the genes encoding the enzymes of amino acid biosynthesis indicated that these were generally induced for growth on minimal medium (Fig. 5; Table 3). The *argA* gene, which encodes *N*-acetylglutamate synthase, the first enzyme of the pathway, and also *ygjG*, a probable ornithine aminotransferase, were expressed at significantly higher levels on minimal medium. Expression of the genes of the branched-chain amino acid biosynthetic pathways (67)was significantly elevated in minimal medium. The first gene of the *ilvGMEDA* operon, which encodes the enzymes of isoleucine and valine synthesis, was expressed at significantly higher levels on minimal medium. Interestingly, the monocistronic gene *ilvC*, which is derepressed exclusively by valine, had a log expression ratio of 0.977 on minimal medium, the highest of any of the amino acid biosynthesis genes. The leucine biosynthetic genes, encoded by the *leuABCD* operon, were all expressed at significantly higher levels on minimal medium. The high expression ratios of the leucine and valine biosynthetic genes are consistent with the relatively high abundance of these two amino acids (third and fourth most abundant, respectively) in *E. coli* cells (45). The genes encoding the first enzymes of the four branches of the aromatic amino acid biosynthetic pathways were all significantly elevated in cells grown on minimal medium (51). The first step of the "common pathway" of chorismate synthesis, encoded by *aroF*, and the first step of tyrosine biosynthesis, encoded by *tyrA*, form an operon, in that order, and had log expression ratios of 0.847 and 0.934, respectively. The *pheA* gene was significantly elevated, as were four of the five genes of the *trpEDCBA* operon; the *trpD* transcript level was high in both minimal and rich media. The gene encoding the first step in serine biosynthesis, *serA*, and the gene which codes for the enzyme that forms glycine from serine, *glyA*, were expressed at significantly higher levels on minimal medium. The *cysK* gene, which encodes cysteine synthase A, was expressed at significantly higher levels on minimal medium, while *cysM*, the gene encoding cysteine synthase B, was expressed at slightly higher levels on rich medium. The *cysE* product, serine transacetylase, forms a multifunctional complex with the *cysK* product, and the relative expression ratios of *cysK* and *cysE* (0.497 versus −0.024) are consistent with the *cysE* product being much less abundant in the enzyme complex (36). The uniquely MetR-regulated methionine synthase gene, *metE*, was expressed at a significantly higher level on minimal medium, in contrast to the remaining MetJ-regulated genes of methionine biosynthesis,

TABLE 3. Genes of nitrogen metabolism and biosynthesis showing significant expression ratios

Functional group	Gene	Gene Product	Log ratio (minimal/rich medium)
Amino acids	*ilvC*	Ketol-acid reductoisomerase	0.977
	leuD	Isopropylmalate isomerase subunit	0.951
	tyrA	Chorismate mutase-T and prephenate dehydrogenase	0.934
	gltD	Glutamate synthase, small subunit	0.889
	aroF	3-Deoxy-D-arabinoheptulosonate-7-phosphate synthase	0.847
	leuA	2-Isopropylmalate synthase	0.809
	leuB	3-Isopropylmalate dehydrogenase	0.756
	serA	D-3-Phosphoglycerate dehydrogenase	0.633
	ygjG	Probable ornithine aminotransferase	0.623
	leuC	3-Isopropylmalate isomerase (dehydratase) subunit	0.566
	trpB	Tryptophan synthase, beta protein	0.563
	glyA	Serine hydroxymethyltransferase	0.541
	gdhA	NADP-specific glutamate dehydrogenase	0.519
	trpC	*N*-(5-Phosphoribosyl)anthranilate isomerase and indole-3-glycerolphosphate synthetase	0.518
	ilvG1	Acetolactate synthase II, large subunit, interrupted	0.513
	trpE	Anthranilate synthase component I	0.511
	metE	Tetrahydropteroyltriglutamate methyltransferase	0.499
	cysK	Cysteine synthase A, *O*-acetylserine sulfhydrolase A	0.497
	aspC	Aspartate aminotransferase	0.482
	trpA	Tryptophan synthase, alpha protein	0.481
	argA	*N*-Acetylglutamate synthase	0.444
	pheA	Chorismate mutase P and prephenate dehydratase	0.439
	serC	3-Phosphoserine aminotransferase	0.401
Vitamins and cofactors	*nrdH*	Glutaredoxin-like protein; hydrogen donor	0.638
	hemC	Porphobilinogen deaminase	0.552
	entE	2,3-Dihydroxybenzoate-AMP ligase	0.543
	grxB	Glutaredoxin 2	0.506
	gst	Glutathionine *S*-transferase	0.501
	folE	GTP cyclohydrolase I	0.489
	ggt	Gamma-glutamyltranspeptidase	0.477
	entF	ATP-dependent serine activating enzyme	0.459
	entB	2,3-Dihydro-2,3-dihydroxybenzoate synthetase, isochorismatase	0.451
	entC	Isochorismate hydroxymutase 2 enterochelin biosynthesis	0.428
	thiH	Thiamine biosynthesis	−0.473
Nucleotides	*pyrI*	Aspartate carbamoyltransferase, regulatory subunit	0.628
	pyrB	Aspartate carbamoyltransferase, catalytic subunit	0.464
	prsA	Phosphoribosylpyrophosphate synthetase	−0.472
	ndk	Nucleoside diphosphate kinase	−0.484
	pfs	ORF, hypothetical protein	−0.515
	gmk	Guanylate kinase	−0.646
	upp	Uracil phosphoribosyltransferase	−0.646
Fatty acid and phospholipid metabolism	*cfa*	Cyclopropane fatty acyl phospholipid synthase	0.618
	fadA	3-ketoacyl-CoA thiolase	0.494
	fabA	β-Hydroxydecanoyl thioester dehydrase	−0.413
	fabD	Malonyl-CoA-[acyl-carrier-protein] transacylase	−0.437
	fabI	Enoyl-[acyl carrier protein] reductase	−0.468
	accC	Acetyl-CoA carboxylase, biotin carboxylase subunit	−0.557
	fabH	3-Oxoacyl-[acyl carrier protein] synthase III	−0.571
	fabF	3-Oxoacyl-[acyl carrier protein] synthase II	−0.576
	fabZ	(3*R*)-Hydroxymyristol acyl carrier protein dehydratase	−0.608

which did not vary significantly (25). The cobalamin-dependent methionine synthase encoded by *metH* was not expressed on minimal or rich media (data not shown). Overall, 8 of the 22 amino acid biosynthesis genes which were significantly elevated on minimal medium corresponded to the first step in the biosynthetic pathway. Thus, significant elevation of the first step in the amino acid biosynthetic pathways in cells grown on minimal medium was a recurring regulatory theme, consistent with the roles of these steps in controlling the flow of precursor metabolites out of the central pathways and into biosynthesis. Increased expression of the amino acid biosynthetic genes on minimal medium was indicative of the need to generate these building blocks from the sole carbon source, glucose.

Biosynthesis of vitamins, cofactors, prosthetic groups and carriers. Expression of the 106 genes involved in biosynthesis of vitamins, cofactors, prosthetic groups, and carriers followed the same trend as the genes of amino acid biosynthesis, although the expression ratios were generally not so large (Fig.

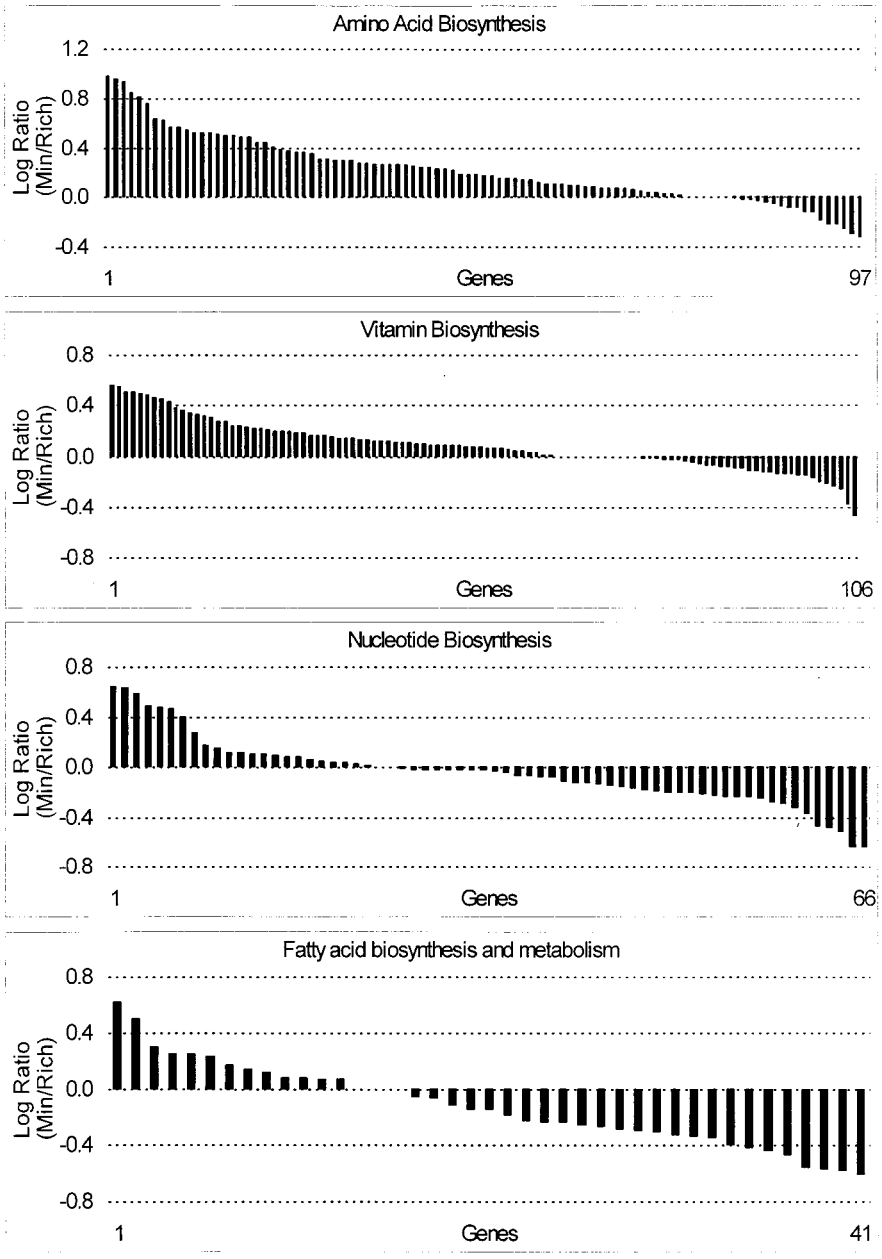

FIG. 5. Log expression ratios of biosynthetic genes were sorted by value for the minimal glucose versus Luria broth plus glucose experiment and are grouped by related pathways.

5; Table 3). Among the genes expressed at significantly higher levels on minimal medium were *hemC*, involved in porphyrin biosynthesis; the first three genes of the *entCEBA* operon and *entF*, involved in enterobactin biosynthesis; *grxB*, encoding glutaredoxin 2; *gst*, encoding glutathione *S*-transferase; *folE*, encoding the first step in tetrahydrofolate biosynthesis; and *ggt*, involved in glutathione biosynthesis. Only one gene, *thiH*, had an expression ratio that was significantly higher on rich medium, but this was in contrast to the remainder of the *thi* genes, which in general were expressed at modestly higher levels on minimal medium. Given that the vitamins and cofactors synthesized by the pathways in this functional group are needed in very small amounts, so small that they are rarely included in chemical composition tables (45), it is not surprising that most of these genes did not have significant expression ratios. Nevertheless, the general trend of higher expression in minimal medium is again indicative of the need to generate these building blocks de novo from glucose.

Nucleotide biosynthesis. While expression of the genes involved in biosynthesis of amino acids, vitamins, enzyme cofactors, and prosthetic groups, etc., was generally elevated on minimal glucose medium, expression of the genes involved in nucleotide salvage and biosynthesis was more evenly divided between the two growth conditions (Fig. 5; Table 3). The *pyrBI* genes, which form an operon encoding the first step of pyrimidine biosynthesis, were expressed at significantly higher levels on minimal medium, perhaps reflecting the presence of uridine in the rich medium, which would tend to repress these genes

(47). There are three enzymes involved in conversion of ribonucleotides to deoxyribonucleotides (33, 47), but of the three corresponding genetic loci only the *nrdHIEF* operon was expressed at significantly higher levels on minimal glucose medium. In previous studies, hyperinduction of *nrdEF* by hydroxyurea was measured, but this was the first experiment comparing *nrdEF* transcript levels under normal growth conditions (33), and this was the first indication that the genes encoding the NrdEF accessory proteins, NrdH and NrdI (34), are coregulated with *nrdEF*. There were five genes that were expressed at significantly higher levels in rich medium: the *prsA* gene, which encodes an enzyme that forms the first precursor of purine biosynthesis, and *upp*, *gmk*, *pfs*, and *ndk*, all of which encode enzymes involved in nucleotide salvage or interconversion, consistent with the availability of nucleotides in the rich medium.

Fatty acid biosynthesis and degradation. The *cfa* gene, which encodes an enzyme responsible for postsynthetic formation of cyclopropane fatty acids from unsaturated fatty acids, had a significantly high expression ratio on minimal medium (Table 3; also see Table 6). Since *cfa* is transcribed from an RpoS-dependent promoter (16), this result is consistent with elevated expression of *rpoS* on minimal medium (see below). All of the genes of the *fad* regulon (13) of fatty acid degradation (except for *fadA* [possibly an erroneous result]) were significantly elevated on rich medium, including *fadB*, which is in the *fadAB* operon, and *fadD*, which together encode the fatty acid oxidation multienzyme complex (Fig. 5). Also significantly elevated on rich medium were *fadR*, the repressor of the *fad* genes, and *fadL*, which encodes a long-chain fatty acid transporter. These results tend to indicate that the cells grown on rich medium were exposed to exogenous long-chain fatty acids, leading to induction of the *fad* regulon (14). Interestingly, the *ato* genes, which are involved in degradation of four-carbon fatty acids, were modestly elevated on minimal medium, and the sensor of the two-component regulator of these genes, encoded by *atoS*, was significantly elevated on minimal medium. These results suggest that the cells grown on minimal glucose medium were exposed to acetoacetate, which is the inducer of the *ato* genes (14). *E. coli* is not known to form acetoacetate from glucose, and it is possible that some closely related compound such as acetolactate, which is formed by *E. coli*, serves as an inducer of the *ato* genes (46, 67).

Expression of the genes of fatty acid biosynthesis was generally elevated on rich medium, and, with the exception of *fabB* and *fabG*, all of the *fab* genes were significantly elevated (Fig. 5; Table 3). The relative expression ratios of the genes in the *fabHDG-acpP-fabF* operon corresponded very closely to measurements of transcript levels by Northern analysis (77). In addition, *accA*, which encodes a component of acetyl coenzyme A (acetyl-CoA) carboxylase, was elevated on rich medium. The transcription rate of *accC* is growth rate dependent; the rate is higher in faster-growing cells (16). With the exception of FadR activation of *fabA*, less is known about the regulation of the *fab* genes (16). The data presented here, indicating that the *fab* genes were generally expressed at higher levels on rich medium, suggest that regulation of the phospholipid biosynthesis genes could be growth rate dependent (Fig. 5). This is a reasonable hypothesis, given that faster-growing cells must make membrane components more rapidly. However, the genomic expression data do not prove this hypothesis, and it is also possible that regulation of the *fab* genes is mediated by a signal molecule(s) in the rich medium. Further research in this area will help to clarify the global regulation of phospholipid biosynthesis.

Carbon and energy metabolism. The cells grown on rich medium showed nothing remarkable with respect to the expression pattern of genes involved in carbon and energy metabolism. Of the 409 genes of carbon catabolism, central metabolism, and energy metabolism, only 8 were expressed at significantly higher levels on rich medium (Table 4). These included *nuoM* and *nuoN* of the large operon encoding NADH dehydrogenase I and *cyoA* of the operon encoding cytochrome oxidase *c* (24), suggesting that aerobic respiration was elevated under this growth condition.

Cells grown on minimal glucose medium expressed 31 of the 409 of the carbon and energy metabolism genes at significantly higher levels. These included genes involved in D-lactate utilization (*dld*), acetate formation (*poxB*), regulation of *poxB* expression (*rpoS*), acetate utilization (*aceA*, *aceB*, *gltA*, *icd*, and *mdh*), and coupling of glucose and acetate cometabolism (*uspA*) (Tables 4 and 5). The elevated expression of these genes implicates metabolism of acetate and D-lactate as being perhaps the prominent feature of glucose metabolism in minimal medium. Under this growth condition, cells first consume glucose, which causes repression of the glyoxylate bypass and tricarboxylic acid cycle (15). Simultaneously, the cells excrete acetate and lesser amounts of D-lactate as overflow metabolites (46). As glucose is consumed and acetate accumulates, cells switch smoothly to cometabolism of glucose and acetate (1, 4, 14). This switch involves induction of the tricarboxylic acid cycle and glyoxylate bypass enzymes required to provide energy and to replenish intermediates used for amino acid biosynthesis (14).

Evidence has been published which suggests that pyruvate oxidase (PoxB) forms acetate from pyruvate during the transition from exponential growth to stationary phase: *poxB* expression requires RpoS and thus is elevated during transition phase (11). That cells grown in glucose minimal medium exhibited elevated *poxB* levels supports the contention that acetate was formed via pyruvate oxidation. The elevated expression of *rpoS* during late logarithmic growth (Table 5) also argues that RpoS may play a crucial role in regulating acetate metabolism.

Mutants lacking *uspA* exhibit diauxic growth on minimal glucose medium. This behavior probably occurs because of a failure to assimilate acetate until glucose becomes completely exhausted (49). In the wild-type cells examined here, expression of *uspA* was significantly elevated during growth on glucose minimal medium (Table 5), supporting the argument that UspA somehow plays a critical role in coupling of glucose and acetate cometabolism.

In summary, the evidence presented here provides some insight into the global control of carbon flow in cells growing on glucose in minimal medium. The data argue that acetate overflow metabolism is an important aspect of growth on glucose as the sole carbon and energy source, RpoS may play a role in regulating carbon metabolism genes in late-logarithmic-phase cells, and the universal stress protein, UspA, may coordinate glucose and acetate cometabolism.

Cellular processes and global regulators. Growth on minimal medium with glucose as the sole carbon and energy source places a burden on the cell to synthesize its amino acids de novo or starve. Thus, cells growing on minimal glucose medium are partially starved for amino acids, certainly a stressful situation and potentially having dramatic consequences on global gene regulation, elevating transcript levels of stress-inducible genes, and invoking the stringent response (8, 9, 29, 32). Several of the genes known to be regulated by the stringent-response signal molecule, ppGpp, were found to be differentially regulated on minimal and rich media (Fig. 6; Table

TABLE 4. Genes of carbon and energy metabolism showing significant expression ratios

Functional group	Gene	Gene product	Log ratio (minimal/rich medium)
Catabolism	*amyA*	Cytoplasmic alpha-amylase	0.679
	nanA	*N*-Acetylneuraminate lyase	0.581
	poxB	Pyruvate oxidase	0.535
	galK	Galactokinase	0.519
	ptr	Protease III	−0.629
	clpP	ATP-dependent proteolytic subunit	−0.689
Central metabolism	*gadA*	Glutamate decarboxylase isozyme	1.569
	gadB	Glutamate decarboxylase isozyme	1.497
	aceA	Isocitrate lyase	0.928
	gltD	Glutamate synthase, small subunit	0.889
	aceB	Malate synthase A	0.871
	gltA	Citrate synthase	0.746
	gpmA	Phosphoglyceromutase 1	0.724
	mdh	Malate dehydrogenase	0.587
	rpiB	Ribose 5-phosphate isomerase B	0.580
	phnJ	Phosphonate metabolism	0.538
	icdA	Isocitrate dehydrogenase	0.506
	zwf	Glucose-6-phosphate dehydrogenase	0.503
	nrdE	Ribonucleoside-diphosphate reductase 2, alpha subunit	0.489
	nrdF	Ribonucleoside-diphosphate reductase 2, beta chain	0.478
	tpiA	Triosephosphate isomerase	0.442
	talA	Transaldolase A	0.439
	pfkB	6-Phosphofructokinase II	0.423
	speE	Spermidine synthase	−0.531
Energy metabolism	*nrfC*	Formate-dependent nitrite reductase	0.805
	dld	D-Lactate dehydrogenase	0.726
	nrfA	Periplasmic cytochrome c(552)	0.617
	glpD	*sn*-Glycerol-3-phosphate dehydrogenase (aerobic)	0.616
	nrfB	Formate-dependent nitrite reductase	0.600
	qor	Quinone oxidoreductase	0.497
	ppc	Phosphoenolpyruvate carboxylase	0.477
	atpG	Membrane-bound ATP synthase, F1 sector, gamma subunit	0.451
	nuoJ	NADH dehydrogenase I chain J	0.419
	dsbE	Disulfide oxidoreductase	0.418
	hyfB	Hydrogenase 4 membrane subunit	0.415
	frdD	Fumarate reductase	0.401
	fdnI	Formate dehydrogenase N, cytochrome b_{556} gamma subunit	0.400
	nuoN	NADH dehydrogenase I chain N	−0.412
	ackA	Acetate kinase	−0.446
	cyoA	Cytochrome *o* ubiquinol oxidase subunit II	−0.461
	nuoM	NADH dehydrogenase I chain M	−0.537
	fdoG	Formate dehydrogenase O, major subunit	−0.967

5). Most notable of these genes was *rpoS*, encoding the stationary-phase sigma factor, which was significantly elevated on minimal medium. In fact it appeared that RpoS-dependent gene expression was a prominent feature of the genomic expression pattern of cells grown on minimal medium (Table 6). It is not clear from these genomic expression assays if the elevated level of the *rpoS* transcript was the result of regulation by ppGpp, although this would be consistent with the positive correlation between ppGpp concentration and RpoS levels (9), because it was also found that expression of *nlpD* (which encodes a lipoprotein and is operonic with *rpoS*) was significantly elevated on minimal medium (Table 2). Thus, these data do not distinguish between the possibilities that the higher level of *rpoS* transcription was driven by the *nlpD* promoter or by the *rpoS* promoters located within the upstream *nlpD* gene (29, 38). Production of RpoS is also subject to complex posttranscriptional and translational regulation, and therefore it cannot be presumed that *rpoS* transcript levels are correlated with RpoS activity (29). However, the number of RpoS-inducible genes that were observed to be expressed at significantly elevated levels on minimal medium (21 of them) argues strongly in this case that the *rpoS* transcript level correlated with RpoS function. Interestingly, of the 21 RpoS-dependent genes which were significantly elevated on glucose minimal medium, more than half are known to be involved in the physiological changes that highlight entry into stationary phase (32). However, the cells used in these experiments were in late logarithmic growth phase, still in steady-state growth. Although most studies have focused on the role of RpoS in preparing cells for entry into stationary phase, it has been suggested that RpoS may play a role in logarithmic phase as well (29), and the results presented here support this idea. Since this question is likely to receive further attention, a time course study of genomic expression in cells growing on minimal glucose medium in batch culture would be invaluable.

Some of the regulatory genes had significant expression ratios that were consistent with the elevated transcript levels of their target genes, such as *fadR*, which was expressed at signif-

TABLE 5. Genes involved in cell processes and global regulation showing significant expression ratios

Functional group	Gene	Gene product	Log ratio (minimal/rich medium)
Cell processes	*osmC*	Osmotically inducible protein	1.204
	katE	Catalase	0.893
	msyB	Acidic protein suppresses mutants lacking function of protein export	0.837
	glgS	Glycogen biosynthesis	0.794
	otsB	Trehalose-6-phosphate phophatase	0.777
	fic	Induced in stationary phase, affects cell division	0.654
	glgC	Glucose-1-phosphate adenylyltransferase	0.649
	osmY	Hyperosmotically inducible periplasmic protein	0.580
	bolA	Possible regulator of murein genes	0.564
	otsA	Trehalose-6-phosphate synthase	0.556
	cspD	Cold shock protein	0.540
	cbpA	Curved DNA binding protein	0.540
	envY	Envelope protein, thermoregulation of porin biosynthesis	0.477
	uspA	Universal stress protein	0.469
	motA	Proton conductor component of motor	0.457
	glgB	1,4-α-Glucan branching enzyme	0.430
	sodC	Superoxide dismutase precursor (Cu-Zn)	0.430
	pbpG	Penicillin binding protein 7	0.406
	acrF	Integral transmembrane protein	0.400
	sodB	Superoxide dismutase, iron	−0.904
Cell structure	*nlpD*	Lipoprotein	0.629
	slp	Outer membrane protein induced after carbon starvation	0.549
Regulatory function	*dps*	Global regulator, starvation conditions	1.402
	rpoS	RNA polymerase, sigma S (sigma38) factor	0.766
	atoS	Sensor protein AtoS for response regulator AtoC	0.580
	rseA	Sigma E factor, negative regulatory protein	0.538
	arsR	Transcriptional repressor *ars* operon	0.530
	molR1	Molybdate metabolism regulator	0.521
	lrp	Regulator for leucine (or *lrp*) regulon	0.511
	srlR	Regulator for *gut* (*srl*), glucitol operon	0.464
	tar	Methyl-accepting chemotaxis protein II	0.463
	phoU	Negative regulator for *pho* regulon	0.457
	narP	Nitrate/nitrite response regulator (sensor NarQ)	0.444
	rpoE	Sigma E factor; heat shock and oxidative stress	0.425
	wrbA	*trp* repressor binding protein	0.420
	rcsB	Positive regulator for colanic capsule biosynthesis (sensor, RcsC)	0.416
	fadR	Negative regulator for *fad* regulon, positive activator of *fabA*	−0.662
	cspA	Cold shock protein, transcriptional activator of *hns*	−0.776
	fis	Site-specific DNA inversion stimulation factor; DNA binding protein	−0.818

icantly higher levels on rich medium (as noted above), and *lrp*, which was expressed at significantly higher levels on minimal medium, correlating well with the elevated expression of several genes of the amino acid biosynthetic pathways (48). Several other regulatory genes showing significant expression ratios in this experiment are pleiotropic, and their roles under the growth conditions reported here are not as well understood (Table 5). Among the regulatory genes that were more highly expressed on rich medium are *cspA*, which encodes a cold shock transcription factor, and *fis*, which encodes a factor involved in site-specific recombination and pleiotropic transcriptional regulation (Table 5). Recent evidence indicates that *cspA* is expressed in cells that have not been subjected to cold stress and that its expression is higher in early logarithmic growth phase (7), a pattern of regulation that is remarkably similar to that of *fis* (3, 21, 70), which is also known to be more highly expressed in rich medium (35). Significantly higher in cells grown on minimal medium was *dps*, which encodes a DNA binding protein induced by starvation (42).

What these general DNA binding proteins, Dps and Fis, together with HN-S, seem to have in common is their involvement in growth rate-dependent regulation of gene expression, and it is nearly impossible to discuss these regulators without mentioning RpoS, which either regulates expression of or is regulated by these other factors (8, 9, 29, 32, 35). Together with RpoS, HN-S-dependent gene expression was prominent in the genomic transcription pattern of cells grown on minimal medium, and in fact the four genes with the highest expression ratios on minimal medium, *hdeA*, *hdeB*, *gadA*, and *gadB* (*dps* was fifth highest [Tables 4 and 6]) are known to be regulated by HN-S (5, 74, 75). Interestingly, *hns* expression was similar in minimal and rich media, suggesting either that expression of the *gad* and *hde* genes was not regulated by HN-S under these conditions or that *hns* expression (or HN-S function) is subject to posttranscriptional regulation by a mechanism which has yet to be described. In fact, expression of *gadB* and *hdeAB* in *Shigella flexneri* (72) and also *gadA* and *gadB* in *E. coli* (10) is RpoS dependent.

The apparent connection between these HN-S-regulated genes is their involvement in acid resistance (10). The unlinked genes, *gadA* and *gadB*, encoding homologous glutamate decarboxylases (62), are thought to be induced during fermentation as a result of acid stress (5, 61, 72). The *gadB* gene appears to form an operon with *xasA* (*gadC*) in *E. coli* (10) and is known

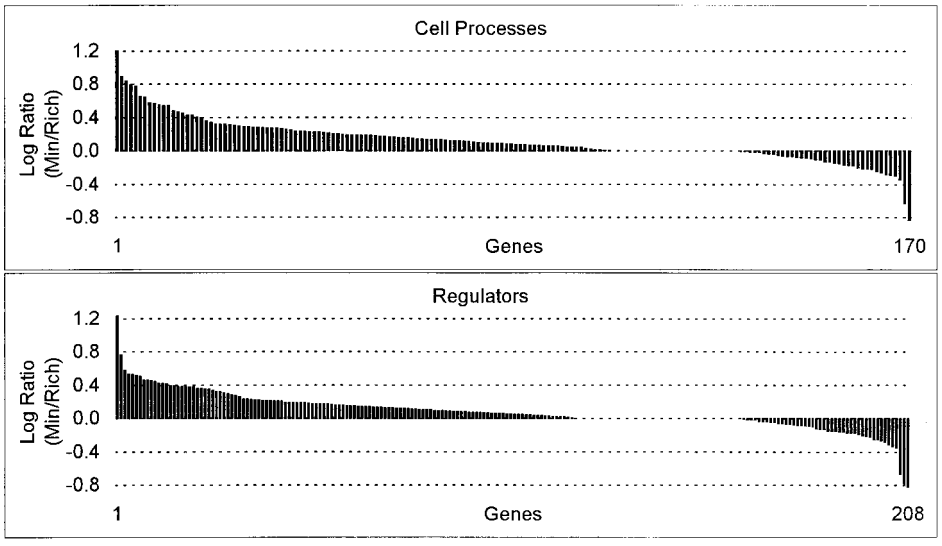

FIG. 6. Log expression ratios of cell process genes and regulatory genes sorted by value for the minimal glucose versus Luria broth plus glucose experiment.

to be cotranscribed with *xasA* (*gadC*) in *S. flexneri* (72); *gadC* mutants of *E. coli* are acid sensitive (30). Not surprisingly, *xasA* (*gadC*) had a significant log expression ratio on minimal medium (0.580 [data not shown]). Clustered together with *gadA* and *hdeAB* are several other genes that showed significantly higher expression ratios on minimal medium, i.e., *hdeD*, *yhiE*, and *yhiX* (log expression ratios of 0.872, 0.852, and 1.096, respectively [data not shown]). The functions of these five genes are all unknown, but it has been shown that *hdeAB* mutants of *S. flexneri* are acid sensitive (72). The *yhiX* gene, which encodes an AraC-like protein, is a likely candidate for regulation of the *gad* and *hde* genes, given its position downstream of *gadA* and its high expression ratio on minimal medium. Furthermore, alignment of the *gadA*, *gadB*, and *hdeD-hdeAB* regulatory regions (200 bp upstream of start codons) revealed a 19-bp sequence which is perfectly conserved in *gadA* and *gadB* and of which 15 bp are conserved in all three (data not shown). In summary, the results suggest that these HN-S/RpoS-dependent genes comprise a system for acid tolerance. It is interesting to speculate that RpoS plays a role in these logarithmic-phase cells of coordinating induction of the acid tolerance genes, together with the genes of organic acid me-

TABLE 6. Genes regulated by RpoS showing significant expression ratios

Functional group	Gene	Gene product	Log ratio (minimal/rich medium)
Catabolism	*galK*	Galactokinase	0.519
	poxB	Pyruvate oxidase	0.535
Cell processes	*bolA*	Possible regulator of murein genes	0.564
	cbpA	Curved DNA binding protein	0.540
	fic	Induced in stationary phase, affects cell division	0.654
	glgS	Glycogen biosynthesis	0.794
	glgC	Glucose-1-phosphate adenylyltransferase	0.649
	glgB	1,4-α-Glucan branching enzyme	0.430
	katE	Catalase	0.893
	osmC	Osmotically inducible protein	1.204
	osmY	Hyperosmotically inducible periplasmic protein	0.580
	otsB	Trehalose-6-phosphate phophatase	0.777
	otsA	Trehalose-6-phosphate synthase	0.556
Energy metabolism	*frdD*	Fumarate reductase, anaerobic	0.401
	glpD	*sn*-Glycerol-3-phosphate dehydrogenase	0.616
FA and PL metabolism	*cfa*	Cyclopropane fatty acyl phospholipid synthase	0.618
Hypothetical	*hdeA*	ORF, hypothetical protein	1.872
	hdeB	ORF, hypothetical protein	1.705
Regulatory function	*dps*	Global regulator, starvation	1.402
	rpoS	Sigma S (sigma 38) factor	0.766
	wrbA	*trp* repressor binding protein	0.420

tabolism, under conditions of glucose overflow metabolite formation.

Conclusion. In the single experiment presented here, the hallmark features of growth on minimal and rich media were revealed. Across the genome, we observed differences in the expression of functionally grouped genes that paralleled the physiology of these two growth conditions. Cells grown in rich medium with a good carbon and energy source, glucose, grew rapidly, turning off the pathways of biosynthesis and elevating the expression of the genes involved in macromolecule synthesis, most prominently protein synthesis. Cells in minimal medium faced the need to synthesize all of their building blocks from a single carbon and energy source, again glucose, and this burden was reflected not just in the turning on of biosynthetic pathways but also in the elevated expression of regulators of cell processes and regulons involved in stress tolerance. The most prominent features of growth on glucose minimal medium were the formation of overflow metabolites, in particular acetate, and protection of the cell from the stress of living in a self-formed acidic environment. All of these aspects of physiology were revealed not by painstaking and careful analysis in the laboratory of each system but, rather, by deduction from the genomic expression patterns of cells grown under these two rather different conditions. These deductions would not have been possible were it not for countless microbial physiology experiments published over the past 50 years (44). On the other hand, from the one simple experiment reported here, the tremendous potential of functional genomics is obvious. As a result of this experiment, several genes were added to functional groups on the basis of coregulation with similar and related genes. Also, several testable hypotheses were generated, in particular those involving the flow of carbon to acetate, coupling of glucose and acetate cometabolism, and acid resistance, the importance of which has been previously pointed to in enteric bacteria (4).

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