JOURNAL OF BACTERIOLOGY, June 2004, p. 3516–3524 Vol. 186, No. 11
0021-9193/04/$08.00+0 DOI: 10.1128/JB.186.11.3516–3524.2004
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Transcriptome Analysis of Crp-Dependent Catabolite Control of Gene Expression in *Escherichia coli*

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Received 7 November 2003/Accepted 22 February 2004

We report here the transcriptome analyses of highly expressed genes that are subject to catabolite repression or activation mediated by the cyclic AMP receptor protein (Crp). The results reveal that many operons encoding enzymes of central carbon metabolic pathways (e.g., Krebs cycle enzymes), as well as transporters and enzymes that initiate carbon metabolism, are subject to direct Crp-mediated catabolite repression. By contrast, few enzyme-encoding genes (direct regulation) but many ribosomal protein- and tRNA-encoding genes (indirect regulation) are subject to Crp-dependent glucose activation. Additionally, Crp mediates strong indirect catabolite repression of many cytoplasmic stress response proteins, including the major chaperone proteins, five ATP-dependent protease complexes, and several cold and heat shock proteins. These results were confirmed by (i) phenotypic analyses, (ii) real-time PCR studies, (iii) reporter gene fusion assays, and (iv) previously published reports about representative genes. The results serve to define and extend our appreciation of the Crp regulon.

A dominant mechanism by which *Escherichia coli* and other related bacteria sense carbon sufficiency involves cyclic AMP and its receptor protein, Crp (15, 35). The mechanisms by which Crp regulates gene expression in response to variable cytoplasmic levels of cyclic AMP have been extensively investigated with primary emphasis on *E. coli* and *Salmonella* strains (5, 34, 35, 41). Dozens of operons have been shown to be subject to Crp-mediated control by using classical approaches (17). Transcriptome and proteome approaches have been used to study the control of various regulons, as well as to facilitate glucose flux analyses (10, 39). Indeed, the transcriptome approach has allowed investigators to challenge established paradigms, and the technology has enjoyed rapid adoption among researchers (7). However, no report has focused on genome-wide analyses of Crp-mediated catabolite regulation in *E. coli*. In this study, we corrected this deficiency by conducting combined transcriptome, phenotypic, and bioinformatic analyses of the Crp-mediated responses of *E. coli* to exogenous glucose availability. We also tabulated comparative data derived from the classical literature and confirmed representative regulatory responses by using alternative approaches. We show here that a variety of stress-related genes, encoding chaperones (1, 4, 38), ATP-dependent proteases (9, 11, 20), and certain temperature shock proteins (12, 31, 40), are regulated in response to the presence of Crp, apparently by an indirect mechanism.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains used in this study were BW25113 and an isogenic *crp* mutant derivative, LJ3017, constructed as described by Zhang et al. (43). Strains were grown at 37°C with agitation at 250 rpm in Luria-Bertani (LB) broth containing 50 mM potassium phosphate, pH 7.4, and 0.2 mM L-cysteine with or without 0.4% glucose. Cells were grown in 25 ml of medium in 250-ml shake flasks starting at an optical density at 600 nm (OD_{600}) of 0.05. Each determination was performed three times, and the results were averaged (6).

Cell harvesting and preparation of RNA. Cells from each triplicate experiment were harvested in the exponential growth phase when cultures reached an OD_{600} of 0.5. The total contents of each shake flask (25 ml) were poured into a Millipore vacuum filtration apparatus (catalog no. 1004700) with a Millipore 0.8-μm-pore-size filter (catalog no. AAWPO4700). The collected cells with filter were immediately transferred to a 200-ml glass beaker containing liquid nitrogen. RNA was extracted from each sample as described by Caldwell et al. (6).

Target preparation. RNA harvested from a given *E. coli* strain and at a given time point was reverse transcribed into biotin-labeled cDNA by the method of de Saizieu et al. (8). Total RNA (18 μg) was incubated at 37°C overnight in an 80-μl reaction mixture consisting of 1× GIBCO first-strand buffer (50 mM Tris-HCl [pH 8.3], 75 mM KCl, 3 mM $MgCl_2$); 10 mM dithiothreitol; 40 μM random hexamer; 0.3 mM concentrations (each) of dCTP, dGTP, and dTTP; 0.12 mM dATP; 0.3 mM biotin-dATP (NEN catalog no. NEL999); and 1,800 U of Super-Script II reverse transcriptase. To remove RNA, the reaction mixture was brought to 0.25 M NaOH and incubated at 65°C for 30 min. The reaction mixture was neutralized with HCl, and the nucleic acid was precipitated at −20°C in ethanol with 2.5 M ammonium acetate and 20 μg of glycogen. The pellet was washed, air dried, resuspended in water, and quantitated by UV spectroscopy. The yield was approximately 10 to 12 μg of biotin-labeled cDNA. This cDNA (10 μg) was fragmented in 33 μl of 1× One-Phor-All buffer (Amersham-Pharmacia no. 27-0901-02) with 3.75 mU of DNase I at 37°C for 10 min. After the DNase had been heat killed, fragmentation was validated by running 1.5 μg of the fragmented cDNA on a 1.2% agarose gel. Biotin-containing cDNA routinely ranged in size from 25 to 400 nucleotides. The remaining 8 μg of cDNA was hybridized to an Affymetrix (Santa Clara, Calif.) *E. coli* GeneChip array.

Array description. The Affymetrix *E. coli* array has been described in detail by Selinger et al. (37). Briefly, each microarray contains 295,936 25-mer oligonucleotide probes. Half of the probes are a perfect match to the corresponding *E. coli* chromosomal sequences, while the other half have a single mismatch at the 13th base position. The array includes 4,327 genes and intergenic regions (2). The complete set of averaged data is provided at our website (www-biology.ucsd.edu/~msaier/supmat; see Table S1).

Hybridization, scanning, and data collection. The hybridization, scanning, and data collection procedures described by Caldwell et al. (6) were followed.

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RT-PCR studies. Bacterial cells were cultured in LB broth with or without 0.4% glucose exactly as for the microarray analyses. When the OD_{600} reached 0.5, cells were collected by centrifugation at 4°C. Total RNA was subsequently isolated with the RNeasy Mini Kit (Qiagen) in accordance with the manufacturer's protocol. Residual DNA present in the RNA preparations was removed by RNase-free DNase (Stratagene). cDNAs were synthesized with the superscript first-strand synthesis kit (Invitrogen) in accordance with the manufacturer's instructions and stored at −20°C prior to use. Real-time PCR (RT-PCR) was carried out on the LightCycler instrument (Roche Diagnosis Corporation) in accordance with the manufacturer's recommended protocol. Primers used for the RT-PCR were as follows: for *aceE*, GAA GAA GGT GTT GAG CGT GC and TTG CGG AAGA CTG GAA GGAC; for *clpB*, CGAC ATC CTG AAA GCA GCAG and CAG ACC TTC AAC GAT GGCAG; for *ibpA* (*hslT*), CG CTT TAC CGT TCT GCT ATT GG and TGC GTT CAA AGT TGC GTT CAG.

Data analysis. Array images were analyzed with Affymetrix Microarray Suite 5.0 software to determine raw expression intensity values, which then were scaled and normalized to a target value of 1,000. These data were imported into a Microsoft Excel spreadsheet for further analysis. The intensity values in the data sets ranged from about 3×10^{-1} to 8×10^{4}. Three data sets were obtained for each of the following experimental conditions: wild type in LB, wild type in LB plus glucose, *crp* mutant in LB, and *crp* mutant in LB plus glucose. The data sets for each strain and condition were compared pairwise to determine the Pearson correlation coefficient with the formula $r = [n(\Sigma XY) - (\Sigma X)(\Sigma Y)/\{[n\Sigma X^2 - (\Sigma X)^2][n\Sigma Y^2 - (\Sigma Y)^2]\}^{1/2}$. For each triplicate data set, the two sets with the highest Pearson correlation coefficient were retained for further analysis. The statistical significance of fold changes between data sets of different strains and conditions was determined as described by Caldwell et al. (6) and also in Results. Briefly, for each pair of replicate data sets, a table was generated from the average of the logs of the two replicate signals (ALS) and their log ratio (LR). From this table, with a series of sliding windows with a size of 201 ALS values, the mean ALS values and the standard deviation values of the corresponding LR were calculated (SD_{LR}).

RESULTS

Glucose-activated Crp-dependent genes. Table 1 summarizes the genes that gave (i) a statistically significant change (the fold change was at least 4.37 times higher than the SD_{LR} for the ALS of the gene on the wild-type and *crp* strains), (ii) consistently large signals (signal intensity, ≥1,000), (iii) a *crp*/wild-type ratio of ≥2.0 following growth in LB broth, and (iv) activation by glucose in a Crp-dependent fashion. Very few enzyme-encoding genes met these criteria, although several genes encoding ribosomal proteins and tRNAs did. Enzyme-encoding genes showing Crp-mediated glucose activation were the *aceE* gene, encoding the pyruvate dehydrogenase E1 component; the *guaB* gene, encoding inosine 5′ monophosphate dehydrogenase; and the *ptsG* gene, encoding the glucose-specific IIBC permease of the phosphotransferase system (PTS). These genes may be subject to repression by the cyclic AMP-Crp complex, although *ptsG* is subject to glucose activation by a distinct Mlc-dependent mechanism (16, 28, 29). A few other enzyme-encoding genes exhibited Crp-mediated glucose activation but did not satisfy all of the criteria cited above (see Table S1 at our website). These genes included, for example, *aceF*, encoding the pyruvate dehydrogenase E2 component; *accC*, encoding acetyl coenzyme A (CoA) carboxylase; *ack*, encoding acetate kinase; and *adk*, encoding adenylate kinase. It is surprising that so few highly expressed enzyme-encoding genes are directly activated by the cyclic AMP-Crp complex compared to enzyme-encoding genes that are subject to repression (see below).

Two other genes, *fis*, encoding the Fis (factor for inversion stimulation) protein, a basic, histone-like, chromatin-binding protein, and the *spf* gene, encoding an antisense regulator of *galK* translation, were subject to strong Crp-dependent glucose activation. Crp-binding sites could be found in or near most of the enzyme-encoding and *fis* operons. On this basis, we assume that these genes are directly regulated by Crp.

Many genes concerned with transcription and translation proved to be subject to Crp-dependent glucose activation. These genes encode tRNAs, ribosomal proteins, elongation factors, and RNA polymerase subunits (see Table S1 at our *E. coli* transcriptome website). Those satisfying the criteria cited above are listed in Table 1. We could not find Crp-binding sites in or near these genes or operons, suggesting that they are indirectly regulated, possibly in response to changes in growth rate due to the presence or absence of Crp and/or glucose availability (14). This suggestion is in agreement with previous reports on select ribosomal protein and tRNA-encoding genes (14).

Glucose-repressed Crp-dependent genes. Table 2 tabulates genes subject to Crp-dependent glucose repression identified by the same criteria cited above for Table 1, except that the *crp*/wild-type strain signal ratio had to be ≤0.5. The majority of these genes encode enzymes and transporters involved in carbon catabolism. These proteins include (i) enzymes of central carbon metabolism (e.g., the Krebs cycle), (ii) transporters and enzymes that initiate the metabolism of an exogenous carbon compound (e.g., galactitol), and (iii) transporters and enzymes that initiate the metabolism of an exogenous amino acid or peptide that can be used as a primary source of both carbon and nitrogen (e.g., aspartate). The second largest class encodes stress response proteins primarily concerned with protein folding and degradation. Thus, 51 of the 72 genes listed in Table 2 are carbon catabolic genes while 18 of these genes are stress response genes. Only three genes fell into the "miscellaneous" category. For all of these genes, the *crp* mutation eliminated or decreased the magnitude of catabolite repression. Residual glucose repression in the *crp* mutant strain is presumably due to effects of other transcription factors.

The prominent carbon catabolic genes include several genes encoding enzymes in central pathways of carbon catabolism. These enzymes include (i) the glyoxalate shunt enzymes (AceA and AceB); (ii) lactaldehyde dehydrogenase A (AldA); (iii) most of the Krebs cycle enzymes, including fumarase (FumA), citrate synthase (GltA), malate dehydrogenase (Mdh), succinate dehydrogenase (Sdh), 2-oxoglutarate dehydrogenase (SucAB), and succinyl-CoA synthetase (SucCD); and (iv) the central gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PckA). However, nearly half of the genes exhibiting Crp-dependent catabolite repression are concerned with the initiation of carbon metabolism. These genes include the enzymes catalyzing catabolism of formate (Fdo), galactitol (Gat), glycerol and α-glycerophosphate (Glp) lactate (Lct and Lld), maltose (Mal), mannose (Man), galactose (Mgl), glucitol (or sorbitol) (Srl), and trehalose (Tre). Four of these carbohydrates, galactitol, mannose, glucitol, and trehalose, are substrates of the *E. coli* PTS, while six of these compounds, formate, glycerol, α-glycerol phosphate, lactate, maltose, and galactose, are non-PTS carbon and energy sources. Many of these genes (i.e., *gat*, *glp*, *mgl*, *lld*, *mal*, and *tre*) showed residual glucose repression in the *crp* mutant. In several of these cases, the *fruR* mutation by itself diminished the repressive effect of glucose while the *crp fruR* double mutant exhibited full resis-

TABLE 1. Genes subject to Crp-dependent glucose activation[a]

Gene name and category	Absolute signal value[b]				Ratio[b]				Function	Crp sequence 1[c]	Position 1[c]	Rank 1[c]	Crp sequence 2[c]	Position 2[c]	Rank 2[c]
	wt	wt + G	*crp*	*crp* + G	wt + G/wt	*crp*/wt	*crp* + G/wt + G	*crp* + G/*crp*							
Enzyme-encoding genes															
aceE	1,880	8,177	4,520	4,447	4.4	2.4	0.5[d]	1.0	Pyruvate dehydrogenase EI component	GGTGGAGGAGGTCACG	−2288	399			
guaB	649	2,749	1,699	2,784	4.2	2.6	1.0	1.6	I-MP dehydrogenase	TGTGAGCGAGATCAAA	−162	106	TTTGATCTCGCTCACA	−147	473
ptsG	735	2,387	1,584	1,390	3.2	2.2	0.6	0.9	Glucose-specific IIBC component of the PTS	CGTGATAGCCGTCAAA	−151	265	TTTGACGGCTATCACG	−136	555
Regulatory nucleic acid binding protein-encoding genes															
fis	828	5,726	2,345	2,999	6.9	2.8	0.5[d]	1.3	Factor for inversion stimulation	TTTTATAGGTGTCACA	−354	436			
spf	2,391	26,801	19,644	23,655	11.2	8.2	0.9	1.2	SpoT 42 RNA; antisense regulator of *galK* translation						
Ribosomal protein-encoding genes															
rplS	2,146	5,704	4,887	3,572	2.7	2.3	0.6	0.7	50S ribosomal protein L19						
rpmE	2,226	8,773	4,828	7,355	3.9	2.2	0.8	1.5	50S ribosomal protein L31						
rpsQ	3,311	8,991	7,320	5,218	2.7	2.2	0.6	0.7	30S ribosomal protein S17						
rpsT	3,616	24,476	11,003	11,054	6.8	3.0	0.5[d]	1.0	30S ribosomal protein S20						
RNA-encoding genes															
[*alaU*	4,865	17,798	10,431	10,955	3.7	2.1	0.6	1.1	Alanine tRNA						
alaW	4,602	16,307	10,351	11,933	3.5	2.2	0.7	1.2	Alanine tRNA						
alaX]	5,564	16,814	11,597	13,422	3.0	2.1	0.8	1.2	Alanine tRNA						
[*cysT*	5,906	13,127	13,776	12,209	2.2	2.3	0.9	0.9	Cysteine tRNA						
glyW	7,967	20,720	15,732	15,795	2.6	2.0	0.8	1.0	Glycine tRNA						
leuZ]	4,937	10,319	13,902	9,908	2.1	2.8	1.0	0.7	Leucine tRNA						
[*glyV*	7,873	22,687	15,590	17,237	2.9	2.0	0.8	1.1	Glycine tRNA						
glyX	5,796	23,340	13,878	15,042	4.0	2.4	0.6	1.1	Glycine tRNA						
glyY]	2,654	12,628	8,174	11,747	4.8	3.1	0.9	1.4	Glycine tRNA						
leuQ	2,340	5,592	6,493	5,074	2.4	2.8	0.9	0.8	Leucine tRNA						
leuX	1,796	3,768	4,344	3,042	2.1	2.4	0.8	0.7	Leucine tRNA5 (amber [UAG] suppressor)						
proK	2,642	4,386	7,934	4,038	1.7	3.0	0.9	0.5[d]	Proline tRNA						
proM	1,613	2,588	7,602	1,657	1.6	4.7	0.6	0.2[d]	Proline tRNA						
selC	649	3,272	1,552	2,753	5.0	2.4	0.8	1.8[d]	Selenocysteyl tRNA						
serT	1,341	4,061	5,390	4,192	3.0	4.0	1.0	0.8	Serine tRNA						
[*trpT*	7,024	12,860	15,467	10,501	1.8	2.2	0.8	0.7	Tryptophan tRNA						
tyrT	2,685	6,067	8,418	5,513	2.3	3.1	0.9	0.7	Tyrosine tRNA						
tyrV]	4,840	12,056	15,615	10,454	2.5	3.2	0.9	0.7	Tyrosine tRNA						

[a] Genes tabulated gave a high level of reproducibility for replicate values, large signals, and *crp*/wt ratios greater than 2.0. Brackets indicate genes included within a single operon or regulon.

[b] Abbreviations: wt, wild type; *crp,* the isogenic *crp*-null mutant; G, grown in LB medium plus glucose. When G is not indicated, cells were grown in LB medium.

[c] Crp binding sites were identified by using the GRASP-DNA program (36). The position relative to the binding site of the translational start site of the gene and the rank of that site relative to all other sites in the genome are provided as detailed by Schilling et al. (36).

[d] Crp-independent glucose repression (*proK* and *proM*) and activation (*selC*) are presumably mediated by another transcription factor. The two-fold decrease observed for three genes, *aceE, fis,* and *rpsT,* when the *crp* mutant is compared with the wild type in the presence of glucose could be due to Crp-mediated activation of gene expression in the presence of glucose in the wild-type strain.

TABLE 2. Genes subject to CRP-dependent glucose repression[a]

Name	wt	wt + G	*crp*	*crp* + G	Ratio of wt + G/wt	Ratio of *crp*/wt	Ratio of *crp* + G/wt + G	Ratio of *crp* + G/*crp*	Function	Crp sequence	Position 1	Rank 1
Carbon metabolic genes												
Central metabolic pathways												
aceA	2,747	498	711	462	0.2	0.3	0.9	0.6	Isocitrate lyase	TGTAATGGATGTCACG	2012	53
aceB	1,432	296	339	237	0.2	0.2	0.8	0.7	Malate synthase A			
aldA	3,668	305	696	231	0.1	0.2	0.8	0.3	Lactaldehyde dehydrogenase A			
fumA	3,046	819	1,057	969	0.3	0.3	1.2	0.9	Fumarate hydratase class I	CGTGAGCAATATCACG	−1191	173
fumA	3,046	819	1,057	969	0.3	0.3	1.2	0.9	Furmarate hydratase class I	CGTGAGCAATATCACG	−1191	173
gltA	5,086	452	1,732	706	0.1	0.3	1.6	0.4	Citrate synthase	AGTGATCCAGGTCACG	−414	186
mdh	4,164	996	1,749	1,220	0.2	0.4	1.2	0.7	Malate dehydrogenase			
pckA	2,488	765	875	929	0.3	0.4	1.2	1.1	Phosphoenolpyruvate carboxykinase			
sdhA	3,503	614	1,591	682	0.2	0.5	1.1	0.4	Succinate dehydrogenase, flavoprotein subunit			
sdhD	2,304	401	1,019	462	0.2	0.4	1.2	0.5	Succinate dehydrogenase, 13-kDa hydrophobic protein			
sucA	3,920	822	1,345	813	0.2	0.3	1.0	0.6	2-Oxoglutarate dehydrogenase, E1 component			
sucB	5,734	802	1,725	923	0.1	0.3	1.1	0.5	Dihydrolipoamide succinyltransferase component (E2)			
sucC	6,684	1,250	1,866	1,700	0.2	0.3	1.4	0.9	Succinyl-CoA synthetase, beta chain			
sucD	3,815	526	1,057	753	0.1	0.3	1.4	0.7	Succinyl-CoA synthetase, alpha chain			
Initiation of carbohydrate metabolism												
fdoG	2,114	805	506	859	0.4	0.2	1.1	1.7	Formate dehydrogenase O, alpha subunit	TGTGACAAATATCACA	−226	16
										TGTGATATTTGTCACA	−241	38
gatA	9,436	523	1,972	574	0.1	0.2	1.1	0.3	Galactitol-specific IIA component of the PTS			
gatB	5,439	174	1,190	233	0.03	0.2	1.3	0.2	Galactitol-specific IIB component of the PTS			
gatC	5,431	281	1,287	347	0.1	0.2	1.2	0.3	Galactitol-specific IIC component of the PTS			
gatD	3,495	77	681	115	0.02	0.2	1.5	0.2	Galactitol-1-phosphate dehydrogenase			
gatR	1,200	500	562	562	0.4	0.5	1.1	1.0	Galactitol utilization operon repressor			
gatZ	13,508	988	4,046	937	0.1	0.3	0.9	0.2	Tagatose 6-phosphate kinase	CGAGATAACGATCACA	946	292
										TGTTTTCGATTTCAAA	901	406
glpA	1,413	414	523	362	0.3	0.4	0.9	0.7	Anaerobic glycerol-3-phosphate dehydrogenase, subunit A	TGTGCGGCAATTCACA	147	307
glpD	2,537	509	1,056	873	0.2	0.4	1.7	0.8	Aerobic glycerol-3-phosphate dehydrogenase	TGTTATACATATCACT	113	103
glpD	2,537	509	1,056	873	0.2	0.4	1.7	0.8	Aerobic glycerol-3-phosphate dehydrogenase	GGTAATTGATTTCACT	873	462
glpF	4,035	175	825	348	0.04	0.2	2.0	0.4	Glycerol facilitator			
glpK	7,164	92	1,310	840	0.01	0.2	9.1	0.6	Glycerol kinase			
glpQ	3,903	164	987	262	0.04	0.3	1.6	0.3	Periplasmic glycerophosphoryl diester phosphodiesterase precursor	CGCGATAGATTTCACG	308	420
glpT	7,330	242	1,596	297	0.03	0.2	1.2	0.2	Glycerol-3-phosphatase transporter	TGTGCGGCAATTCACA	−126	307
glpX	2,098	1,327	757	1,201	0.6	0.4	0.9	1.6	Glycerol-inducible fructose-1,6-bisphostphatase homologue phosphatase			
lctD	2,464	94	648	91	0.04	0.3	1.0	0.1	L-Lactate dehydrogenase operon regulator			
lctR	2,060	136	258	118	0.1	0.1	0.9	0.5	L-Lactate dehydrogenase operon regulator			
lldP	1,556	236	247	206	0.2	0.2	0.9	0.8	L-Lactate permease			
malE	3,090	454	955	342	0.1	0.3	0.8	0.4	Periplasmic maltose-binding protein precursor	TGTAACAGAGATCACA	−129	39
										TGTGATCTCTGTTACA	−114	81
malK	1,725	123	332	131	0.1	0.2	1.1	0.4	ATP-binding cassette protein for maltose uptake	TGTAACAGAGATCACA	236	39
										TGTGATCTCTGTTACA	251	81
lamB	3,180	429	990	431	0.1	0.3	1.0	0.4	Phage lambda receptor protein			

Continued on following page

TABLE 2—*Continued*

Name	wt	wt + G	*crp*	*crp* + G	Ratio of wt + G/wt	Ratio of *crp*/wt	Ratio of *crp* + G/wt + G	Ratio of *crp* + G/*crp*	Function	Crp sequence	Position 1	Rank 1
lamB	3,180	429	990	431	0.1	0.3	1.0	0.4	Phage lambda receptor protein			
malM	1,581	532	765	446	0.3	0.5	0.8	0.6	Periplasmic maltose operon protein			
manX	2,021	608	785	546	0.3	0.4	0.9	0.7	Mannose enzyme IIAB component of the PTS			
manY	2,004	600	944	582	0.3	0.5	1.0	0.6	Mannose enzyme IIC component of the PTS			
mglA	1,258	165	197	200	0.1	0.2	1.2	1.0	ATP-binding cassette protein for galactose uptake			
mglB	4,417	490	658	493	0.1	0.1	1.0	0.7	Periplasmic galactose-binding protein precursor	TGTGAGTGATTTCACA TGTGAAATCACTCACA	−267 −252	26 137
mglC	1,683	73	400	91	0.04	0.2	1.3	0.2	Galactose permease protein			
srlA	2,303	317	596	380	0.1	0.3	1.2	0.6	Glucitol-specific IIBC component of the PTS			
srlB	1,354	307	376	229	0.2	0.3	0.7	0.6	Glucitol- and sorbitol-specific IIA component of the PTS			
srlD	2,308	436	573	322	0.2	0.2	0.7	0.6	Glucitol-6-phosphate dehydrogenase			
treB	3,977	340	961	341	0.1	0.2	1.0	0.4	Trehalose-specific IIBC component of the PTS			
treC	3,795	158	688	280	0.04	0.2	1.8	0.4	Trehalose-6-phosphate hydrolase			
Initiation of amino acid metabolism												
aspA	2,611	718	1,004	778	0.3	0.4	1.1	0.8	Aspartase	GGTGATCTATTTCACA CGTAATCTGGATCACT	−158 −93	22 571
dsdA	1,695	186	324	165	0.1	0.2	0.9	0.5	D-Serine dehydratase			
prlC	1,602	572	476	584	0.4	0.3	1.0	1.2	Oligopeptidase A			
putP	2,136	569	784	570	0.3	0.4	1.0	0.7	Sodium/proline uptake symporter	TGCTACGCATGTCACA	321	425
tnaA	14,210	210	540	214	0.01	0.04	1.0	0.4	Tryptophanase	TGTGATTCGATTCACA TGTGAATCGAATCACA TGTATTCTGCTTCACG	−371 −356 750	28 42 542
tnaL	13,125	159	655	187	0.01	0.05	1.2	0.3	*tna* operon leader peptide	TGTGATTCGATTCACA TGTGAATCGAATCACA	−91 −76	28 42
Chaperone and stress protein-encoding genes												
Chaperones												
dnaJ	3,025	2,312	1,423	910	0.8	0.5	0.4	0.6	DnaJ chaperone			
dnaK	14,241	4,238	3,251	1,654	0.3	0.2	0.4	0.5	DnaK chaperone (heat shock protein 70)			
grpE	11,811	3,392	3,774	3,109	0.3	0.3	0.9	0.8	GrpE chaperone			
mopA	13,764	3,581	4,191	2,358	0.3	0.3	0.7	0.6	GroEL protein			
mopB	12,188	5,577	4,935	3,847	0.5	0.4	0.7	0.8	GroES protein			
ATP (GTP)-dependent proteases												
clpB	3,413	411	606	349	0.1	0.2	0.8	0.6	ClpB ATP-hydrolyzing regulator of ClpP			
clpP	2,172	667	858	756	0.3	0.4	1.1	0.9	ClpP ATP-dependent protease, proteolytic subunit			
clpX	2,098	1,259	1,126	1,069	0.6	0.5	0.8	0.9	ClpX ATP-hydrolyzing regulator of ClpP			
hflB	4,831	2,532	2,022	3,001	0.5	0.4	1.2	1.5	Cell division protein FtsH (ATP-GTP-dependent protease)			
hflK	2,304	1,230	646	1,475	0.5	0.3	1.2	2.3	HflK regulator of HflB protease			
hflX	2,755	1,103	928	1,662	0.4	0.3	1.5	1.8	HflX GTP-binding regulator of HflB protease			
hslU	5,819	1,795	1,183	1,335	0.3	0.2	0.7	1.1	ATP-dependent protease, ATP-binding subunit			
hslV	3,788	1,270	709	896	0.3	0.2	0.7	1.3	ATP-dependent protease, protease subunit			
htpX	1,817	754	895	702	0.4	0.5	0.9	0.8	Heat shock protein HtpX (putative protease)			
lon	2,983	1,135	1,094	1,056	0.4	0.4	0.9	1.0	Lon protease			

Continued on following page

TABLE 2—*Continued*

Name	wt	wt + G	*crp*	*crp* + G	Ratio of wt + G/wt	Ratio of *crp*/wt	Ratio of *crp* + G/wt + G	Ratio of *crp* + G/*crp*	Function	Crp sequence	Position 1	Rank 1
Heat shock proteins												
[*hslS*	7,011	552	454	340	0.1	0.1	0.6	0.7	Small heat shock protein HslS (IbpB)			
[*hslT*	7,515	581	705	378	0.1	0.1	0.7	0.5	Small heat shock protein HslT (IbpA)			
htpG	2,497	266	534	143	0.1	0.2	0.5	0.3	Heat shock protein HtpG			
Miscellaneous genes												
deoD	3,446	1,465	1,863	881	0.4	0.5	0.6	0.5	Purine nucleoside phosphorylase			
ftsJ	3,506	932	778	1,547	0.3	0.2	1.7	2.0	Cell division protein FtsJ (ribosomal large-subunit methyltransferase)			
miaA	4,117	1,587	1,660	2,025	0.4	0.4	1.3	1.2	tRNA isopentenylpyrophosphate transferase			

[a] Brackets indicate genes included within a single operon or regulon. Other conventions are as described in the footnotes to Table 1.

tance to glucose repression. The genes that most clearly showed dual control of catabolite repression by Crp and Cra were *gat*, *mgl*, *tre*, and *dsd* (data not shown). These observations suggest that in several cases, catabolite repression depends on both Crp and Cra (32, 33). The fact that many genes show residual glucose repression in the *crp* mutant is not surprising since multiple mechanisms of catabolite repression are recognized in *E. coli* (35).

Crp also regulates genes encoding enzymes, permeases, and regulators that initiate the catabolism of amino acids as both carbon and nitrogen sources. Representative genes encode (i) aspartase (AspA), (ii) D-serine dehydratase (DsdA), (iii) the primary proline uptake permease (PutP), (iv) tryptophanase (TnaA), and (v) the *tna* operon leader peptide (TnaL). One gene encoding an oligopeptidase (PrlC) is also listed. Many additional carbon metabolic genes not meeting our rigorous criteria for inclusion in Table 2 were similarly found to be subject to Crp-dependent catabolite repression, as expected on the basis of published results, as well as our phenotypic data (see below; see also Table S1 at our website).

Several additional interesting enzyme- or transporter-encoding genes proved to be subject to strong glucose repression mediated at least partially by Crp (see our website). For example, the *nmpC* and *tsx* outer membrane porin-encoding genes fell into this category. Both exhibited only partial relief from catabolite repression in the *crp* mutant, but a *crp-fruR* double mutant (32, 33) showed no glucose repression (unpublished results). The same behavior was observed for the *dadA* and *dadX* genes encoding D-amino acid dehydrogenase and alanine racemase, respectively, as well as the genes encoding the dicarboxylate transporters DctA and DcuA. Crp-binding sites could be identified in the control regions of some of these genes but not others. It therefore appears that Crp and the *fruR* gene product, Cra, cooperate in the regulation of many operons in *E. coli*.

Phenotypic analyses. We conducted phenotypic analyses, measuring the oxidation of various carbon and nitrogen sources with 96-well Biolog microtiter plates (3). For carbon sources of interest that were not included in the Biolog plates, or where the responses were ambiguous, we conducted studies of growth on minimal agar plates containing the carbon source at 0.2%. Table 3 summarizes some of the results. For a more complete compilation of the phenotypic data for carbon and nitrogen sources found to be under Crp control, see our website (Table S2).

Almost all of the carbon sources tested (e.g., acetate, fumarate, D- and L-malate, succinate, α-ketoglutarate, lactate, glycerol, D,L-α-glycerol phosphate, maltose, maltotriose, and glucitol) gave positive wild-type responses but negative *crp* mutant responses. Of the potential nitrogen sources, L-aspartate, sev-

TABLE 3. Oxidation or utilization of various carbon sources by isogenic wild-type and *crp* mutant strains of *E. coli*

Carbon source	Strain	
	wt[a]	*crp*
Acetate		−
Fumarate	+	−
D,L-Malate	+	−
Succinate	+	−
Formate[b]	+	−
Galactitol[b]	+	−
D-α-Ketoglutarate	+	−
D,L-Lactate	+	−
Glycerol	+	−
D,L-α-Glycerol phosphate	+	−
Maltose	+	−
Maltotriose	+	−
Mannose[b]	+	±
Galactose[b]	+	±
D-Glucitol	+	−
Trenalose[b]	+	±
L-Aspartate	+	−
Glycyl-L-aspartate	+	−
Glycyl-L-glutamate	+	−
L-Proline	±	−
Tryptophan[b]	+	−
D-Serine	+	±

[a] wt, wild type.
[b] Compound studied by substrate utilization on minimal agar plates. All others were determined with Biolog 96-well plates, which measure substrate oxidation.

TABLE 4. Correlation between the transcriptome results of this study and gene expression analyses reported in the literature

Method of assay	Condition	Reference	Fold change	Array result
ptsG-lacZ	Minimal medium + glucose/minimal medium + glycerol	30	7.5	3.2
fis (Northern blotting)	*crp*/wt	22	2.0	2.8
sucA-lacZ	LB + glucose/LB	23	0.6	0.2
mdh-lacZ	LB + glucose/LB	24	0.6	0.2
fumA-lacZ	LB + glucose/LB	25	0.2	0.3
gltA-lacZ	LB + glucose/LB	26	0.4	0.1
tnaA (tryptophanase sp act)	Minimal medium + tryptophan + glucose/minimal medium + tryptophan	13	0.2	0.01

eral peptides, and proline also gave positive wild-type responses but negative *crp* mutant responses. D-Serine showed a decreased but appreciable response in the mutant relative to that in the wild type. Oxidation of galactitol, galactose, mannose, trehalose, and tryptophan did not show differences between the wild type and the *crp* mutant on the Biolog plates, but when these compounds were assayed for growth on minimal plates, the wild type, but not the *crp* mutant, proved to use galactitol, trehalose, and tryptophan while both strains utilized galactose and mannose, with the mutant growing less well than the wild type. Thus, the transcriptome data are in agreement with the phenotypic data in all of the cases tested.

Correlation between transcriptome and classical approaches. Table 4 summarizes the results of our transcriptome analyses and compares them with corresponding published data obtained by classical approaches. In most cases, the fold effects of glucose on gene expression in a wild-type background were examined. In every such case, the direction of the effect (repression or activation) was the same. Similar agreement has been reported for transcriptome analyses versus traditional methods conducted with *Bacillus subtilis* (21). Quantitative differences may be within the range of experimental error considering that in all cases different strains were used and that in several cases different media were used. The different genetic backgrounds could have contributed to the observed differences. However, for all but one of the glucose-repressed genes, the fold repression by glucose was greater when assayed by microarray technology than when *lacZ* reporter gene fusions were used. The *lacZ* fusion technology may introduce artifacts affecting gene expression, as discussed previously (27).

Crp-mediated control of stress response genes. The stress response proteins whose synthesis proved to be under Crp control include the major chaperone proteins DnaJK, GrpE, and GroEL/ES (MopAB) (Table 2). Five cytoplasmic ATP-dependent proteases or protease complexes (ClpPBX, HflBKX, HslUV, HtpX, and Lon) also proved to be subject to strong Crp-mediated catabolite repression. In the case of the *hfl* genes, the *crp* mutation reversed glucose repression (Table 2) while the *fruR* mutation abolished it (data not shown). Finally, three biochemically ill-defined heat shock genes (*hstS*, *hstT*, and *htpG*) are listed in Table 2. We also identified putative stationary-phase- or carbon starvation-inducible genes (e.g., *cspD*, and *yjiY*) that are subject to strong Crp-dependent glucose repression (42). Crp-binding sites could be found upstream of both the *cspD* and *yjiY* genes. It is therefore clear that many stress response genes in *E. coli* are subject to Crp-dependent catabolite repression, in agreement with the fact that *crp* mutants are sensitive to certain types of stress and starvation conditions (18, 19).

From a mechanistic standpoint, it is important to note that we were not able to find Crp-binding sites in the control regions of most of these stress genes and operons. The regulatory consequences of inclusion of glucose in the medium and of the loss of the Crp protein are therefore presumed to be indirect in most cases. Sigma-32 is known to be under Crp control, but its contribution to the rates of gene transcription should be minimal under the conditions used in our studies (19). Of the three miscellaneous genes (Table 2), one is a purine nucleoside phosphorylase while two are involved in translational regulation.

Confirmation by RT-PCR. Three of the genes exhibiting Crp-dependent glucose regulation were selected for confirmation by RT-PCR. Table 5 summarizes the results and compares them with the transcriptome data reported in Tables 1 and 2. The three genes analyzed are the *aceE* gene, which is subject to catabolite activation (Table 1), and the stress response genes *clpB* and *hslT*/*ibpA* (Table 2). Excellent agreement between the two methods is apparent. The only minor discrepancies are observed for the *clpB* and *hslT*/*ibpA* genes after growth of the *crp* mutant in LB plus glucose. In these two instances, the differences between the two methods are only twofold. Since the repressive effect of glucose was about 10-fold in both cases, this difference may be within the range of experimental error.

DISCUSSION

In this paper, we have provided a comprehensive analysis of Crp-mediated catabolite control in *E. coli*. The transcriptome data (Tables 1 and 2, as well as Table S1 at our website) were confirmed and extended by (i) citing published data for representative genes (Table 4), (ii) conducting phenotypic analyses

TABLE 5. Comparison of RT-PCR and microarray analyses of gene expression in response to glucose and a *crp* mutation[a]

Gene	RT-PCR				Microarray			
	(wt + G)/wt	*crp*/wt	*crp* + G/wt + G	*crp* + G/crp	(wt + G)/wt	*crp*/wt	*crp* + G/wt + G	*crp* + G/crp
aceE	2.5	1.6	0.6	0.9	4.4	2.4	0.5	1.0
clpB	0.1	0.2	1.8	0.8	0.1	0.2	0.8	0.6
hslT/*ibpA*	0.1	0.1	1.5	0.7	0.1	0.1	0.7	0.5

[a] The values shown are signal ratios. wt, wild type; G, growth in LB medium plus glucose.

that reflect gene expression control (Tables 3 and S2), and (iii) conducting RT-PCR experiments (Table 5). The agreement between the different methods was striking, allowing us to conclude that the transcriptome data are reliable.

Far more highly expressed genes proved to be subject to direct catabolite repression (Table 2) than catabolite activation (Table 1) (see also Table S1 at our website). Most of the carbon metabolic genes subject to catabolite control showed responses to glucose and the loss of Crp as expected. Thus, many genes that function in the initiation of carbon utilization proved to be strongly repressed, as were several central carbon metabolic genes (Table 2). By contrast, genes that showed glucose activation included the glucose transporter gene *ptsG*, those that encode specific metabolic enzymes that are required for growth under glucose fermentative conditions such as *aceE* and *guaB* (Table 1), a few regulatory nucleic acid-binding protein-encoding genes such as the *fis* and *spf* genes, and many genes subject to a positive growth rate response. Only in the former genes were Crp-binding sites identified in the control regions (Table 1).

Perhaps of greatest interest was the surprising number of stress genes that are subject to catabolite repression (Tables 2 and 5). These genes encode many chaperone proteins (1, 4, 38), all of the important cytoplasm ATP-dependent protease complexes that can function both as general chaperones and for protein degradation (9, 11, 20), and both heat shock and cold shock genes (12, 31, 40). Sometimes the repressive effects were very large (Table 2), even though a Crp-binding site could not be identified in the control regions of the encoding operons. Indirect mechanisms mediated by transcription factors under Crp control are therefore likely. Further experiments are required to ascertain what these mechanisms are.

ACKNOWLEDGMENTS

We thank Ron Sapolsky for assistance in the data analysis and Mary Beth Hiller for assistance in the preparation of the manuscript.

This work was supported by National Institutes of Health grant GM64368 from the National Institute of General Medical Sciences. G.G. acknowledges a sabbatical fellowship from the Dirección General de Asuntos del Personal Académico de la Universidad Nacional Autónoma de México.

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