JOURNAL OF BACTERIOLOGY, Apr. 1993, p. 2026–2036
0021-9193/93/072026-11$02.00/0

Vol. 175, No. 7

Global Regulation of Gene Expression in *Escherichia coli*†

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Received 2 November 1992/Accepted 29 January 1993

Global transcription responses of *Escherichia coli* to various stimuli or genetic defects were studied by measuring mRNA levels in about 400 segments of the genome. Measuring mRNA levels was done by analyzing hybridization to DNA dot blots made with overlapping lambda clones spanning the genome of *E. coli* K-12. Conditions examined included isopropyl-β-D-thiogalactopyranoside (IPTG) induction, heat shock, osmotic shock, starvation for various nutrients, entrance of cells into the stationary phase of growth, anaerobic growth in a tube, growth in the gnotobiotic mouse gut, and effects of pleiotropic mutations *rpoH*, *himA*, *topA*, and *crp*. Most mapped genes known to be regulated by a particular situation were successfully detected. In addition, many chromosomal regions containing no previously known regulated genes were discovered that responded to various stimuli. This new method for studying globally regulated genetic systems in *E. coli* combines detection, cloning, and physical mapping of a battery of coregulated genes in one step.

Escherichia coli is a facultative anaerobe. Continuous shifts from one condition to another are its way of life (40). A shift in conditions often requires an extensive adaptive response for growth or even for survival. Usually a group of genes, termed a stimulon, is involved in the response to a particular stimulus. A stimulon may consist of many regulons, each of which is defined by a group of genes under the control of the same regulatory factors. A regulon consists of operons, each of which is under the control of the same promoter(s) (34).

A traditional method of examining cell response has been the use of O'Farrell two-dimensional electrophoresis gels (38) to resolve individual proteins of cells grown under different conditions. Induced or repressed synthesis of proteins can be detected by comparing the patterns. Heat shock proteins and starvation-induced proteins, for example, were originally identified by this method. Since the genetic origin of a particular protein spot on a gel is generally not known, subsequent mapping and/or cloning of a possibly large set of genes for the affected proteins is not straightforward.

Another widely used approach is to screen a collection of bacteria carrying a reporter gene inserted at random locations in the genome by transposon mutagenesis (18, 27, 32, 45). By assaying or selecting for the reporter gene before and after a treatment, genes affected by the treatment can be mapped and cloned easily by using the linked transposon as a marker. One of the drawbacks of this method is that it is limited to unessential genes, unless some genetic tricks are undertaken (46).

In this report, we present a new technique in which detection, cloning, and mapping of a responding gene is achieved in one step and which is applicable to all categories of genes. This technique employs hybridization to measure mRNA levels expressed from various genetic regions using an overlapping clone set in λ vectors (11, 21). Comparison of mRNA levels transcribed from each cloned region between control and experimental situations allows us to detect the induced or repressed genes contained in the λ clones. Our technique can be applied to *E. coli* grown under the influence of a variety of chemical, physical, or physiological treatments. Growth under nutrient starvation, heat shock, or osmolarity variation can mimic conditions outside the animal gut. Studying gnotobiotic animals with gut *E. coli* populations can shed light on gene expression in the natural host (51, 52), and the resulting gene expression pattern can be compared with that under anaerobic conditions in a test tube. In addition, mutations of *E. coli* genes, especially those which show pleiotropic effects, can be studied in the laboratory to uncover the molecular mechanisms behind the adaptive responses in *E. coli*. Examples include *rpoH*, *himA*, *topA*, and *crp*, which code for the heat shock-specific transcriptional initiation factor σ^{32}, the α subunit of the integration host factor (IHF), DNA topoisomerase I, and the cyclic AMP (cAMP) receptor protein CRP, respectively.

MATERIALS AND METHODS

Media, buffers, and reagents. MOPS minimal medium contains 0.4% glucose, 10 mM NH_4Cl, 1.32 mM potassium phosphate, 0.53 mM $MgCl_2$, 0.28 mM K_2SO_4, 0.01 mM $FeSO_4$, 0.5 μM $CaCl_2$, 50 mM NaCl, 4 mM Tricine, 40 mM MOPS [3-(*N*-morpholino)propanesulfonic acid] (pH 7.2), and trace elements (35). NZC broth contains 1% NZ amine (Sheffield Products), 0.5% NaCl, 0.1% Casamino Acids, and 8 mM $MgSO_4$. Luria-Bertani medium (LB) is 1% Bacto Tryptone, 0.5% yeast extract, and 1% NaCl. PBS (phosphate-buffered saline) contains 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.5.

Bacterial strains. *E. coli* K-12 strain W3110 was from our laboratory stock. *rpoH* mutant strain CAG9301 (MG1655 *rpoH120*::*kan zhg-21*::Tn*10* [53]) was kindly provided by C. A. Gross's laboratory. *crp* mutant strain RZ1331 (MG1655 Δ*crp rpsL* [12]) and *lacI* mutant strain RZ4502 (MG1655 *lacI42*::Tn*10*) were kindly provided by W. S. Reznikoff's laboratory. The *himA* mutant strain K2691 (K37 *himA*Δ*sma*, carrying an intragenic deletion of *himA* [16]) and its isogenic wild-type strain K37 were kindly provided by D. I. Friedman. Mutant *topA10* strain DPB923 and its isogenic wild-type strain DPB924 (3) were kindly provided by S. Cohen's laboratory.

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Bacterial growth and treatments. Isopropyl-β-D-thiogalactopyranoside (IPTG) induction was done by adding IPTG to a final concentration of 1 mM in a mid-log culture of W3110 in NZC broth for 60 min before harvesting. For the severe heat shock treatment, a flask of W3110 culture in MOPS minimal medium was grown at 37°C to mid-log phase and was moved to a 50°C water bath shaker for 7 min before harvesting. For the mild heat shock treatment, a flask of MG1655 culture in LB was grown at 16°C to mid-log phase and was then moved to a 42°C water bath shaker for 7 min before harvesting. Phosphate starvation was achieved by growing W3110 cells in MOPS minimal medium with a decreased concentration of phosphate (0.1 [experimental] or 1.32 mM [control]). After 8 h of growth, the cells were harvested at an optical density at 550 nm (OD_{550}) of 0.4. The culture was still growing upon harvesting but at a much lower rate. Nitrogen starvation was done by growing W3110 cells in MOPS minimal medium with a decreased concentration of ammonium chloride (2.7 [experimental] or 10 mM [control]). Glucose starvation was achieved by growing W3110 or *crp* cells in MOPS minimal medium with a decreased concentration of glucose (0.05 [experimental] or 0.4% [control]).

For ammonium and glucose starvation, the growth rate was only slightly lower than that of the control until an abrupt transition into the stationary phase upon depletion of the limiting nutrient at an OD_{550} between 0.4 and 0.5. Cells were harvested 50 min after the onset of starvation. For W3110 cells entering the stationary phase in full-strength MOPS minimal medium, the transition in the growth curve was also sharp and occurred at an OD_{550} of 0.97. Cells were harvested 50 min after entering stationary phase. In contrast to the sharp transition in the growth curve of cells in minimal medium, W3110 cells entering the stationary phase in the complex medium NZC was gradual and smooth. Cells were harvested during the transition at an OD_{550} of 0.97.

For anaerobic growth, W3110 cells were grown in 8 ml of MOPS minimal medium with air excluded from the tightly capped tube which was rotated at 37°C. Cells were harvested at mid-log phase at an OD_{550} of 0.25. Under this growth condition, bacteria entered the stationary phase at an OD_{550} of 0.45 with a viable cell titer of 5×10^8/ml, which was much lower than that of the aerobically grown cells. W3110 failed to grow in NZC broth anaerobically. For W3110 to grow in the mouse gut, 5×10^{10} bacterial cells in 10 ml of PBS were spread onto the food and put into the drinking water for 11 germfree female BALB/c mice. This practice was continued for 3 weeks before sacrifice of the mice and recovery of bacterial cells. One germfree mouse that had not had bacteria introduced into its food and water was sacrificed, and no bacteria or nucleic acids were found in its cecum. The germ-free cecum contained about 1.5 ml of semisolid material and was about fivefold larger than the normal nongermfree cecum. After the introduction of bacteria, the cecal size remained the same and the viable bacterial density in the cecum reached 1.3×10^{10}/ml, which was much higher than that grown in a flask.

Osmotic shock was done by adding 8 ml of prewarmed 3 M NaCl (in NZC broth) into 40 ml of W3110 culture in NZC at mid-log phase (an additional 0.5 M NaCl to NZC) and harvested 20 min later. The *rpoH* mutant CAG9301 and its isogenic wild-type strain MG1655 were grown in LB at 16°C with vigorous shaking and harvested in the mid-log phase. The *himA* mutant and its isogenic wild-type strain K37 and the *topA10* mutant DPB923 and its isogenic wild-type strain DPB924 were all grown in NZC broth at 37°C aerobically to mid-log phase before harvesting.

Total RNA extraction from *E. coli* cells. For cells grown in flasks, 10 ml of cell culture was immediately mixed with an equal volume of crushed ice at −70°C and spun at 4°C in a 50-ml centrifugation tube. The cell pellet was resuspended in 0.6 ml of ice-cold buffer (10 mM KCl, 5 mM $MgCl_2$, 10 mM Tris; pH 7.4) and then immediately added to 0.6 ml of hot lysis buffer (0.4 M NaCl, 40 mM EDTA, 1% β-mercaptoethanol, 1% sodium dodecyl sulfate [SDS], 20 mM Tris; pH 7.4) containing 100 μl of water saturated phenol. This mixture was incubated in a boiling water bath for 40 s. The debris was removed by centrifugation. The supernatant was extracted with phenol-chloroform five times, ethanol precipitated, and dried. The dried RNA pellet was dissolved in water before use.

To recover *E. coli* cells growing in the mouse gut, the whole cecum was taken out and immediately dispersed in 6 ml of ice-cold PBS by vigorous agitation. The cells were filtered through a 5-μm-pore-size Millipore membrane filter, spun down, resuspended in 0.2 ml of lysis buffer, added into a new tube containing 0.2 ml of hot lysis buffer and 0.2 ml of water-saturated phenol, and incubated in a boiling water bath for 40 s. RNA was recovered following the same steps described above. Between 50 and 125 μg can be recovered from each mouse, depending on how much was lost during the filtration. We always used a glass fiber pad before the Millipore filter to facilitate filtration. No nucleic acids was recovered from the germfree mouse that did not have bacteria introduced into its food and water.

Preparation of ^{32}P-labeled total cDNA. The random hexamer priming method was used (13). The reaction mixture contained 15 μg of total RNA, 5 μg of $pd(N)_6$ (random hexamers from Pharmacia, Inc.), 0.5 mM dATP, 0.5 mM dGTP, and 0.5 mM dTTP, 5 μM dCTP, 100 μCi of [α-^{32}P]dCTP (3,000 Ci/mmol), 50 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, 40 mM KCl, 0.5 U of RNasin (Promega, Inc.) per μl, and 50 U of avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc.) in a total volume of 50 μl. The reaction was allowed to continue overnight at room temperature. EDTA and NaOH were then added to final concentrations of 50 mM and 0.25 M, respectively, and the mixture was incubated at 65°C for 30 min to degrade the RNA templates. The cDNA was then ready to use after neutralization by adding HCl and Tris buffer. Free nucleotides were not removed. Usually, more than one half of the isotope was incorporated.

Preparation of DNA dot blots of the *E. coli* Genomic encyclopedia. First, DNA concentration of each clone was determined by hybridization of blots of clone DNAs and of a λ concentration standard with a λ DNA probe. Hybridization was quantified by the Betascope 603 blot analyzer (Betagen Corp.), which collects beta particles directly from the blot with high efficiency. Then, 0.5 μg of each clone DNA was incubated in 0.25 M NaOH and 10 mM EDTA at 65°C for 60 min to denature DNA and degrade residual RNA contaminants. By using a homemade manifold filtration system (21 by 21 wells), each clone DNA was blotted onto a GeneScreen Plus nylon membrane (19 by 19 cm) in the alkaline solution. After neutralization, the blots were baked at 85°C for 2 h under vacuum. Twelve spots of λ (strain *c*I875*S*am7) DNA were also included in all blots as a control. No signal was seen, indicating that no expression from the cryptic lambdoid genes was detectable.

Hybridization and quantification. The DNA dot blot was hybridized to ^{32}P-labeled total cDNA in a solution containing

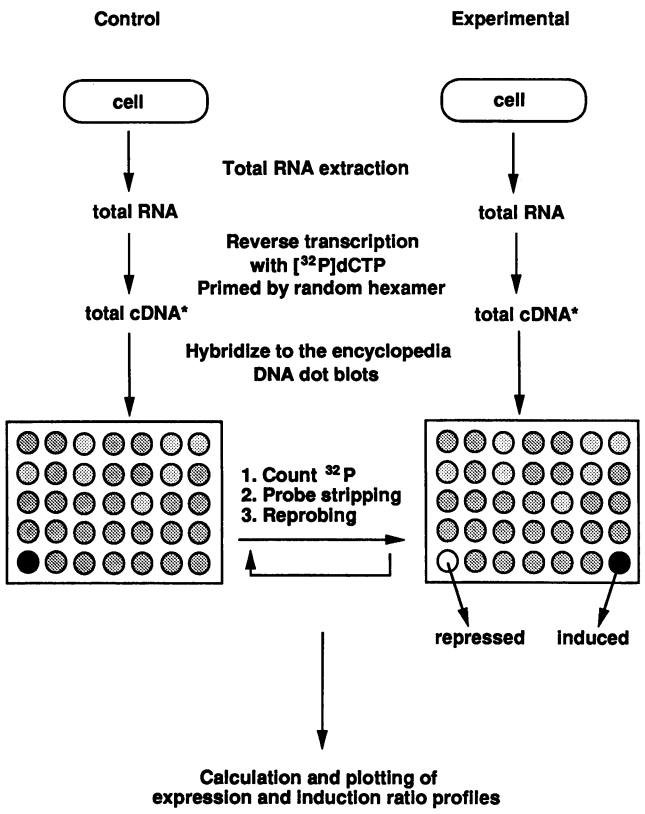

FIG. 1. Experimental design.

0.1% polyvinylpyrrolidone, 0.1% Ficoll, 0.1% sodium PP_i, 0.1% bovine serum albumin, 0.5% SDS, 1 M NaCl, and 0.1 M sodium citrate, pH 7.2, at 65°C for 2 days and then washed with a solution containing 0.1% SDS, 100 mM NaCl, and 10 mM Na-citrate, pH 7.2. The same dot blot was used for hybridization with both control and experimental cDNAs, with an alkaline probe stripping procedure (soaked twice in 0.25 M NaOH–0.75 M NaCl at room temperature, 30 min each, neutralized, and completely dried at 65°C for at least 30 min) between the two hybridizations. We found that the procedure was more reproducible when the same blot was used for comparison. We have been able to reprobe the GeneScreen Plus nylon membranes more than 15 times. Quantification was done with the Betascope 603 blot analyzer (Betagen Corp.).

RESULTS

Experimental design and data analysis. Figure 1 illustrates the experimental design. Control and experimental cultures were grown, and total RNA was isolated by the hot phenol method. ^{32}P-labeled cDNA was prepared in vitro with reverse transcriptase primed with mixed hexanucleotides. The λ clones from the *E. coli* DNA encyclopedia were used to prepare DNA dot blots which were analyzed with a λ-specific probe to ensure equal DNA quantities for the clone spots (see Materials and Methods). Each control and experimental hybridization was performed at least twice by reusing the same blot. Radioactivity of beta-particle emission was measured directly from the blot for each hybridization with the Betascope 603 blot analyzer (Betagen Corp.). The machine was programmed to output the counts per minute for each spot automatically. After subtracting the background level, which was taken from the blank area of each blot, the activity of each clone relative to the total counts per minute for all clones was calculated. Ratios of experimental to control values were taken from all possible combinations to determine the overall mean induction ratio and standard error. In 6,431 determinations of induction ratios, the standard error averaged 12% and was less than 20% in 82% of cases and less than 30% in 93% of cases. Thus, we regard induction ratios of >1.3 or <0.7 as significant and ratios of >2 or <0.5 as highly significant. We used the same blot with stripping, because hybridization ratios obtained from comparisons between different blots had standard errors about twice as high. Figure 2 shows four examples of typical hybridization patterns. Figure 3 shows the baseline transcription profile (Fig. 3-1) and 17 induction ratio profiles (Fig. 3-2 to 3-18). The original data for each clone are in the Ph.D. thesis of S.-E. Chuang (8) and are available upon request. Genes genetically mapped (2) and physically located (41, 42) in all individual miniset clones are also described in reference 8.

Phage DNA encyclopedia. The miniset of λ clones of the genomic encyclopedia that Kohara et al. (21) made from *E. coli* K-12 W3110 was obtained from F. C. Neidhardt, who received them from K. Isono's laboratory. These clones were single plaque purified. Each λ clone contains an insert of bacterial DNA between 9 and 21 kb. Strain W3110 has an inversion of 800 kb between the *rrnD* (min 71.9) and *rrnE* (min 90.5) operons (22). The 13 *rrn* (ribosomal RNA operon)-containing clones were excluded from the blots, because their signals are so strong that they prevent the detection of differences of their cocloned genes and blacken out the adjacent clones on the autoradiograph. These *rrn* clones were initially identified by hybridization with total cDNA probe (data not shown). The *rrn* clones are as follows: overlapping clones 124 and 125 (clones 124/125) (*rrnH* [min 5.1]), 436/437 (*rrnG* [min 56.2]), 530/531 (*rrnD-rrnE* fusion [min 71.9]), 534 (*rrnB* [min 89.7]), 548/549 (*rrnA* [min 87.3]), 557/558 (*rrnC* [min 84.6]), and 629/630 (*rrnD-rrnE* fusion [min 90.5]). Besides these seven inevitable gaps, there are eight additional small gaps not covered by Kohara's original ordered DNA library (less than 1% of the genome [21]). Some of these gaps were filled by our own clones (names starting with EC) which were constructed from strain MG1655 in Charon vectors. About 12% of this minimum set are redundant clones and were not included in the blots to yield room for full coverage.

After the data were collected, we found in connection with another project that some of the Kohara isolates had unexpected restriction maps. We therefore exhaustively mapped all clones, finding that a total of 28 of the 476-clone miniset did not correspond to the correct map of the *E. coli* genome, namely, clones 103, 112, 118, 147, 160, 176, 179, 240, 249, 266, 307, 314, 322, 323, 335, 337, 342, 357, 370, 373, 378, 380, 404, 519, 528, 609, 665, and 674. The correct clones were not found in the isolates from the original samples. A new set of clones obtained directly from Japan had similar problems. Therefore, data for all these clones were excluded. Overall, there is about 92% coverage. The gaps are indicated directly above the map axes in Fig. 3. The problems with the miniset have now been solved in the commercially available blots sold by Takara Shuzo Co. (37). We now also have all the corrected 28 Kohara clones and clones of our own collections which cover most of these *E. coli* chromosome regions in strain MG1655, which are available from us upon request.

Baseline expression profile of *E. coli*. The average abun-

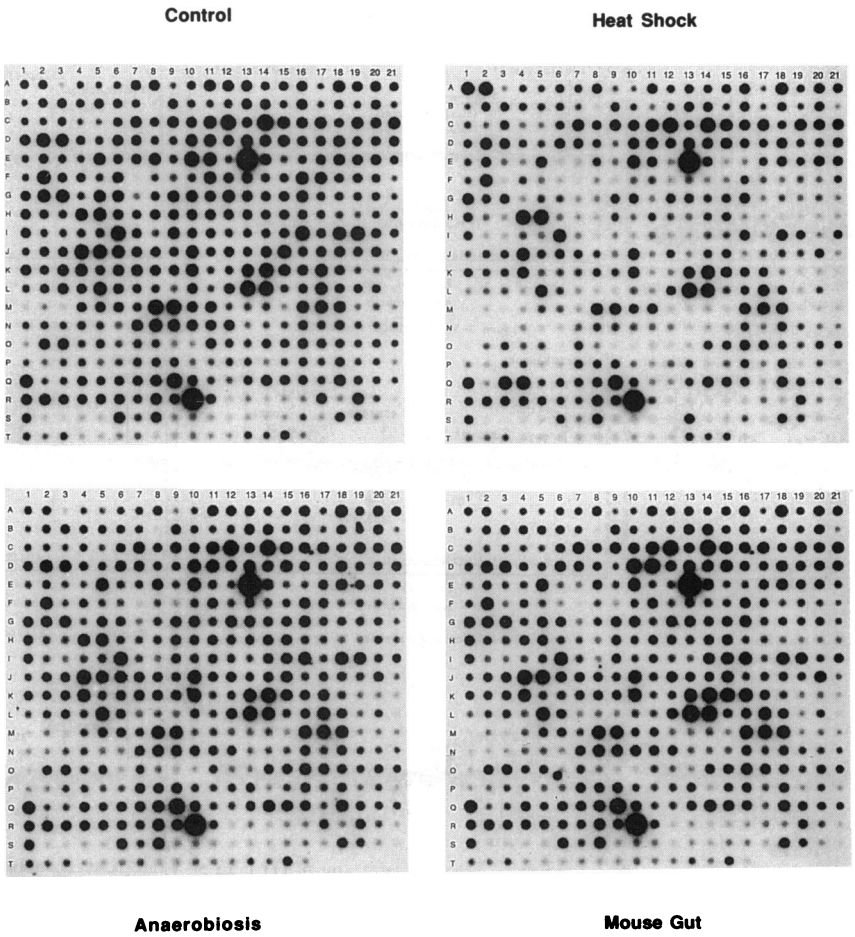

FIG. 2. Hybridization pattern examples. In these four examples (control, heat shock, anaerobiosis, and growth in the mouse gut) all clones were from Kohara's collection arranged with clone 101 in the top left position and clone 676 in the bottom right position. The picture was taken directly from the Betascope's printout.

dance of mRNA transcribed from various intervals of the *E. coli* genome as a whole are plotted against the map position in Fig. 3-1. To obtain this basal expression pattern, we averaged the data for the 27 controls used in all of the experiments. The clones that contain genes which are most active, for example, the 12 most active clones shown in Fig. 3-1, are scattered around the genome. The activity of the most active clone was about 2 orders of magnitude higher than those of the least active ones.

IPTG induction. Figure 3-2 shows the induction ratio profile of *E. coli* W3110 cells induced in mid-log phase with 1 mM IPTG for 1 h before harvesting. The two *lac* sequence-containing clones, clones 138 and 139 (min 8) showed 2.5 times more hybridization signal in the presence of IPTG than in its absence. When these clones were examined in repressor-negative cells (*lacI*), signal was not detected (data not shown). These observations validate the experimental design of our system. The induction ratio of 2.5 for this experiment is much less than the 700-fold increase of β-galactosidase enzymatic activity we obtained. This result is not unexpected, since the background level of a hybridization experiment is much higher than the basal level of enzyme activity for a β-galactosidase assay. Moreover, only one-fifth of the DNA of clones 138 and 139 consists of *lac* sequences. Constitutive expression of neighboring genes on each clone will dilute the induction effect. Nevertheless, our profile assay is clearly sensitive enough to allow detection of *lac* operon induction.

In addition to the *lac*-containing clones, clones 645 and 646 (min 94) also showed significant induction. This induction did not require the *lac* repressor (data not shown). This signal probably came from the *mel* (melibiose) operon which resides in this region. This operon codes for α-galactosidase and thiomethylgalactoside permease II. While the *lac* operon is under negative control of *lacI*, the *mel* operon is under positive control of *melR* (49). It is not clear whether MelR has any role in mediating IPTG induction of *mel*. Interestingly, melibiose, the inducer of the *mel* operon, can also induce the *lac* operon (33). There are two other regions, at min 20.6 (clones 217/218) and 27.5 (clones 251/252/253), which have an induction ratio significantly higher than 1.4.

Two adjacent clones at min 46.5, clones 365 and 366, in a region containing the *mgl* operon are slightly repressed. It is most interesting that clone 365 which contains the intact *mgl* operon, has blue plaques on X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) plates without inducer IPTG and that this phenotype requires the host *lac* operon (data not shown). The *mgl* operon codes for the methylgalactose transport system and is involved in galactose taxis. It is intriguing to consider that a DNA sequence in *mgl* (or a

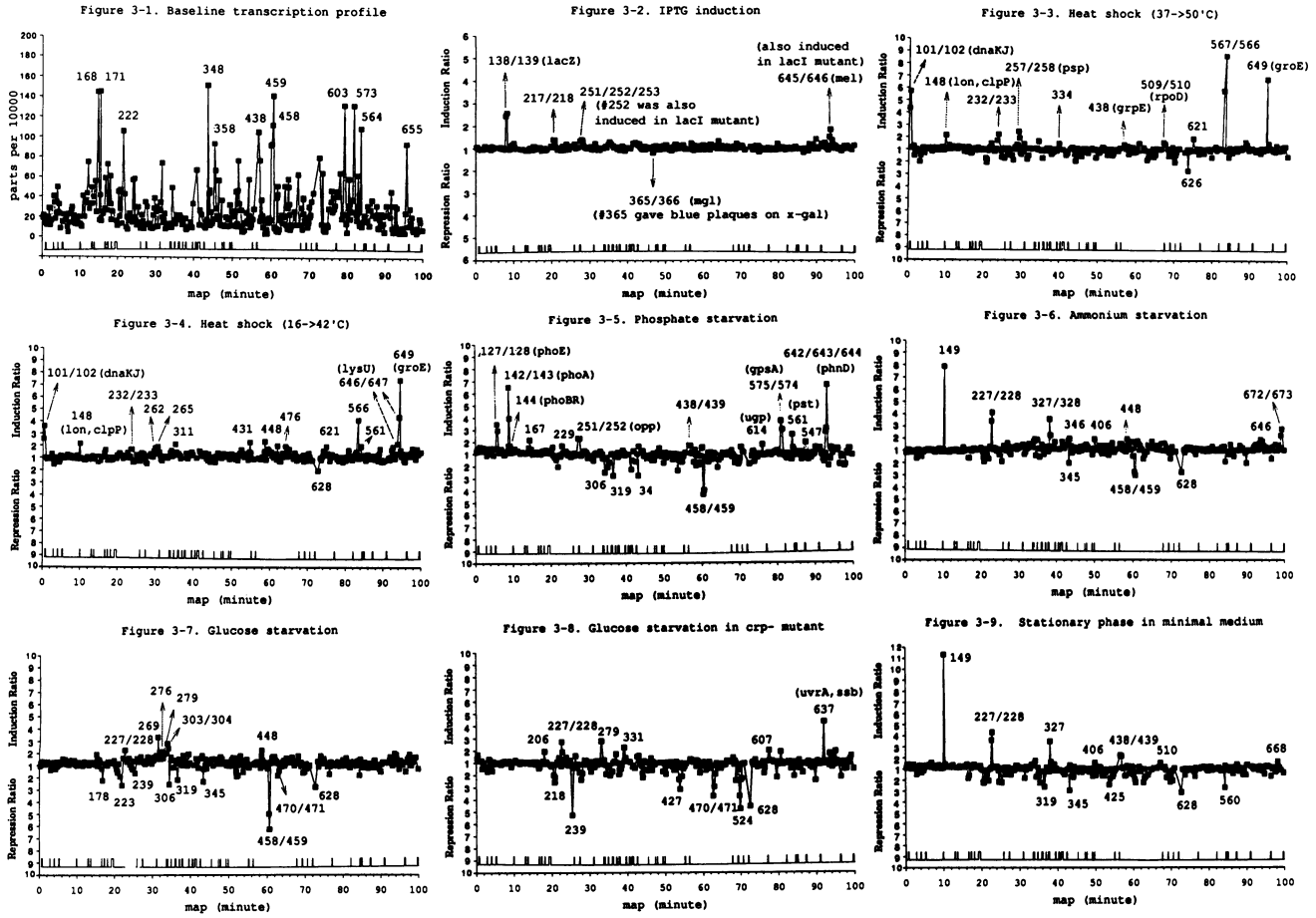

FIG. 3. Baseline transcription profile and induction ratio profiles. The average RNA level for each clone from all the control experiments is plotted with the Microsoft spread sheet program Excel (Fig. 3-1). Units are parts per 10,000 parts versus map (in minutes). The 12 most actively expressing clones are labeled. They are distributed randomly around the genome. The bars above the x axis indicate the gaps in the data sets. The 17 sets of induction ratio profiles are plotted in Fig. 3-2 to 3-18. The effects of various treatments on gene expression (Fig. 3-2 to 3-13) are expressed as the ratios of transcription with a treatment to that without a treatment. The effects of various gene products on gene expression (Fig. 3-14 to 3-18) are expressed as the ratios of transcription in the wild type to that in the mutant. For repression, the reciprocal of the induction ratio was plotted. The x axis shows the genetic map position (in minutes). The most induced or repressed clones, and clones discussed in the text were labeled. Known or possible responsible genes are indicated in parentheses.

protein product encoded by *mgl*) might interact with and titrate out the *lac* repressor to give blue plaques.

Heat shock. The second model system we studied is the response of *E. coli* W3110 to heat shock from 37 to 50°C (for reviews of the heat shock system in *E. coli*, see references 9, 15, and 36) (see Fig. 3-3 for the induction profile for this experiment). Figure 2 (top panels) shows a reproduction of dot blot hybridization patterns. Three very strong signals (greater than fourfold) are readily apparent in both Fig. 2, top panels, and Fig. 3-3. Two of them, *dnaKJ* at min 0.3 (clones 101 and 102) and *groESEL* at min 94.1 (clone 649) are well known. The highest signal, however, came from clones 567 and 566 at min 83, which contain no previously known heat shock genes. We have characterized, among other new heat shock proteins, a 15-kDa protein expressed from these clones in vivo and in vitro (8a). We have found that this region contained a bicistronic operon encoding two proteins of similar molecular masses (15 kDa), which was under the control of a $E\sigma^{32}$ promoter and was transcribed counterclockwise (8b).

Most of the previously mapped heat shock genes were detected in this study, for example, *dnaK* and *dnaJ* of clones 101 and 102, *lon* and *clpP* of clone 148, *rpoD* of clone 509, and *groEL* and *groES* of clone 649. The *htpG*-containing clone 152, the *grpE*-containing clones 438/439, and the *lysU*-containing clones 646/647 failed to show significant signal. ClpP, the catalytic subunit of the ATP-dependent protease Ti, was recently recognized as the heat shock protein F21.5 detected earlier in two-dimensional gels (23). We also confirmed the observation (31) that ClpP is a processed protein (data not shown). From the two overlapping clones 257 and 258, we identified a heat shock-inducible protein of 28 kDa. Synthesis of this protein was not stimulated by the heat shock RNA polymerase $E\sigma^{32}$ in an in vitro expression system (data not shown). This 28-kDa protein is probably the recently identified protein PspA (phage shock protein, mapped at min 28.8) (6, 7, 50). The two proteins match both in the locations of the encoding genes and the sizes of the proteins and were both inducible by a severe heat shock treatment at 50°C, but not by a milder heat shock treatment at 42°C (see below), and the induction was not σ^{32}-dependent. The *htrB* gene (20) is not responsible for the

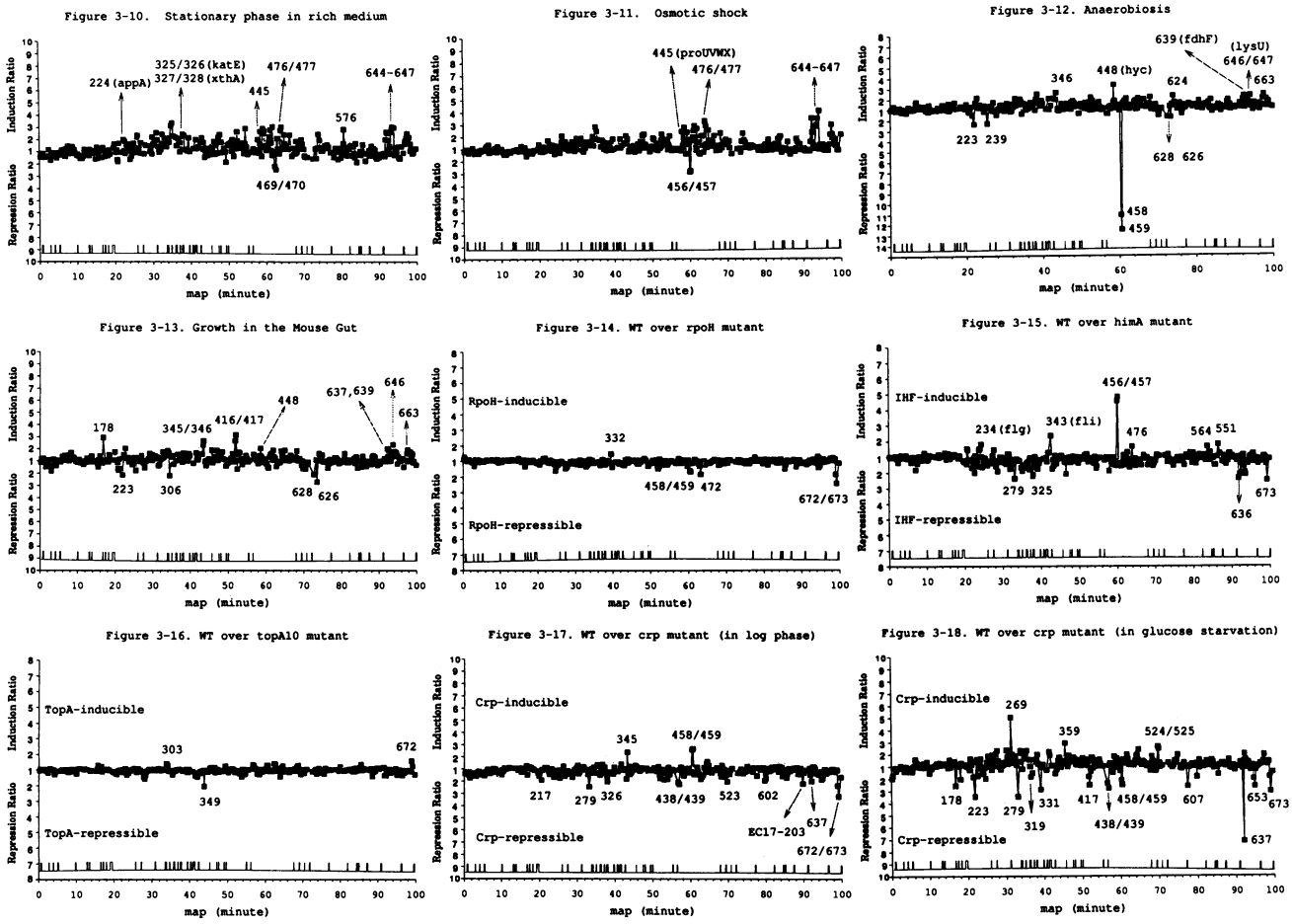

FIG. 3—*Continued.*

induction seen in clones 232 and 233 (unpublished results). Other significantly induced genetic regions (for example, clones 334 and 621) are also previously uncharacterized. The region showing the most repression was found at min 73.4 (clone 626).

In another experiment we examined heat shock of strain MG1655 in the rich medium LB from 16 to 42°C. Figure 3-4 shows the induction ratio profile. In addition to all strong signals detected in the first condition, other clones were found to be induced only in this second heat shock condition, for example, clones 262/263/264/265, 311, 431, 448, 476, 561, and the *lysU*-containing clones 646/647, which missed detection in the high-temperature heat shock treatment. Three known heat shock genes, *htpG*, *grpE*, and *rpoD* (clones 152, 438/439, and 509, respectively), which gave weak signals in the first heat shock condition, escaped detection in the second heat shock condition. *pspA*-containing clones 257/258 gave no signal for the 16 to 42°C shift, which was consistent with the reported property of the *pspA* gene. The clone most repressed by this condition was found at min 72.6 (clone 628). This clone contains the operons for most of the ribosomal proteins and is also among the most repressed by many treatments (see below).

In summary, we were able to detect almost all known heat shock loci plus many new ones with the new method. We have detected previously uncharacterized heat-inducible synthesis of 26 proteins from 12 loci, and these results will be published elsewhere.

Phosphate starvation. The effects of phosphate starvation are shown in Fig. 3-5. Most of the mapped Pho regulon genes (47) were among the signals we detected. Examples include clones 127 and 128 (*phoE* [5.8 min]), 142 and 143 (*phoA* [8.8 min]), 144 (*phoBR* [9.1 min]), 614 (*ugp* [76 min]), 561 (*pst* [84 min]), and 642/643/644 (*phnD* [93 min]) (29). Some strong new signals are found including clones 574 and 575 at min 81.2. This region contains gene *gpsA* which codes for *sn*-glycerol-3-phosphate dehydrogenase [NAD(P)$^+$]. This enzyme acts on the same substrate as the *ugp* gene products (*sn*-glycerol-3-phosphate transport system), which are known members of the Pho regulon and are among the detected clones. We suggest that *gpsA* also belongs to the Pho regulon. Preliminary results showed that synthesis of a 39-kDa protein was induced by phosphate starvation from this region (data not shown).

The second strongest new signal induced by phosphate starvation came from clones 251/252 at min 27.8. The *oppA-BCDE* operon fully occupies the 5-kb overlapping region of these two clones. This operon (encoding the oligopeptide transport system) is therefore likely to be the new member in the Pho regulon. Other new regions we detected, for example, clones 167 (min 14.4), 229 (min 23), 438/439 (min 56.8), and 547 (min 87.5) are likely to contain the remaining uncharacterized *psi* (phosphate starvation induction) genes or previously unknown ones. The strongest repression occurred in clones 458/459 at min 60.5. This region was also the strongest repressed by starvation for ammonium and glucose

and by anaerobiosis, and the repression by glucose starvation was found to be *crp* dependent (see below). Repression at min 43.3 (clone 345) also showed a similar response except in anaerobiosis.

Ammonium starvation. The ammonium starvation profile of W3110 is shown in Fig. 3-6. At least four gene regions (clone 149 [min 10.2], clones 227/228 [min 22.7], clones 327/328 [min 38.2], and clones 672/673 [min 99.1]) were strongly activated and 10 others were mildly activated by deprivation of ammonium. Induction in clone 149 was dramatic. *ffs*, coding for the 4.5S RNA, is the only gene mapped so far to this region. Three regions were strongly repressed under ammonium starvation (clone 345 [min 43.3], clones 458/459 [min 60.5], and clone 628 [min 72.6]).

Although high levels of glutamine synthetase are observed when cells are grown with limiting amounts of ammonium (the ntr system [for reviews, see references 28 and 39]), in this experiment we did not detect a RNA level increase in the region of *glnA* (clones 547/546 [min 87.6]). This result is consistent with the hypothesis that induction of *glnA* by limiting ammonium was diminished by high levels of NtrC after adaptation (44).

Glucose starvation. The ratio of RNA levels 50 min after the onset of starvation for glucose to that in log phase is shown in Fig. 3-7. For comparison, the glucose starvation behavior in a *crp* mutant is shown in Fig. 3-8. Most of the genes in the cAMP-CRP regulon, for example, the *lac* operon, were not unambiguously detected, probably because in the absence of specific inducers, induction signals from catabolite derepression caused by glucose starvation alone are not large enough to be detected by our technique. Nevertheless, some genes are induced and some are repressed in both strains, and most of them can be grouped into six categories as follows: (i) *crp*-dependent induction (induced only in the wild type, for example, clones 269, 276, 303/304, and 448); (ii) *crp*-independent induction (induced in both strains, for example, clones 227/228, and 279); (iii) *crp*-dependent repression (repressed only in the wild type, for example, clones 178, 223, 319, 345, and 458/459); (iv) *crp*-independent repression (repressed in both strains, especially, for example, clone 628); (v) induced only in the *crp* mutant (for example, clones 206, 331, 607, and 637); (vi) repressed only in the *crp* mutant (for example, clones 218, 427, and 524). In addition, some clones were more repressed in the *crp* mutant than in the wild type, for example, clones 239, and 470/471. Notice that the extreme repression, which occurs in clones 458/459 at min 60.5, was *crp* dependent. Also, the most induced region in *crp* mutant, which occurs in clone 637 at min 92.1, was not affected in the wild type. Since there is no induction in either of the flanking clones 636 and 638, the responsible gene(s) must lie in the gap region between the two, and according to the current map, the adjacent *uvrA* and *ssb* are the only genes located here.

Stationary phase. Figure 3-9 shows the induction ratio profile of *E. coli* cells 50 min into stationary phase in MOPS minimal medium compared with that of the mid-log phase. The pattern is strikingly similar to the ammonium starvation pattern discussed above. The most strongly induced clones 149, 227/228, and 327, are common to both situations. This fact indicates that this medium is probably limiting for ammonium at the end of log-phase growth. A few differences, e.g., up-regulation of clone 448 in ammonium starvation, could have resulted from the different cell densities at cessation of growth in the two experiments (see Materials and Methods).

When W3110 was allowed to grow into stationary phase in rich medium (NZC broth), the transition was not sharp and the induction ratio profile (Fig. 3-10) during the transition shows a complex response with many genes altering their level of expression. This pattern is not like the previous one but is quite similar to the osmotic shock pattern discussed below. The known stationary phase-specific expression of *appA*, *katE*, and *xthA* were detected in clones 224 (22.5 min), 325/326 (37.8 min), and 327/328 (38.2 min), respectively, but they were not among the most strongly induced. Clones 469/470 at min 62.5 were the most repressed.

Osmotic shock. Osmotic shock was produced by raising the NaCl concentration from 85 to 585 mM in a mid-log-phase culture grown in rich medium (NZC broth) and harvested 20 min later (Fig. 3-11). The pattern is complex and similar to that of the stationary-phase culture grown in rich medium. The majority of clones were induced or repressed in parallel, including 20 of the 30 most induced clones (data not shown). We observed that the osmotic shock condition actually caused the cells to cease to grow. We propose that this osmotic shock may thus mimic cells entering stationary phase. Recent studies show that *rpoS*, coding for a putative novel sigma factor, plays a major role in controlling the stationary-phase response (24). Some of the osmotically regulated genes are also stimulated by *rpoS* in response to entering stationary phase (19).

Several known osmolarity-regulated genes (for a review, see reference 10) were detected. For example, clone 445 contains *proU*, which encodes the transport system for the osmoprotectants glycine betaine and proline. Clones 456 and 457 were the only ones substantially repressed by osmotic shock. The major differences between the patterns of osmotic shock and stationary phase occur in two regions of the genome: (i) while clones 456 and 457 were the most repressed by osmotic shock, they were not affected in stationary phase; (ii) while clones 469 and 470 were the most repressed in stationary phase, they were not significantly affected by osmotic shock. Notice also that these two osmotically repressed clones 456/457 were the most dramatically up-regulated by IHF (see below).

Anaerobiosis. The most striking effect of anaerobiosis (Fig. 3-12) was the dramatic repression of genes on two adjacent clones 458/459 at min 60.5. Most likely the responsible genes lie in the 7-kb overlapping region held in common between them. This region was also the most affected by starvation for various nutrients (see above) and by CRP (see below). Two other genetic regions (clones 223 and 239) were also repressed. Some of the known anaerobically expressed genes were detected. For example, the most induced clone 448 contains the *hyc* operon (probably the same as the *ant* genes, which encode Na^+/H^+ antiporter activity [26 and 52a]. The ant_{up} mutation at 0.5 min probably represents a regulatory gene [15a]), and clone 639 contains *fdhF* (formate dehydrogenase [26]). Clones 646/647 contain *lysU* (alternative lysyl-tRNA synthetase, also a known heat shock protein [25]).

Growth of *E. coli* in the mouse gut. The expression pattern of *E. coli* cells grown in the gut of gnotobiotic mice was compared with that of cells grown aerobically in a tube (Fig. 3-13). We expected to see some similarity to anaerobiosis response (Fig. 3-12), and indeed, the responses were strikingly parallel. The most striking difference in response was the lack of significant repression of clones 458/459 in the mouse gut, in contrast to the dramatic repression of this region under anaerobic conditions in tubes in the laboratory. These clones probably contain genes that are stimulated by the mammalian host in some way, and which are possibly

implicated in the symbiosis between the bacteria and host. The detergent shock proteins recently reported by Adamowicz et al. (1) could be the proteins encoded by these genes.

Analysis of pleiotropic Mutations *rpoH*, *himA*, *topA10*, and *crp*. *rpoH* codes for the heat shock-specific transcription factor σ^{32}. Mutant CAG9301, carrying an *rpoH* null mutation, and its isogenic wild-type strain MG1655 were grown in LB at 16°C, which was the optimal growth temperature for this mutant, to mid-log phase (53). The wild-type expression to mutant expression ratio profile is shown in Fig. 3-14. The effect of *rpoH* was not dramatic. No clones were up-regulated in the presence of *rpoH* to any significant extent. Instead, a few regions were down-regulated by *rpoH*, especially clones 672/673 at min 99.1. Although RpoH is not required for growth at low temperatures, apparently it has at least some transcriptional effect on cells growing in that condition.

himA codes for the α subunit of the IHF (for a review, see reference 14). We compared *himAΔsma*, a mutant carrying an intragenic deletion of *himA*, with its isogenic wild-type strain K37 (Fig. 3-15). Clones 456/457 at min 60 and clone 343 at min 42.7 contain clear-cut examples of genes whose transcription is stimulated by IHF. Five other regions were slightly up-regulated by IHF: clone 234 at min 24.4, clone 476 at min 64, clone 564 at min 83.6, and clone 551 at min 86.5. It is worth mentioning that the regions containing the two flagellum operons were among them: the *flg* operon in clone 234 and the *fli* operon in clone 343. A few genes were slightly down-regulated by IHF, for example, clones 279, 325, 636, and 673.

topA codes for DNA topoisomerase I. We compared mutant *topA10* strain DPB923, carrying a nonsense mutation of *topA*, with its isogenic wild-type gene in strain DPB924 (Fig. 3-16). Overall, the effect of the *topA10* mutation on the transcription profile is not pronounced and could be due to the possibility that increased DNA superhelicity affects all regions equally. The most significant effect found was the down-regulation of clone 349 at min 43.8. Since the two adjacent clones were not affected, the responsible gene(s) is probably located in the 6-kb gap region between clones 348 and 350. There are no physically mapped genes in this region.

crp codes for the cAMP receptor protein CRP. The ratio of transcription of W3110 (*crp*$^+$) to MG1655 (*crp*) in log phase is shown in Fig. 3-17. The ratio in glucose starvation is shown in Fig. 3-18. During log phase, the cAMP level is low and CRP protein is likely acting alone to assert its effect. Although no cAMP-independent function of CRP has been reported (5), two gene regions were clearly more strongly transcribed in *crp*$^+$ clones during log phase: clone 345 at min 43.3 and clones 458/459 at min 60.5. Notice also that clones 458/459 were the most repressed clones by both starvation for glucose, ammonium, and phosphate and in response to anaerobiosis, and its repression by glucose starvation is *crp* dependent (Fig. 3-5 to 3-8 and Fig. 3-12). Clone 345 had a similar situation, except in anaerobiosis. Several gene regions were down-regulated, for example, clones 217, 279, 326, 438/439, 523, 602, EC17-203, 637, and 672/673.

During glucose starvation, cAMP levels increase. The increase in the *crp* mutant possibly is even higher because of the absence of the feedback repression on the *cya* gene (coding for adenylate cyclase which synthesizes cAMP) by the CRP-cAMP complex. The effect of a *crp* mutation during glucose starvation is likely to be mediated by the CRP-cAMP complex. As shown in Fig. 3-18, the effect of CRP is more pronounced than it is in the log phase. The most CRP-stimulated regions include clones 269, 359, and 524/525. The most CRP-down-regulated regions include clones 178, 223, 279, 331, 417, 438/439, 458/459, 607, 637, 653, and 673. Clone 637 had the most dramatic response, and it was this clone that was most stimulated by glucose starvation, as mentioned above, which occurred only in the *crp* mutant background (Fig. 3-7 and 3-8).

To summarize the CRP effects in both log phase and glucose starvation, the most affected clones can be grouped into six categories and will be discussed in relation to a most simplified model considering only CRP. (i) There were clones that were up-regulated in log phase only, for example, clone 345. This clone might be induced by CRP, but not affected by CRP-cAMP complex during glucose starvation. This model also explains its *crp*-dependent down-regulation by glucose starvation [Fig. 3-7]), (ii) There were clones that were down-regulated in log phase only, for example, clones 217, 523, 602, and EC17-203. These clones might be repressed by CRP, but not affected by the CRP-cAMP complex. (iii) There were clones that were up-regulated in starvation only; all of the 20 CRP-up-regulated clones in starvation with a ratio higher than 2 were found to be in this group, especially for example, clones 269, 359, and 524/525. These clones might be induced by the CRP-cAMP complex, but CRP alone has no effect, which is the conventional model of action by CRP and cAMP. This model also explains the *crp*-dependent induction by glucose starvation of clone 269 (Fig. 3-7). The other three *crp*-dependent glucose-starvation-inducible clones mentioned in the glucose starvation section, clones 303, 304, and 448 (Fig. 3-7) all can be explained by this model. (iv) There were clones that were down-regulated in starvation only, for example, clones 178, 223, 319, 331, and 607. These clones might be repressed by the CRP-cAMP complex, but CRP alone has no effect. This model also explains the *crp*-dependent repression of clones 178, 223, and 319 by glucose starvation (Fig. 3-7). (v) There were clones that were down-regulated in both phases, for example, clones 279, 438/439, 637, and 673. These genes might be repressed by CRP with or without cAMP. This model would predict no effect by glucose starvation in either the wild type or *crp* mutant (Fig. 3-7 and 3-8), and it was the case for clones 438/439 and 673. (vi) There were clones that were up-regulated in log phase and down-regulated in starvation, for example, clones 458/459. These clones might be induced by CRP but repressed by the CRP-cAMP complex. This model also explains quantitatively well the *crp*-dependent five-fold repression of clones 458/459 (Fig. 3-7) by glucose starvation in the wild type (a combination of a 2.5-fold induction by CRP [Figure 3-17] and a 2-fold repression by the CRP-cAMP complex [Fig. 3-18]).

Cross induction and repression. Some genetic regions were found to be induced by more than one treatment. For example, genes located on clones 227/228 at min 22.7 were induced by glucose starvation in both *crp* mutants and *crp*$^+$ strains, by ammonium starvation, and in response to the stationary phase in minimal medium. This region has been sequenced and characterized by Ni et al. (36a). They found that this region contained a bicistronic operon which was induced when cells entered stationary phase in an *rpoS* (also known as *katF*)-dependent manner.

Some clones were repressed by more than one treatment. For example, the *crp*-dependent, glucose starvation repressed clones 458/459 were also the most repressed under all starvation situations, including oxygen. Clones 458 and 459 were also the most up-regulated by CRP during log-phase growth. This gene region was among the most active

regions of overall transcription (Fig. 3-1). In addition, the gene regions most repressed by starvation for glucose, ammonium, and phosphate were generally common, although most of their induced ones were not. The most up-regulated clones by the *himA* gene, clones 456 and 457, were also the most repressed by osmotic shock. It may be coincidental that different genes in the affected region were involved, but on the other hand some genes are likely controlled by more than one system (48).

DISCUSSION

A most appealing feature of the global technique we have developed is that identification, cloning, and physical mapping of a regulated gene or a battery of coregulated genes can all be achieved at the same time. The method is simple, fast, direct, and not restricted to nonessential genes. To illustrate and validate this technique, we examined two classic regulatory systems, induction of the *lac* operon by IPTG and the heat shock induction system. Even with the classic systems, some interesting new results were found. IPTG induction had significant effects outside the classic *lac* operon. Heat shock induction revealed many previously uncharacterized heat shock genes. We also found that expression patterns were strikingly similar between anaerobic growth and growth in the mouse gut. The responses to a strong osmotic shock and to entering stationary phase were also unexpectedly parallel, indicating that the osmotic shock condition used here was strong enough to trigger a response similar to that of entering stationary phase (cells ceased to grow in both conditions). The similarity between responses to ammonium starvation and to stationary phase in the MOPS minimal medium indicates that it was ammonium which was depleted first and provoked a similar response to ammonium starvation. The glucose starvation pattern is different in a *crp* mutant. While some affected clones responded similarly, others responded in an opposite way or in only one strain. Induced clones in the *crp* mutant were not simply a subset of clones induced in the wild type. It is then likely that CRP plays other roles in response to glucose starvation, in addition to mediating the CRP/cAMP-dependent transcription (4, 17, 30, 43). It is informative to look at the responses in different genetic backgrounds when they are known to be relevant. In addition, we also observed positive and negative effects of CRP on global gene expression both in log phase (low cAMP level) and during glucose starvation (high cAMP level).

Perspectives. The average insert size of a λ clone in this encyclopedia ranges from 9 to 21 kb, which may easily contain more than a dozen genes. It is inevitable that individual genes of a clone may have different activities and respond to a given stimulus differently which results in a reduced signal. To address this limitation, a bigger and finer structured gene encyclopedia containing about 4,000 to 6,000 ordered clones of the size of a gene is required. A DNA dot blot (80 by 80) could be managed. Since a project to sequence the whole genomic DNA of *E. coli* is actively under way in our laboratory, the numerous M13 clones constructed for sequencing are immediately available for this purpose, or specially selected regions could be amplified by PCR (polymerase chain reaction).

Once a clone of interest is found, further studies are needed to (i) determine the responsible gene's precise location in the λ clone, (ii) identify its gene product(s), and (iii) ascertain the function(s) of the genes. The first task is straightforward. By taking advantage of the restriction map available for each clone, we can conduct Southern hybridization analysis using cDNA probes made from control and experimental total RNA. Restriction fragment(s) which hybridize more cDNA in response to a particular stimulus can be identified. We have done this successfully for the heat shock clones (8a). Single-gene sized M13 clones constructed for sequencing purpose or small PCR fragments can be similarly used in a dot blot analysis. To identify gene products, the UV-irradiated host system and the coupled transcription-translation system can be used. Twenty-six previously uncharacterized heat shock-inducible proteins have been successfully identified by this approach. Sixteen of these proteins were found to be σ^{32}-inducible in vitro (8a). As for the third task, importance of a particular gene can be studied by (i) sequence analysis, and (ii) examination of the phenotypes of mutants which can be constructed by in vitro mutagenesis and subsequent homologous recombination in vivo.

ACKNOWLEDGMENTS

We thank S. Cohen, D. I. Friedman, C. A. Gross, Y. Kohara, F. C. Neidhardt, and W. S. Reznikoff for the gifts of bacterial and phage strains. We thank E. Balish and J. A. Croft for help in using the gnotobiotic facility, and K. Rudd for the use of consolidated *E. coli* sequence database. We also thank W. S. Reznikoff and V. Burland for critically reading this manuscript.

This work is supported in part by NIH grant GM35682 to D.L.D. and by a scholarship to S.-E.C. from the Ministry of Education, Republic of China (Taiwan).

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