

Adaptation to famine: A family of stationary-phase genes revealed by microarray analysis

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Contributed by Rowena G. Matthews, August 23, 2002

Bacterial adaptation to nutrient limitation and increased population densities is central to survival and virulence. Surprisingly, <3% of *Escherichia coli* genes are known to play roles specific to the stationary phase. There is evidence that the leucine-responsive regulatory protein (Lrp) may play an important role in stationary phase, so this study used microarrays representing >98% of *E. coli* genes to more comprehensively identify those controlled by Lrp. The primary analysis compared isogenic Lrp⁺ and Lrp⁻ strains in cells growing in steady state in glucose minimal medium, either in the presence or absence of leucine. More than 400 genes were significantly Lrp-responsive under the conditions used. Transcription of 147 genes was lower in Lrp⁺ than in Lrp⁻ cells whether or not leucine was present; most of these genes were tightly coregulated under several conditions, including a burst of synthesis on transition to stationary phase. This cluster includes 56 of 115 genes already known to play roles in stationary phase. Our results suggest that the actual number of genes induced on entrance into stationary phase is closer to 200 and that Lrp affects nearly three-quarters of them, including genes involved in response to nutrient limitation, high concentrations of organic acids, and osmotic stress.

Bacteria rarely live in environments that permit long episodes of exponential growth. Instead growth is typically limited by nutrient exhaustion. This period, during which biomass does not show a net increase, is termed stationary phase. As might be expected for organisms that spend most of their time between meals, bacteria prepare intensively for stationary phase, in some cases going through complex differentiation processes such as sporulation. Survival of a nonspore-forming bacterium like *Escherichia coli* also requires extensive reprogramming, but only 2.3% of all *E. coli* genes (115/4,290) have a known association with stationary phase as judged by annotation in GenBank.

Lrp, the leucine-responsive regulatory protein, is a global transcription regulator highly conserved in enteric bacteria (1, 2). Leucine is a coregulator of Lrp activity and, depending on the target gene, can be neutral, potentiating, or antagonistic. Lrp is thought to mediate transitions between “feast and famine” because of its reciprocal regulation of amino acid metabolism: biosynthetic genes are activated and catabolic genes are repressed. Expression of Lrp itself is induced by guanosine tetraphosphate (ppGpp), a nucleotide formed when ribosomes are exposed to uncharged tRNAs (3). Earlier studies in *E. coli* revealed that Lrp affects the expression of >70 genes, but surprisingly only six stationary-phase genes were known to be regulated by Lrp (Table 1).

Three observations suggest that our understanding of the physiological role of Lrp is incomplete and that Lrp may play a major role in stationary-phase transitions. First, Lrp levels vary with growth phase, being lowest in late exponential phase when ppGpp levels are low (3), so Lrp-repressed genes should show a burst of expression during the transition to stationary phase. Second, the transcription of the stationary phase-induced gene *osmY* is regulated by Lrp and it was noted that Lrp appears to control additional stationary phase-induced genes (4). Third,

mutations in *lrp* confer a growth advantage in stationary phase (GASP) phenotype (5). Strains with GASP-conferring *lrp* mutations proliferate at the expense of less-fit cells, in part because such mutations enhance the ability to grow on certain amino acids.

In this study we used DNA microarrays containing >98% (4,221/4,290) of annotated ORFs in the *E. coli* K-12 genome, to define the set of genes controlled by Lrp (6). These studies compared the expression profiles of isogenic strains containing or lacking Lrp during exponential growth in media containing or lacking leucine. Our studies indicate a major role for Lrp in the regulation of stationary phase-induced genes and also suggest that the *E. coli* genome includes twice as many stationary phase-induced genes as current annotations indicate.

Methods

Bacterial Strains and Media. To compare strains containing or lacking Lrp, cells of WT *E. coli* K-12 strain W3110 and its isogenic derivative BE1 (*lrp::Tn10*) (7) were grown in glucose minimal Mops medium (8) supplemented with 0.4 mM L-isoleucine, 0.4 mM L-valine, 10 μ M thiamine, and, where indicated, 10 mM L-leucine. Isoleucine and valine were added to minimize the difference in growth rate between *lrp*⁺ and *lrp*⁻ strains (7). Plating media for strain BE1 contained tetracycline (20 μ g/ml). For studies on the expression of Lrp-regulated genes on entrance into stationary phase, strain W3110 was grown in Luria broth (9).

Growth Conditions. Frozen stocks were streaked out and used to inoculate overnight cultures the next day. Cultures were grown overnight aerobically at 37°C before diluting 100-fold into 100 ml of medium. Growth was monitored via OD₄₂₀ and cultures were maintained in exponential growth for at least 10 generations by dilution before harvesting at an OD of 0.3. Fifty milliliters of cells was mixed with 6.25 ml of ice-cold 5% water-saturated phenol (pH <7.0) in ethanol. This treatment immediately stops synthesis and breakdown of RNA (10) and is important for analysis of bacteria such as *E. coli* in which the average mRNA half-life is about 2 min. After 5 min on ice, treated samples were pelleted, the supernatant was removed, and pellets were frozen in liquid nitrogen and stored at -80°C.

Isolation of Total RNA. Frozen cell pellets were lysed by resuspension in 800 μ l of Tris-EDTA (pH 8.0) containing 0.5 mg/ml lysozyme, addition of 80 μ l of 10% SDS, and incubation for 1–2 min at 64°C. Sodium acetate (88 μ l of 1 M, pH 5.2) was added, followed by 1 ml of water-saturated phenol (pH <7.0). Samples were inverted 10 times and incubated 6 min at 64°C while

Abbreviations: Lrp, leucine-responsive regulatory protein; ppGpp, guanosine tetraphosphate.

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Table 1. Stationary-phase genes regulated (directly or indirectly) by Lrp

| Gene | ORF | Fold | Gene | ORF | Fold | Gene | ORF | Fold | Gene | ORF | Fold |
|--|-------|------|-------------|-------|------|-------------|-------|------|-------------|-------|------|
| Prior association with Lrp (6 genes) | | | | | | | | | | | |
| <i>aidB</i> | b4187 | 3.3 | <i>osmC</i> | b1482 | 5.9 | <i>poxB</i> | b0871 | 8.0 | | | |
| <i>csiD</i> | b2659 | 2.3 | <i>osmY</i> | b4376 | 8.9 | <i>yjbJ</i> | b4045 | 1.7 | | | |
| Association with Lrp revealed by this study (47 genes) | | | | | | | | | | | |
| <i>adhE</i> | b1241 | 2.9 | <i>frdA</i> | b4514 | 2.8 | <i>osmB</i> | b1283 | 3.2 | <i>yciG</i> | b1259 | 4.6 |
| <i>aldB</i> | b3588 | 3.3 | <i>gadA</i> | b3517 | 12.8 | <i>osmE</i> | b1739 | 4.4 | <i>ydcS</i> | b1440 | 2.3 |
| <i>amyA</i> | b1927 | 8.9 | <i>gadB</i> | b1493 | 11.2 | <i>otsA</i> | b1896 | 10.4 | <i>yeaG</i> | b1783 | 7.0 |
| <i>appY</i> | b3515 | 2.1 | <i>gadC</i> | b1492 | 6.0 | <i>otsB</i> | b1897 | 7.2 | <i>ygaU</i> | b2665 | 6.4 |
| <i>bfc</i> | b4149 | 3.7 | <i>ggt</i> | b3447 | 10.9 | <i>slp</i> | b3506 | 4.9 | <i>yhiV</i> | b3514 | 3.2 |
| <i>bola</i> | b0435 | 2.2 | <i>grxB</i> | b1064 | 2.3 | <i>sodC</i> | b1646 | 3.1 | <i>yigB</i> | b4269 | 3.0 |
| <i>cbpA</i> | b1000 | 3.6 | <i>hdeA</i> | b3510 | 5.7 | <i>tam1</i> | b1519 | 3.6 | <i>yncC</i> | b1450 | 2.7 |
| <i>cfa</i> | b1661 | 3.1 | <i>hdeB</i> | b3509 | 6.1 | <i>treA</i> | b1197 | 6.7 | <i>yohF</i> | b2137 | 3.1 |
| <i>csiE</i> | b2535 | 4.9 | <i>hdeD</i> | b3511 | 3.4 | <i>treF</i> | b3519 | 2.2 | | | |
| <i>dacC</i> | b0839 | 2.4 | <i>katE</i> | b1732 | 14.5 | <i>wrbA</i> | b1004 | 15.7 | | | |
| <i>dps</i> | b0812 | 4.7 | <i>kch</i> | b1250 | 2.1 | <i>yahO</i> | b0329 | 4.9 | | | |
| <i>fbaB</i> | b2097 | 8.4 | <i>ldcC</i> | b0186 | 2.3 | <i>ycgB</i> | b1188 | 13.0 | | | |
| <i>fic</i> | b3361 | 2.7 | <i>mlrA</i> | b2127 | 5.0 | <i>yciF</i> | b1258 | 2.4 | | | |

Genes indicated in bold are annotated in GenBank or reported in the literature as being regulated by RpoS, others are associated with stationary phase but not necessarily controlled by RpoS. Underlining indicates coregulation of the highlighted genes (Pearson's correlation coefficients of >0.5). Fold indicates the reduction of expression in the Lrp⁺ relative to the Lrp⁻ strain, measured in cells grown in the absence of leucine.

inverting 6–10 times every 40 s, then chilled on ice and centrifuged. The aqueous layer was transferred to a new tube and an equal volume of chloroform was added. After samples were inverted 6–10 times and centrifuged, the aqueous layer was distributed into fresh tubes and 1 vol of 3 M sodium acetate (pH 5.2)/1 mM EDTA and 2.5 vol of ice-cold 100% ethanol were added to each tube. Tubes were incubated at -80°C and spun in a microcentrifuge for 25 min. Supernatants were removed and the pellets were washed with 1 ml of cold 80% ethanol before being air-dried. Tubes were then reloaded after resuspension in 100 μ l of water.

DNase Treatment of RNA Samples. Each RNA sample was treated with 1 μ l ribonuclease-free DNase (10 units/ μ l, Roche Diagnostics) after addition of 0.5 μ l ribonuclease inhibitor (40 units/ μ l, Roche Diagnostics), and 50 μ l 5 \times DNase I buffer (50 mM MgCl₂/50 mM Tris-HCl, pH 7.5/5 mM EDTA/5 mM DTT). Samples were incubated 30 min at 37°C, extracted with phenol, phenol/chloroform, and chloroform (twice), then ethanol-precipitated as described above. Finally, the pellet was washed once with 80% ethanol and resuspended, after drying, in 50 μ l of water. Samples were tested for purity by measuring the 260-nm/280-nm absorbance ratio. Ratios between 1.8 and 2.1 indicated samples were sufficiently pure for use in the microarrays. The concentration of RNA was derived from measurements of the absorbance at 260 nm; an absorbance of 1 unit at 260 nm corresponds to 40 μ g RNA per ml.

Labeling of cDNA and Hybridization to DNA Array. cDNA preparations were labeled with fluorescent Cy3 or Cy5 fluorophores as described (6). One microgram pdN6 primer (Amersham Pharmacia) was mixed with 25–30 μ g RNA in 16 μ l water on ice. After 10 min at 65°C and 2 min on ice, FluoroLink Cy3, or Cy5 dUTP (Amersham Pharmacia), 3 μ l of a 1 mM stock, was added along with the transcription mix [3 μ l of 0.1 mM DTT, 6 μ l first-strand buffer, 0.6 μ l deoxyribonucleoside triphosphates (25 mM each of dATP, dCTP, dGTP, and 10 mM dTTP (Invitrogen)), and 2 μ l of the reverse transcriptase SuperscriptII (Invitrogen)]. Samples were incubated 10 min at room temperature and 110 min at 42°C. Thirty microliters was added to 470 μ l of water and spun in a Microcentrifuge 30 (Millipore) at maximum speed for 8 min or until the volume was reduced to 3–5 μ l. Both the Cy3 and Cy5

concentrates were combined into a final volume of 10 μ l. Two microliters of 20 \times SSC (9), 2 μ l water, and 0.35 μ l of 10% SDS were added to the sample mix before incubation at 100°C for 2 min followed by brief centrifugation and incubation 2–3 min at room temperature.

Relative mRNA levels were determined by parallel two-color hybridization to spotted DNA microarrays (6) that contained 4,221 of 4,290 recognized ORFs in the *E. coli* genome (11). Hybridization was carried out for 4–6 h at 64°C in 16 μ l of solution containing, in addition to labeled probes, 40 μ g denatured salmon sperm DNA in 2 \times SSC and 0.2% SDS. Arrays were washed according to a standard protocol (<http://cmgm.stanford.edu/pbrown/mguide/index.html>). Arrays were analyzed by using GENPIX 3.0 (Axon Instruments, Union City, CA). Replicate hybridization experiments from two independent sets of cultures were performed for each condition. To control for possible, but not yet identified, dye-specific artefacts, the labeling dyes were also switched between the two samples.

Data Analysis. Microarray data were imported into an EXCEL (Microsoft) spreadsheet. Replicate hybridizations and duplicate spots on the array were averaged, and SDs were calculated.

Results and Discussion

Lrp Affects Transcription of About One-Tenth of All *E. coli* Genes. Our first major observation was that the transcription of the great majority of genes is not significantly affected by Lrp, at least under the growth conditions used. Fig. 1A shows a histogram of the distribution of Lrp⁻/Lrp⁺ mRNA ratios for all of the genes present on the microarray, with the presence or absence of exogenous leucine indicated by color, and Fig. 1B shows these Lrp⁻/Lrp⁺ ratios with results in the absence of leucine on the y axis and results in the presence of leucine on the x axis. In Fig. 1B genes on which Lrp has no effect appear at the coordinates 1,1; the actual mean x,y coordinates for all analyzed genes are 1.13, 1.12. The distribution is skewed by a population of genes subject to strong negative regulation by Lrp (see below). The dotted lines in Fig. 1A and the square shown in Fig. 1B contain the genes showing a less than 2-fold change in expression. More than 90% of all tested genes show less than 2-fold response to Lrp under the conditions used. (For these data, a 2-fold response corresponds roughly to 2 SDs from the mean.)

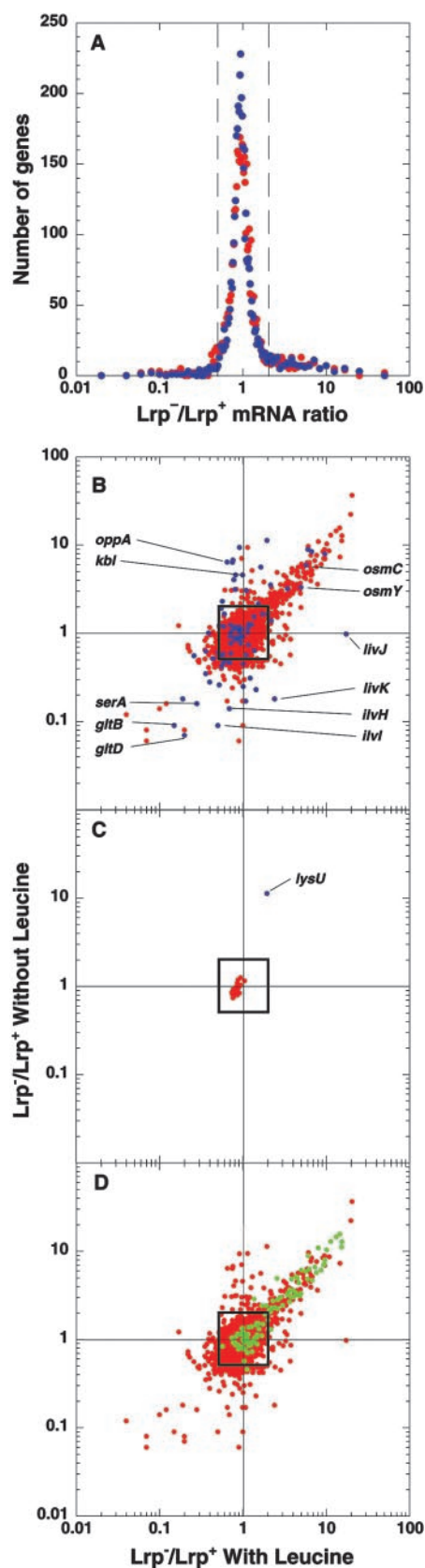


Fig. 1. (A) Histogram of Lrp^-/Lrp^+ mRNA ratios for the 4,221 genes represented on the microarray that gave detectable hybridization signals. Ratios obtained from cells grown without leucine are shown in blue and with leucine in red. The dotted lines represent 2-fold changes in transcript abundance. (B) Lrp^-/Lrp^+ gene expression ratios, in the presence (x axis) vs. the absence of

leucine (y axis). Spots corresponding to genes previously known to be Lrp-responsive are shown in blue; some are labeled. The square corresponds to a 2-fold change in transcript abundance in response to *lrp* mutation. (C) Results for the aminoacyl-tRNA synthetases produced by *E. coli*. Colors and square are as described in B. (D) Results for all genes as in B, but spots shown in green correspond to genes known to be associated with stationary phase.

The nonresponders inside the square include some genes known to be regulated by Lrp, which is not surprising. For example, *yjbJ* is known to be repressed by Lrp only in stationary phase; during exponential growth Lrp has no detectable effect (12). Another example is *ompF*; OmpF polypeptide levels are Lrp-responsive, because *ompF* translation is modulated by the antisense RNA *micF*, expression of which is controlled by Lrp (13). In addition, any gene whose transcript abundance is modulated less than 2-fold by Lrp is considered a nonresponder by this analysis.

Lrp is highly abundant compared with most regulatory proteins, which has led to suspicion that it acts as a histone-like protein with broad nonspecific effects. The fact that Lrp affects transcription of only 10% of genes more than 2-fold seems incompatible with Lrp acting primarily as a chromosome-organizing protein with generalized effects, but the 10% figure nevertheless represents a surprisingly large regulatory network. The methods we used do not distinguish between direct and indirect effects of Lrp. Among the Lrp-responsive genes outside the square in Fig. 1B is *glnA*, previously shown to be indirectly regulated by Lrp (7).

Our second observation is that the slight growth rate reduction associated with the *lrp* mutation (7) has not biased our results. Many genes vary their level of expression in proportion to the growth rate, so a *lrp*-associated reduction in growth rate could make it appear as if Lrp were affecting these genes. Fig. 1C shows the results for the aminoacyl-tRNA synthetases. Expression of these genes varies with growth rate (14), yet all but one lie within the square demarcating 2-fold change in expression. The one exception is *lysU*, an alternative lysyl-tRNA synthetase known to be repressed by Lrp (15).

Patterns of Response to Lrp. Many known Lrp-regulated genes (blue spots in Fig. 1B) show the expected patterns of expression in our microarray analyses. Theoretically there are four possible extremes of leucine dependence for Lrp-responsive genes. Genes on the line $y = 1$ are responsive to Lrp only in the presence of leucine; this group includes the branched-chain amino acid transport gene *livJ*, which is known to be repressed by Lrp only in the presence of leucine (16). Genes on the line $x = 1$ are Lrp-responsive only in the absence of leucine; this is a larger group that includes the peptide transport gene *oppA*, known to be repressed by Lrp only when leucine is absent (17). Genes on the descending diagonal ($y = 1/x$) show opposite Lrp responses in the presence and absence of leucine; this very small group includes the leucine transport gene *livK*, which is known to be activated by Lrp in the absence of leucine and repressed by Lrp in the presence of leucine (12). The fourth group of genes lies along the rising diagonal ($y = x$); these genes are Lrp-responsive irrespective of leucine and include the glutamate synthase gene *gltB*, which is known to be activated by Lrp irrespective of leucine (7), and *osmY*, which is known to be repressed by Lrp irrespective of leucine (18).

The biggest surprise in Fig. 1B is the abundance of leucine-independent Lrp-responsive genes; by far the majority of these (148 total) are negatively regulated by Lrp, forming a “comet tail” to the upper right of the nonresponders. The relative distribution of colors in Fig. 1B indicates that this comet tail contains a much lower proportion of previously identified Lrp-controlled genes (blue) than do the other regulatory classes. The

leucine (y axis). Spots corresponding to genes previously known to be Lrp-responsive are shown in blue; some are labeled. The square corresponds to a 2-fold change in transcript abundance in response to *lrp* mutation. (C) Results for the aminoacyl-tRNA synthetases produced by *E. coli*. Colors and square are as described in B. (D) Results for all genes as in B, but spots shown in green correspond to genes known to be associated with stationary phase.

apparently biased sampling of genes in previous studies, many of which relied on the sensitivity of *lrp*-regulated genes to leucine, may have distorted our understanding of the Lrp regulon.^{††}

Lrp and Stationary Phase. Many of the genes represented in the comet tail in Fig. 1B are annotated as being functionally associated with stationary phase, as being induced in stationary phase, or both. These genes are shown in green in Fig. 1D. Of 115 *E. coli* genes with annotated links to stationary phase, nearly half are in the comet tail, meaning that a third of the 148 genes in the comet tail have already been associated with the stationary phase (Table 1). A large fraction of these genes is also known to be under the control of σ^S , the stationary phase and general stress-specific alternative sigma factor RpoS (20), as indicated by bold type in Table 1.

Many Lrp-regulated comet-tail genes with known functions may have roles in reprogramming the cell to survive nutrient limitation, high concentrations of organic acids, and osmotic stress (Table 1). These genes include *fbaB*, which specifies an isozyme of fructose-bisphosphate aldolase, and *adhE*, which specifies an alcohol/aldehyde dehydrogenase that permits growth on ethanol (21); *fbaB* is induced during growth on substrates like acetate that require gluconeogenic metabolism (22). The genes *csiD* and *csiE* are induced by carbon starvation, although their precise roles are not known (23). MlrA is required for production of the fibrous surface adhesin curli and extracellular matrix synthesis in association with biofilm formation. Other comet tail genes are induced by acid stress; these include *hdeAB*, *osmY*, *dps*, *gadA*, *gadBC*, and *sodC* (24–26). Genes involved in the transport and metabolism of osmoprotectants include *treA*, *treF*, and *otsAB*, and osmotic stress also leads to the induction of *osmC*, *osmY*, *osmB*, and *osmE*. The annotations for all genes shown in Table 1 can be found in Table 2, which is published as supporting information on the PNAS web site, www.pnas.org.

The genes represented in the comet tail are tightly coregulated under a variety of conditions. To date, microarrays have been used to study *E. coli* gene expression in more than 35 protocols, using different chromosomal mutations and growth conditions (A.K. and P.O.B., unpublished data). We pooled these results and used cluster analysis to identify genes that are coregulated under a variety of conditions. Using a Pearson correlation coefficient of 0.5 as the lower limit, most of the genes in the comet tail appear to be coregulated (Fig. 2, Table 1, and Table 3, which is published as supporting information on the PNAS web site). Subsets of the genes in this cluster show correlation coefficients above 0.75, in the same range as genes within an operon. In one particularly telling experiment, details of which are shown in Fig. 3, most of the genes listed in Tables 1 and 3 were found to be coincided in rich medium on the transition from exponential growth to stationary phase. Thus, the genes that are transcribed at elevated rates in the absence of Lrp during steady-state growth, irrespective of leucine, also show a burst of transcription coincident with the transient drop in Lrp levels seen in during transition to stationary phase. These observations suggest that the comet tail contains a substantial number of previously unrecognized stationary phase-inducible genes.

Two of the most strongly Lrp-responsive genes are *talA* and *tktB*, which specify transaldolase and transketolase isozymes involved in the pentose phosphate pathway. FbaB is also required for the operation of this pathway. As the growth rate slows and ppGpp levels rise when cells are starved for carbon,

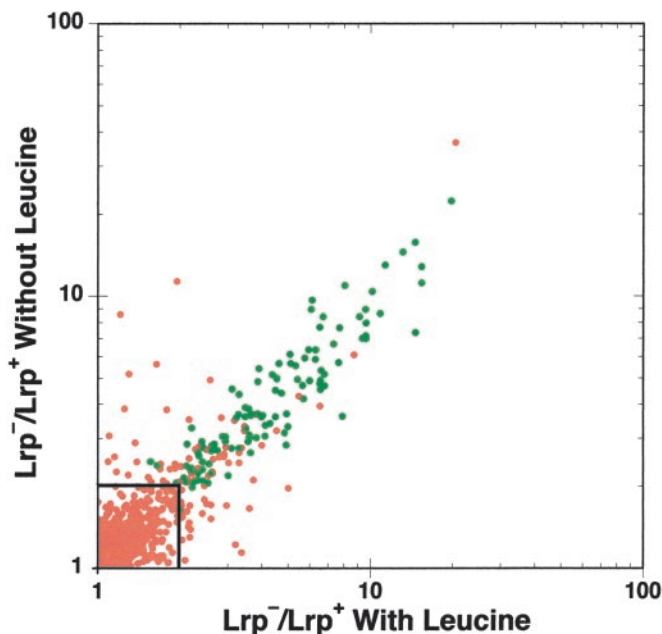


Fig. 2. Cluster analysis of regulatory patterns shown under diverse conditions by leucine-independent, Lrp-responsive genes. The area shown corresponds to the upper right quadrant of Fig. 1B, and the square indicates the 2-fold response limit. Spots shown in green correspond to genes that are coregulated. In an initial correlation analysis of 57 known genes in the Lrp regulon, four genes (*osmC*, *osmY*, *fbaB*, and *poxB*) were identified with very high Pearson's correlation coefficients (>0.7) with respect to one another, but not with other members of the regulon. Subsequently an all vs. all correlation analysis was performed for the genes lying outside the square in this quadrant. Genes with correlation coefficients averaging >0.5 to *osmC*, *osmY*, *fbaB*, and *poxB* were considered to be coregulated.

ribosomes are disassembled and their proteins and RNA are recycled (27, 28). RNA catabolism requires nucleotidases and nucleoside hydrolases or pyrophosphorylases. RihA (formerly YbeK) specifies a pyrimidine nucleotide hydrolase (29) that is Lrp-responsive. Ribose catabolism requires the operation of the pentose phosphate pathway (Tal and Tkt), as well as fructose-bisphosphate aldolase (FbaB) (30). YhjD is annotated as a tRNA processing ribonuclease and YhbO as a putative intracellular protease. The single most strongly Lrp-depressed gene (37-fold) in Table 2 is *ybeJ* (*gltI*). This gene specifies a putative periplasmic amino acid binding protein and may be part of a *ybeJ-gltJKL-rhiA* operon thought to specify a glutamate-aspartate transport complex in addition to RhiA. One of the growth advantage in stationary-phase (GASP) mutations (31) is a genomic rearrangement that activates this operon. Table 3 lists candidate stationary-phase genes in the comet tail.

GadX (YhiX) is an activator of the glutamate decarboxylase genes *gadA* and *gadBC* (32). It is part of the large gene cluster *slp yhiFD hdeBAD yhiEUVWX gadA*, the components of which are repressed by Lrp, and most of which are known to be induced by acid stress and/or entrance into stationary phase. Without further experimentation, generalized conclusions may be premature, but there clearly appears to be a common theme: a shift in metabolism from growth on plentiful nutrients to scavenging and protection under adverse conditions.

Lrp and Growth Rate. One puzzling feature of our data is the mild phenotype associated with the *lrp* mutation, at least during growth in glucose minimal Mops medium. Previous studies have shown that *lrp* strains grow more slowly than isogenic *lrp+* parents, but the effect is fairly small and much of the difference

^{††}Formally a regulon is a set of genes directly controlled by a regulator, and the term stimulon refers to the set of genes that respond to a given stimulus (19). As our studies do not distinguish between direct and indirect effects of Lrp on transcription, we use the term regulon with the caveat that further experimentation will be required to demonstrate direct regulation of target genes by Lrp.

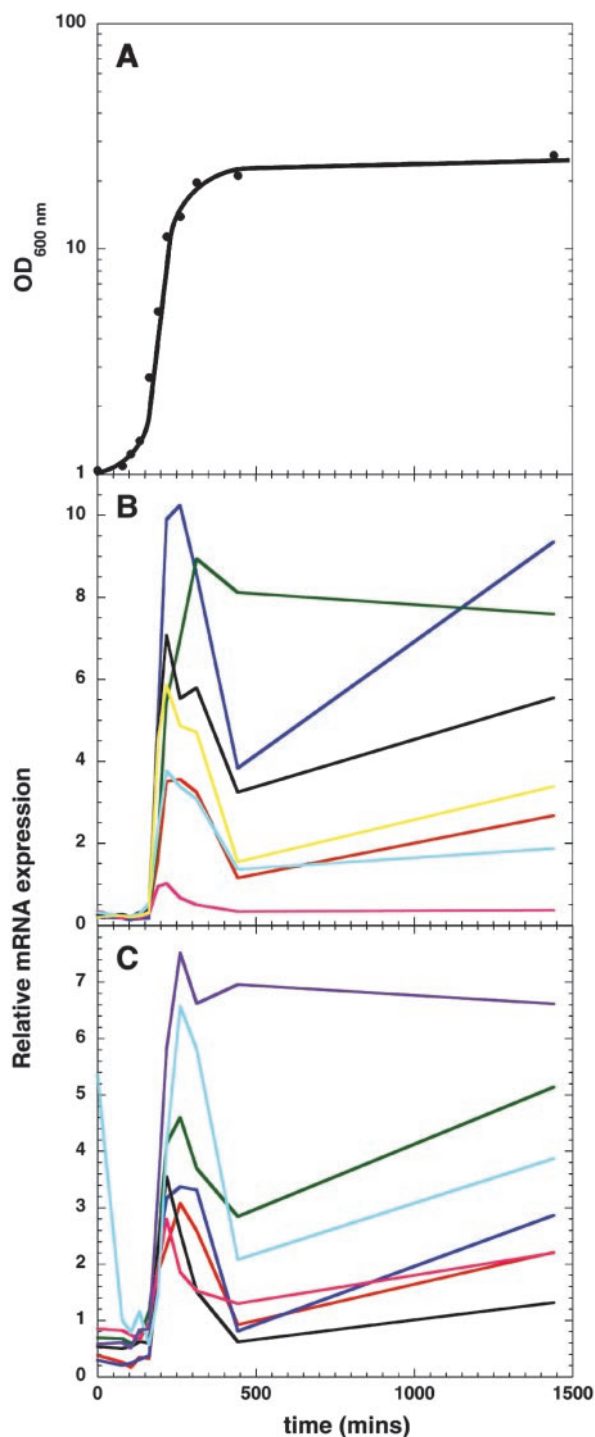


Fig. 3. Expression of selected target genes during growth transitions. *E. coli* strain MG1655 was grown in Luria broth containing 0.2% glucose. (A) Plot of log OD₆₀₀ vs. the time after initiation of growth. (B) Expression of selected Lrp-regulated stationary-phase genes during growth transitions. mRNA levels were determined by microarray analysis and expressed as a ratio against the level of expression of the same gene in cells grown to an OD₆₀₀ of 0.5 in Vogel-Bonner minimal medium containing 0.2% glucose. Red line, *fbaB*; green line, *dps*; dark blue line, *gadA*; yellow line, *hdeB*; pink line, *katE*; light blue line, *otsA*; black line, *hdeA*. These genes show a pulse of expression at an OD₆₀₀ of about 15, as the cells are making the transition from exponential growth to stationary phase. (C) Expression of selected candidate stationary-phase genes (drawn from those listed in Table 3) during growth transitions. Black line, *qor*; light blue line, *sufC*; red line, *talA*; dark blue line, *tktB*; green line, *yhiX*; pink line, *yhbO*; purple line, *ygiW*.

(in glucose minimal media) can be eliminated by providing isoleucine and valine (7), as we have done. One might expect that constitutive elevated expression of well over 100 stationary-phase genes would in itself slow cell growth, in addition to the possible metabolic burden of futile cycling as degradative and biosynthetic pathways function simultaneously. One possibility is that a combination of translational and allosteric controls maintain the cell's physiological balance despite the severity of the transcription-level dysregulation.

Lrp and ppGpp. The alarmone ppGpp plays a major role in transitions to stationary phase. It would be reasonable to suspect that many of the 148 Lrp-responsive, leucine-independent, stationary-phase (comet tail) genes are controlled by ppGpp. First, Lrp levels are themselves positively controlled by ppGpp (3). Second, many comet tail genes are responsive to RpoS (underlined entries in Table 1), as noted above, and many RpoS-dependent promoters only function in the presence of ppGpp (33). However, the comet tail genes appear to respond to Lrp irrespective of ppGpp. We base this assertion on results from the exponential growth experiments shown in Fig. 1. An analysis of 45 ribosomal protein genes included in our microarrays revealed an average difference of expression of only $\approx 20\%$ between the Lrp⁺ and Lrp⁻ strains, despite the fact that transcription of these genes is strongly inhibited by ppGpp (34). The actual means \pm standard errors for these genes, normalized to the Lrp⁺ culture grown in the absence of leucine, are: 1.27 ± 0.06 (Lrp⁺, +Leu), 0.99 ± 0.07 (Lrp⁻, +Leu), and 0.81 ± 0.05 (Lrp⁻, -Leu).

Conclusions

The observed changes in expression suggest that when Lrp concentrations are lowest, during the transition to stationary phase (3), some of the induced genes prepare the cell to mobilize internal nutrient reserves and metabolize fermentation products. Similar changes may be required to survive in the stark competition revealed by Kolter and his colleagues (5, 35), where cells with growth advantage in stationary-phase (GASP) mutations take over the stationary-phase population because they are better able to scavenge nutrients from their dying neighbors. Our results rationalize why loss of Lrp function could enhance scavenging capabilities; in addition to increased catabolism of serine, glycine, and threonine, *lrp* mutants have increased expression of enzymes involved in gluconeogenesis and the pentose phosphate pathway and in tolerance of acid and osmotic stresses.

Adding the comet tail genes to those with previously recognized stationary-phase associations, there now appear to be as many as 215 genes induced on entry into stationary phase, and more than 70% (155 total) are Lrp-responsive. These findings substantially shift our view of the main physiological role of Lrp, in addition to doubling the number of genes that may play roles in stationary phase. Lrp appears to limit maximal expression of many genes in the transition between exponential and stationary phases. The return of Lrp to its high pregrowth concentration explains why, in late stationary phase, *lrp* mutation could lead to a growth advantage. The roles of the many candidate stationary-phase genes need to be determined, and it is important to explore the functional relationship between Lrp and RpoS first noted by Hengge-Aronis and colleagues (4, 18), a much more extensive relationship than has been appreciated until now.

Note. After this article had been written and reviewed, a study appeared that focused on statistical analysis of microarray data, using the *E. coli* Lrp regulon as a model system (36). Although that study and ours compare Lrp⁺ and Lrp⁻ strains growing exponentially in glucose minimal Mops media, they differ in strain background, microarrays used, and whether or not the effects of leucine were determined. Despite

these differences, a preliminary analysis of the data in the two manuscripts reveals general agreement between the cultures grown in the absence of leucine. The comet tail stationary-phase genes were not detected as a group by Hung *et al.* (36), as this required analysis of leucine effects.

This work was supported in part by National Science Foundation Grant MCB9807237 (to R.G.M. and R.M.B.), the Howard Hughes Medical Institute (to P.O.B.), funds from the University of Minnesota (to A.K.), and National Institutes of Health Cellular Biotechnology Training Grant GM08353 (to T.H.T.).

1. Calvo, J. M. & Matthews, R. G. (1994) *Microbiol. Rev.* **58**, 466–490.
2. Newman, E. B., D'Ari, R. & Lin, R. T. (1992) *Cell* **68**, 617–619.
3. Landgraf, J. R., Wu, J. & Calvo, J. M. (1996) *J. Bacteriol.* **178**, 6930–6936.
4. Lange, R., Barth, M. & Hengge-Aronis, R. (1993) *J. Bacteriol.* **175**, 7910–7917.
5. Zambrano, M. M., Siegele, D. A., Almiron, M., Tormo, A. & Kolter, R. (1993) *Science* **259**, 1757–1760.
6. Khodursky, A. B., Peter, B. J., Cozzarelli, N. R., Botstein, D., Brown, P. O. & Yanofsky, C. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 12170–12175.
7. Ernsting, B. R., Atkinson, M. R., Ninfa, A. J. & Matthews, R. G. (1992) *J. Bacteriol.* **174**, 1109–1118.
8. Neidhardt, F. C., Bloch, P. L. & Smith, D. F. (1974) *J. Bacteriol.* **119**, 736–747.
9. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
10. Lin-Chao, S. & Cohen, S. N. (1991) *Cell* **65**, 1233–1242.
11. Blattner, F., Plunkett, G. R., Bloch, C., Perna, N., Burland, V., Riley, M., Collado-Vides, J., Glasner, J., Rode, C., Mayhew, G., *et al.* (1997) *Science* **277**, 1453–1474.
12. Bhagwat, S. P., Rice, M. R., Matthews, R. G. & Blumenthal, R. M. (1997) *J. Bacteriol.* **179**, 6254–6263.
13. Ferrario, M., Ernsting, B. R., Borst, D. W., Wiese, D. E., II, Blumenthal, R. M. & Matthews, R. G. (1995) *J. Bacteriol.* **177**, 103–113.
14. Grunberg-Manago, M. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. & Umberger, E. (Am. Soc. Microbiol., Washington, DC), Vol. 2, pp. 1432–1457.
15. Lin, R., Ernsting, B., Hirshfield, I. N., Matthews, R. G., Neidhardt, F. C., Clark, R. L. & Newman, E. B. (1992) *J. Bacteriol.* **174**, 2779–2784.
16. Haney, S. A., Platko, J. V., Oxender, D. L. & Calvo, J. M. (1992) *J. Bacteriol.* **174**, 108–115.
17. Andrews, J. C. & Short, S. A. (1986) *J. Bacteriol.* **165**, 434–442.
18. Colland, F., Barth, M., Hengge-Aronis, R. & Kolb, A. (2000) *EMBO J.* **19**, 3028–3037.
19. Neidhardt, F. C. & Savageau, M. A. (1996) in *Escherichia coli and Salmonella*, eds. Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M. & Umberger, H. E. (Am. Soc. Microbiol., Washington, DC), Vol. I, pp. 1310–1324.
20. Hengge-Aronis, R. (2000) in *Bacterial Stress Responses*, eds. Storz, G. & Hengge-Aronis, R. (Am. Soc. Microbiol., Washington, DC), pp. 161–178.
21. Clark, D. & Cronan, J. E., Jr. (1980) *J. Bacteriol.* **141**, 177–183.
22. Thomson, G. J., Howlett, G. J., Ashcroft, A. E. & Berry, A. (1998) *Biochem. J.* **331**, 437–445.
23. Weichart, D., Lange, R., Henneberg, N. & Hengge-Aronis, R. (1993) *Mol. Microbiol.* **10**, 407–420.
24. Choi, S. H., Baumler, D. J. & Kaspar, C. W. (2000) *Appl. Environ. Microbiol.* **66**, 3911–3916.
25. Foster, J. W. (2000) in *Bacterial Stress Responses*, eds. Storz, G. & Hengge-Aronis, R. (Am. Soc. Microbiol., Washington, DC), pp. 99–115.
26. Waterman, S. R. & Small, P. L. (1996) *Mol. Microbiol.* **21**, 925–940.
27. Bessarab, D. A., Kaberdin, V. R., Wei, C. L., Liou, G. G. & Lin-Chao, S. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 3157–3161.
28. Kuroda, A., Nomura, K., Ohtomo, R., Kato, J., Ikeda, T., Takiguchi, N., Ohtake, H. & Kornberg, A. (2001) *Science* **293**, 705–708.
29. Petersen, C. & Møller, L. B. (2001) *J. Biol. Chem.* **276**, 884–894.
30. Sprenger, G. A. (1995) *Arch. Microbiol.* **195**, 324–330.
31. Zinser, E. R. (2000) Ph.D. thesis (Harvard Medical School, Boston).
32. Shin, S., Castanie-Cornet, M. P., Foster, J. W., Crawford, J. A., Brinkley, C. & Kaper, J. B. (2001) *Mol. Microbiol.* **41**, 1133–1150.
33. Kvint, K., Farewell, A. & Nystrom, T. (2000) *J. Biol. Chem.* **275**, 14795–14798.
34. Cashel, M., Gentry, D. R., Hernandez, V. J. & Vinella, D. (1996) in *Escherichia coli and Salmonella*, eds. Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M. & Umberger, H. E. (Am. Soc. Microbiol., Washington, DC), Vol. I, pp. 1458–1496.
35. Zinser, E. R. & Kolter, R. (1999) *J. Bacteriol.* **181**, 5800–5807.
36. Hung, S.-P., Baldi, P. & Hatfield, G. W. (2002) *J. Biol. Chem.* **277**, in press.