# The *E. coli* molecular phenotype under different growth conditions

Mehmet U. Caglar, John R. Houser, Craig S. Barnhart, Daniel R. Boutz, Sean M. Carroll, Aurko Dasgupta, Walter F. Lenoir, Bartram L. Smith, Viswanadham Sridhara, Dariya K. Sydykova, Drew Vander Wood, … other authors? … Christopher J. Marx, Edward M. Marcotte, Jeffrey E. Barrick, Claus O. Wilke

Affiliations

## Abstract

Modern systems biology requires extensive, carefully curated measurements of cellular components in response to different environmental conditions. While high-throughput methods have made transcriptomics and proteomics datasets widely accessible and relatively cheap to generate, systematic measurements of both mRNA and protein abundances under a wide range of different conditions are still relatively rare. Here we present a detailed, genome wide transcriptomics and proteomics dataset of *E. coli* grown under 34 different conditions. We manipulate concentrations of sodium and magnesium in the growth media, and we consider four different carbon sources glucose, gluconate, lactate, and glycerol. Moreover, samples are taken both in exponential and stationary phase, and we include two extensive time-courses, with multiple samples taken between 3 hours and 2 weeks. We find that exponential-phase samples systematically differ from stationary-phase samples, in particular at the level of mRNA. Regulatory responses to different carbon sources or salt stresses are more moderate, but we find numerous differentially expressed genes for growth on gluconate and under salt and magnesium stress. Our data set provides a rich resource for future computational modeling of *E. coli* gene regulation, transcription, and translation.

## Introduction

A goal of systems biology has been to understand how phenotype originates from genotype. The phenotype of a cell is determined by complex regulation of metabolism, gene expression, cell signaling Understanding the connection between phenotype and genotype is crucial to understanding disease and for synthetic engineering of biology (Botstein and Risch 2003). Computational models are particularly well suited to studying this problem, as they can synthesize and organize diverse and complex data in a predictive framework, but detailed experimental studies including many samples are needed to understand interactions between different types of omics data (Zhang, Li, and Nie 2010). Much effort is currently being spent on understanding how to best integrate information collected about multiple cellular subsystems and derived from different high-throughput methods. For example, there are many proposed approaches for relating gene expression and protein abundances, focusing on integrative, whole-cell models (Joyce and Palsson 2006; Zhang, Li, and Nie 2010; Ideker et al. 2001; Vogel and Marcotte 2012).

Given the growing interest in integrative modeling approaches, there is a pressing need for high quality genome-scale data that is comparable across cellular subsystems under many different external conditions. *E. coli* is an ideal organism to study genome-wide, multi-level regulatory effects of external conditions, since it is well adapted to the laboratory environment (Lee 1996) and was one of the first organisms studied at the whole-genome level (Blattner et al. 1997). There have been a number of studies of the *E. coli* transcriptome and/or proteome in response to different growth conditions. For example, if cells are grown at high density, expression of most amino-acid biosynthesis genes is down-regulated and expression of chaperones is up-regulated, suggesting that high cell density is stressful for cells (Yoon et al. 2003). Exposure of *E. coli* to reduced temperature leads to changes in gene-expression patterns consistent with reduced metabolism and growth (Gadgil, Kapur, and Hu 2005). At the single-cell level, the *E. coli* transcriptomes and proteomes are noisy, and mRNA and protein copy numbers tend to be uncorrelated (Taniguchi et al. 2010). Under long-term glucose starvation, mRNAs are generally down-regulated while the protein response is more varied (Houser et al. 2015). Copy numbers of proteins involved in energy-intensive processes decline whereas those of proteins involved in nutrient metabolism remain constant, likely to provide the cell with the ability to jump-start metabolism when nutrients become available again. Larger-scale studies that measure mRNA and/or protein abundances under multiple different conditions include (Soufi et al. 2015; Schmidt et al. 2015; Lewis et al. 2010; Lewis et al. 2009).

Here, we provide a systematic analysis of *E. coli* gene expression under a wide variety of different conditions. We measure both mRNA and protein abundances, at exponential and stationary phases, for growth conditions including different carbon sources and different salt stresses. We find that mRNAs and proteins display divergent responses to the different growth conditions. Further, growth phase yields more systematic differences in gene expression than does either carbon source or salt stress, though this effect is more pronounced in mRNAs than in proteins. We expect that our data set will provide a rich resource for future modeling work.

## Results

### Experimental design and data collection

We grew multiple cultures of *E. coli* REL606, from the same stock, under a variety of different growth conditions. We measured RNA abundances under all conditions and matching protein abundances for approximately 2/3 of the conditions (Figure 1). Results from one of these conditions, long-term glucose starvation, have been presented previously (Houser et al. 2015). Conditions not previously described include one additional starvation experiment, using glycerol instead of glucose as carbon source, exponential and stationary phase cultures using either gluconate or lactate as carbon source, and conditions varying Mg2+ and Na+ stress.

Measurements of RNA and protein abundances were carried out as previously described (Houser et al. 2015). All resulting data sets were carefully checked for quality, normalized, and log-transformed. Our final data set consisted of 152 RNA samples, 105 protein and 20 flux samples associated with high Mg+2 and high Na+ experiments (Supplementary Table 1).

Our raw RNA-seq data covers 4279 distinct mRNAs, our protein data covers 4201 distinct proteins and metabolism flow data covers 13 different fluxes. All raw data files are available in appropriate repositories (see Methods for details), and final processed data are available as Supplementary Tables 3, 4 and 6.

The most preferable state for *E. coli* with respect to different Na+ concentrations is 5mM Na+. There are two different favorable concentrations (0.05mM and 100mM) for *E.coli* with respect to Mg2+. Most favorable carbon source is glucose and least favorable carbon source is lactate (Figure 2).

**Broad trends of gene expression differ between mRNA and proteins**

To identify broad trends of gene expression among the different growth conditions, we performed hierarchical clustering on both mRNA and protein abundances (Figures 3 and 4). For mRNA, we found that differences in gene expression were primarily driven by the growth phase (exponential vs. stationary/late stationary). Nearly all-exponential samples clustered together in one group, separate from the vast majority of stationary and late-stationary samples (Figure 3). Mg2+ levels, Na+ levels, and carbon source had less influence on the clustering results. We also found a similar result for protein abundances (Figure 4). The exponential-phase samples grouped together, separated from stationary and late stationary samples. Similarly, Na+ levels and carbon sources also seemed to be grouped together upon clustering.

To quantify the clustering patterns of mRNA and protein abundances, we defined a metric that measured how strongly clustered a given variable of the growth environment (growth phase, Mg2+ level, Na+ level, carbon source) was relative to the random expectation of no clustering. For each variable, we calculated the mean cophenetic distance between all pairs corresponding to the same condition (e.g., for growth phase, all pairs sampled at exponential phase and all pairs sampled at stationary/late stationary phase). The cophenetic distance is defined as the height of the dendrogram produced by the hierarchical clustering from the two selected leafs to the point where the two branches merge. We then converted each mean cophenetic distance into a *z*-score, by resampling mean cophenetic distances from dendograms with reshuffled leaf assignments. A *z*-score below −1.96 indicates that the mRNA or protein abundances are clustered significantly by the corresponding variable.

### We found that mRNA abundances were significantly clustered by growth phase, with a *z*-score of −23.99 (Table 1). Na+ and Mg2+ levels displayed the next-largest *z*-scores by magnitude, of −1.54 and −1.46, but these were not significantly different from zero. The *z*-score for carbon source was 1.16, which implies that there is no significant clustering by carbon source in the mRNA data. Importantly, when we calculated a *z*-score for batch number, we found that batch effects also significantly influenced mRNA abundances, with *z* = −2.82. Batch number here represents cultures grown at the same time, in parallel. Thus, batch effects may represent fluctuations in incubator temperatures, slight differences in growth medium composition or water quality, or effects of reviving the initial inoculum of cells, among other possibilities.

### For protein abundances, the variables Na+ level, growth phase, and carbon source were all significantly clustered, with *z*-scores of −4.78, −4.21, and −3.15, respectively (Table 1). Batch number had a *z*-score of −23.29, which implies that there were strong batch effects present in the protein data.

### In summary, the largest effect in mRNA abundances, growth phase, was similarly present in proteins. However, protein abundances clustered also by Na+ and carbon source, effects that weren’t present in the mRNA data. Finally, both mRNA and protein data were influenced by batch effects, and the effect was much more pronounced for proteins than for mRNA (Table 1).

### Identification of differentially expressed genes

We next asked under which conditions and to what extent RNA and protein expression were altered. To identify differentially expressed mRNAs and proteins, we used DESeq2 (Love, Huber, and Anders 2014). Since our data clustered significantly by growth phase, we analyzed RNA and protein expression separately for exponential and stationary phase. For each growth phase, we defined the reference condition to be glucose as carbon source, with 5mM Na+ and 0.8mM Mg2+. This is the baseline formulation of media used in the glucose time-course samples (Houser et al. 2015). We then compared RNA and protein abundances between this reference condition and the alternative conditions (different carbon sources, elevated Na+, and elevated or reduced Mg2+). Note that a detailed comparison of reference exponential phase vs. reference stationary phase has already been published (Houser et al. 2015).

We defined significantly differentially expressed genes as those whose abundance had at least a two-fold change (log2 fold change > 1) between the reference condition and a chosen experimental condition, at an FDR-corrected *P* value < 0.05. We found that the number of significantly differentially expressed mRNAs and proteins varied substantially between exponential and stationary phase and between mRNAs and proteins (Figure 5). In general, there were fewer differentially expressed genes in stationary phase than in exponential phase. Further, protein abundances showed the most differential regulation for high Na+ and for the carbon source glycerol, whereas mRNA showed the most differential regulation for altered Mg2+ levels and again for the carbon source glycerol (Figure 5).

Next, we asked how much overlap there was among differentially expressed genes between the various growth conditions. To simplify this analysis, we did not distinguish between up- or down-regulated genes, and we combined low and high Mg2+ into one group “Mg stress” and glycerol, lactate, and gluconate into one group “carbon source”. (Note that differentially expressed genes were still identified for individual conditions, as described above, and were combined into “Mg stress” and “carbon source” only for the final comparison.) At the mRNA level, there was little overlap between Na stress and either Mg stress or carbon source, and moderate overlap between Mg stress and carbon source, in particular in exponential phase (Figure 6). By contrast, at the protein level, there was more overlap between Na stress and either Mg stress or carbon source, in particular in exponential phase (Figure 6).

We also identified significantly altered biological pathways and molecular activities of gene products. We use the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000) for biological pathways and annotations from the Gene Ontology (GO) Consortium for molecular functions (Ashburner et al. 2000). Figure 7 and Supplementary Table 7 show the top 5 significantly altered biological pathways molecular functions under different conditions, as determined by DAVID (Huang, Sherman, and Lempicki 2008). In all cases, we used a cutoff of 0.05 on FDR-corrected *P* values to identify significant annotations.

Finally, we looked at individual, differentially expressed genes associated with specific pathways and/or functions (Supplementary Figures in HTML file). As an example, the differentially expressed mRNAs associated with significantly altered KEGG pathways under high Mg2+ concentrations in exponential phase are shown in figure 8. Almost all of the most altered genes are significantly down-regulated in both situations. By contrast, using glycerol instead of glucose in exponential phase caused up-regulation of many pathways and genes (Figure 9).

**Broad trends of metabolism flow**

The metabolism flow experiments were done for both high Mg+2 and high Na+ concentrations and for both exponential and stationary phase. For each of 20 samples 13 different fluxes were measured 3 times with technical replicates. Average values of those measurements were used in analyze. Trends with respect to changing Mg+2 and Na+ can be classified in 2 groups almost half of the fluxes are not changing with changing concentration and the other half responds to changes in Mg+2 and Na+ concentrations (Supplementary Figure 2-3).

## Discussion

We have studied the regulatory response of *E. coli* under a wide variety of different growth conditions. The experimental conditions we have considered include four different carbon sources, different levels of Na+ and Mg2+ stress, and growth into deep stationary phase, up to two weeks post inoculation. We have found that gene regulation changes the most with respect to growth phase; in general, the exponential phase under one condition is more similar to the exponential phase under another condition than to the stationary phase under the same condition. Further, we have found that the smallest number of differentially expressed genes for different carbon sources and larger numbers for different salt stresses. Under salt stress, most genes tend to be down-regulated, with the exception of elevated Mg2+ in stationary phase, where we have found over twice as many up-regulated mRNAs as down-regulated mRNAs. Finally, our measurements provide a comprehensive dataset that will be useful for future modeling of *E. coli* metabolism.

Our data provides a comprehensive picture of *E. coli* in terms of number, range, and depth of different stresses, comparable and complementary to other recently published datasets. For example, (Schmidt et al. 2015) considered 22 unique conditions and measured abundances of >2300 proteins. mRNA abundances were not measured. (Soufi et al. 2015) considered 10 unique conditions and also measured abundances of >2300 proteins. They were interested primarily in up- and down-regulated proteins under different ethanol stresses. However, similar to our study, they found down-regulation of genes associated with arginine biosynthesis and metallic ion binding under stress. (Lewis et al. 2010) considered only 3 different carbon sources but measured mRNA and protein abundances in different strains adapted to these growth conditions. Finally, (Lewis et al. 2009) compiled a database of 213 mRNA expression profiles covering 70 unique conditions, including different carbon sources, terminal electron acceptor, growth phase, and genotype. In comparison, we considered 34 unique conditions, measured 143 mRNA expression profiles and 101 protein expression profiles, and used the exact same *E. coli* genotype throughout.

Similar to our prior study (Houser et al. 2015), we observed clear differences in the differential expression of mRNAs and proteins. In particular, (Houser et al. 2015) reported that mRNAs are widely down-regulated in stationary phase whereas only select proteins are down-regulated. Consistent with that observation, we found here that mRNAs were significantly and strongly clustered by Na+ level (z=-4.34) and growth phase (*z* = −23.21) whereas proteins were not (*z* = −1.26). By contrast, at the protein level we saw significant clustering by carbon source (*z* = −2.80), which we didn’t see at the mRNA level. More specifically, (Houser et al. 2015) found that energy-intensive processes were down-regulated and stress-response proteins up-regulated in stationary phase. Similarly, we observed here that stress conditions also lead to the down-regulation of energy-intensive processes. In particular, we saw down-regulation of translation, flagellar assembly, nucleotide related pathways such as RNA degredation, and aminoacyl-tRNA biosynthesis. These pathways seem to be broadly down-regulated under many different stress conditions (Yoon et al. 2003; Gadgil, Kapur, and Hu 2005).

For all growth conditions we observed patterns of altered gene expression consistent with the known function of specific differentially expressed genes. For example, under Na+ stress we saw up-regulation of *acnB* and *talB*, as previously reported (Weber, Kögl, and Jung 2006). In particular, low Mg2+ concentrations caused a decrease in the *fec* operon also associated with ABC transporters involved in iron transport. A possible explanation is that magnesium transporters are permeable to ferrous iron at low magnesium concentrations (Hantke 1997), and a down-regulation of iron transporters may be needed to compensate. The fact that many ABC transporters were affected by salt concentrations may be indicative of other transporters that are leaky to off-target ions.

Providing glycerol instead of glucose as the sole carbon source increased expression of the *glp* operon, which is involved in glycerol uptake (Weissenborn, Wittekindt, and Larson 1992). Gluconate as a carbon source increased expression of the *gtn* operon, involved in gluconate metabolism (Fujita et al. 1986; Bausch et al. 1998). Finally, for both altered salt concentrations and altered carbon sources we saw differential expression in a number of genes involved in amino-acid and nucleotide biosynthesis, as well as genes encoding ribosomal proteins. These regulatory changes may reflect the overall metabolic activity of *E. coli*, and how the bacteria generally respond to more or less challenging conditions, rather than any specific induction caused by experimental conditions.

It is well known from large-scale, high-throughput gene-expression studies that measurements are frequently confounded by batch effects (Leek et al. 2010). We saw such effects in our study as well. In our data, the batch number indicates bacterial samples that were grown at the same time. Not unexpectedly, our data showed significant clustering by batch number, and more so in protein data than in mRNA data (*z* scores of −20.54 and −2.11, respectively). Batch effects are not inherently a problem, as long as we are aware of their existence and analyze the data accordingly. To address the batch effects we add batch as a distinct variable into DeSeq model, which is the suggested way to address the batch effect (“Differential Analysis of Count Data – the DESeq2 Package” 2016). But if one wants to investigate further Increasingly sophisticated methods are being developed to separate batch effects from real signal in an automated fashion (Kim, Zorraquino, and Tagkopoulos 2015).

Given the many cellular changes observed in mRNA and protein levels, we turned to 13C labeling techniques (Zamboni et al. 2009; Houser et al. 2015) to examine the extent to which these changes effected the relative flow of metabolism through central metabolic pathways during exponential growth. For this work we concentrated upon growth on glucose during Na+ and Mg2+ stresses. Across the salt concentrations utilized growth changed over nearly a two-fold range, with the doubling time changing from approximately 50 to 95 minutes. Na+ levels decreased growth by a third, getting monotonically worse with higher levels, yet there were no significant changes observed in central metabolism. Intermediate Mg2+ levels permitted the fastest growth, with ~10% slower growth at 8 mM and below, and ~50% slower growth at 400 mM. Although the relative flux at several key splits in metabolism was unchanged across this range, several changed significantly. The proportion of oxaloacetate generated from phospoenolpyruvate and pentose-5-phosphate from glucose-6-phosphate both decreased (and thus pentose-5-phosphate from glyceraldehyde-3-phosphate decreased) when growth was slow at either high or low concentrations. As these changes were not observed during Na+ stress that led to similar growth defects, they do not simply arise as a consequence of slowed growth and we speculate that they may be specific to Mg2+ stress. With the exception of those changes, however, the general picture was that homeostasis in central metabolism was sufficient to ward off significant changes in relative pathway use despite large effects upon overall growth rate and the pools of mRNA and proteins.

In summary, our study provides a large and comprehensive data set investigating the gene-regulatory response of *E. coli* under different growth conditions, both at the mRNA and the protein level. We have found systematic differences in gene-expression response between exponential and stationary phase, and between mRNAs and proteins. Our data set provides a rich resource for future modeling of the *E. coli* metabolism.

## Materials and Methods

### Cell Growth, RNA seq, and proteomics (This part need something related with flux measurements)

Growth and harvesting of *E. coli* B REL606 cell pellets for the multiomic analysis was as previously described (Houser et al. 2015) with the following additional details. For tests of different carbon sources, the Davis Minimal (DM) medium used was supplemented with 0.5 g/L of the specified compound (glycerol, lactate, or gluconate) instead of glucose. Mg2+ concentrations were varied by changing the amount of MgSO4 added to DM media from the concentration of 0.83 mM that is normally present. For tests of different Na+ concentrations, NaCl was added to achieve the final concentration. The base recipe for DM already contains ~5 mM Na+ due to the inclusion of sodium citrate, so 95 mM NaCl was added for the 100 mM Na+ condition, for example. Exponential phase samples were taken during growth when the OD600 reached 20-60% of the maximum achieved after saturating growth. Stationary phase samples were collect 20-24 hours after the corresponding exponential sample. The exact sampling times for each condition are provided in Supplementary Table S1.

After sample collection, RNA seq and mass-spec proteomics were performed exactly as described in (Houser et al. 2015).

### Data Preparation

Our raw input data consisted of RNA and protein counts. Protein counts can be fractional, because some peptide spectra cannot be uniquely mapped to a single protein. We rounded all protein counts to the nearest neighbor for subsequent analysis. We set the counts of all unobserved proteins to zero. Subsequently, all RNA and protein counts were analyzed in the same manner.

We next performed quality control, by checking replicates of the same condition for consistency. For all pairs of replicate samples, we made histograms of the log-differences of RNA or protein counts. If the two samples differ only by experimental noise, then the resulting histogram should have a mode at 0 and be approximately bell-shaped. If a sample consistently shows deviations from this expectation when compared to other samples, then there are likely systematic problems with this sample, and we excluded all such samples. In total, we excluded 6 RNA and no protein samples.

After quality control, we normalized read counts using size-factors calculated via DESeq2 (Love, Huber, and Anders 2014). Because we had many RNAs and proteins with counts of zero at some condition, we added pseudo-counts of +1 to all counts before calculating size factors. However, we then used those size factors to normalize raw counts (i.e., without pseudo-counts).

### Clustering

We clustered normalized counts based on their Euclidian distance, using the complete linkage method implemented in the hclust function in R. This method defines the cluster distance between two clusters as the maximum distance between their individual components (Madhulatha 2012). At every stage of the clustering process, the two closest clusters are merged into the next bigger cluster. The final outcome of this process is a dendogram that measures the closeness of different samples to each other.

To assess whether the clustering process significantly grouped similar samples together, we employed a reshuffling test. For any category that we tested for significant clustering (e.g., carbon source, Na stress, or batch number), we calculated the mean cophenetic distance in the clustering dendogram between all pairs belonging to the same level of the categorical variable tested (e.g., same carbon source). We then repeatedly reshuffled the labeling within each category and recalculated the mean cophenetic distance each time. Finally, we calculated *z* scores of the original cophenetic distance relative to the distribution of reshuffled values.

### Identifying differentially expressed genes

We used DESeq2 (Love, Huber, and Anders 2014) to identify differentially expressed mRNAs and proteins across conditions. We used two reference conditions in our comparisons, one for exponential phase and one for stationary phase. The reference conditions always had glucose as carbon source and base NaCl and MgSO4 concentrations. We did not compare exponential phase to stationary phase samples, since this comparison was done in depth by (Houser et al. 2015) for samples grown on glucose and with base NaCl and MgSO4 concentrations.

We considered genes as differentially expressed between two conditions if their log2 fold change was >1 and their FDR-corrected *P* value <0.05. We subsequently annotated differentially expressed genes with DAVID (Huang, Sherman, and Lempicki 2008), considering both KEGG pathways (Kanehisa and Goto 2000) and GO annotations (Ashburner et al. 2000).

### Statistical analysis and data availability

All statistical analyses were performed in R. The relevant R scripts and processed data are available on github: XXX Raw RNA reads and peptide spectra are also available under accession numbers XXX and YYY.

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