**Controlled measurements of multiple cellular components**

Here we outline our approach to measure multiple cellular components under identically controlled experimental conditions. Under a particular environmental condition we grow multiple cultures from the same stock and in the same media and the cultures are distributed to the different labs to measure RNA, protein, lipids, and metabolic flux ratios (Figure 1A). We present data on a long term glucose starvation of *E. coli* in a time course lasting 2 weeks. Freezer stocks of the *E. coli REL606* strain where revived for 24 hrs, diluted and preconditioned for another 24 hrs, and diluted again at the start of the experimental time course (Figure 1B). In a pilot experiment a growth curve was taken to determine informative time points for analysis (Figure 1C). Time points spanning 3 hrs-2 weeks where collected and equitably distributed to measure RNA (via RNAseq), proteins via LC/MS, Lipids via Maldi-TOF MS and ESI MS, and Metabolic Fluxes via U-13C labeled glucose and GC-MS (Figure 1D). Below we characterize our data outlining major trends and comparing and contrasting the response of different cellular subsystems.

**General trends of RNA and Protein time course**

To elucidate the general trends of differential gene expression during glucose starvation we performed *K*-means clustering separately on the RNA and protein profiles, averaged across all biological replicates. Below we discuss how we identified expression profiles based upon general behaviors and then perform a GO term enrichment. For now we focus on comparing and contrasting general trends between the RNA and protein profiles. In *K*-means clustering it is up to the user to pick the number of clusters that is appropriate such that the profiles are well separated into groups with unique and distinct behaviors.

We varied the number of clusters for both RNA and protein profiles to , and we found the best clustering performance around 15 clusters for the RNA profiles and 25 clusters for the protein profiles. Thus, the RNAs are responding in a much more uniform manner than the proteins, as shown by a heatmap of the cluster centroids of RNA and protein, respectively (Figure 2A and B). A large number of the mRNAs are down regulated, while the protein response is much more diverse. or both

It is important to note that the above clustering of the RNA and protein are independent and thus we cannot directly compare individual clusters between figures 2A and B. The next section addresses the correlation between absolute and relative changes in abundance of individual proteins and their transcripts.

**Correlations between individual mRNAs and proteins**

There is generally poor correlation between the absolute abundances of mRNAs and their corresponding proteins <refs>. This trend also holds in our data, as can be seen by the scatter plot of protein and RNA levels (Figure 2F) for the first time point (*t*=3 hr). Both protein and RNA levels in the plot are averaged across all three biological replicates. To put proteins and RNA, within a given sample, on comparable scales the protein counts were normalized using the apex method<ref> for absolute quantification and the mRNA was normalized to the length of each transcript. All proteins and mRNAs were then scaled by the average of the protein and mRNA, respectively, across the entire sample. The strongest correlation between RNA and Protein was at three hours (Spearman ρ=0.59, *P*=…). This number is in-line with measurements of correlations between RNA and protein for other organisms <ref>.

In addition to the correlation of absolute abundance, we also tested if the relative amounts of transcripts and proteins, across the time course, show any correlation. We account for the underlying dynamics of the time courses by considering two limiting cases. At one extreme we assume each protein has a degradation rate slower than the time scale of the experiment. At the other extreme we assume each protein is degraded on a time scale that is fast compared to the time scale of the experiment. In the first limiting case proteins integrate their transcript levels over time. In the second limiting case (relative) protein levels track with their (relative) transcript level. If a protein is not integrally or proportionally related to its transcript it could be due to an intermediate protein degradation rate, saturation effects, or some other post-translational regulation or nonlinearity. Plotted in figure 2C and D are histograms of the Spearman correlation coefficients (ρ) calculated for protein vs. the integral of its transcript and protein vs. its transcript, respectively. Approximately 15% of the proteins correlated highly (ρ>0.75) with the integral of their transcripts whereas approximately 20% correlated highly with proportional levels of RNA. There s no overlap between the two sets.

**Correlation of expression between genes in the same operon**

Genes within an operon are co-transcribed as a single RNA and thus are likely to be under the same transcriptional control. Our RNAseq data showed high correlation between pairs of genes within an operon (figure 3A), demonstrating nearly identical transcriptional regulation within operons. In contrast to the mRNA, pair-wise correlation between protein profiles of genes within the same operon showed very little correlation overall (figure 3B). As an example, proteins in an operon that have relatively high correlation, show differences in their changing expression over time (figure 3C,D). On the other hand, mRNAs within an operon are nearly indistinguishable in their profiles, as seen by the representative emrKY operon (figure 3E). Differences in translational efficiency between genes, localization, feedback, or other post-translational modifications could contribute to the lack of correlation seen in protein profiles of genes in the same operon<ref>. Our data demonstrates that genes closer together within an operon are more likely to have correlated protein profiles (see figure 3C) evidence that distance between genes is a strong indicator of translational regulation.

**RNA and Protein time course classification**

Typical analysis of RNA expression data often involves performing a hierarchical clustering of profiles followed by a term enrichment of subsets of genes found in the emerging patterns. In this approach the patterning that comes from hierarchical clustering can be arbitrary, dependent on what level of the hierarchy one chooses to look at. Thus, we sought to sort the time courses into general behaviors in an unbiased manner. To accomplish this we fit each individual mRNA and protein to a piecewise continuous curve (figure 4A). This curve is defined by 4 free time parameters and 3 free amplitude parameters. To fit the curve we used a population based differential evolution (DE) algorithm. Furthermore the fitness function used in minimization is scaled to the experimental error (see methods). Thus, our algorithm provides confidence intervals for our fit based upon the variability in biological replicates. To demonstrate the effectiveness of our fitting strategy we randomly selected five mRNA profiles and their respective fits (figure 2B-F). Green circles show the average of three biological replicates with their standard deviations (green bars) and the blue line and bar show the average and standard deviation of the population of fits respectively. Both the data and fit have been normalized to the average of the time course.

As can be seen in figure 4B-E, for mRNAs we generally get good agreement between the data and model. Thus, the fits give us reasonable estimates of the distribution of time scales involved in the response. Figure 4F shows the distribution of *t*1, the time to first inflection. Most of the mRNAs begin responding between 3-8 hrs, during the transition between exponential to stationary growth. To better understand the regulation of cellular processes (and mRNAs) in our dataset we sorted the mRNA profiles, based upon the estimated parameters of our fit, into five general categories: up regulated, down regulated, temporarily up regulated, temporarily down regulated, or unknown. The confidence intervals for our fits allowed sorting individual mRNAs into these five categories with high confidence. The mRNAs in the categories “down regulated” and “up regulated” showed significant enrichment for GO terms. The average of the mRNAs in each of these terms can be seen in figure 5A, B.

To characterize the protein response we followed the same general strategy of fitting, classification, and GO enrichment as we did for the RNA profiles. The distribution of the time to first inflection for the proteins is a little broader than the mRNAs. However, they are still mostly in the range between 3-8 hrs with very few proteins that have not responded by the time the cells enter stationary phase. There are many proteins that are turned on for the duration of the time course, compared to the mRNAs where very few remain on for the entire duration of the experiment. Figure 5D shows the average of the proteins in a given GO term that are enriched in the set of proteins that are being up regulated. The average of proteins in GO terms being down regulated have a much wider distribution of decay times compared to the RNAs being down regulated likely due to differing protein stabilities (figure 5C).

As a complementary approach we also averaged all proteins in a given Kegg pathway regardless of their behavior. Many pathways showed little to no differential regulation, on average, in their protein levels. Pathways that did change cohesively are plotted in Figure 5E and F depending on if they were down or up regulated, respectively.

**Flux ratio analysis.**

We used flux ratio analysis to measure the relative metabolic fluxes passing through different branches of the central metabolism. The only significant changes in the flux ratio that occur during the time course are in P5P from G3P and S7P and P5P from G6P lower branch (Figure 6G). P5P from the G6P lower branch decreases from 1 to 0.5 into the second week. P5P from G6P and S7P is one minus the flux from P5P from the G6P lower branch. All other measured flux ratios showed little change through the course of the experiment (figure 6A-I). We also compared the flux ratios with the corresponding ratio of enzymes responsible for catalyzing those reactions both at the level of RNA and protein. Generally speaking the protein ratios changed little, consistent with the low change in flux ratio <Note: I'm not sure if this is true, Viswanadan will have to speak to this>. The flux ratio that did change (P5P from G6P lower branch), however, showed high correlation with its corresponding protein ratio (ρ=0.85, *P*=…) and low correlation between its corresponding mRNA ratio (ρ=0.5, *P*=…).

**Lipid profiles**

Using negative ion MALDI-TOF and ESI mass spectrometry we analyzed Lipid A and phospholipids, respectively. Around 8hr the ms peaks associated with an addition of the C16 chain to lipid A and the presence of a ~702.5 m/z corresponding to cyclopropanation of one unsaturated double bond within a PE molecule containing acyl chains increased. Both modifications to lipid A and phospholipid continued to increase up to the 2 week period with the 702.5 m/z peak associated with phospholipid modification dominating, by 2 weeks, over the basal major peak at ~688.5 m/z. The enzymes relevant to the above lipidA and phospholipid modifications are pagP and cycloproponated fatty acid synthase (cfa), respectively. PagP transcript levels do not change significantly during the time course, neither did PhoP levels that drive transcription of PagP. Neither PagP or PhoP was observed at the protein level. CFA synthase protein levels increase between 3-6 hrs before decreasing again and CFA synthase RNA levels increase again around 1 week (figure 7B).