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

To elucidate the general trends 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 found ~15 clusters to work well for the RNA profiles while ~25 clusters worked better for the protein profiles. From the RNA and protein data we found ~1900 significantly changing profiles each. Thus, the RNAs are responding in a much more uniform manner than the proteins. Figure 2 A and B show a heatmap of the cluster centroids of RNA and protein, respectively. On the y-axis is the cluster number sorted arbitrarily and on the x-axis is the log10 of the time (normalized to t0=3hr). The darker blue colors represent higher relative expression. As can be seen most of the mRNas are down regulated and a few are transiently up regulated. Much fewer of the proteins are down regulated compared to the mRNAs with many permanently up regulated. It is important to note that the above clustering of the RNA and protein are independent and thus we can not directly compare individual clusters between figures 2 A 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**

In general, there is poor correlation between the absolute abundances of mRNAs and their corresponding proteins <refs>. This is a trend seen in our data. Plotted in figure 2F is a scatter plot of protein and RNA levels, on a log10 scale, for the first time point (t=3hr). Both protein and RNA levels are averaged across all three biological replicates. The protein counts are normalized using the apex method for absolute quantification and the mRNA is normalized to the length of each transcript. All proteins and mRNAs are then scaled by the average of the protein and mRNA respectively across the entire sample in order to put the x and y axis on similar scales. The strongest correlation between RNA and Protein was at three hours and had a ρ=0.59. This number is in-line with measurements of correlations between RNA and protein for other organisms. 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 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 2 C 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 correlate highly (ρ>0.75) with the integral of their transcripts where as approximately 20% correlate highly with proportional levels of RNA. There is no overlap between the two sets. The terms enriched in the set of proteins that are proportionally controlled are Peptidyl-cysteine modification, locomotion and taxis, and others(see Table1). GO terms enriched in the set of integrally controlled proteins are nitrogen compound biosynthetic process, peptidoglycan biosynthetic process, and others (see Table 1).

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

Genes within an operon are cotranscribed as a single RNA and are thus are likely under the same transcriptional control. Our RNAseq data shows 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 show very little correlation overall(figure 3B). 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. We also see evidence for different degradation rates of proteins within an operon that have high pair-wise correlation.(figure 3E)

**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, however, 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 differential evolution (DE) algorithm that is population based. 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 (fig 2 B-F). Green circles show the average of three biological replicates with their std 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.

**RNA profiles**

As can be seen in figure 4 B-E we in general get good agreement between the data and model. Thus, the fits give us reasonable estimates of the distribution of times scales involved in the response. Figure 4 F shows the distribution of t1, 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 RNAs) in our dataset we sorted the RNA 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 RNAs into these five categories with high confidence. The RNAs in the categories down regulated and temporarily up regulated showed significant enrichment for GO terms. The average of the RNAs in each of these terms can be seen in figure 5A,B.

**Protein Profiles**

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 5 D 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 time course behavior. Many pathways showed little to no differential regulation, on average, in their protein levels. The pathways that did change are plotted in Figure 5 E and F depending on if they where 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 6) P5P from the G6P lower branch decreases from 1 to 0.5 into the second week where as P5P from G6P and S7P increases to 0.5 from ~0 at the same time. Thus, it seems P5P production is shifted from the G6P lower branch to G3P and S7P branches at 1-2 weeks of glucose starvation.

**Lipid profiles**

Using negative ion MALDI-TOF and ESI mass spectrometry to analyze Lipid A and phospholipids respectively. Around 8hrs we saw an increase in 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. 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 do 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 1week (figure 7 B).