Transcriptional regulation is only half the story

Joshua B Plotkin*

Department of Biology, University of Pennsylvania, Philadelphia, PA, USA

* Corresponding author. Department of Biology, University of Pennsylvania, 433 S. University Avenue, Philadelphia, PA 19104, USA.
Tel./Fax: +1 617 571 9591; E-mail: jplotkin@sas.upenn.edu

Molecular Systems Biology 6: 406; published online 24 August 2010; doi:10.1038/msb.2010.63

This is an open-access article distributed under the terms of the Creative Commons Attribution Noncommercial Share Alike 3.0 Unported License, which allows readers to alter, transform, or build upon the article and then distribute the resulting work under the same or similar license to this one. The work must be attributed back to the original author and commercial use is not permitted without specific permission.

The central dogma of molecular biology, articulated by Francis Crick, posits a flow of information from DNA to RNA to protein. Although the Human Genome Project has helped elucidate the first step in this cascade, the relationship between mRNA abundance and protein abundance has resisted systematic quantification, especially in higher eukaryotes. In their recent publication in Molecular Systems Biology, Vogel et al (2010) use a combination of microarrays and shotgun proteomics to quantify absolute mRNA and protein levels for over 1000 genes in a human cell line. Their analysis identifies sequence features related to translation and protein degradation that are as important as transcription in determining steady-state protein levels. This work provides an unprecedented, system-wide accounting of how information stored in our DNA determines the eventual state of our cells.

Molecular biologists have traditionally focused on transcriptional regulation as the main determinant of protein levels and, thus, cellular function. This focus is due, in part, to the historical sequence of discoveries following the work of Jacques Monod, and, more recently, to the development of microarrays (Schena et al, 1995) and sequencing-based technologies for large-scale mRNA quantification. The detailed view of transcription that has emerged from high-throughput mRNA measurements, including the inference of global regulatory networks (e.g. Lee et al, 2002), has shaped our understanding of how a healthy cell works, and also how pathways arise and might be remedied. In fact, our knowledge of transcriptional regulation has improved so rapidly over the past decade that it is easy to forget the diverse array of post-transcriptional processes—including mRNA processing and modification, miRNA modulation, translation initiation, elongation, termination, and protein degradation—that also influence steady-state protein levels.

Not so fast, say Vogel et al (2010). Systematic studies of post-transcriptional processes have come of age, owing again to the development of technologies for high-throughput measurements. In this study, the authors used microarrays to quantify mRNA levels, together with a sophisticated mass-spectrometry-based proteomics method called APEX (Lu et al, 2007) to quantify soluble protein levels in a tumor cell line. The APEX method, originally developed in yeast and Escherichia coli, is the work-horse behind the present study. Under this protocol, proteins are digested into peptides, which are separated by liquid chromatography, and then ionized and sequenced with tandem mass spectrometry. In principle, protein amounts are then quantified simply by counting the numbers of corresponding peptides observed in repeat runs. In practice, the APEX method critically corrects for factors, such as efficiency of ionization, that influence the a priori probability of peptide detection. As a result, APEX provides reliable quantification of protein levels over five orders of magnitude.

Vogel et al. analyzed about 200 sequence features as potential determinants of the steady-state protein levels they measured. The correlates considered include features such as coding-sequence length, amino-acid composition, predicted mRNA structure, putative miRNA target sites, and the presence of upstream start codons. The authors observed a lognormal distribution of protein-per-mRNA ratios—suggesting that many independent factors together contrive to determine translational efficiency and protein degradation rates. Some of the strongest individual correlates of protein abundance identified in the study are unsurprising: longer coding sequences typically produced less protein, controlling for mRNA levels, consistent with the idea that long transcripts are translated inefficiently and are prone to protein misfolding. Similarly, amino-acid content is also correlated with protein abundance, controlling for mRNA levels, consistent with variable costs associated with the depletion of different amino acids and different propensities for protein misfolding as a function of amino-acid composition. Furthermore, strong 5' mRNA secondary structure or the presence of upstream start codons both reduced protein levels, again controlling for mRNA. However, several features had a surprisingly small role: codon adaption and miRNA target sites did not significantly influence protein abundance. The most important take-home message, furnished by a non-linear multiple regression, is that features related to post-transcriptional processes, especially those found in the coding sequence, together explained as much variation in protein levels as mRNA levels themselves did (Figure 1). Thus, transcriptional regulation is only half the story.

Aside from generating the largest dataset to date of protein and mRNA concentrations in human cells, this study
systematically quantifies the importance of translation and protein degradation regulatory processes, both individually and in aggregate. This work extends similar analyses performed in bacteria (Nie et al, 2006) and yeast (Brockmann et al, 2007; Wu et al, 2008), and it is preferable to analyses that are based on mRNA and protein measurements obtained from separate experiments. Nonetheless, this study is still limited to about 1000 soluble proteins, measured in an asynchronous, log-phase population of a tumor cell line, which contains chromosomal and methylation irregularities. Moreover, the strict separation of sequence features into those that determine steady-state mRNA levels and those that act post-transcriptionally is problematic: some nominally post-transcriptional features, such as those that influence ribosomal initiation, may feed back to influence steady-state mRNA levels as well (Iost and Dreyfus, 1995). Nonetheless, future studies in multiple cell lines, ideally including membrane proteins and synchronized populations, should elucidate how protein levels differ between and, indeed, define alternative cellular states. Such studies will be especially powerful when combined with high-throughput techniques for measuring ribosomal occupancy (Ingolia et al, 2009), allowing us to compare protein levels with direct estimates of translational efficiency, and to quantify protein stabilities as well.

The quantification and analysis of protein levels for 1000 human genes is a remarkable technical feat and is emblematic of the system-wide approach to studying basic questions in molecular biology. Without doubt, the growing literature based on high-throughput mass spectroscopy will continue to inform our understanding of post-transcriptional regulation, much as microarrays revolutionized our understanding of transcriptional regulation. Such measurements performed in relatively natural cellular conditions on endogenous genes will nicely complement manipulative experiments that interrogate protein production using synthetic, heterologous gene constructs (e.g. Voges et al, 2004). Together, these systematic approaches promise to elucidate the operational details of Crick’s central dogma.

**Conflict of interest**

The author declares that he has no conflict of interest.

**References**

Brockmann R, Beyer A, Heinisch JJ, Wilhelm T (2007) Post-transcriptional expression regulation: what determines translation rates? *PLoS Comput Biol* 3: e57

Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS (2009) Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling. *Science* 324: 218–223

Iost I, Dreyfus M (1995) The stability of *Escherichia coli* lacZ mRNA depends upon the simultaneity of its synthesis and translation. *EMBO J* 14: 3252–3261

Lee TI, Rinaldi NJ, Robert F, Odom DT, Bar-Joseph Z, Gerber GK, Nannah NM, Harbison CT, Thompson CM, Simon I, Zeitlinger J, Jennings EG, Murray HL, Gordon DB, Ren B, Wyrick JJ, Tagne JB, Volkert TL, Fraenkel E, Gifford DK et al (2002) Transcriptional regulatory networks in *Saccharomyces cerevisiae*. *Science* 298: 799–804

Lu P, Vogel C, Wang R, Yao X, Marcotte EM (2007) Absolute protein expression profiling estimates the relative contributions of transcriptional and translational regulation. *Nat Biotechnol* 25: 117–124

Nie L, Wu G, Zhang W (2006) Correlation of mRNA expression and protein abundance affected by multiple sequence features related to translational efficiency in *Desulfovibrio vulgaris*: a quantitative analysis. *Genetcs* 174: 2229–2243

Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270: 467–470

Vogel C, de Sousa RA, Ko D, Le S-H, Shapiro BA, Burns SC, Sandhu D, Bourtz DR, Marcotte EM, Penalva LO (2010) Sequence signatures and mRNA concentration can explain two-thirds of protein abundance variation in a human cell line. *Mol Syst Biol* 6: 400

Voges D, Watzele M, Nemetz C, Wizemann S, Buchberger B (2004) Analyzing and enhancing mRNA translational efficiency in an *Escherichia coli* *in vitro* expression system. *Biochem Biophys Res Commun* 318: 601–614

Wu G, Nie L, Zhang W (2008) Integrative analyses of post-transcriptional regulation in the yeast *Saccharomyces cerevisiae* using transcriptomic and proteomic data. *Curr Microbiol* 57: 18–22

**Molecular Systems Biology** is an open-access journal published by *European Molecular Biology Organization* and *Nature Publishing Group*. This work is licensed under a Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported License.