Questions about: Ingolia et al. - Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling

Question set 1

This manuscript is mainly about the establishment of the method. Why would one use ribosome profiling? What applications did the authors have in mind when they set to do this? Can you envision others? Contrast proteomics-based measurement of protein levels with that based on ribosome profiling.

Question set 2

The authors point out that there must be regulation at the level of translation. What is the evidence for that?

Question set 3

What were the main steps that needed to be worked out for developing this method? How where they addressed? What evidence do the authors provide that these steps worked adequately? What is the evidence that the sequenced footprints reflect the translated sequence?

Question set 4

What are the authors' arguments that the obtained ribosome profiles reveal different phases of translation? In particular, what does figure S12 show?

Question set 5

How is translation efficiency estimated? What do Figures 2E and S8 show about the translation efficiency of different genes?

Questions about: Li et al. - Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources

Question set 1

In the introduction the authors mention design principles governing protein production within cells. What regulation targets are discussed? How do they fit with those you read about in the Drummond-Wilke study?

Question set 2

In the first section of the results the authors describe their approach to obtaining absolute numbers of protein molecules per cell. What is this approach? They further argue that, at least in rich media, translation elongation proceeds at roughly constant speed, irrespective of the relative abundance of individual tRNAs. What evidence supports this statement?

Question set 3

What is the principle of proportional synthesis of proteins? What do the authors show to be the main mechanism? Why would it make sense to implement proportional synthesis? Does this have anything to do with gene structures in bacteria? At what level is proportional synthesis regulated? What factors are known so far to regulate translation initiation?

Question set 4

What are the implications of proportional synthesis on the cell function in the presence of aneuploidy? Can you envision a mechanism that would allow the cell to better cope with aneuploidy?

Question set 5

What do the authors mean by 'hierarchical expression of functional modules'? The authors argue that this applies to many systems, including toxin-antitoxin, sigma factor-antisigma, two-component systems (substrate-kinase). They further mention that an excess of substrate relative to enzyme ensures robustness against variation in both of these factors. Why would that be?

Question set 6

What principle have the authors uncovered concerning the abundance and mode of action of transcription factors? What is shown in Figure 6? Does it fit with, for example, the regulation of the Lac operon?

Question set 7

The authors analyze in detail the Met biosynthesis pathway. How do they estimate the demand for the metabolite produced in this pathway and how do they estimate the production rate? What does Figure 7B show about the optimality of MetE levels?

Question set 8

In the discussion, the authors mention that proportional synthesis is achieved by finely tuned rates of translation initiation. What evidence was presented for this in the manuscript? Do you find it convincing?