

Insights provided by ribosome profiling With these advantages and disadvantages in mind, the application of ribosome profiling to specific biological questions has confirmed much of what we know about translation mechanism from decades of elegant structural, biochemical and genetic studies⁵⁰. Quantitative measurement of protein synthesis rates over multiple time points of a dynamic process can also provide information about specific gene function. The application of this method to numerous organisms and cellular states has illuminated fundamental aspects of cell biology that were previously challenging to probe experimentally, providing measurements for how much of each protein is synthesized, how translation is regulated, where synthesis starts and stops and what is being synthesized. Assuming that the average translation elongation rate is similar for different genes, ribosome profiling provides direct, global and quantitative measurements of rates of protein synthesis, thereby capturing information that has been largely invisible to gene expression measurements of mRNA levels alone. Analysis of the positions of mRNAs in polysome gradients provides valuable complementary information to that obtained with ribosome profiling, but again, this method is laborious and typically yields only a qualitative measure of protein synthesis. Other recent studies in disparate systems -- from the *Drosophila melanogaster* oocyte-to-embryo transition⁵⁵ to the *Trypanosome* life cycle⁵⁶ to the mammalian cell cycle⁵⁷ to plants under hypoxic conditions²⁷ -- have used ribosome profiling to identify specific proteins that drive these complex processes. The latter category includes cellular differentiation, organismal development and dynamic responses to cellular stress, which are all cases in which the instantaneous and downstream gene expression measurements provided by ribosome profiling are particularly illuminating for understanding molecular control. Cases in which ribosome profiling data provide markedly different information than can be obtained by traditional mRNA abundance measurements for gene expression tend to fall into two categories: systems in which transcriptional regulation is minimal^{26,54,55}; and dynamic cellular programmes^{11,12,27,35,57-59}. This property of proportional synthesis, by which subunits of multiprotein complexes are synthesized at rates that are proportional to their stoichiometry in the complex, turns out to be generally true for *Escherichia coli* and was also observed for some (but not all) complexes in budding yeast (*Saccharomyces cerevisiae*). Subsequent morpholino knockdown experiments showed that specifically blocking translation of these three factors resulted in a shutdown of the first wave of zygotic transcription and development, indicating that they are the key factors responsible for the initiation of the zygotic developmental programme⁵⁴. As is the case for many protein complexes in bacteria, the eight different subunits of the FoF₁-ATP synthase are expressed from a single polycistronic mRNA, and thus measurements of mRNA levels would suggest that the subunits are all expressed at very similar levels. For example, hierarchical clustering of patterns of new protein synthesis for each gene over the dynamic process of meiosis in budding yeast resulted in an intricate map of gene expression that

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