Insights provided by ribosome profiling With these advantages and disadvantages in mind, the application of ribosome profiling to specific biologi- cal 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 regu- lated, 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 quantita- tive 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 labo- rious and typically yields only a qualitative measure of protein synthesis.Other recent studies in disparate systems –– from the Drosophila melanogaster oocyte–to–embryo transi- tion55 to the Trypanosome life cycle56 to the mamma- lian 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 develop- ment 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 dif- ferent information than can be obtained by traditional mRNA abundance measurements for gene expression tend to fall into two categories: systems in which tran- scriptional regulation is minimal26,54,55; and dynamic cellular programmes11,12,27,35,57–59.This property of proportional synthesis, by which sub- units of multiprotein complexes are synthesized at rates that are proportional to their stoichiometry in the com- plex, 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 tran- scription 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 Fo F1 –ATP syn- thase 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 pro- tein synthesis for each gene over the dynamic process of meiosis in budding yeast resulted in an intricate map of gene expression that provided highly detailed functionalHow much?3b).