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Ribosome profiling and dynamic regulation of translation in mammals

Cédric Gobet and Felix Naef

Protein synthesis is an energy-demanding cellular process. Consequently, a well-timed, fine-tuned and plastic regulation of translation is needed to adjust and maintain cell states under dynamically changing environments. Genome-wide monitoring of translation was recently facilitated by ribosome profiling, which uncovered key features of translation regulation. In this review, we summarize recent ribosome profiling studies in mammals providing novel insight in dynamic translation regulation, notably related to circadian rhythms, diurnal feeding/fasting cycles, cell cycle progression, stress responses, and tRNA landscapes. In particular, recent results show that regulating translation initiation and elongation represent important mechanisms used in mammalian cells to rapidly modulate protein expression in dynamically changing environments.

Address
The Institute of Bioengineering (IBI), School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015, Switzerland

Corresponding author: Naef, Felix (felix.naef@epfl.ch)

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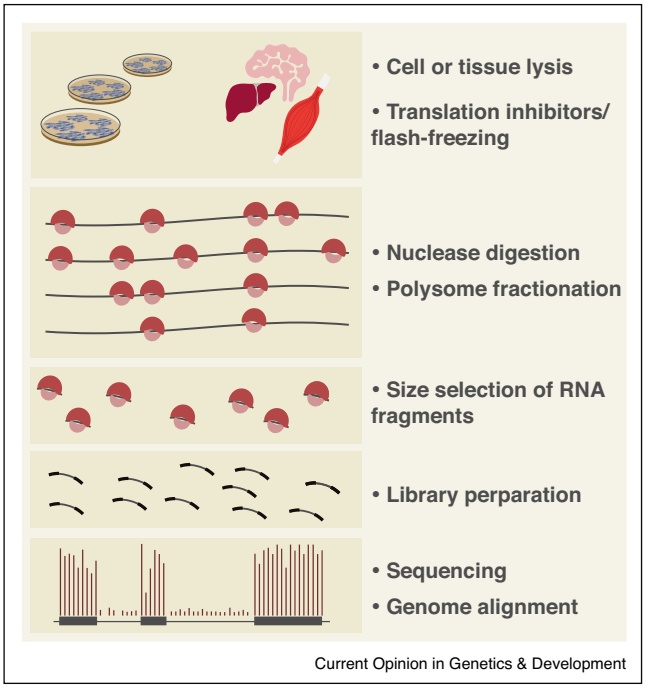
Introduction

Protein levels in cells are controlled by the dynamic balance between production and degradation processes underlying many steps from gene transcription to protein translation. Today, a diversity of technologies to probe the complex molecular networks involved in gene expression is available [1]. Development of high-throughput sequencing, mass spectrometry and bioinformatics tools, has allowed integrative analysis to yield genome-wide and fairly complete views of the many cellular steps regulating protein levels. Surprisingly, such analyses have revealed hidden and intricate layers of regulation, and allowed estimating the respective contributions of transcription, mRNA accumulation-degradation, translation and post-translational events to the steady-state protein levels [2]. For example, such efforts showed how translation explains part of the reported deviation in mRNA versus protein accumulation. However, the contribution of translation regulation is recently debated [3,4], in particular, since it significantly depends on the systems and conditions [5,6]. Notably, the regulation of translation has emerged as an efficient mechanism to dynamically control protein abundance, which can do so more rapidly than other gene regulatory steps [7].

In 2009, Ingolia *et al.* [8] invented an ingenious method called "Ribosome profiling" (RP) (Figure 1), enabling genome-wide monitoring of translation. Briefly, ribosomes are halted by translation inhibitors or alternatively by flash freezing the cells. Messenger RNA (mRNAs) bound by ribosomes are isolated and undergo ribonuclease digestion. In addition, ribosomal proteins can be used to isolate ribosome-associated mRNAs through pull-downs in order to perform cell-type specific ribosome profiling [9–11]. Thereafter, polysomes are fractionated and monosomes are isolated. The mRNA fragments protected by the ribosomes are size selected to target a typical footprint of about 30 nucleotides. Subsequently, the RNA fragments are used to prepare a DNA library, which is then sequenced. The resulting reads, computationally mapped on the transcript databases or genome assemblies, determine the exact positions with nucleotide resolution of the respective translating ribosomes. After piling up all the reads, the obtained ribosome occupancy profiles along mRNAs reflect the combined effects of translation initiation, ribosome elongation, and translation termination rates [12]. In many models of translation, it is assumed that average ribosome dwell times (also called codon residence time) are equal for each gene, and that ribosomes density on transcripts is low, such that effects caused by traffic jams of ribosomes can be neglected [13]. Under these assumptions, the ratio of normalized RP and RNA-Seq signals is typically used as a proxy for the relative translation efficiency (defined as the rate of protein production per mRNA) and differential expression analysis [14,15]. However, RP signals reveal large fluctuations over the coding DNA sequence of a gene, which indicate elongation regulation and positional bias [16]. Various models and analyses in different organisms attempted to explain such properties with different molecular features [17–19,12,20–22] (*e.g.* tRNA abundance, tRNA-aminoacylation, wobble base pairing, mRNA secondary structure, amino acid properties, mRNA sequence, nascent protein folding and chaperones), and revealed as well technical or analytical biases

Figure 1



Overview of the ribosome profiling protocol.
Cells or tissues are treated with translation inhibitors or flash frozen in liquid nitrogen. Nuclease treatment is applied and unprotected RNA is digested. Ribosomes are recovered and mRNA footprint fragments are selected in function of their size. A library is prepared from the subsequent fragments and deep sequenced. Alignment of the reads to the genome reveals the respective position of ribosomes.

[23–27], (translation inhibitors, restriction enzymes, library preparation, fragment size selection and identification of ribosome sites). Because of this complexity, the inference of initiation and ribosome elongation rates is still highly challenging and not settled. Here, we will not discuss the various factors that determine position-specific RP signals, but focus on recent RP studies in mammals, highlighting dynamical aspects of translation regulation. For excellent and more general reviews on RP, we refer to Refs. [28] and [29].

Regulation of translation initiation

Translation follows three consecutive steps: initiation, elongation and termination, and is thought to be mainly regulated at the initiation step via global or mRNA-specific mechanisms [30]. In the canonical model of translation, a 43S pre-initiation complex (PIC), involving methionyl-tRNAi, the small ribosomal subunit and eukaryotic initiation factor 2 (eIF2) among other factors, is recruited to the m^7G-capped 5′ ends of mRNAs mainly through the regulation of the eIF4F complex (eIF4G, eIF4E and eIF4A). The PIC scans the 5′ untranslated regions (UTR) of mRNAs until recognizing the initiation codon. Consequently, the 60S ribosomal subunit is recruited and the resulting 80S complex is ready for elongation [31]. Various initiation factors are involved in this initiation phase, which provide a direct way for translation regulation, mainly through their phosphorylation. Trans-acting factors and mRNA features, mainly in the UTRs, add an mRNA-specific layer on this regulation [32]. Upstream signaling pathways sensitive to external/internal cues (*e.g.* stress, nutrients, or mitogens) directly regulate initiation factors and related proteins. In particular, the mammalian target of rapamycin complex 1 (mTORC1) pathway is known as the master regulator of translation [33]. mTORC1 modulates translation mostly through phosphorylation of eIF4E binding proteins (4E-BPs) and their subsequent release from eIF4E, allowing the formation of the eIF4F complex. In addition, mTORC1 regulates other factors (*e.g.* ribosomal protein S6 kinase) as well as ribosome biogenesis and tRNA synthesis. Recently, the genome-wide translational landscape under the control of mTOR was unveiled with RP [34,35]. In mouse embryonic fibroblasts treated with Torin 1 (mTOR inhibitor *i.e.*) [34], down regulation of genes related to the translation machinery and harboring 5' terminal oligopyrimidine (TOP) sequence motifs, or TOP-like motifs, was identified. Similarly, monitoring of translation in human prostate cancer cells [35] revealed high sensitivity of TOP mRNAs to mTOR inhibitors.

Feeding state dependent translation

Energetic state and nutrient availability play a central role in translation, mainly through mTORC1 and eIF2alpha regulation and their respective nutrient-sensitive signaling pathways [36]. Notably, in their pioneering ribosome footprinting study in yeast [7], Ingolia *et al.* showed that transcripts related to ribosome biogenesis were translationally affected under amino acid starvation, which extended previous experiments in amino acid-deprived yeasts [37]. More recently, polysome profiling in mouse revealed a temporal and feeding state dependent regulation of signaling pathways controlling translation initiation [38]. In the context of cross-talk between feeding/fasting cycles and circadian clocks, recent work used RP to study translation regulation occurring recurrently (rhythmically) in function of daily time in mouse liver [39•,40]. Under both ad libitum and night restricted feeding conditions, rhythmic translation efficiency was detected in two groups of genes. First, a cluster of transcripts related to the translation machinery showed up to ten fold increased ribosome occupancy during the night, coinciding with the animal feeding state. This group of transcripts was enriched for TOP motifs [39•,40]. Secondly, transcripts enriched for nuclear-encoded mitochondrial genes, and functionally related to oxidative phosphorylation (OXPHOS), exhibited increased translation during the day (fasted state). However, these transcripts showed lower translational amplitudes compared to the TOP transcripts, and were slightly affected in clock-deficient mice. Notably, these genes