Quantitative genomic analysis of translational control

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Short Abstract **— Translational control plays critical roles in gene expression and is mechanistically interwound with mRNA degradation. The RNA-dependent ATPase Dhh1 has been characterized as a key factor to elicit translation repression, which leads either to degradation or to storage of mRNA. To comprehensively identify modes of Dhh1-mediated regulation, we have quantified levels of mRNA expression and translation of wild-type and *dhh1*Δ yeast strains under exponential growth conditions on a genome scale. Our analysis has identified novel Dhh1-regulated mRNAs that are likely to be controlled by multiple distinct mechanisms.**

Keywords **— translational control, RNA-binding proteins, Dhh1, ribosome profiling.**

I. Introduction

REEGULATION of translation plays important roles in numerous biological processes, such as embryonic development, memory formation and innate immunity, and its dysregulation is associated with various human diseases [1]. Translation is a complex process and is influenced by a number of factors, including RNA-binding proteins (RBPs), which bind target mRNAs with various levels of specificity and affinity. The mechanisms of action of a RBP could vary depending on the target mRNA and on the environmental/developmental conditions and, therefore, have to be analyzed with a defined mRNA substrate under a defined condition in a quantitative manner. Although a number of RBPs have been shown to function in translational control by low-throughput analyses, attempts to comprehensively identify and quantitatively characterize their mechanisms of action are still scarce.

II. Approach and Results

We have set out to establish experimental and data analysis pipelines to quantitatively analyze roles of an RBP in translation control on a genome scale, using a model system, *Saccharomyces cerevisiae*. As a case study, we focus on the evolutionarily conserved RNA-dependent ATPase Dhh1, whose metazoan homologs are implicated in crucial biological processes, such as maternal mRNA storage, germ cell development, synaptic plasticity and miRNA-mediated translation repression [2-4]. Although genetic and biochemical analyses using engineered model mRNAs under various conditions have characterized Dhh1 as a general factor to elicit translation repression, which leads either to degradation or to storage of mRNA, the precise mechanisms of action of Dhh1 in regulation of each endogenous mRNA remain elusive. To comprehensively identify modes of Dhh1-mediated regulation, we have quantified levels of mRNA expression and translation in rapidly growing wild-type and *dhh1*Δ strains by mRNA-seq as well as by ribosome profiling, which measures levels of mRNAs that are engaged in translation [5]. Our initial analysis led to the following observations. First, as has been observed in previous gene-specific studies, Dhh1 preferentially regulates specific classes of mRNAs rather than uniformly affects all genes to a similar extent. Second, the overall changes in levels of ribosome profiling signals by deletion of *DHH1* mirror those in mRNA levels, consistent with the idea that Dhh1-mediated translation repression mainly results in rapid degradation of mRNA in dividing cells. We nevertheless identified a small fraction of genes whose translation rates are modulated by Dhh1 independently of their mRNA levels.

III. Outlook

Our results suggest that Dhh1-mediated translation repression mainly leads to degradation rather than storage of mRNA in dividing yeast cells. Nevertheless a small fraction of mRNAs is likely to be routed to mRNA storage after translation repression. Quantitative kinetic analyses of the Dhh1-regulated mRNAs identified by our initial analysis will provide a better picture of the mechanisms of actions of Dhh1.

References

[1] Sonenberg N, Hinnebusch AG (2007) New modes of translational control in development, behavior, and disease. *Mol Cell* **28**, 721-729.

[2] Nakamura A *et al.* (2001) Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during Drosophila oogenesis. *Development* **128**, 3233-3242.

[3] McCann C *et al.* (2011) The Ataxin-2 protein is required for microRNA function and synapse-specific long-term olfactory habituation. *PNAS* **108**, E655-662.

[4] Chu CY, Rana TM (2006) Translation repression in human cells by microRNA-induced gene silencing requires RCK/p54. *PLoS Biol* **4**, e210.

[5] Ingolia NT *et al.* (2009) Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science* **324**, 218-223.

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