From differential transcription of ribosomal proteins to differential structure of ribosomes

About a decade ago, I observed that as the cell growth rate increases, mRNAs coding for ribosomal proteins are transcriptionally induced to varying degrees. This observation puzzled me as it defied my expectation that faster growing cells meet their demands for increased protein synthesis by simply inducing all ribosomal proteins to the same degree to make more ribosomes. These initial data were limited to mRNA levels and thus too indirect to make concrete conclusions about ribosomal structure and function. This commentary outlines my trajectory investigating this puzzle in search of more direct data.

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From differential transcription of ribosomal proteins to differential structure of ribosomes

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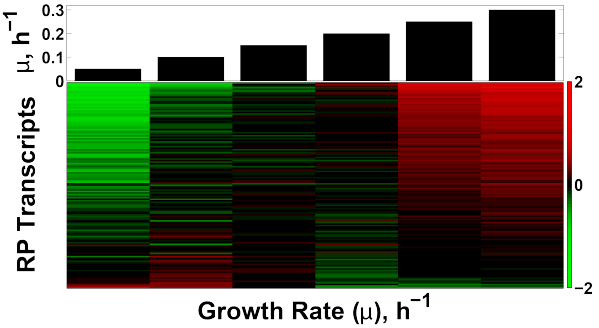
As growth rate increases, ribosomal proteins (RPs) are transcriptionally induced to varying degrees. This observation puzzled me as it defied my expectation that faster growing cells simply make more ribosomes by inducing all RPs equally. This commentary outlines my trajectory investigating this puzzle.

The results in our Cell report (Slavov *et al*, 2015) are particularly satisfying to me since they bring clarity to a puzzle that I have pursued for almost a decade. The puzzle started with an observation that I made while a beginning graduate student in the Botstein laboratory at Princeton University. I studied the transcriptional responses of yeast cells growing across a wide range of growth rates (Slavov and Botstein, 2011).

I found that as growth rate increases, mRNAs coding for ribosomal proteins (RPs) are transcriptionally induced to varying degrees; some are even repressed (Slavov *et al*, 2012; Slavov and Botstein, 2013).



These data allowed us to evaluate a suggestion that Ole Maaløe had proposed for bacteria over 30 years earlier: cells growing faster should induce the transcription of ribosomal proteins since they need to make more ribosomes that can meet the increased demands for protein synthesis. While most mRNAs coding for ribosomal proteins (RPs) exhibited this logical trend (their levels increased with the growth rate), others did not. The RP transcript levels that deviated from the expectation were reproducible across biological replicas and even across different

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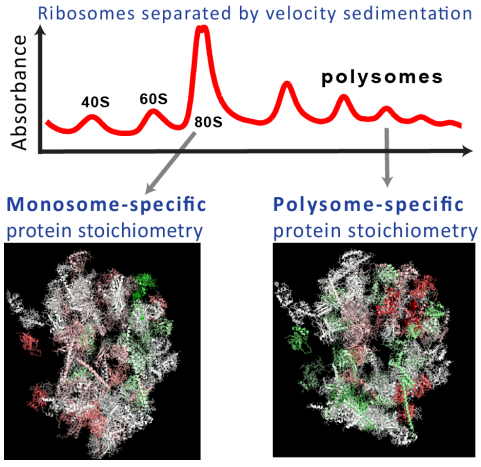
nutrient limitations used to control the cell growth rate. Furthermore, the number of the RP transcripts defying the expectations was even larger when I grew the yeast cells on ethanol carbon source (Slavov and Botstein, 2011). I also observed uncorrelated variability in RP transcripts across human cancers (Slavov and Dawson, 2009), but this observation was based on public data without biological replicates and with many confounding factors.

My observations of differential RP transcriptional induction puzzled me deeply. According to the decades-old model of the ribosomes, each ribosome has exactly one copy of each core RP. Thus, the simplest mechanism for making more ribosomes is to induce the transcription of each RP by the same amount, not to induce some RPs and repress others. Still, biology often defies simplistic expectations; one can easily imagine that RP levels are controlled mostly post-transcriptionally. Transcript levels for RPs were enough to pick my curiosity but ultimately too indirect to serve as evidence for the protein composition of the ribosomes. Thus, I neglected the large differences in RP transcriptional responses and interpreted our data with the satisfyingly simple framework suggested by Ole Maaløe. Many other research groups have also reported differential transcription of RP genes but these observations have many of the limitations inherent in my transcriptional data (Bévort and Leffers, 2000; Xue and Barna, 2012). The puzzle remained latent in my mind until years later I quantified the yeast proteome by mass-spectrometry as part of investigating trade-offs of aerobic glycolysis (Slavov *et al*, 2014). This time, the clue for altered protein composition of the ribosomes was at the level of the ribosomal proteins, not their transcripts. While still indirect and inconclusive, I found this observation compelling, especially since it resonated with an exciting opinion article by Gilbert (2011). My inconclusive observations motivated me to design experiments specifically aiming to find out if the protein composition of the ribosome can vary within a cell and across growth conditions.

The data from these experiments showed that unperturbed cells build ribosomes with different protein compositions that depend both on the number of ribosomes bound per mRNA and on the growth conditions (Slavov *et al*, 2015).



I find this an exciting result because it opens the door to conceptual questions such as: What is the extent, scope and specificity of ribosome-mediated translational regulation? What

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are the advantages of regulating gene expression by modulating the ribosomal composition as compared to the other layers of gene regulation, from histone modifications through RNA processing to protein degradation? Do altered ribosomal compositions offer trade-offs, such as higher translational accuracy at the expense of lower translation-elongation rate via more kinetic proofreading? Some of these question may (hopefully will) reveal general principles. These questions are fascinating to speculate about but they can also be answered by direct measurements. Designing experiments that can rigorously explore and discriminate among different conceptual models should be a lot of fun!

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