HIGH-RESOLUTION PHENOTYPIC PROFILING OF A EUKARYOTIC RIBOSOME

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Abstract

All living cells contain ribosomes, complex macromolecular assemblies of ribonucleic acid (RNA) and proteins, responsible for synthesizing polypeptides in a process called translation. High-resolution three-dimensional ribosomal structures coupled with biochemical studies have paved the way in understanding the various mechanistic events during protein synthesis. However, a systematic study dealing with the individual components and features of the ribosome is lacking. Specifically, the functional connection of each ribosomal protein and individual ribosomal RNA (rRNA) modification to cellular processes outside the domains of translation has not been fully explored.

Using the yeast *Saccharomyces cerevisiae* as a model eukaryotic organism, I investigated the contribution of individual ribosomal proteins and rRNA modifications to cellular fitness during growth in optimal and stress environments. I performed high-resolution phenotypic profiling on isogenic yeast strains with individual deletions in 110 cytoplasmic ribosomal protein (cRP) genes, 67 mitochondrial ribosomal protein (mRP) genes and 65 small nucleolar RNA genes (snoRNA). SnoRNAs facilitate site-specific rRNA modifications hence, their removal results to the absence of modification on the corresponding target site on the rRNA. I utilized a high-throughput phenotyping approach wherein the growth behaviors of individual deletion mutants were monitored in liquid micro-culture environmental arrays. Three physiologically-relevant growth variables, growth lag, growth rate and growth efficiency, extracted from high-resolution growth curves allowed for the precise quantification of marginal phenotypic consequences of deletions not detectable by standard, more qualitative approaches.

Detailed analysis of growth dynamics in a plethora of perturbed environments revealed a surprising range of hitherto unknown phenotypic diversity in the ribosome ranging from extreme sensitivity to extreme resistance in essentially every tested environment. Identification of dominant trends in the chemogenetic landscape allowed for the separation of the deletion mutants into distinct stress sub clusters, resolving the phenotypes into effects on different aspects of cellular physiology. Notable was the identification of phenotypes in all the sets of deletion mutants (cRP, mRP and snoRNA knockouts) exhibiting varying degrees of sensitivity to alternative carbon sources, strongly linking many of them to respiratory functions. The mix of cRP, mRP and snoRNA deletion mutants in some stress-specific sub cluster, such as the oxidative stress sub cluster, also indicated greater than expected functional overlap. The functional specialization within the yeast ribosome revealed a new level of translational complexity suggesting the existence of compositionally distinct ribosomes which are customized according to environmental cues. The direct participation of some ribosomal protein components and snoRNAs in cellular stress response was also envisioned. The implication of these hypotheses is significant not only in the basic understanding of the inner workings of the ribosome but also in providing new avenues in explaining the underlying mechanisms in the growing number of human diseases linked to defective components of the translational machinery.

Keywords: box C/D snoRNA, box H/ACA snoRNA, *2'-O*-ribose methylation, pseudouridylation, high-resolution phenotypic profiling, ribosome, ribosomal protein genes, chemogenetic profiling, *Saccharomyces cerevisiae*

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