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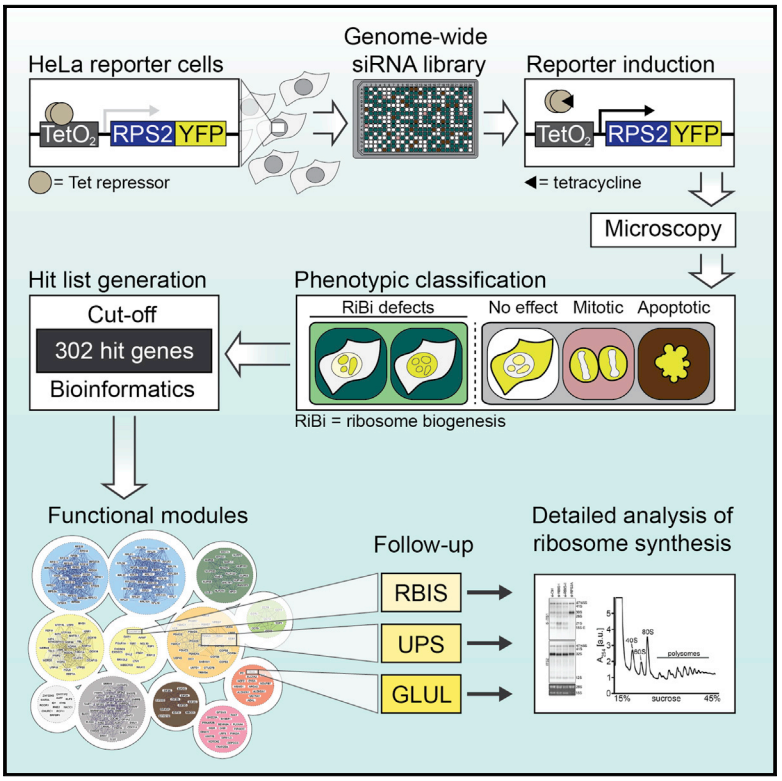
# Genome-wide RNAi Screening Identifies Protein Modules Required for 40S Subunit Synthesis in Human Cells

## Graphical Abstract




## Highlights

- Genome-wide RNAi screening identifies ~300 factors required for 40S subunit production
- The E3 ubiquitin ligase CRL4 is required for nucleolar steps of ribosome maturation
- Intracellular Gln synthesis supports efficient nuclear maturation of 40S subunits

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## In Brief

Badertscher et al. perform an image-based, genome-wide RNAi screen on 40S ribosomal subunit biogenesis and identify ~300 factors that support this process in human cells. Among these are many factors belonging to essential protein modules, including the ubiquitin-proteasome system, vertebrate-specific ribosome synthesis factors such as RBIS, and the glutamine synthetase GLUL.

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## SUMMARY

**Ribosome biogenesis is a highly complex process requiring many assisting factors. Studies in yeast have yielded comprehensive knowledge of the cellular machinery involved in this process. However, many aspects of ribosome synthesis are different in higher eukaryotes, and the global set of mammalian ribosome biogenesis factors remains unexplored. We used an imaging-based, genome-wide RNAi screen to find human proteins involved in 40S ribosomal subunit biogenesis. Our analysis identified ~300 factors, many part of essential protein modules such as the small subunit (SSU) processome, the eIF3 and chaperonin complexes, and the ubiquitin-proteasome system. We demonstrate a role for the vertebrate-specific factor RBIS in ribosome synthesis, uncover a requirement for the CRL4 E3 ubiquitin ligase in nucleolar ribosome biogenesis, and reveal that intracellular glutamine synthesis supports 40S subunit production.**

## INTRODUCTION

Ribosomes are conserved molecular machines dedicated to protein synthesis in all kingdoms of life. They are built from a small and a large subunit, both of which are sophisticated ribonucleoprotein particles composed of rRNA and ribosomal proteins (RPs). Although the ribosome structure and mode of operation follow the same principles in all species, ribosome synthesis has become more complex during evolution. The reasons for this greater complexity include increases in ribosome size, the establishment of nucleo-cytoplasmic compartmentalization, and the evolution of elaborate signaling networks that control ribosome synthesis and function, especially in higher eukaryotes. Importantly, deregulated ribosome production is associated with various ribosomopathies and cancer (Bywater et al., 2013; Hannan et al., 2013; Stumpf and Ruggero, 2011; Teng et al., 2013).

In vertebrates, the small 40S ribosomal subunit contains one rRNA (18S) and 33 ribosomal proteins (RPSs), whereas the large 60S subunit is built of three rRNAs (28S, 5.8S, and 5S) and 47 ribosomal proteins (RPLs). Ribosomal subunit biogenesis begins in specialized nuclear regions, called nucleoli, that are organized around repeats of rRNA genes. RNA polymerase I (Pol I) transcribes these genes as long precursor rRNAs (pre-rRNAs). Each pre-rRNA contains rRNA segments (18S, 5.8S, and 28S) that are surrounded by RNA regions that are removed in a number of temporally and spatially controlled rRNA processing reactions. Concomitant with rRNA transcription and processing, the pre-rRNA is modified by pseudouridylation and methylation, mostly catalyzed by a large number of small nucleolar ribonucleoprotein (snoRNP) particles. The process of rRNA maturation is tightly coupled to the ordered deposition of ribosomal proteins onto the pre-rRNA. An endonucleolytic cleavage step splits the nascent pre-ribosome into the precursors of 40S and 60S subunits in the nucleolus, which, thereafter, follow separate biogenesis pathways. Pre-60S maturation also includes the incorporation of 5S rRNA, which is synthesized independently by RNA Pol III. Following further nuclear biogenesis steps and nuclear export, pre-40S and pre-60S particles are finally matured in the cytoplasm, where both subunits join to form the translationally competent 80S ribosome. In yeast, the whole process of ribosome production is assisted by >200 non-ribosomal proteins known as *trans*-acting factors (Henras et al., 2008; Thomson et al., 2013; Tschochner and Hurt, 2003; Turowski and Tollervey, 2015; Woolford and Baserga, 2013).

Ribosome synthesis is an energy-demanding process that is tightly regulated and occupies a central position in growth control (Drygin et al., 2010; Warner, 1999). To adapt ribosome production to cellular needs, vertebrate cells integrate a large variety of upstream cues, including nutrient availability. Defects in