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Ribosomal proteins Rpl10 and Rps6 are potent regulators of yeast replicative life span

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Abstract

The yeast ribosome is composed of two subunits, the large 60S subunit (LSU) and the small 40S subunit (SSU) and harbors 78 ribosomal proteins (RPs), 59 of which are encoded by duplicate genes. Recently, deletions of the LSU paralogs *RPL31A* and *RPL6B* were found to increase significantly yeast replicative life span (RLS). RPs Rpl10 and Rps6 are known translational regulators. Here, we report that heterozygosity for *rpl10*Δ but not for *rpl25*Δ, both LSU single copy RP genes, increased RLS by 24%. Deletion of the SSU *RPS6B* paralog, but not of the *RPS6A* paralog increased replicative life span robustly by 45%, while deletion of both the SSU *RPS18A*, and *RPS18B* paralogs increased RLS moderately, but significantly by 15%. Altering the gene dosage of *RPL10* reduced the translating ribosome population, whereas deletion of the *RPS6A*, *RPS6B*, *RPS18A*, and *RPS18B* paralogs produced a large shift in free ribosomal subunit stoichiometry. We observed a reduction in growth rate in all deletion strains and reduced cell size in the SSU *RPS6B*, *RPS6A*, and *RPS18B* deletion strains. Thus, reduction of gene dosage of RP genes belonging to both the 60S and the 40S subunit affect lifespan, possibly altering the aging process by modulation of translation.

Keywords: Yeast replicative aging; Longevity; Translational capacity; Ribosomal protein; Rpl10; Rps6; Rps18

1. Introduction

The present and future enormous social and economic costs of the aging society have raised much interest in the study of the aging process in model systems, like the yeast *Saccharomyces cerevisiae*, the worm *Chaenorhabditis elegans*, the fly *Drosophila melanogaster* and the mouse, *Mus musculus*. So far, experimental evidence suggests that reduced nutrient intake or calorie restriction (CR) is a potent strategy to increase life span in yeast, worms, flies and mammals. In particular, genetic interventions to reduce the activity of conserved components of the nutrient sensing insulin/IGF-1 signaling pathway, including the yeast AKT homologue, Sch9, have been found to increase lifespan in all these species (reviewed in Longo, 2004). Also, genetic alterations in the ancient nutrient and growth factor sensing *TOR* (target of rapamycin) pathway have been found to simulate CR (Jorgensen et al., 2002, 2004), making components of this pathway a promising tool for the study of cellular aging. Indeed, recently deletions of *TOR* and *TOR* target genes have been shown to modulate replicative and chronological life span in yeast (Kaeberlein et al., 2005; Powers et al., 2006). In yeast, replicative life span or mother cell specific ageing is defined by the number of daughter cells produced by a newly born yeast cell (virgin cell) before senescence, i.e., the count of mitotic cell cycles an individual mother cell can undergo. On the other hand, chronological, or post-mitotic, life span is measured

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by the time a non-dividing cell population remains viable in spent media.

Kaeberlein et al. (2005) from a large-scale analysis of 564 non-essential single gene deletion strains of yeast (EUROSCARF) identified 6 gene deletions that increased replicative life span and which corresponded to genes encoding components of the *TOR* pathway. Together with *TOR*, Ras/Protein kinase A (PKA) signaling conduits regulate the expression of common downstream targets, among them the rRNA, ribosomal protein (RP) and ribosome biogenesis (Ribi) regulons (Jorgensen et al., 2004; reviewed in Dann and Thomas, 2006). Interestingly, the candidate proteins include Tor1 (encoded by the non-essential paralog of the two yeast *TOR* genes), the *TOR* transcriptionally upregulated proteins, ribosomal proteins L6b and L31a and Sch9. Sch9, like PKA, is a nutrient responsive kinase, previously shown by genetic analysis to modulate replicative aging in yeast (Jackson and Groves, 2004). *TOR* is thought to act upstream or in parallel to PKA, whereas Sch9 is thought to act in a pathway parallel to PKA and *TOR* (Davis et al., 2003; Lee et al., 2005). The work of Jorgensen et al. (2004) and Kaeberlein et al. (2005) suggest a link between the observed effect of *SCH9* deletion on reduction of growth rate, reduction in cell size and increase in replicative aging. The authors show that Sch9 and the transcription factor Sfp1, a downstream target of *TOR*, are negative regulators of the Start event of the yeast cell cycle (Jorgensen et al., 2002) and are also nutrient sensitive activators of the RP and Ribi regulons (Jorgensen et al., 2004). Thus, a fundamental connection emerged between regulation of ribosome biogenesis and Start, as *TOR* and Sch9, in a nutrient dependent mode, operate in parallel to drive the ribosome biosynthetic machinery, and thus translational capacity, to attainment of the critical cell size for cell division.

In the context of nutrient dependent regulation of translational capacity it is necessary to reflect that protein synthesis is one of the most energy consuming cellular processes, occupying an estimated 50% of the total cellular energy (Hay and Sonenberg, 2004; Martin and Hall, 2005).

An individual eukaryotic ribosome, and thus a yeast ribosome, is composed of a small 40S subunit, harboring the mRNA decoding center and a large 60S subunit, which harbors the peptidyltransferase center. In yeast, the 40S subunit is built from the 18S rRNA and from 32 ribosomal proteins, 24 of which are encoded by duplicated genes. The 60S subunit is built from the 25/28S rRNA, the 5.8 rRNA and the 5S rRNA and harbors 46 ribosomal proteins, 35 of which are encoded by duplicate genes (Planta and Mager, 1998). Ribosomal proteins, in addition to their structural role as components of the ribosome are increasingly recognized as regulatory molecules in cell growth and differentiation (Mazumder et al., 2003; Amsterdam et al., 2004; Ruvinsky et al., 2005). Previously, we have described ribosomal proteins L10 and S6 as regulators of translational capacity and differential mRNA expression in yeast (Koller et al., 1996; Karl et al., 1999; Oender et al., 2003; Pachler et al., 2004). Both, Rpl10 and Rps6, are multifunctional ribosomal proteins. Rpl10 in the nucleolus is involved in the processing of precursor – rRNA molecules (Zuk et al., 1999 and our unpublished observations), together with the 60S subunit export factor Nmd3 mediates 60S subunit export from the nucleus (Gadal et al., 2001) and in the cytoplasm functions in subunit joining of the large 60S and the small 40S subunits (Eisinger et al., 1997). Rps6 in mammals is the target of the S6Kinase (S6K), which is one of the targets of mammalian *TOR* (Ruvinsky and Meyuhas, 2006). The proposal that ribosomes decorated with phosphorylated Rps6 are preferentially recruited into polysomes (Fumagalli and Thomas, 2000) is still under debate (Ruvinsky and Meyuhas, 2006). In yeast, we have shown that the LSU ribosomal protein Rpl10 and the SSU ribsosomal protein Rps6 genetically and functionally interact in differential mRNA translation, apparently targeting mRNAs encoding key regulators of energy metabolism like pyruvate kinase (Pachler et al., 2004).

Here, we show that Rpl10, and Rps6 are potent regulators of yeast replicative aging and that Rps18 is a moderate, but significant modulator of yeast RLS. Interestingly, heterozygosity for *rpl10*Δ, but not heterozygosity for another LSU single copy RP gene, *rpl25*Δ, confers a reduction of the translating ribosome population, i.e., the translational capacity of the cell. In contrast, single deletions of the SSU paralogs *RPS6A*, *RPS6B*, *RPS18A*, and *RPS18B* do not alter the translating ribosome complement, but rather alter the stoichiometry of the free ribosomal subunits. Furthermore, a reduction in growth rate was observed in all deletion strains and cell size was reduced in the SSU *RPS6A*, *RPS6B*, and *RPS18B* deletion strains. The data support the hypothesis that distinct ribosomal proteins, both of the LSU and the SSU, can contribute to an increase in yeast replicative life span, possibly by modulating quantitatively and qualitatively the synthesis of proteins operative in the aging process.

2. Materials and methods

2.1. Materials and strains

Components for preparation of media to culture yeast cells were obtained from Gibco-BRL (Paisley, Scotland) and Merck (Darmstadt, Germany). Yeast media, culture conditions and the manipulation of yeast strains were as described by Rose et al. (1990). Glass beads were obtained from B. Braun Biotech International, Darmstadt, Germany.

Strains were obtained from the MAT **a**/α and MAT α yeast ORF deletion collection (EUROSCARF). The deletions were generated in the parental strains MAT **a**/α BY4743 and MAT α BY4742 (see Table 1).

2.2. Ribosomal profile analysis

It is important to note that all profiles were obtained from yeast cells cultivated at 24 °C, the same temperature