D.3. M. Tamame

Functional analysis of L19: a regulatory role of the 60S ribosomal subunit in translation?

Ana Jiménez-López, Álvaro Gil-Hernández and **Mercedes Tamame**
Instituto de Biología Funcional y Genómica, IBFG. CSIC/ Universidad de Salamanca. Campus Unamuno. Salamanca 37007. Spain. Phone: 923121673. e-mail: tamame@usal.es

L19 is an essential r-protein component of the 60S ribosomal subunit, whose precise roles in ribosome biogenesis and function remain unknown mainly due to the unavailability of conditional mutants in genetically amenable organims. Recent findings indicate that r-protein activity is highly regulated to impart a new layer of specificity in the control of gene expression and development in mammalians (4). Moreover, defective 60S function or availability reduce 60S/40S joining and translation rates, and are cause of human diseases (2, 8). Given the growing interest on a regulatory role of the 60S subunit in global and gene-specific translation (4), we have undertaken a mutational analysis to investigate L19 functions in *Saccharomyces cerevisiae.* The impact of r-protein alterations on initiation and reinitiation of translation can be evaluated in this yeast using the translational regulation of *GCN4* as a tool (3). In fact, *gcd17* mutations that derepress *GCN4* translation allowed us uncover two esential functions of L33 in ribosome biogenesis and 60S/40S joining (5).

According to the current structural models of the 80S ribosome, a long carboxy-terminal α-helix of L19e penetrates the 40S subunit, contacting an expansion segment of the 18S rRNA (ES6) and forming the novel eukaryotic bridge (eB12) (1,7). The structural information led to the hypothesis that L19e may interact with translation factors at the entrance or exit of the 60S tunnel, facilitate ribosomal subunit joining and/or regulate the *ratcheting* movement during translation (1).

In *S. cerevisiae* L19e is encoded by paralogous *RPL19A* and *RPL19B* genes that encode identical L19 proteins but differ in their introns and flanking regulatory sequences. We have mutagenized a functional *RPL19BΔi* allele (650nt) devoid of its long intron (384nt) that fully complements the growth phenotypes of Δ*rpl19B* or/and Δ*rpL19A* deletions when cloned in a centromeric *LEU2* vector. Two libraries of ~2000 independent clones were transformed into a Δ*rpL19A* Δ*rpL19B leu2Δ* (*pGAL::RPL19BURA3*) mutant, and 25 uracil-auxotrofic/ leucine-prototrophic transformants selected on the basis of exhibiting slow growth (*slg*) or increased termosensitivity at high or low temperatures (37º*ts,* 16º*cs*). The mutations leading to more severe *ts* and *cs* phenotypes predict amino acid changes in the carboxy-terminal domain of L19, and some leading to *slg* in the amino-terminal half of the protein. We are analyzing how *rpL19B* mutations affect pre-rRNA processing, interactions of L19 with some r-proteins and ribosome biogenesis factors, nucleo-cytoplasmic transport and production of mature 60S, general translation rates and *GCN4* translational regulation. Very recent data indicate that introns of yeast r-proteins may play a role in ribosome synthesis and function (6). Therefore, selected mutations identified in this work will be now generated in intron-containing *RPL19A* and *RPL19B* to analyze possi differences with Δ*in*-alleles, and to investigate potential paralogue-specific phenot

1. Ben-Shem A., Jenner L., Yusupova G., Yusupov M. 2010. Crystal structure of the eukaryoyic ribosome. S 1209