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For RNase protection, specific probes were generated for each tRNA and a biotin-labelled antisense oligo-dT-primed RNA probe was prepared as previously described (Horowitz et al., 2007). U-2 OS cells were washed in phosphate-buffered saline (PBS) and RNAs were extracted using the QIAGEN RNeasy RNA isolation kit, based on the manufacturer's instructions. Cells were lysed in RIPA buffer and then treated with 1unit/ml RNase-free recombinant RNase A (Roche) for 15min at room temperature. Following phenol/chloroform extraction, samples were ethanol-precipitated and reverse transcribed using AMV reverse transcriptase (Roche) using a 5'-biotinylated primer. RNA probes were amplified using Dynabeads Oligo(dT)-25 (Invitrogen) and a PCR protocol consisting of 94C for 1min, 32C for 1min then 75C for 1min and a final extension step of 75C for 10min. The denaturing alkaline gel electrophoresis was performed according to the manufacturers' instructions, using an equal volume of native alkaline loading buffer for the top and bottom gels. The bottom gels were prestained using 0.1ng/ml Molecular Weight marker VI (Roche), followed by transfer to a 0.45 um nitrocellulose membrane. Streptavidin-HRP detection was then performed according to the manufacturer's instructions, using the SuperSignal West Pico Chemiluminescent Substrate kit (Pierce). Images of the blots were taken using a UVITEC Alliance Q9 documentation system or the GE ImageQuant LAS 500 imager. Densitometry analysis of bands was performed using Fiji software. For non-denaturing gel analysis, gels were transferred to a 0.45 um nitrocellulose membrane overnight at 40mA in a cold room and the uncut product, which served as an estimate of the degraded product, was quantified using Fiji software. In all experiments, 3% of the lysate/ribosome fraction was loaded in the input lane, and 90% was used for immunoprecipitation. The RNase protection was performed in biological duplicates, and one of the two repeats is shown in the figures.

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R, the most widely used statistical analysis and graphics software, is an imperative tool for data analysis and research in statistics, economics, engineering, social science, and the life sciences. R is a free software environment that includes an integrated development environment to author and execute statistical code. The R environment integrates sophisticated graphical functions into a traditional UNIX environment. This software makes it easy to create a variety of analytical files and to manipulate and view those files in one place. To install the R IDEs, you first need to install the R software. Next you will need to download RStudio, an Integrated Development Environment. RStudio is an open source integrated development environment (IDE) for the R language. A web server is a computer that is connected to the Internet. A web server is usually connected to a different network. Web servers can contain software, such as an operating system and a web server application, that is running on the computer. A web server has a specified port on which the web server software runs. Live-cell imaging: HeLa shNT and shIP6K1 cells stably expressing the luciferase reporter pRL-3XB were cultured on glass bottomed Ibidi dishes (1.5% gelatin, 20n4 2-mercaptoethanol) and incubated at 37C in 5% CO2. When cells were 80% confluent, cultures were treated with 100g/ml cycloheximide (CHX) for 15min, trypsinised, washed with ice-cold PBS containing 10g/ml CHX, and lysed in polysome lysis buffer [20mM Tris CI (pH 7.4), 100mM KCI, 5mM MgCl2, 0.5% Nonidet P-40, 100g/ml CHX, 1mg/ml heparin, supplemented with protease inhibitor cocktail]. Clarified lysate containing 175g RNA was loaded on top of a 10-50% sucrose gradient prepared in gradient buffer [20mM Tris CI (pH 7.4), 140mM KCI, 5mM MgCl2, 100g/ml CHX and 1mg/ml heparin, supplemented with protease inhibitor cocktail] and centrifuged at 221,000g for 3h and 15min at 4C. After centrifugation, the gradients were analysed by measuring absorbance at 254nm using a density gradient fractio

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