Supplementary Materials and Methods

Preparation of cell extracts

Cells were lysed either in RIPA buffer (150mM NaCl, 1% NP-40, 0.5% Na-deoxycholate, 0.1% SDS, 50mM Tris pH 7.5 and Complete^{TM} (Roche) protease inhibitor cocktail) for western blots or buffer X (150mM KCl, 50mM HEPES pH 7.5, 1mM EDTA, 2mM DTT, 0.2% Tween-20 and Complete^{TM}) for the m^7GTP resin precipitation assay.

Viability

Cells were resuspended in PBS containing 10% FBS and 0.2 µg/ml propidium iodide (PI). Flowcytometric records were obtained on a FACS Calibur (Beckton Dickinson) and analyzed with CellQuest software.

Polysomal fractionation and analysis

Cells were treated with 0.1 mg/ml cycloheximide (CHX) for 3min at 37°C, washed twice with ice cold PBS/CHX and scraped in lysis buffer (1% Triton X-100, 0.3M NaCl, 15mM $MgCl_2$, 15mM Tris (pH 7.4), 0.1mg/ml CHX, 100 units RNAse-In (Ambion)). 200ug/ml heparin was added to the cytoplasmic lysate and cellular debris was removed by centrifugation. The lysate was layered on a 10ml continuous sucrose gradient (20-50% sucrose in 15mM $MgCl_2$, 15mM Tris (pH 7.4), 0.3M NaCl). After 90min of centrifugation at 39.000 rpm in an SW41-Ti rotor at 4°C, the absorbance at 254nm was measured continuously as a function of gradient depth in a BioRad UV monitor. Background signals were subtracted from these profiles and the overall signal normalized for differences in loading. To estimate overall translation efficiency, we integrated the fractional area under the curve representing polysomal RNA. The average number of ribosomes per mRNA in the polysomes (i.e. with 2 or more ribosomes attached) was estimated for each culture condition. The polysomal area was first sub-divided into ~50 fractions and then we summed the product of the fractional area at a given depth multiplied by the mean number of ribosomes per mRNA at that depth. The mean number of ribosomes per mRNA as a function of depth was estimated based on the positions of

recognizable absorbance peaks in the polysome profiles. Error bars represent the standard error of the mean from 2-3 experimental replicates.

RNA isolation and reverse transcription

Samples were collected in 0.55M final concentration of guanidinium-HCl and subjected to a total of 4 precipitation steps (ethanol, sodium acetate and lithium chloride) and one phenol-chloroform extraction. RNA quantity and quality was checked using the ND-1000 spectrometer (NanoDrop Technologies) and RNA Nano LabChip kit on the 2100 Bioanalyzer (Agilent) respectively. The RNA was reverse transcribed using random decamers (Ambion) and MML reverse transcriptase (Sigma-Aldrich).

Quantitative PCR analysis – primers and probes

The abundance of the following genes were detected with SYBR® Green I (Eurogentec/Applied Biosystems) using the indicated forward and reverse primers: h/m18S: F-agtccctgccctttgtacaca and R-gatccgagggcctcactaaac; hActin: F-accatggatgatgatatcgcc and R-gccttgcacatgccgg; hATF4: F-tggccaagcacttcaaacct and R-gttgttggagggactgaccaa; hCHOP: F-ggagcatcagtcccccactt and R-tgtgggattgagggtcacatc; hGADD34: F-cccagaaacccctactcatgatc and R-gcccagacagccaggaaat; hCAIX: F-catcctagccctggtttttgg and R-gctcacacccccttttggtt. The following genes were measured using TaqMan® Gene Expression Assays (Applied Biosystems): mCHOP: Mm00492097_m1; mActin: Mm00607939_s1; mATF4: mm00515324_m1; mCAIX: mm 00519870_m1; mMyd116: Mm00435119_m1.

Western blotting – primary antibodies

Primary antibodies included monoclonal anti-actin (C4, ICN Biomedicals), polyclonal anti-eIF2α, polyclonal anti-eIF2α phosphorylated at serine 51 (Research Genetics), polyclonal anti-ATF4 (SC-200 Santa-Cruz Biotechnology), monoclonal anti-eIF4E (clone 87, BD Transduction Laboratories), anti-4E-BP1 (11208, described in (Gingras et al., 1996)), anti-eIF4GI (16206, a gift from L. Carrasco), anti-eIF4GII (SS 0217, described in (Gradi et al., 1998) and anti-4E-T (KS1, described in (Dostie et al., 2000)).

Immunofluorescence – antibodies

Slides were incubated with polyclonal anti-eIF4E (5853, described in (Frederickson et al., 1991)) or monoclonal anti-eIF4E (BD Transduction Laboratories) together with polyclonal anti-4E-T (KS1, described in (Dostie et al., 2000)). All antibodies were diluted in 3%BSA, 0.05% Na-azide and 0.1% Triton X-100 in PBS. Slides incubated with secondary antibodies (Alexa Fluor^{R} 488 goat anti-rabbit IgG and 568 goat anti-mouse IgG) and DAPI FluoroPure^{TM} grade nucleid acid stain (all from Molecular Probes).

References

Dostie, J., Ferraiuolo, M., Pause, A., Adam, S.A. and Sonenberg, N. (2000) A novel shuttling protein, 4E-T, mediates the nuclear import of the mRNA 5' cap-binding protein, eIF4E. *Embo J*, **19**, 3142-3156.

Frederickson, R.M., Montine, K.S. and Sonenberg, N. (1991) Phosphorylation of eukaryotic translation initiation factor 4E is increased in Src-transformed cell lines. *Mol Cell Biol*, **11**, 2896-2900.

Gingras, A.C., Svitkin, Y., Belsham, G.J., Pause, A. and Sonenberg, N. (1996) Activation of the translational suppressor 4E-BP1 following infection with encephalomyocarditis virus and poliovirus. *Proc Natl Acad Sci U S A*, **93**, 5578-5583.

Gradi, A., Imataka, H., Svitkin, Y.V., Rom, E., Raught, B., Morino, S. and Sonenberg, N. (1998) A novel functional human eukaryotic translation initiation factor 4G. *Mol Cell Biol*, **18**, 334-342.