RNA binding assay

An RNA oligonucleotide containing a pyrimidine tract to which PSF binds (native oligo: 5'-UUCGUGCUGACCCUGUCCCUUUUUUUUCCACAG-3') and a scrambled oligonucleotide which PSF is unable to bind (5'-GCUGUUACUGCCCUGUCCCUUUUUUUUCCACAG-3')[60,61] were deprotected as recommended by the manufacturer and radiolabelled at the 5'-end using [32P]ATP and T4 polynucleotide kinase. Purified PSF (0.25 µg) and ALK (0.4 µg) proteins were pre-incubated at 30°C for 30 min in kinase assay reaction buffer, and then incubated with 0.2 pmol labelled oligonucleotides for 30 min at 30°C in binding buffer (25 mM Tris pH 7.5, 50 mM NaCl, 1 mM EDTA, and 50 ng/µl yeast tRNA). RNA-protein complexes were resolved by continuous 7.5% PAGE.

Real-Time Analysis

To detect the effect of PSF and NPM/ALK on GAGE6 expression, 293T cell line were transiently transfected with pcDNA3.1 alone (Invitrogen, Carlsbad, CA), with the same plasmid encoding PSF or cotransfected with PSF and NPM/ALK by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) following a standard protocol. After 60 hours, RNA was extracted with TRIZOL (Invitrogen, Carlsbad, CA) following a standard protocol. cDNA was synthesized from 100ng of total RNA, using 'TaqMan Reverse Transcription Reagents' (Applied Biosystems, Foster City, CA, USA). Quantitative Real Time RT-PCR was performed using 'Brilliant SYBR Green QPCR Master Mix' (Stratagene, La Jolla, CA) on a '7900HT Sequence Detection System' (Applied Biosystems, Foster City, CA, USA) under standard conditions. The housekeeping gene GAPDH was used as an internal reference to normalize the expression of GAGE6. The forward and reverse primers for GAGE6[62] and GAPDH were respectively:

GAGE6-For 5’-GCCTCCTGAAGTGATTGGGCCTA-3’ and GAGE6-rev 5’-CAGGCGTTTTCACCTCCTCTGGA-3’, GAPDH-SybrFor 5’-TGCACCACCAACTGCTTAGC-3’ and GAPDH-SybrRev 5’-GGCATGGACTGTGGTCATGAG-3’. All the Real Time RT-PCR experiments were performed in triplicate.