**Protocols regularly used in Chen Lab**

**RNA Isolation, qRT-PCR and Northern Blotting**

Total RNA from each cultured cell line or cultured cells with different treatments was extracted with Trizol Reagent (Invitrogen) according to the manufacturer’s protocol. For qRT-PCR, after treatment with DNase I (Ambion, DNA-freeTM kit), the cDNA synthesis was carried out using SuperScript III (Invitrogen) with oligo (dT) and random hexamers. QPCR was done using SYBR Green Realtime PCR Master Mix (TOYOBO) and a StepOnePlusTM real-time PCR system (Aplied Biosysterms). The relative expression of different sets of genes was quantified to *actin* mRNA.

Northern blotting was carried out according to the manufacturer’s protocol (DIG Northern Starter Kit, Roche). RNA was loaded on native Agarose gels or denatured PAGE gels. Digoxigenin (Dig) labeled antisense probes were generated using T7 RNA polymerase by in vitro transcription with the RiboMAX™ Large Scale RNA Production System (Promega).

**Isolation of Nucleoli**

Nucleoli isolation in PA1 cells was performed as described (Hacot et al., 2010) with modifications. 2 × 107 PA1 cells were used for nucleoli isolation. Cell pellet was suspended by gentle pipetting in 200 μl lysis buffer (10 mM Tris pH 8.0, 140 mM NaCl, 1.5 mM MgCl2, 0.5% Igepal, 2 mM Ribonucleoside Vanadyl Complex), and incubated on ice for 10 min. During the incubation, one tenth of the lysate was added to 1 ml Trizol for total RNA extraction. The rest of the lysate was centrifuged at 1000 rpm for 3 min at 4°C to pellet the nuclei and the supernatant was the cytoplasmic fraction. To fractionate nuclear fractions, nuclei pellet was resuspended with 200 μl 340 mM sucrose solution containing 5 mM MgCl2. One tenth of the lysate was added to 1 ml Trizol for nuclei RNA extraction. To prepare nucleoplasmic and nucleolar fractions, nuclei were broken by sonication until intact nuclei cannot be detected in suspension by microscope. 200 μl 880 mM sucrose solution containing 5 mM MgCl2 was gently added to sonicated nuclei and then centrifuged 20 min at 2,000 rpm, 4°C to pellet nucleoli, and the supernatant was the nucleoplasmic fraction. Fractionated RNAs from the same amount of cells were used for cDNA synthesis and RT-PCR.

**RNA *In Situ* Hybridization and Immunofluorescence Microscopy**

To detect *SLERT*, RNA FISH was carried out as previously described with in vitro transcribed Dig-labeled antisense probe (Yin et al., 2012). Briefly, cells were fixed with 3.6% PFA and 10% acetic acid for 15 min, followed by permeabilization with 0.5% Triton X-100 for 5 min on ice. Then cells were subjected to incubation with denatured Dig-labeled FISH probes in hybridization buffer (50% formamide in 2xSSC) at 50°C overnight. After hybridization, anti-Dig primary antibody and fluorescent secondary antibody were sequentially added to visualize signal with DeltaVision Elite imaging system (GE Healthcare). The nuclei were counterstained with DAPI.

**RNA/DNA Double FISH**

Sequential RNA/DNA double FISH experiments were carried out after RNA FISH. Cells were denatured at 80°C for 5 min in prewarmed 2×SSC and 70% deionized formamide, pH 7.0. Next, cells were hybridized with denatured DNA probe prepared from Nick Translation (Abbott) overnight. After hybridization, two washes of 10 min at 37°C with 50% formamide in 2×SSC were performed, followed by two washes of 15 min at 37°C with 1×SSC and two washes of 15 min at 37°C with 2×SSC. Slides were then mounted with ProLong Gold antifade reagent (Thermo Fisher SCIENTIFIC) with DAPI. Analysis was performed on single Z stacks acquired with a DeltaVision Elite imaging system (GE Healthcare).

**tRSA RNA Pull-down Assay and Western blotting**

tRSA RNA pull-down assays were carried out as described (Iioka et al., 2011) with modifications. *SLERT* full-length or internal sequence was cloned into pcDNA3 plasmid with the tRSA tag at its 5’ end. RNA products were *in vitro* transcribed using the T7 RiboMAX™ Large Scale RNA Production System (Promega). 10 μg per reaction of synthetic RNAs were denatured for 5 min at 65°C in RNA Structure buffer (10 mM HEPES pH 7.0, 10 mM MgCl2) and slowly cooled down to room temperature. Then, folded RNAs were incubated with 50 μl of streptavidin Dynabeads (Invitrogen) for 20 min at 4°C in the presence of 2 U/ml RNasin (Promega). PA1 cells (1 ×107) were harvested and resuspended in 1 ml of lysis buffer [10 mM HEPES pH 7.0, 200 mM NaCl, 1 mM DTT, 1% Triton X-100, protease inhibitor cocktail (Roche)] followed by sonication for 4×10 sec with an interval of 1 min on ice and then centrifuged at 13,000 rpm for 10 min at 4°C. The supernatant was pre-cleared with 50 μl of streptavidin Dynabeads for 20 min at 4°C followed by the addition of 20 μg/ml yeast tRNA for 20 min at 4°C. Then the pre-cleared lysate was added to folded RNAs and incubated for 3.5 h at 4°C followed by washing 4×5 min with wash buffer [10 mM HEPES pH 7.0, 400 mM NaCl, 1 mM DTT, 1% Triton X-100, protease inhibitor cocktail (Roche), 2 mM RVC]. To harvest the protein complex, 50 μl of 1× SDS loading buffer was added and boiled for 10 min at 100°C.

**Native RNA-Protein Complex Immunoprecipitation**

PA1 cells (1×107) were harvested, resuspended in 1 ml lysis buffer [50 mM Tris pH 7.4, 150 mM NaCl, 0.05% Igepal, 0.5% NP-40, 0.5 mM PMSF, 2 mM RVC, protease inhibitor cocktail (Roche)] followed by 4×10 sec sonication with an interval of 1 min on ice. After centrifuging at 13,000 rpm for 10 min at 4°C, the supernatant was pre-cleared with 15 μl Dynabeads® Protein G (Invitrogen). The pre-cleared lysates were further incubated with 2 μg antibody (Proteintech) for 2 h at 4°C. Then 15μl Dynabeads® Protein G beads (blocked with 1% BSA and 20μg/ml yeast tRNA) were added to the mixture and incubated for another 1 h at 4°C followed by washing with wash buffer [50 mM Tris pH 7.4, 300 mM NaCl, 0.05% Sodium Deoxycholate, 0.5% NP-40, 0.5 mM PMSF, 2 mM RVC, protease inhibitor cocktail (Roche)]. The RNA-protein complex was eluted with elution buffer (100 mM Tris pH 6.8, 4% SDS) at room temperature for 15 min. For qRT-PCR, each RNA sample was treated with DNase I (Ambion, DNA-freeTM kit) and then reverse transcription was performed with SuperScript® III Reverse Transcriptase (Invitrogen) with oligo (dT) and random hexamers followed by qRT-PCR analysis.

**Formaldehyde Crosslinking RNA Immunoprecipitation**

PA1 cells (1×107) were harvested and suspended in 10 ml PBS with 1% formaldehyde to fix 10 min at room temperature. Crosslinking was stopped by the addition of glycine to a final concentration of 0.25 M followed by incubation at room temperature for 5 min. After pelleting cells at 1,000 rpm for 5 min, the cell pellet was resuspended in 1 ml RIPA buffer [50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% Sodium Deoxycholate, 1 mM PMSF, 2 mM RVC, protease inhibitor cocktail (Roche)] followed by 8×10 sec sonication with an interval of 1 min on ice. After centrifuging at 13,000 rpm for 10 min at 4°C, the supernatant was pre-cleared with 15 μl Dynabeads® Protein G (Invitrogen) and 20 μg/ml yeast tRNA at 4°C for 30 min. Then the pre-cleared lysate was incubated with beads that were pre-coated with 2 μg anti-DDX21 antibody (Proteintech) for 4 hr at 4°C. The beads were washed 4×5 min with washing buffer I (50 mM Tris pH 7.4, 1 M NaCl, 1% NP-40, 1% Sodium Deoxycholate), and 4×5 min with washing buffer II (50 mM Tris pH 7.4, 1 M NaCl, 1% NP-40, 1% Sodium Deoxycholate, 1 M Urea). The complex was eluted from beads by adding 140 μl elution buffer (100 mM Tris pH8.0, 10 mM EDTA, 1% SDS). To reverse crosslinking, 4 μl 5 M NaCl and 2 μl 10 mg/ml proteinase K were added into the RNA samples, and incubated at 42°C for 1 h followed by another hour inculation at 65°C. RNA was then extracted, digested with DNase I (Ambion) and used to synthesize cDNA with SuperScript® III Reverse Transcriptase (Invitrogen) with oligo (dT) and random hexamers followed by qRT-PCR analysis.

**Co-Immunoprecipitation**

PA1 cells (2×107) were harvested and suspended in 1 ml lysis buffer [50 mM Tris pH 7.4, 150 mM NaCl, 0.05% Igepal, 0.5% NP-40, 0.5 mM PMSF, 2 mM RVC, protease inhibitor cocktail (Roche)] followed by 3×10 sec sonication. After incubation with 2 μg antibody (Santa Cruz) or anti-flag M2 antibody (Sigma), the beads were washed 4×5 min with wash buffer [50 mM Tris pH 7.4, 300 mM NaCl, 0.05% Sodium Deoxycholate, 0.5% NP-40, 0.5 mM PMSF, 2 mM RVC, protease inhibitor cocktail (Roche)]. To harvest the protein complex, 50 μl of 1× SDS loading buffer (62.4 mM Tris pH 6.8, 10% glycerol, 2% SDS, 0.0012% bromophenol blue) was added, boiled for 10 min at 100°C, and analyzed by Western blotting.

**Chromatin Immunoprecipitation**

PA1 cells (1×107) were harvested and suspended in 10 ml PBS with 1% formaldehyde to fix 10 min at room temperature. Crosslinking was quenched by the addition of glycine to a final concentration of 0.25 M followed by incubation at room temperature for 5 min. After pelleting cells at 1,000 rpm for 5 min, the cell pellet was resuspended in 1 ml lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% Sodium Deoxycholate) followed by sonication with S220 Focused-ultrasonicators (Covaris) to achieve 300-500 bp DNA fragments. After centrifugation at 16,000 g for 10 min at 4°C, the supernatant was pre-cleared with 15 μl Dynabeads® Protein G (Invitrogen) with 100 μg BSA and 100 μg ssDNA. Then the precleared lysates were incubated overnight at 4°C with 2 μg antibody. The beads were washed with 600 μl lysis buffer, 600 μl high salt wash buffer (1% Triton X-100, 0.1% Sodium Deoxycholate, 50mM Tris-HCl at pH8.0, 0.5 M NaCl, 5 mM EDTA), 600 μl LiCl immune complex wash buffer (0.25 M LiCl, 0.5% Igepal, 0.5% Sodium Deoxycholate, 10 mM Tris pH 8.0, 1 mM EDTA) sequentially, followed by two washes with 600 μl TE Buffer (10 mM Tris pH 8.0, 1 mM EDTA) at 4°C. The complex was eluted by adding 200 μl fresh-prepared elution buffer (1% SDS, 0.1 M NaHCO3) with rotation at room temperature for 15 min. Then the reverse crosslinking was carried out by adding 8 μl 5 M NaCl and incubated at 65°C for 4 h, followed by supplemented with 4 μl 0.5 M EDTA and 10 μl proteinase K (10 mg/ml) at 55°C for 2 h. DNAs were purified by phenol/chloroformextraction extraction and ethanol precipitation by adding 20 μg yeast tRNA. The pellets were dissolved in 100 μl ddH2O for qRT-PCR.

**Cell lysate preparation and ds- or ss-RNA pull-down assay**

Biotinylated RNA pull-down was performed as described ([Wu et al., 2016](#ENREF_8)) with slight modifications. In brief, 4 μg biotinylated RNAs were heated for 5 minutes at 65 °C in PA buffer (10 mM Tris HCl pH 7.5, 10 mM MgCl2, 100 mM NH4Cl) and slowly cooled down to room temperature. To prepare cell lysate for pull-down with single-stranded (ss) RNA (*efgp* or *Alu*) or double-stranded (ds) inverted repeated RNAs (IR*egfp*s or IR*Alu*s), 1×107 PA1 cells were resuspended in 2 ml PBS, 2 ml nuclear isolation buffer (1.28 M sucrose, 40 mM Tris-HCl pH 7.5, 20 mM MgCl2, 4% Triton X-100) and 6 ml DEPC-water, followed by incubation on ice for 15 minutes. Nuclei were pelleted by centrifugation at 1,000 rpm for 5 minutes at 4 °C, and resuspended in 1 ml binding buffer (100 mM HEPES pH 7.0, 50 mM KCl, 10% glycerol, 1 mM EDTA, 1 mM DTT, 0.5% Triton X-100) supplemented with tRNA (0.1 mg/ml), heparin (0.5 mg/ml) and RNasin (1unit/μl). The nuclei were sonicated and centrifuged at 13,000 rpm for 10 minutes at 4 °C. The supernatant was pre-cleared with Streptavidin Dynabeads (Invitrogen) for 30 minutes at 4 °C, followed by incubation with folded RNAs for 2 hours and with beads for 1 hour at 4 °C. Beads were washed five times with the binding buffer, and boiled in 1 x sample buffer for 10 minutes. The retrieved proteins were subjected to NuPAGE 4-12% Bis-Tris Gel (Invitrogen). Then the gel was stained using Pierce® Silver Stain for Mass Spectrometry kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. After silver staining, specific bands were cut and sent to Core Facility of Molecular Biology (Institute of Biochemistry and Cell Biology, Shanghai, CAS) for mass spectrometry (MS) analysis.