Supplemental Experimental Procedures

Cell culture and transfection

Cultured Huh7 NNeo/C-5B cells that continuously replicate genotype 1b HCV RNA were maintained in DMEM and supplemented with 10% FBS, 1% nonessential amino acids, 200 μM L-glutamine and G418 selection marker.

For miRNA (luciferase) assays, DNA constructs were transfected into Huh7 cells in 60 mm tissue culture plates using Lipofectamine 2000 (Invitrogen), according to the manufacturer's protocol. Firefly luciferase (pLUC-122x2 was previously described by (Jopling et al., 2008) and Renilla luciferase (pRL-SV40) plasmids were transfected at 1 μg and 0.1 μg per well, respectively. The Renilla luciferase plasmid was used as a control for transfection efficiency and samples were normalized to Renilla luciferase expression. Transfected cells were cultured for 24 h and harvested in 1x Passive Lysis Buffer (Promega). Luciferase assays were performed according to the manufacturer's instructions.

Two of the siRNA oligonucleotides for Xrn2 were synthesized by the Stanford Protein and Nucleic Acids facility. The siRNA sequences are as follows: siXrn2 #1, 5'-GGAUGCAGCUGAUGAGAAAdTdT-3' and siXrn2 #2, 5'-GGGAAAGCAUGAUGAACUUUU-3'. Oligonucleotides were resuspended as described in Experimental Procedures. Oligonucleotide siXrn2 #3 was purchased from Dharmacon (siGENOME Human XRN2 (22803) D-017622-03-0005). Oligonucleotides were resuspended according to the manufacturer's protocol.

Small RNA Northern blot analysis

To examine small RNAs, 10 μg TRIzol-extracted RNA was resuspended in loading buffer (95% formamide, 1% EDTA), denatured for 5 min at 95 °C and separated in 15% polyacrylamide-

6.75 M urea gels (Sequagel, National Diagnostics). Gels were run in 1× Tris–borate buffer at 1,000 V, 250 mA and 30 W for 1 h 20 min. Subsequently, RNA was transferred to a membrane (Hybond) for 1 h at 1,000 V, 250 mA and 30 W using Owl Semi Dry Electroblotter (Thermo Scientific). MiR-122 and U6 RNA were detected using the UltraHyb Oligo buffer (Ambion) and oligonucleotide probes. MiR-122 and U6 oligonucleotide probes were 5′-end labeled with PNK kinase and γ-^{32}P dATP. Oligonucleotide sequences of miR-122 and U6 probes are 5′-CAAACACCATTGTCACACTCCA-3′ and 5′-CACGAATTTGCGTGTCATCCTTGC-3′ respectively. Autoradiographs were quantitated using ImageQuant (GE Healthcare).

Puromycin density gradients

Cells were lysed in buffer containing 500 mM KCl, 15 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, 1 mg/ml heparin, and 1% Triton X-100. Following incubation on ice for 15 min, polysomal subunits were separated at 37 °C for 10 min in the presence of 2 mM puromycin. Following centrifugation, cleared lysates were layered onto 10–60% sucrose (500 mM KCl, 15 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, and 1 mg/ml heparin) gradients with a 70% sucrose cushion. Gradients were centrifuged at 35,000 rpm at 4 °C in an SW41 rotor for 2 h 45 min and then fractionated with an Isco Retriever II/UA-6 detector system. Polysomal mRNAs were prepared as described in Experimental Procedures.

MTT Cytotoxicity Assay

Cell viability was determined by using a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) cell proliferation assay kit (Roche, 11465007001) according to the manufacturer's protocol. The percentage of cell viability was calculated as the ratio of absorbance in treated cells compared with that in untreated controls. All experiments were performed in triplicate and repeated three times.

PARP Western Blot

Huh7 cells were transfected with siRNAs, infected with JFH1 HCV, and lysates were analyzed by Western Blot as described in Experimental Procedures. PARP primary antibody (1:1000, Cell Signaling 9542) was used for Western blot analysis. Donkey anti-rabbit-HRP (sc-2313) was purchased from Santa Cruz Biotechnologies and used at 1:10,000.

Supplemental References

Jopling, C.L., Schutz, S., and Sarnow, P. (2008). Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. Cell host & microbe *4*, 77-85.