Molecular Cell, Volume *28*

Supplemental Data

U2 snRNP Binds Intronless Histone Pre-mRNAs to Facilitate U7-snRNP-Dependent 3′ End Formation

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Supplemental Experimental Procedures

U7 snRNP-Dependent Cleavage Constructs

For the mouse H3 U7-snRNP substrates, the **histone pre** plasmid (Huang et al., 2003) was digested with Eco RI and Eco RV. Insertion of the four copies of the 22-nt RNA element, control element, or the various antisense elements was done by ligating annealed oligonucleotides into the plasmid: 4x22(W): [5'-AATTC(ACAACAAGAAGACGCGCATCAT)$_{x4}$GAT and 5'-ATC(ATGATGCGCGTCTTCTTGTTGT)$_{x4}$G], 4x22(C): [5'-AATTC(ACAACAACTAGCCACGGATCAT)$_{x4}$GAT and 5'-ATC(ATGATCCGTGGCTAGTTGTTGT)$_{x4}$G], U1AS: (5'-AATTCCAGGTAAGTAGAT and 5'-ATCTACTTACCTGG), U2AS: (5'-AATTCCAGATACTACGAT and 5'-ATCGTAGTATCTGG), U11AS: (5'-AATTCAGCCCTTTTTGAT and 5'-ATCAAAAAGGGCTG), and U12AS: (5'-AATTCTTCCTTACTCGAT and 5'-ATCGAGTAAGGAAG). A 20-nt spacer was introduced between each antisense element and the histone stem-loop by inserting annealed DNA oligonucleotides into the Eco RV restriction site: (5'-AGTCTAGGGCCCACAGGGGC and 5'-GCCCCTGTGGGCCCTAGACT).

The mouse H2a U7-snRNP substrates were generated by PCR. The H3 U7-snRNP substrate-containing plasmids were used as the templates for PCR (T7 primer: 5'-TAATACGACTCACTATAGGGAGCCCGGGCTGCAGGAATTC and 5'-GGTGGCTCTGAAAAGAGCCTTTG) to introduce a T7 RNA polymerase promoter in the products. The PCR products were gel purified and amplified using the T7 primer and the reverse primer (5'-GTGACACAACTCTTTATCTGATTCAGTGGGTGGCTCTGAAAAGAGCCTTT). This reverse primer includes the sequence for the H2a U7-snRNP substrate (Bond et al., 1991).

H3-derived U7-snRNP substrate-containing plasmids were linearized using Xho I for in vitro transcriptions. U7-snRNP-dependent cleavage of the transcribed RNAs yields either a 193-nt 5' fragment and 116-nt 3' fragment (for 4x22-nt constructs) or a 135-nt 5' fragment and 116-nt 3' fragment (for AS element-containing constructs).

U7-snRNP-dependent cleavage of the H2a-derived substrates yields either a 122-nt 5' fragment and 25-nt 3' fragment (for 4x22-nt constructs) or a 46-nt 5' fragment and 25-nt 3' fragment (for AS element-containing constructs).

CPA Constructs

Plasmids were generated containing four copies of the 22-nt RNA element or the control element upstream of the SV40 late polyadenylation signal. The pSVL plasmid (Wilusz and Shenk, 1988) was digested with Eco RI and Nco I. Insertion of four copies of the RNA element or control element was done by ligating annealed oligonucleotides into the plasmid: 4x22(W): [5'-AATTC$(ACAACAAGAAGACGCGCATCAT)_{x4}$C and 5'-

CATGG(ATGATGCGCGTCTTCTTGTTGT)$_{x4}$G] and 4x22(C): [5'-AATTC(ACAACAACTAGCCACGGATCAT)$_{x4}$C and 5'-CATGG(ATGATCCGTGGCTAGTTGTTGT)$_{x4}$G].

Plasmids were linearized using Dra I for in vitro transcription. CPA cleavage for both 22-nt RNA element-containing constructs results in a 228-nt 5' fragment and a 62-nt 3' fragment. RNAs with four copies of the 22-nt RNA element and control element were produced from the same plasmids linearized with Nco I. Transcription produced 95-nt RNAs, with the extra 7-nt introduced in the cloning steps.

SF3b/hPrp43 Cross-linking Constructs

The 101-nt H2a RNA, Δ22, and Δ7 RNAs were transcribed from PCR products generated using the **histone pre** plasmid as a template. All PCR reactions used the same forward primer: 5'-TAATACGACTCACTATAGGATACATG. The reverse primers were: 101-nt H2a RNA: 5'-CTGCAGGTGGCGCGGG; Δ22: 5'-CTGCAGGTGGCGCGGGCGCGGGCCG; and Δ7: 5'-CTGCAGGTGGCGCGGGATCATGCGC. The 7nt construct was generated by annealing two DNA oligonucleotides [5'-TAATACGACTCACTATAGGGAGGAGGCGCCGGGCGCTGTTCAAGAAGTGCGCGTAGTAGGGC and 5'-GCCCTACTACGCGCACTTCTTGAACAGCGCCCGGCGCCTCCTCCCTATAGTGAGTCGTATTA] and performing in vitro transcription from this template.

In Vitro Processing and Extracts

Substrate RNAs for these studies were in vitro transcribed in the presence of [^{32}P]-αUTP and gel purified. U7-snRNP-dependent cleavage extracts and CPA extracts were prepared from HeLa cells as reported (Kolev and Steitz, 2005; Ryan et al., 2004). Uncoupled in vitro CPA reactions were performed using 5 fmol of substrate RNA in 50% extract, 1.5 mM $MgCl_2$, 0.25 mM ATP, 1 mM cordycepin, 20 mM creatine-phosphate, and 2.5% polyvinyl alcohol in 15 μL final volume. Coupled CPA reactions were performed without cordycepin but with 0.8 mM ATP. U7-snRNP-dependent cleavage reactions were performed using 1 fmol of substrate RNA in 50% extract, 10 mM EDTA, and 4 μg of yeast total RNA in a 10 μL final volume. Freshly prepared nuclear extract was used since stimulation of U7-snRNP-dependent cleavage and of CPA was not observed in extract stored for more than 2–3 months at -80ºC.

Cleavage reactions were quenched by the addition of 200 μL of G50 buffer (20 mM Tris, pH 7.5, 2 mM EDTA, 300 mM NaOAc, and 0.25% SDS). Samples were digested with proteinase K, extracted with phenol:chloroform:isoamyl alcohol (25:24:1) solution (PCA), and precipitated with ethanol and run on a denaturing polyacrylamide gel for visualization. All quantitations were from three independent experiments and performed on a STORM 840 phosphorimager.

Micrococcal Nuclease Treatment and pCp-Labeling

50 μL of HeLa nuclear extract was supplemented to 1 mM $CaCl_2$. 15 U of micrococcal nuclease (Roche) was added, and the reactions were incubated at 28ºC for 15 min (Ryner and Manley, 1987). Reactions were quenched with 2 mM EGTA; a fraction of the extract was used for UV cross-linking analysis. To the other fraction, 200 μL of G50

buffer were added. Samples were digested with proteinase K, extracted with PCA, and precipitated with ethanol. pCp-labeling was as described (England and Uhlenbeck, 1978).

In Vitro snRNA Knockdown

snRNA knockdown in extract (Lamond et al., 1989; Seiwert and Steitz, 1993) and in oocytes (Hamm et al., 1989; Yu et al., 1998) was performed as described. The oligonucleotide used to knock down U12 snRNA was 5'-GTTGTTATTTTCCTTACTC.

RNA Immunoprecipitation (RIP)

The RIP protocol was as described previously (Niranjanakumari et al., 2002; Vasudevan and Steitz, 2007) with the following modifications. 40 million HeLa cells at a concentration of 2 million cells/mL were treated with 0.3% formaldehyde in medium for 10 min at 37ºC. Control cells were incubated without formaldehyde. 1.25 M glycine dissolved in PBS was added to a concentration of 0.125 M, and the sample was incubated for 5 min at room temperature. Cells were then washed twice in cold PBS, resuspended in 20 mL of PBS for 10 min on ice, and pelleted. The pellet was resuspended in 1 mL of cell lysis buffer (5 mM PIPES, pH 8.0, 85 mM KCl, 0.5% NP-40, and 0.5 mM PMSF) and Dounce-homogenized with ten strokes of a Wheaton Type A pestle. After spinning at 2700*g* for 5 min at 4ºC, the supernatant was discarded, and the purified nuclei were resuspended in 1 mL of binding buffer (10 mM HEPES, pH 7.0, 150 mM KCl, 1 mM EDTA, 0.5 mM DTT, 10% glycerol, and 0.5% NP-40). 100 µL of the resuspended nuclei were added to each immunoprecipitation reaction (antibodies were immobilized on

protein A-sepharose). Samples were incubated for 4 hrs at 4ºC and washed four times in binding buffer, then twice in RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% NP-40, and 0.5% sodium deoxycholate). The beads were resuspended in G50 buffer and treated with proteinase K at 45 ºC for 40 min. Samples were extracted with PCA, and the RNA/DNA mix was precipitated with ethanol. DNase treatment was performed using DNase I (Roche) for 30 min at 37ºC. Samples were extracted with PCA a second time, and the immunopurified RNA was precipitated with ethanol.

RNAs were analyzed by Northern analysis (snRNAs and mgU2-25/61) or RT-PCR (replication-dependent histone mRNAs). The RT-PCR primers for the replication-dependent histone genes have been described (Barcaroli et al., 2006). RT-PCR primers for RPL-15 are: 5'-GTGGATCACCAAACCAGTCC and 5'-TGGAGCTGGAGAGTATTGCG. The RT-PCR primers to detect *Xenopus* U12 snRNA were: 5'-ATGCCTTAAACTAATGAGTAAGG and 5'-CGGGCAGATCACTGCACC. The Northern probes for 7SK (Wassarman and Steitz, 1991), U1 snRNA, U2 snRNA, U11 snRNA, U12 snRNA (Wassarman and Steitz, 1992), and mgU2-25/61 (Tycowski et al., 2004) have been described.

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