Supporting Data

The initial U3 snoRNA:pre-rRNA base-pairing interaction required for pre-18S rRNA folding revealed by in vivo chemical probing

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Running head: Initial base-pairing of U3 snoRNA to pre-rRNA

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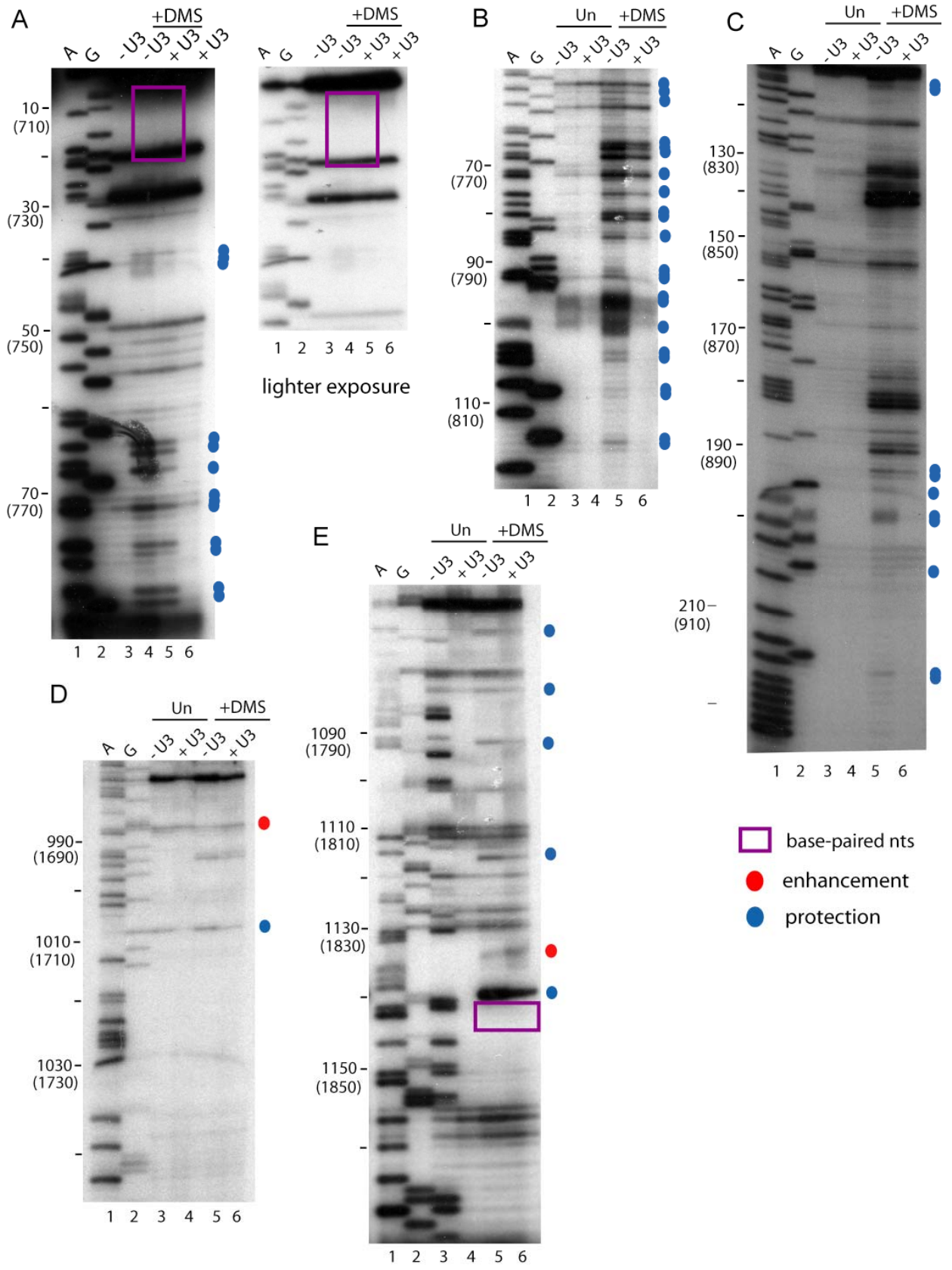
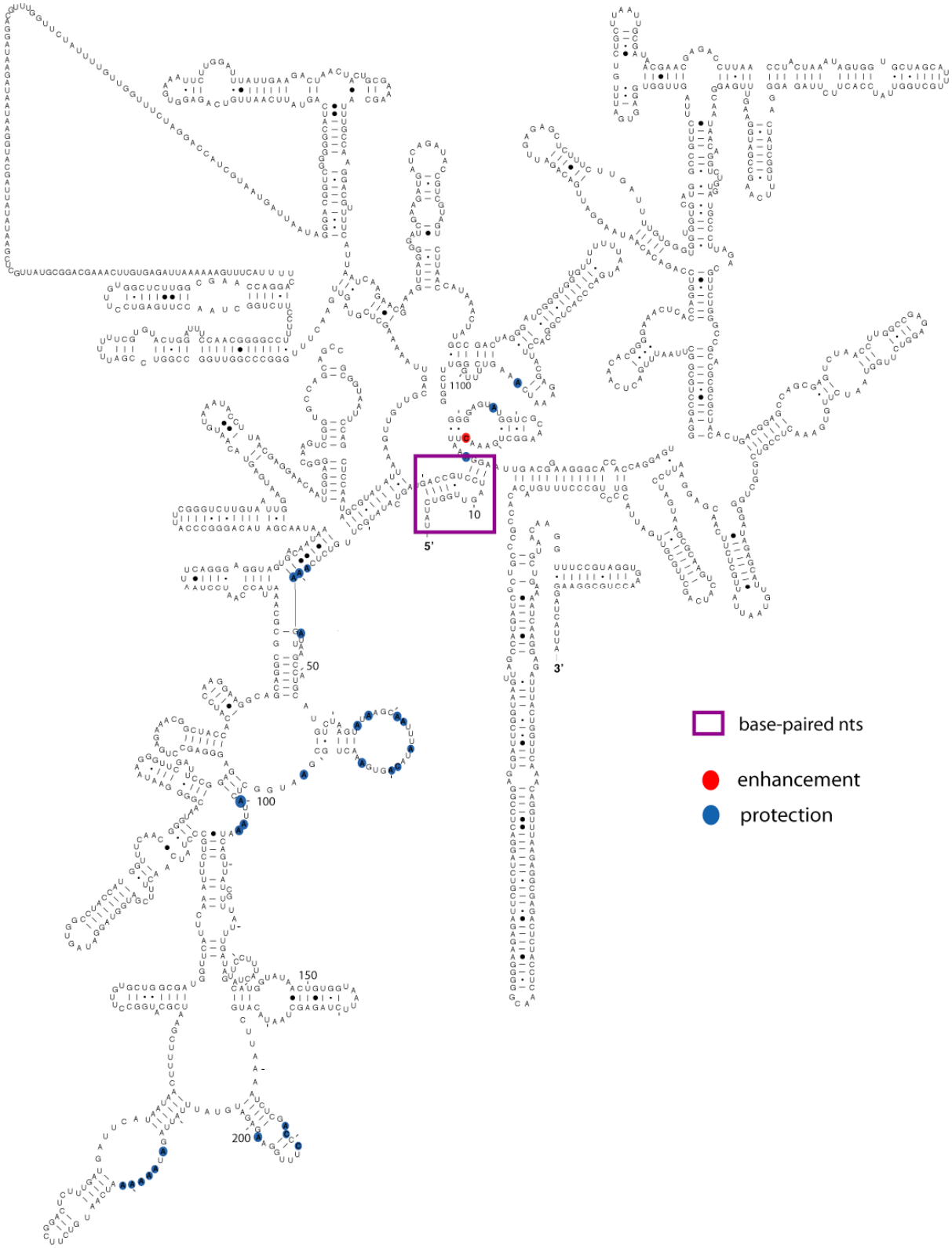
Supplementary Figure S1. Expression of the U3 snoRNA results in altered in vivo DMS reactivity of the nucleotides near the 5'-end pseudoknot of the 18S rRNA. DMS was added at 16 h after shifting the yeast to dextrose to deplete the endogenous U3 snoRNA. Yeast either contained a plasmid that expressed the tagged U3 snoRNA (+U3; U3 WT) or an empty vector (-U3; EV). Total RNA was extracted from unmodified (Un) and modified (+DMS) yeast and analyzed by primer extension with the following primers (A) 82-18S, a dark and a light exposure are shown; (B) 129-18S; (C) 227-18S; (D) 1063-18S and (E) 1169-18S. Lanes A and G are dideoxy sequencing lanes, and the nts are indicated on the left side of the gels. The numbering starts at the 5' end of the 18S rRNA. Parentheses indicate numbering from the start site of transcription. The purple boxes highlight the nts involved in the formation of the 5'end pseudoknot.

Supplementary Figure S2. Summary of the results from in vivo DMS modification of the 18S rRNA sequences near the 5' pseudoknot with and without the U3 snoRNA (data shown in Figure 4). Nucleotides with altered reactivity as a result of the presence of the U3 snoRNA, as compared to absence of the U3 snoRNA, are shown on the secondary structure of the 18S rRNA "http://www.rna.ccbb.utexas.edu/". Blue circles denote nts with lower reactivity, while red circles denote nts with higher reactivity. The size of the dot represents the intensity of the change. The purple boxes highlight the nts involved in the formation of the 5'end pseudoknot.

Supplementary Figure S1



Supplementary Figure S2



Supplementary Table S1. List of plasmids expressing the mutant U3 snoRNAs and the pre-rRNA used in these studies. All the U3 snoRNA genes were cloned into either pRS313 (HIS marker) or pRS314 (TRP marker). The U3 snoRNAs carry a unique sequence for detection purposes (see Figure 1 and Experimental Procedures).

Plasmid	Mutations in U3 snoRNA	Reference
pRS31X (EV) pRS313 (HIS marker) pRS314 (TRP marker)		
pRS313 U3WT pRS314 U3WT	Tagged U3 snoRNA	pRU3* (Samarsky and Fournier, 1998; Wormsley et al., 2001) pTRP-*U3^{tag} [wt]* (Wehner et al., 2002)
pRS313 U3 5H pRS314 U3 5H	nt 41-46 mutated from GAGAAA to ACTTCT	based on (Beltrame and Tollervey, 1995)
pRS313 U3 BoxA pRS314 U3 BoxA	nt 15 to 31 mutated from TAGGATCATTTCTATAG to ATCCTAGTAAAGATATC	pRU3*[box A:subst](Wormsley et al., 2001) this study
pRS313 U3-63 pRS314 U3-63	63 nt from 5'end deleted	pRU3*[-63](Wormsley et al., 2001) this study
pRS313 U3-72 pRS314 U3-72	72 nt from 5'end deleted	pRU3*[-72] (Samarsky and Fournier, 1998; Wormsley et al., 2001) this study
pRS314 U3 3H7	nt 64 to 70 mutated from ACTGAAT to GTCATTA	this study
pRS313 U3 3H11 pRS314 U3 3H11	nt 62 to 72 mutated from CCACTGAATCC to AGGTCATTAGG	this study
YEP24 (URA marker)		
pNOY102 (URA marker)		(Nogi et al., 1991)
pNOY102 ETS3H11	Pre-rRNA nts 281-286 and 289-291 mutated from GGAUU and GUGG to CCTAA and AUCT	this study

Supplementary Table S2. List of oligonucleotides used for site directed mutagenesis, northern blotting and primer extension.

Oligonucleotide	Sequence	Reference
5ETS6U3s	5'-TACTTCATAGGATCATTTCTATAGGAATCGTCATGA AGAGCTCTTCAAAAGAGCCACTGAATCCAACTTGG-3’	
5ETS6U3as	5'-CCAAGTTGGATTCAGTGGCTCTTTTGAAGAGTCTCTTCAT GACGATTCCTATAGAAATGATCCTATGAAGTA-3'	
U3hingemut7s	5'-GAATCGTCACTCTTTGACTCTTCAAAAGAGCCGTCAT TACCAACTTGGTTGATGAGTCCCATAACCTTTGT-3'	
U3hingemut7as	5’-TGTTTCCAATACCCTGAGTAGTTGGTTCAACCATTAC TGCCGAGAAAACTTCTCAGTTTCTCACTGCTAAG-3’	
U3hingemut9s	5'-GAATCGTCACTCTTTGACTCTTCAAAAGAGCCGTCATT AGGAACTTGGTTGATGAGTCCCATAACCTTTGT-3'	
U3hingemut9as	5’-TGTTTCCAATACCCTGAGTAGTTGGTTCAAGGATTACT GCCGAGAAAACTTCTCAGTTTCTCACTGCTAAG-3’	
SD74	5’-ATCAAGATCATCGCGCCGGTTTCTCACTCTGG GGTAC-3’	(Samarsky and Fournier, 1998)
SD13	5’-GCGGCTTAGGCTAAGCTAAGGCCAGC	(Samarsky and Fournier, 1998)
U14	5’-CGATGGGTTCGTAAGCGTACTCCTACCGTGG-3’	
157-5’ETS	5’- CTCAATACGCATCAACCCATGTC-3’	
276-5’ETS	5’-GCTAGTAATCCACCAAATCCTTC-3’	
400-5’ETS	5’-GGAATGGTACGTTTGATATCGCT-3’	
500-5’ETS	5’-TTACACTCTTGACCAGCG-3’	
542-5’ETS	5’TTCAGGTCTCTCTGCTGCCGG-3’	
611-5’ETS	5’-CCACCTATTCCCTCTTGCTAGAAG-3’	
693-5’ETS	5’-GGATCAACCAGATAACTATCTTAA-3’	(Beltrame and Tollervey, 1992)
82-18S	5’-AATGAGCCATTCGCAGTTTC-3’	
129-18S	5’-CCACGGTTATACCATGTAGTAAAGG-3’	
227-18S	5’GAATCATCAAAGAGTCCGAAGAC-3’	
388-18S	5’-TATCGCTGATTTGAGAGG-3’	
1063-18S	5’-TCGTAAGGTGCCGAGTGGGTC-3’	
1169-18S	5’-GTGTTGAGTCAAATTAGCCGCAGG-3’	

Supplementary Table S3. Summary of changes in chemical reactivity of 5'ETS nts in the presence of the mutated U3 snoRNAs, as compared to the absence of the U3 snoRNA as a reference point. E indicates an enhancement and its relative strength is indicated by e or EE, from weakest to strongest. P indicates a protection and its relative strength is indicated by p or P, from weakest to strongest.

Nucleotide	U3 WT	U3 5H	U3 Box A	U3 3H7	U3 3H11
A 295	EE	EE	EE	EE	
C300	p				
A303	E	E	E	E	
C311	EE	EE	EE	EE	
A313	e			e	
C317	E	E	E	E	
A319	e	e	e	e	
C330	p				
A452	e			e	
C454	e			e	
A461	p		p	p	
C469	P		p	P	
C471	p		p	p	
A472	p		p	p	
A473	p		p	p	
A474	p		p	p	
A476	p		p	p	
A605	E		e	e	
A638	p		p	p	
A639	P	p	P	P	
A640	p		p	p	

Supplementary Table S4. Changes in chemical reactivity of the 18S rRNA region of the 35S pre-rRNA when U3WT is expressed. The numbers in parenthesis correspond to numbering from the start site of transcription. Strength of modification is indicated as in Table S3. The numbering is from the 5' end of the 18S rRNA.

Nucleotide	Reactivity
A39 (739)	p
A40 (740)	P
A41 (741)	P
A43 (743)	P
A65 (765)	P
A67 (767)	P
A71 (771)	P
A72 (772)	p
A76 (776)	p
C79 (779)	p
A80 (780)	P
A84 (784)	P
A92 (792)	P
A100 (800)	PP
A103 (803)	P
A104 (804)	P
A138 (838)	P
A188 (888)	p
C189 (889)	p
C191 (891)	p
A215 (1015)	P
A217 (1017)	p
A218 (1018)	p
A219 (1019)	p
A220 (1020)	p
A221 (1021)	p
A1091 (1791)	P
A1116 (1816)	P
C1134 (1834)	e
A1139 (1839)	P