Antisense RNA associated with biological regulation of a restriction-modification system

Running title: Restriction modification and RNA

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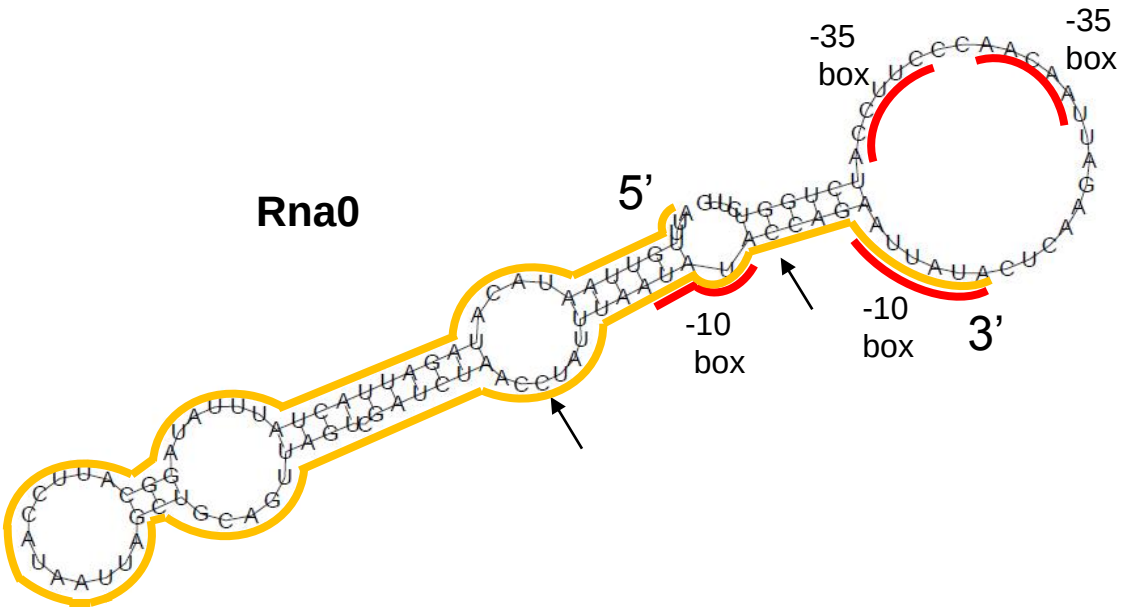
TABLE S1. Primers and probes

Primer	***Oligonucleotide sequence (5'→3')***	***Used for***
944f	AACGATATCTAGAGGTGCGTACAATTAAG	*lacZ* fusions, cloning
982f	AATCTAGAGACATATGTAAC	
1536f	TGCTCTAGATCCTTTTCTTAGAGGGGTCT	
1588f	AATATCTAGACCAGATGGAAGGG	
1588bf	AATATCTAGACCAGAGGGATGGGAAGTTAATCTTGAG	
1629f	ATTGATATCTAGAAAATAGGTTAGATCGAC	
1640r	ACCAGAATTATACTCAAG	
1662f	CTGTCTAGAATTATGGAATGCCTA	
1675f	CGGAATGTCTAGAAATAGTAATCTATGTA	
1692r	CTAAAGCTTGGATATCCATAATTAGCTGCA	
1731r	TTGTCTAGAAAGCTTACAAATTTGTTAATAC	
1800r	ATGAAGCTTGCATGCCACTCCCTCCCATC	
1801r	ATGTCTAGAGCATGCCACTCCCTCCCATC	
1892r	CGAATCTAGATCACTTAGATGTAAGCTG	

Mr	AAGCTCTAGACGAAGCTAATGATCTC	pIMRM cloning
Rf	CGGTCTAGAGTGGTCGACACCATCTGGTTGC	
lacp	CGGGATCGATCACAGAAGAAGTAGT	primer extension
1128f	ATGCCCATGGTCTAGATCTCGCTGTTGGTG	northern blotting
1601r	CATACGATTTAGGTGACACTATAGAATACTGGTCTTGTTATTGA	
1536f	TGCTCTAGATCCTTTTCTTAGAGGGGTCT	
1748r	CATACGATTTAGGTGACACTATAGAATACTGCATGCCAAATTTGTTAATACATAG	
1536f	TGCTCTAGATCCTTTTCTTAGAGGGGTCT	RNase protection assay
1800r	ATGAAGCTTGCATGCCACTCCCTCCCATC	
1425f	TCCTCGTGCGGCCAAGAGGAGATCAAGATTTA	
1634r	CAGATCTAGACTAAAGATTAACAACCCTTC	
colef	GCGGTCGACATATGCGGTGCTACAGAGTTC	
coler	TGAGTCGACGATATCGTAGAAAAGATCAAAG	
loading control	ATCTAGATAAATGTGAGCGGATAACATTGACATTGTGAGCGGATAAC AAGATACTGAGCATG	
PREV0-10mutf	TTGTAACCCGGGAAGACAAAAGCATTCTGCT	P_{REV0} substitutions
PREV0-10mutr	TTGTCTTCCCGGGTTACAAATTTGTTAATAC	
PREV0-35upmutf1	ACAAAAGCATTATGTGTCAAGCAGCATCTATAT	
PREV0-35upmutr1	TTGACACATAATGCTTTTGTCTTTATGA	
PREV0-35upmutf2	AAGCATTATGTTACAAGCAGCATCTATAT	
PREV0-35upmutr3	TGCTTGTAACATAATGCTTTTGTCTTTAT	
PREV0-35mutf	ACAAAAGCATTATGGGGCAAGCAGCATCTATAT	
PREV0-35mutr	TTGCCCCATAATGCTTTTGTCTTTATGA	
PREV0-35downmutf	ATTATGCTAGCCGCAGCATCTATATATAC	
PREV0-35downmutr	GATGCTGCGGCTAGCATAATGCTTTTGTC	
PM1/M2-10mutf	GCGGTCTGGTAAAGCAAATAGGTTAGATCGACTAAC	$P_{M1,2}$ substitutions
PM1/M2-10mutr	ATTTGCTTTACCAGACCGCTACTCAAGATTAACAAC	
sRNAmutf	GCCCATCAACAGTAACCTATGTATTAACAAAT	making pIM24
sRNAmutr	TAGGTTACTGTTGATGGGCATTCCATAATTAG	

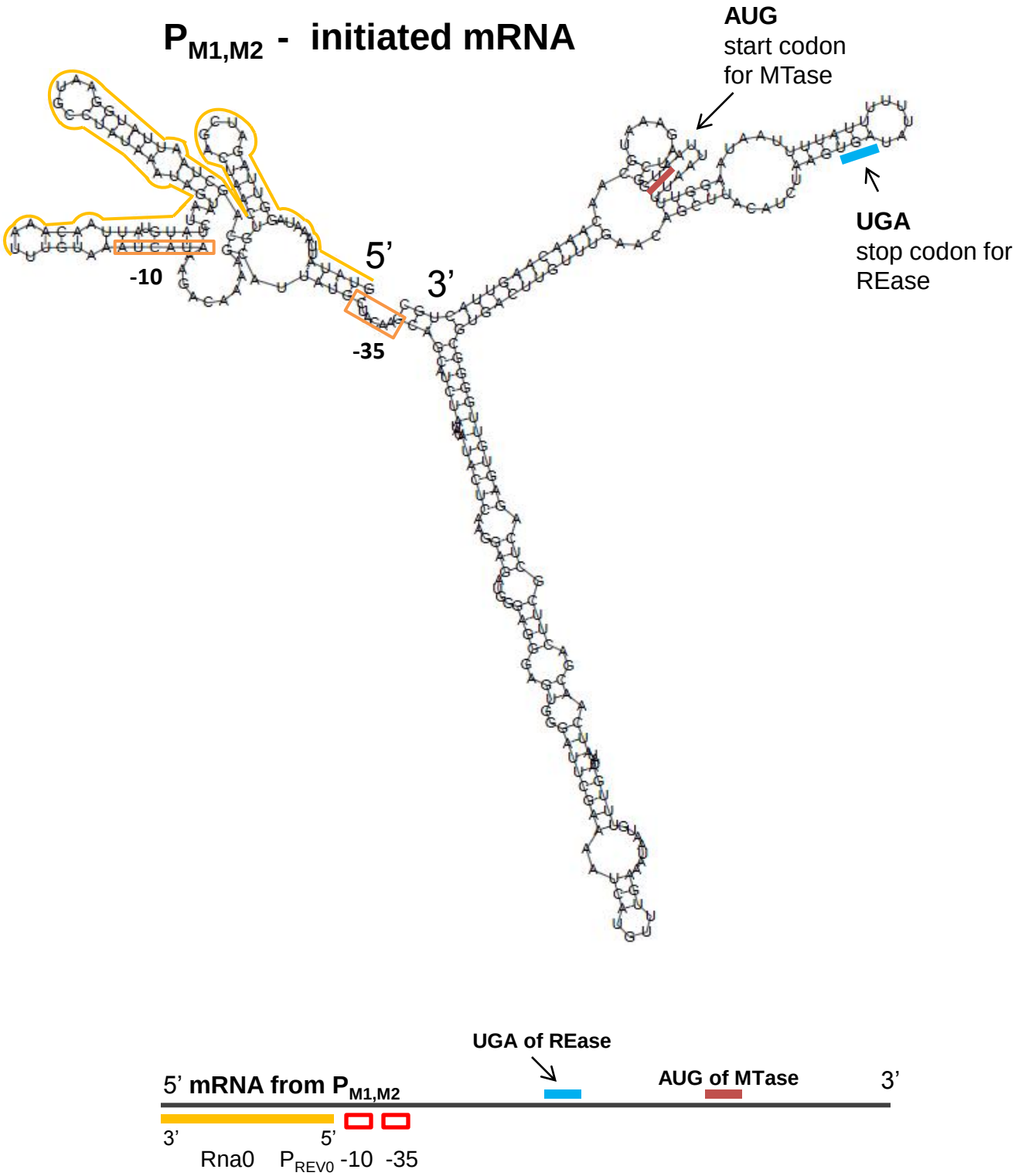
Figure S1. Predicted secondary structure for the antisense RNA (Rna0).



This was generated by RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi).

The optimal RNA fold was generated with a minimum free energy of **-14.79** kcal/mol. Rna0 is indicated by a yellow line with its 5’ and 3’ end. Complementary sequences corresponding to -10 and -35 hexamers for promoters P_{M1} and P_{M2} are indicated by red lines. Two transcription start sites from P_{M1} and P_{M2} are marked by arrows.

Figure S2. Predicted secondary structure for the 5’ end of mRNA for modification enzyme initiated from $P_{M1,M2}$ promoter.



Sequence complementary to the antisense RNA (Rna0) is in yellow, while those corresponding to -10 and -35 hexamers of its (reverse) promoter (P_{REV0}) are boxed. Initiation codon, ATG, for translation of the modification enzyme is underlined in red. Stop codon for translation of restriction endonuclease, UGA, is underlined in blue. This structure was generated by RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi). The optimal RNA fold was generated with a minimum free energy of **-63.20** kcal/mol.

Bottom: a linear representation.