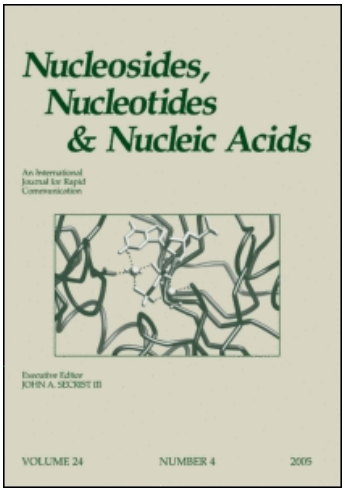

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Template Activity of Oligoribonucleotides Synthesized by the Phosphoramidite Method Using 2'-O-Tetrahydrofuranyl and 2'-O-Tetrahydropyranyl Protecting Groups

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TEMPLATE ACTIVITY OF OLIGORIBONUCLEOTIDES SYNTHESIZED BY THE PHOSPHORAMIDITE METHOD USING 2'-O-TETRAHYDROFURANYL AND 2'-O-TETRAHYDROPYRANYL PROTECTING GROUPS

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Abstract. Solid-phase synthesis and functional activity of oligoribonucleotides containing native and modified translation initiation region (TIR) of phage MS2 and fr RNA replicase gene have been investigated.

Solid-phase synthesis of 15-24 membered oligoribonucleotides was carried out with a Pharmacia Gene Assembler. Tetrahydropyranyl (Thp) and tetrahydrofuranyl (Thf) groups were employed for the 2'-hydroxyl protecting. We combined them with the use of 5'-dimethoxytrityl (DMTr) groups. Nucleoside phosphoramidite units were prepared from 2'-O-tetrahydropyranyl (or tetrahydrofuranyl) suitable base-protected nucleoside derivatives using bis(diisopropylamino)methoxyphosphine. CPG-550 and Bio-Glas-500 served as solid supports for the synthesis of oligoribonucleotides. Till now the combination of the acid-labile 2'-OThp (or 2'-OThf) and 5'-ODMTr was recommended only for the synthesis of short oligomers due to their unstability during chain elongation.[1]

The longer oligoribonucleotides (21 mers) were obtained by means of phosphoramidite approach using the levulinyl group for the protection of 5'-OH group and Thf for 2'-OH.[2,3]

We performed the coupling in CH_3CN during 3 min in the presence of 1-H-tetrazole. Dichloroacetic acid (1%) in dichloromethane was used for the removal of the DMTr group.

After cleaving from the support and deprotection with conc. aqueous NH_3 oligomers were separated by FPLC (C_8 column) and after treating with 80% CH_3COOH on a C_{18} column. All synthetic templates were sequenced enzymatically.

The synthetic oligoribonucleotides were used to investigate the specific interaction between ribosome and TIR of the prokariotic mRNA. Based on the previous studies of template activity of short fragments of phage MS2 and fr RNA containing TIR of the replicase gene, the approximate structure of the initiation region has been determined.[4] The latter contains the initiation codon and a 5'-sequence from it not exceeding 17 nucleotides and containing the Shine-Dalgarno sequence. We also studied oligoribonucleotides containing native and modified TIR's of phage MS2 and fr replicase gene. Functionally active 20mer frR(-17 → 3) have been selected for the template modifications with nucleotide substitutions and deletion. This RNA fragment contains the initiator AUG, the native SD region and 7 adjacent 5'-terminal nucleotides (Table).

Nucleotides within this region were changed by enzymatic or chemical synthetic methods. Oligomers 1 and 2 with the base changes in the spacer region of TIR and at the 5'-terminus had high template activity. The shortening of a sequence at the 5'-end of frR(-17 → 3) up to 16 mer frR(-13 → 3) also has no influence on the effectivity of translation

TABLE. Template activity of phage fr RNA fragments containing TIR of replicase gene

Oligoribonucleotide	Presence of TIR elements structure				Relative activity, %
	Actual AUG	SD sequence	Out-of-frame AUG	Terminator UGA	
Sequence of TIRs in native:					
fr R(-17→3)	+	+	+	+	100
5'CAACAUGAGGAAUACCC**AUG**3'					
MS2 R(-17→3)	+	+	+	+	100
5'AAACAUGAGGAUUACCC**AUG**'3'					
Synthetic:					
1 AAACAUGAGGAAUACCC**AUG**	+	+	+	+	100
2 AAACAUGAGGAAAACCC**AUG**	+	+	+	+	95
3 AAACAUGAAGAAUACCC**AUG**	+	-	+	+	27
4 AAACAUGAAGAAAACCC**AUG**	+	-	+	+	27
5 AAACACGAGGAAAACCC**AUG**	+	+	-	-	11
6 AACAUGAGGAAUACCC**AUG**	+	+	+	+	100
7 AUGAGGAAUACCC**AUG**	+	+	+	+	100
8 UGAGGAAUACCC**AUG**	+	+	-	+	59
9 UCACCAAUACCC**AUG**	+	-	-	-	9

Ribosome binding activities of oligoribonucleotides were compared at 40 pmol of input template.

Actual initiation codon of replicase gene is bold, the putative SD sequence is underlined, sequence AUGA of overlapping out-of-frame AUG and terminator codons is marked with upper line.

Oligoribonucleotides 1-5 were products of enzymatic synthesis, templates 6-9 were obtained from solid-phase synthesis.

Nucleotide substitutions are in bold type.

initiation complex formation. Loosing of the next 5'-terminal nucleotide (oligonucleotide 8) causes a decrease in activity. Base changes (G → A) leading to the modification of SD region (oligomers 3 and 4) sharply decrease the template activity. Single base changes (U_{-12} to C_{-12}) completely inactivate the template. In the latter case the out-of-frame initiation codon AUG and terminator UGA in AUGA sequence close to the 5'-end of SD sequence were eliminated.

These results suggest that the 70S ribosomal initiation complex formed by template oligonucleotide with start codon and the native Shine-Dalgarno tetranucleotide AGGA can be enhanced by presence of the AUGA sequence of overlapping initiation and termination codons.

References

1. S.Iwai, E.Ohtsuka, Nucleic Acids Res., 1988, **16**, 9443-9456.
2. S.Iwai, T.Sasaki, E.Ohtsuka, Tetrahedron, 1990, **46**, 6673-6688.
3. R.Kierzek, M.H.Caruthers, C.E.Longfellow, D.Swinton, D.H.Turner, S.W.Freier, Biochemistry, 1986, **25**, 7840-7846.
4. V.Berzin, I.Cielens, J.Jansone, E.Gren, Nucleic Acids Res., 1982, **10**, 7763-7775.
5. R.Renhof, I.Cielens, T.Nikitina, L.Sherina, Z.Shomstein, E.Gren, FEBS Letters, 1985, **185**, 277-281.