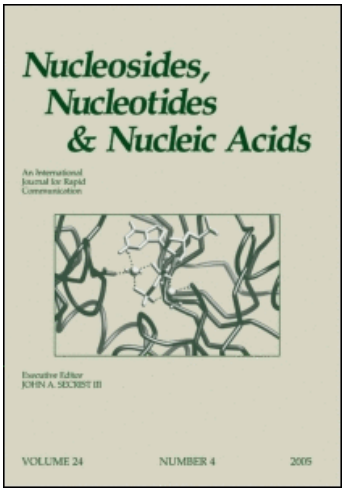

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Interaction of Complementary Oligonucleotides with the 3'-End of Yeast tRNA^{PHE}

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INTERACTION OF COMPLEMENTARY OLIGONUCLEOTIDES WITH THE 3'-END OF YEAST tRNA^{PHE}.

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ABSTRACT: Interaction of yeast tRNA^{Phe} with oligodeoxyribonucleotides (ONs), complementary to the nucleotides 62-76 was investigated. Results of gel-mobility shift assay and RNase A probing evidence that the ONs containing the sequence complementary to the tRNA ACCA end can easily invade the hairpin structure under physiological conditions. The limiting step of association process is the tRNA unfolding.

Structure of RNA is a crucial factor determining binding of oligonucleotides to these molecules. In some cases oligonucleotides can bind to folded RNAs by invading into certain elements of RNA structure. Thus, complementary oligonucleotides can bind to some sequences in yeast tRNA^{Phe} in spite of tight folding of this molecule [1,2].

We investigated binding of ONs to yeast tRNA^{Phe} using gel-mobility shift assay. We have found that oligonucleotides complementary to the 3'-end of the molecule including the ACCA sequence bind to the tRNA. At 20°C the tRNA-ON duplex formation requires 6 hours incubation (Fig. 1). The association rate constant for oligonucleotide is k_{+eff}= 1,78 ±0,03 $M^{-1}s^{-1}$. Independence of the ON concentration suggests that the rate of the process is determined by a rearrangement of the tRNA structure.

Probing of the tRNA structure by RNase A in the course of interaction with ON has shown that immediately after addition of ON to the tRNA, sensitivity of phosphodiester bonds adjacent to C72, C74 and C75 to the enzyme decreases and the bond adjacent to C13 becomes available to the cleavage (Fig. 2). At longer incubation times new cuts appear at pyrimidine sites in the TΨC hairpin, anticodon stem and D-loop, reflecting the

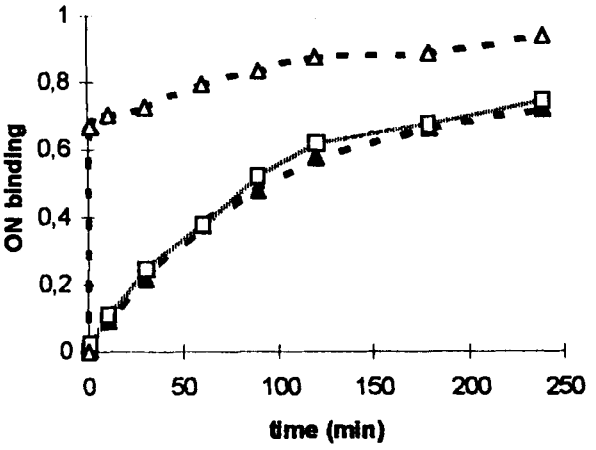
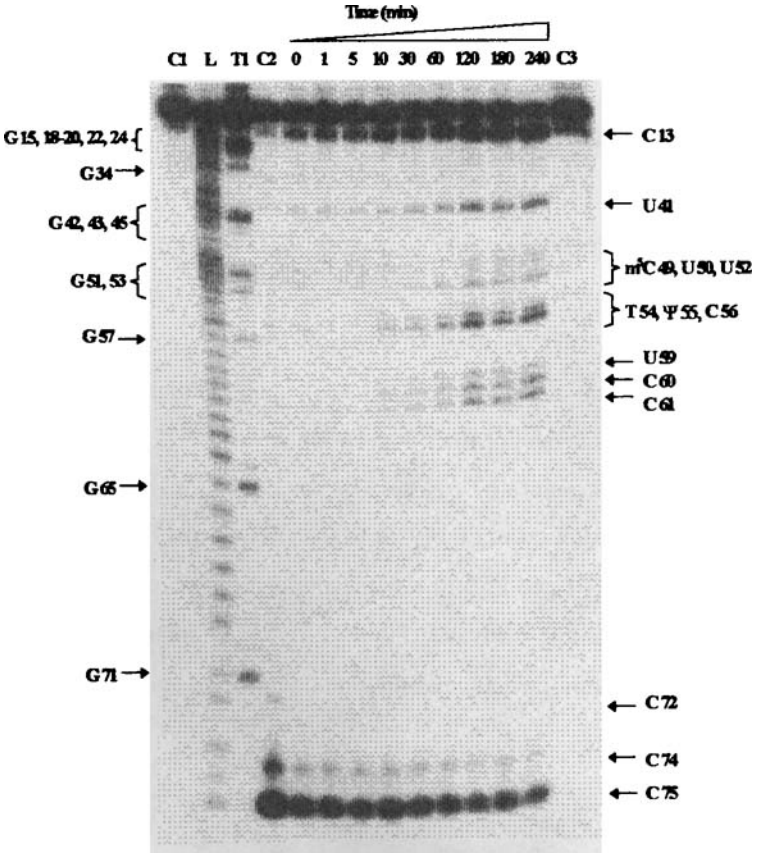


FIG.1. Kinetics of ON binding with tRNA: □ - gel-mobility shift assay data; Δ and ▲ triangles - RNAse A probing data for binding with ACCA sequence and TψC-stem, respectively.



tRNA unfolding. The region of the ON binding (positions 63-76) was completely protected from RNase A cleavage except for C74, where "fraying" of the base pairs at the end of the helix can occur. The kinetics of the TΨC hairpin unfolding detected by RNase A probing is similar to that observed using gel mobility shift assay.

Results of this study evidence that binding of oligonucleotides to the 3'-end of tRNA^{Phe} occurs through an intermediate complex formation with the single-stranded ACCA sequence and following strand displacement leading to unfolding of the tRNA.

ACKNOWLEDGEMENT.

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FIG.2. RNAse A probing of the 3' end [^{32}P]-labeled yeast tRNA^{Phe} in the course of complex formation with ON TGGTGCGAATTCTGT. Autoradiograph of 18% denaturing PAAG. Lanes L and T1 are OH^- and RNase T1 ladders, respectively. Lane C1 - intact tRNA, C2-tRNA digested by RNAse A without ON, C3 - incubation control. Lanes 0-240 corresponds to the tRNA samples incubated with ONs for the indicated times (min) and probed with RNase A. tRNA was incubated with the oligonucleotide (70 μM) at 20^{0}C in 50mM HEPES-KOH pH 7,5, containing 200mM KCl, 0.1mM EDTA. The cleavage was performed by addition of $6 \cdot 10^{-5}$ units of RNase A and incubation for 1 min.