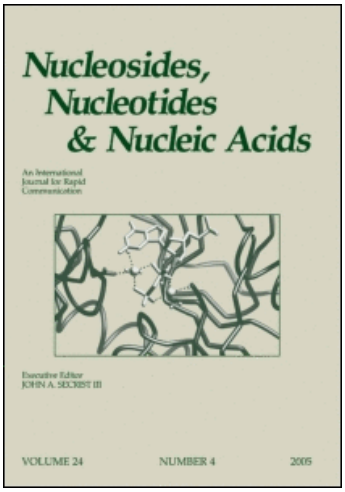

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Translation Arrest by RNase h Incompetent Antisense Oligonucleotides

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TRANSLATION ARREST BY RNase H INCOMPETENT ANTISENSE OLIGONUCLEOTIDES

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ABSTRACT: Most second-generation ON antisense analogs do not activate RNase H. Alternative strategies to arrest translation in a reticulocyte cell-free assay programmed by VSV mRNAs have been explored.

Activation of RNase H by antisense oligonucleotides (ON) is generally accepted to be the most efficient strategy to interfere with mRNA translation. Many ON analogs, *e.g.* alpha-anomeric analogs, or peptide nucleic acids, possess interesting pharmacological properties, but are incapable of activating RNase H. We have assessed the extent with which high-affinity hybridization, covalent linkage and activation of alternative degradative pathways can be used to arrest translation elongation, using a reticulocyte lysate programmed with vesicular stomatitis virus (VSV) mRNA[1,2].

Phosphodiester ON targeted to various portions of the VSV N protein mRNA coding region specifically arrest translation in the presence of exogenous *E. coli* RNase H, leading to the accumulation of truncated peptides. RNase H-incompetent ON are unable to block translation in this assay, despite their high affinity *e.g.* a Tm superior to 70° C for N 3' phosphoramidates or PNAs[3]. Mixed-backbone antisense ON comprising a central phosphodiester window (to activate RNase H) and modified arms (to provide nuclease resistance and improved affinity for the target) represent an interesting

compromise. New methods for the synthesis of PNA-DNA chimeras[4] have allowed the implementation of this strategy to PNAs. Preliminary experiments have indicated that certain PNA-DNA chimeras possess the capacity to activate RNase H and as a consequence lead to *in vitro* translation inhibition (Robbins *et al* in preparation). However, PNA-RNA duplexes have unusual helical structures[5] creating distorsions. This may account for the low Tm of these chimeras with respect to their PNA content and might limit the application of this strategy.

Steric hindrance through covalent attachment *via* a trans-platin modified antisense ON[6] has allowed a specific and RNase H-independent blockade of VSV N protein syntheseis at doses as low as 10nM, *e.g.* at nearly stoichiometrc ratios[3].

Alternatively alpha-anomeric ON analogues can be converted into sequence-specific nucleases upon conjugation to 2-5A oligomers. However, target selection appears critical to promote the assembly of the appropriate ternary complex between the mRNA target, the antisense ON 2-5A chimera and the RNase L effector[7].

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