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Specific inhibition of retroviruses and retrovirus activated oncoproteins by antisense oligonucleotides.

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Murine retroviruses such as Friend leukemia Virus (F-MuLV) and Moloney murine leukemia Virus (Mo-MuLV) display a much lower rate of mutation than HIV. New types of antiretroviral sequence specific agents can therefore be evaluated on these models. Our interest has been focused on oligonucleotides (oligos) rendered resistant to extracellular nucleases. We have therefore used either α anomeric oligos or ordinary oligos (ß anomeric) encapsulated in pH sensitive liposomes. 1) A 15 mer ß oligo complementary of the AUG initiation codon of F-MuLV envelope gene was shown to specifically inhibit synthesis of env protein in a wheat germ lysate. This oligo was then encapsulated in pH sensitive liposomes which unexpectedly penetrate only in retrovirus infected murine fibroblasts. The result was a fully specific 100 % inhibition of retrovirus proliferation at 0.3 μM oligo (added in two steps). 2) An α oligo complementary of the primer binding site of F-MuLV was shown to bind to the retroviral RNA and then to prevent the binding of the $tRNA^{pro}$. It displayed a 30 % specific inhibition of retrovirus proliferation when using electropermeabilization. We suggest that this oligo might act during the extension of the minus DNA strand or could interfere with the second jump. 3) ß oligos were shown to inhibit with specificity the synthesis of the Mo-MuLV activated oncoprotein PIM in a lysate.

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Effects of anti-FMLV triple helix forming oligonucleotides on FMLV production in culture.

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Anti-viral triple helix forming oligonucleotides (TFOs) have been designed to a DNA duplex target located within the *gag* gene of Friend Murine Leukemia Virus (FMLV). This region of the viral genome contains a purine rich stretch of nucleotides which has a high percentage of guanine residues. The specific TFOs are designed to bind to the major groove of the duplex target in an anti-parallel configuration. The TFOs are composed entirely of guanine and thymidine nucleosides with a standard phosphodiester backbone and either a propylamine or cholesterol moiety at the 3' end. The specificity and avidity of the TFOs in recognizing and binding to the target duplex was determined by mobility shift and DNAse foot printing analysis. The results indicated that the TFOs bound to the specific target duplex with dissociation constants ranging from 10^{-7} to 10^{-10}M depending on the TFO and the 3' modification. The ability of these TFOs to attenuate transcription in-vitro was determined by insertion of the FMLV target duplex into a bacterial expression vector. The TFOs were then used to inhibit transcription directed by the bacterial T3 and T7 promoters in-vitro. Inhibition of FMLV production in culture was determined using an NIH3T3 cell line which had been chronically infected with FMLV. In these assays FMLV infected cells were treated with various concentrations of TFOs and then assayed for the presence of virus at various time points post -TFO treatment. To do this, culture supernatants from treated and untreated cells were monitored for the presence of viral reverse transcriptase. Results from these assays indicate that the gaunine rich oligonucleotides were capable of significantly reducing FMLV in the culture supernate.