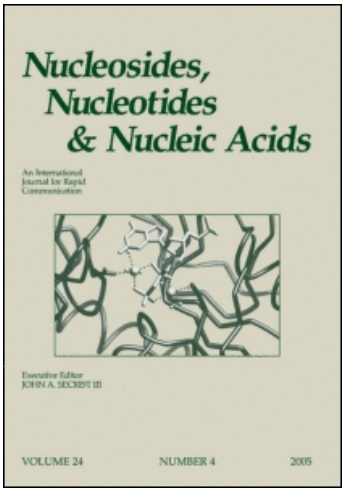

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Targeting HIV mRNA for Degradation: 2,5-A Antisense Chimeras as Potential Chemotherapeutic Agents for AIDS

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TARGETING HIV mRNA FOR DEGRADATION: 2,5-A ANTISENSE CHIMERAS AS POTENTIAL CHEMOTHERAPEUTIC AGENTS FOR AIDS

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ABSTRACT We have identified a region within a 1 kb HIV *gag* RNA which can be ablated *in vitro* using a 2,5-A antisense chimera. The cleavage was specific and almost complete at a concentration of 100 nM chimera.

Conjugation of a 5'-phosphorylated-2',5'-oligoadenylate tetramer (2,5-A) to a 3',5'-deoxyribonucleotide complementary to a targeted sequence in an RNA provides a novel reagent which effects the selective and specific cleavage of the RNA. [*Science* **265**, 789 (1994)]. The chimeric nucleic acid-induced recruitment of the chosen RNA and subsequent activation of the ubiquitous RNase L which then cleaves the targeted RNA, provides a novel approach for the targeted ablation of an mRNA and the protein which it specifies. The general structure of these 2,5-A antisense chimeras is pA2'p5'A2'p5'A2'p5'ApBupBup5'dN3'[p5'dN3']$_{16\text{-}22}$p5'dN, where Bu is 1,4-butanediol and dN is any common deoxyribonucleoside.

HIV *gag*, *rev* and *tat* have been chosen as the targets most likely to afford *in vitro* inhibition of HIV replication. An analysis of the theoretical secondary structure of the appropriate regions of HIV full and partial transcripts using the algorithm developed by Zuker was performed. Using these structures as guides, a series of 2,5-A antisense chimeras directed against a variety of specific sequences were synthesized using newly modified solid-phase technology followed by ion-pair HPLC purification, RP desalting, cation exchange and dialysis.

The study of such chimeras to induce *in vitro* cleavage of a ~1 kb labeled transcript of HIV infectious plasmid (pNL4-3) which encompasses each targeted sequence was employed. Recombinant human RNase L, expressed in SF21 cells from a baculovirus vector and purified to homogeneity *via* FPLC, was used in the assays. A chimera targeting a region in *gag* was most active, resulting in 96% ablation of *gag* transcript at a concentration of 100 nM chimera. Controls used included a mismatched 2,5-A antisense chimera which lacks sequence specificity

and would not anneal, as well as non-5'-phosphorylated or "core" 2,5-antisense chimera which would be incapable of activating RNase L. Neither was able to cleave *gag* transcript. Also, this anti-*gag* 2,5-A antisense chimera did not cleave an unrelated RNA (*bcr-abl* 5'-transcript).

We are developing 5'-exonuclease and phosphatase stable 2,5-A chimera analogues in order to more effectively explore this potential therapeutic principle in acutely and chronically HIV infected cells.