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SUMMARY OF WORK ACCOMPLISHED

Triple Helix Design Principles. The first research priority of the program of Navy support was to refine our understanding of triple helix forming oligonucleotides (TFOs). Binding affinity and strand orientation of triplex forming oligonucleotides were measured as a function of base composition. Based upon that work, which was published in <u>Biochemistry</u> (ref. 3), we showed that triple helices containing GGC and TAT triplets were stable at physiological pH and prefer to bind with an antiparallel strand orientation. This study and the accompanying patent application provided the first evidence that TFOs can bind in a site selective fashion at physiological pH and the first explicit evidence for a new (antiparallel) class of triple helix.

This work also served as the basis for the filing of a continuation in part to a patent application, filed 12/89.

Evidence that TFO binding can modulate somatic gene expression. In parallel to an analysis of TFO structure and affinity, a program was initiated to determine if TFOs could enter the nucleus, bind to as a result of triple helix formation, inhibit DNA then, transcription initiation from human genes in cultured cells. The first two test cases of that kind were performed on the interleukin 2 receptor gene (IL2-r), Nucleic Acids Research (ref. 1), and on the c-myc gene, Proceedings of the National Academy of Science (ref. 2). In both studies, we have provided evidence that TFOs are efficiently transported into the nucleus, remain stable for several hours and, as a result of site-selective triple helix formation, appear to be capable of selective inhibition of target gene expression.

These two papers have provided the first published evidence for site directed TFO binding in living cells, and the first evidence that intracellular binding of TFOs can be used to usefully manipulate the function of cells.

Evidence that TFO binding can modulate viral gene expression. In order to extend these preliminary studies of TFO technology, we determined if TFO binding to a viral promoter could be used to block virus growth in living cells. For the first test case, we chose to study the HIV-1 virus in cultured monocytes and T cells.

The outcome of this work was published in the <u>Journal of</u> <u>Biological Chemistry</u> (ref. 4). In this study, we have confirmed the stability and efficient delivery of TFOs to the nucleus of cultured cells. We have also provided evidence that a TFO targeted to a triplet of Sp1 sites in the HIV-1 LTR appears to selectively inhibit viral mRNA synthesis, and as a result of that mRNA inhibition, blocks viral growth in chronically infected cells and in one acutely infected cell line.



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This study has provided the first suggestion that TFOs can be used to manipulate the growth of a virus. Work is in progress to extend this study: by enhancement of the binding properties of the TFO which was employed, and by extending the work to HSV-1, a double stranded virus.

Defining the distribution characteristics of TFOs in animals. In order to be used as tools to manipulate gene expression in living creatures, methods must be developed which can stabilize TFOs with respect to metabolism and which lead to efficient delivery of TFOs into target tissues. In order to provide preliminary data to address the question, we have monitored the distribution and stability of a model TFO in the Balb-c mouse. In this study, <u>Nucleic Acids Research</u> (ref.5), we employed a TFO with a standard phosphodiester linkage, but end-capped by addition of a propyl amine linker the 3' terminus.

Surprisingly, we have found that this minimally modified TFO was efficiently retained in the mouse, subsequent to IP or IV injection. The compound remained stable for several hours and was well-distributed into most tissues. Tissues of the brain were the one exception, suggesting that minimally modified TFOs will not pass the blood brain barrier.

This study has shown that pharmaceutical or other <u>in vivo</u> uses of TFOs are feasible, using currently available chemical technology. The work is also being used by us as a bench mark to compare the effect of more elaborate modifications of TFO structure, especially those which might enhance TFO delivery to the brain.

SUMMARY OF WORK IN PROGRESS

One original goal of the program of Navy support was to initiate structural studies of triple helix formation, with the goal of supporting subsequent activity in molecular design. During the last six months of Navy support, that work has begun to become successful.

Crystallography. In collaboration with Dr. Kurt Krauss, who directs the University of Houston crystallography facility, we have initiated a program to grow crystals of self complementary triple helices (30bases long) which contain GGC and TAT triplets. Conditions which foster orderly crystal growth to 0.5mM have been obtained and data collection will begin shortly to determine the space group of these crystals. Once it has been determined that diffraction occurs to at least 3A, this program will be submitted to the Navy, to the NIH and to the NSF for independent support. In the initial publications, The Navy will be cited for its preliminary support of this work

NMR. Preliminary CD and 2DNMR studies have also been completed on 30base self complementary triple helices at 500mHZ on a facility at Rice University. High resolution data have been obtained and are being analyzed. As for the crystallography, the focus of the work is to drive our search of novel bases to be included at sites where



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or

simple polypurine triplexes are interrupted by a CG or TA inversions in the duplex.

Support for this continuing study has been obtained from the NIH, in collaboration with B. Montgomery Pettitt, (University of Houston) who is performing the molecular modeling which is crucial to the study. Again, in the first publications, the Navy will be cited for its preliminary support of this work.

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- M.E. Hogan, R. Revankar, R. Varma, T.S. Rao. Nucleosides and oligonucleosides with a phosphatefree internucleoside backbone and process for preparing same. Submitted 3/91
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Triplex forming oligonucleotide reagents targeted to the <u>neu</u> oncogene promoter and methods of use. Submitted 10/13/91