

AD-A250 969

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Form Approved
OMB No. 0704-0188

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Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

RT DATE

05/18/92

3. REPORT TYPE AND DATES COVERED

FINAL REPORT 12/01/88 - 11/30/91

4. TITLE AND SUBTITLE The Design of Oligonucleotides Which Attack Specific Gene Targets		5. FUNDING NUMBERS N00014-89-J-1167	
6. AUTHOR(S) Hogan, Michael, E.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <i>Baylor Coll of Medicine</i> Center for Biotechnology 4000 Research Forest Drive The Woodlands, Texas 77381		8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research 800 N. Quincy St. Arlington, VA. 22217-5000		10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES			
12a. DISTRIBUTION/AVAILABILITY STATEMENT DISTRIBUTION UNLIMITED		12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) 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.			
14. SUBJECT TERMS DNA Recognition Triple helix formation		15. NUMBER OF PAGES 4	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT UL

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JUN 03 1992

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DEPARTMENT OF THE NAVY
FINAL TECHNICAL REPORT
M.E. HOGAN
526-98-3070

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 Biochemistry (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 DNA then, as a result of triple helix formation, inhibit 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 Journal of Biological Chemistry (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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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.

INDEX OF PUBLICATIONS

1. F.M. Orson, D.W. Thomas, W.M. McShan, D.J. Kessler, and M.E. Hogan (1991). **Triplex forming oligonucleotide modulation of IL2R α mRNA transcription.** Nucleic Acids Research 19:3435-3441.
2. E.H. Postel, S.J. Flint, D.J. Kessler, M.E. Hogan (1991). **Evidence that a Triplex-forming oligodeoxynucleotide binds to the c-myc promoter in HeLa cells, thereby reducing c-myc mRNA levels.** Proc. Natl. Acad. Sci. USA 88:8227-8213
3. R.H. Durland, D.J. Kessler, S. Gunnell, M. Duvic, M.B. Pettitt, M.E. Hogan (1991). **Binding of triple helix forming oligonucleotides to sites in gene promoters;** Biochemistry 30:9246-9255
4. W.M. McShan, R.D. Rossen, A.H. Laughter, J. Trial, D. Kessler, J.G. Zendegui, M.E. Hogan & F.M. Orson (1992) **Inhibition of HIV-1 transcription by oligonucleotides designed to form collinear DNA triplexes.** J. Biol. Chem. 267:5712-5721
5. J.G. Zendegui, K.M. Vasquez, J.H. Tinsley, D.J. Kessler & M.E. Hogan (1992) **In vivo stability and kinetics of absorption and disposition of 3' phosphopropyl amine oligonucleotides.** Nucleic Acids Research 20:307-314

PATENTS PENDING OR FILED

M.E. Hogan, D.J. Kessler.

Method for making synthetic oligonucleotides which bind specifically to target sites on duplex DNA molecules, by forming a collinear triplex, the synthetic oligonucleotides and methods of use.
Submitted 12/88. C.I.P. filed on 12/89

M.E. Hogan, R. Revankar, R. Varma, T.S. Rao.

Nucleosides and oligonucleosides with a phosphate-free internucleoside backbone and process for preparing same. Submitted 3/91

M.E. Hogan, R. Revankar, T.S. Rao

Purine base modified 2' deoxyribonucleotides, use in triple helix forming oligonucleotides and process for preparing same. Submitted 5/91

M.E. Hogan

Triplex forming oligonucleotide reagents targeted to the neu oncogene promoter and methods of use.
Submitted 10/13/91