

AD _____

GRANT NUMBER: DAMD17-94-J-4339

TITLE: A Genetic Approach to Identifying Signal Transduction
Mechanisms Initiated by Receptors for TGF-B-Related
Factors

PRINCIPAL INVESTIGATOR: F. Michael Hoffmann, Ph.D.

CONTRACTING ORGANIZATION: University of Wisconsin
Madison, Wisconsin 53706-1490

REPORT DATE: October 1995

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

19960405 050

THIS QUANTITY INVESTIGATED 1

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE October 1995	3. REPORT TYPE AND DATES COVERED Annual 1 Oct 94 - 30 Sep 95		
4. TITLE AND SUBTITLE A Genetic Approach to Identifying Signal Transduction Mechanisms Initiated by Receptors for TGF- β -Related Factors			5. FUNDING NUMBERS DAMD17-94-J-4339	
6. AUTHOR(S) F. Michael Hoffmann, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Wisconsin Madison, Wisconsin 53706-1490			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) Genetic screens for new mutations affecting the signaling pathway by which cells respond to TGF- β -related factors in Drosophila were carried out. Two of the genetic strategies employed were unsuccessful and were abandoned. Two other genetic backgrounds that were sensitized using mutations in the Type I receptor gene and in a candidate transcription factor on the signaling pathway have been used successfully to recover new mutations affecting the phenotypes caused by the sensitized genetic backgrounds. The new mutations were recovered at frequencies that are consistent with a limited number of genes (2-3) being present in which mutations can alter the chosen phenotypes. These 10 new mutations are currently being characterized and mapped. Additional genetic screens to recover more mutations are in progress. The genetic mapping of these new mutations will indicate which cloning strategies should be employed for the molecular identification of the affected genes.				
14. SUBJECT TERMS Transforming growth factor beta; Genetics; Signal transduction; Drosophila; mutations, breast cancer			15. NUMBER OF PAGES 7	
			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 Unlimited	

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to *stay within the lines* to meet *optical scanning requirements*.

Block 1. Agency Use Only (Leave blank).

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C - Contract	PR - Project
G - Grant	TA - Task
PE - Program Element	WU - Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. (If known)

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."

DOE - See authorities.

NASA - See Handbook NHB 2200.2.

NTIS - Leave blank.

Block 12b. Distribution Code.

DOD - Leave blank.

DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.

NASA - Leave blank.

NTIS - Leave blank.

Block 13. Abstract. Include a brief (*Maximum 200 words*) factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code (*NTIS only*).

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

JMH Where copyrighted material is quoted, permission has been obtained to use such material.

JMH Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

JMH Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

JMH In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

 For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

JMH In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

JMH In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

JMH In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

J. Michael Hoff 10.24.95
PI - Signature Date

TABLE OF CONTENTS

Introduction	Page 2
Results	Page 2
Conclusions	Page 4
References	Page 4

INTRODUCTION:

The TGF- β growth and differentiation factors have been implicated in the regulation of breast epithelial cell proliferation and in the invasive behavior of metastatic breast cancer (for example: Pierce et al., 1995). The goal of our research is to identify the molecules involved in the signal transduction cascades activated by TGF- β . The initial molecules in the cascade are the TGF- β receptors, transmembrane serine kinases (Kingsley , 1994; Massagué et al., 1994). During the past year several reports have indicated that defects in the type II TGF- β receptor are associated with different human tumors (for example: Markowitz, S. et al., 1995). We predict that other, currently unknown, molecules in the signaling cascades may be affected during tumor progression.

In order to apply a genetic approach to the dissection of this signaling pathway, we have proposed to use the TGF- β -related genes and related receptor genes in the fruit fly *Drosophila*. Because of the high degree of evolutionary conservation, we predict that the *Drosophila* genes identified in our screens will provide the necessary molecular probes for the identification of the homologous genes in the human genome. This will permit the analysis of these new genes during the process of breast cancer.

RESULTS:

The strategy proposed in our original application was to identify sensitized genetic backgrounds including mutations in the known genes of the TGF- β -related signaling pathway such that additional mutations in novel genes encoding molecules on the pathway will cause detectable phenotypes. Thus far, we have primarily used mutations in the *Drosophila* gene *thickveins*, which encodes a type I receptor essential for signaling by the TGF- β -related *Drosophila* ligand, *dpp*. We and others have also identified the *Drosophila* gene *schnurri* as encoding a molecule on the signaling pathway (Staehling-Hampton et al., 1995). *Drosophila schnurri* encodes a large (250kD) protein with seven zinc finger motifs related to the human transcription factors HIV-EP1 and HIV-EP2. Mutations in *schnurri* disrupt *dpp* signaling and we are now using *schnurri* mutations to sensitize the signaling pathway during our screens. During the first year of the project we have tested several different

genetic backgrounds and mutagenesis strategies which are described below.

Screen #1. An F1 screen for second-site non-complementing mutations to the adult viable mutant allele of the receptor gene *thickveins*.

Mutagen- ethyl nitrosourea

100,000 flies scored

11 new alleles of *thickveins* recovered

3 second-site noncomplementing mutations mapped to the gene *Hairless*.

Screen #2. An F1 screen for dominant enhancer or suppressor mutations of the wing venation caused by the hypomorphic *thickveins* mutant background of *tkv5/tkv6*.

Mutagen- ethyl nitrosourea or gamma rays

24,000 flies scored

No suppressor mutations recovered

10 flies with enhanced wing vein phenotypes recovered but all were sterile so induced mutations were not recovered

Screen #3. An F2 screen for dominant enhancer or suppressor mutations of the wing venation caused by the hypomorphic *thickveins* mutant background of *tkv5/tkv6*.

Mutagen- ethyl nitrosourea

3500 flies scored (second round of similar size currently in progress)

Eight candidate enhancer mutations: five cause lethality, three cause morphological defects.

Seven mutations segregate with chromosome 2; one mutation segregates with chromosome 3

Three of the mutations generate intriguing adult cuticle defects similar to phenotypes observed in flies with mutations in the TGF- β -related gene *dpp*: defects in distal leg segments and split thorax.

The new mutations complement *schnurri* alleles, however the addition of *schnurri* mutant alleles into the *tkv5/tkv6* genetic background produces similar defects in the legs and thorax.

Screen #4. An F1 screen for dominant suppressors of the wing venation phenotype caused by mutations in both the *thickveins* receptor and the putative transcription factor on the signaling pathway, *schnurri*.

Mutagen- ethyl nitrosourea

5000 flies scored (still in progress)

2 flies with putative suppressor mutations

CONCLUSIONS:

Although the first two mutagenesis screens were not successful, the candidates from the third and fourth strategy that are currently being analyzed are promising. These are being mapped by meiotic recombination and complementation tests with known deletions. Further phenotypic characterization of these mutations and localization of the lesions in the genome over the next 2-3 months should permit the initiation of molecular strategies for cloning these genes. This is consistent with the goals and time table initially proposed. We are also continuing to explore other combinations of mutant alleles on the signaling pathway in order to generate additional sensitized genetic backgrounds for further screens.

In summary, our progress thus far is on track with the Statement of Work (Appendix G, pg. 23) of our original proposal. We have made good progress in the first 12 months on Task #1 and are beginning Task #2 on schedule.

REFERENCES

- Kingsley, D.M. (1994) The TGF- β superfamily: new members, new receptors, and new genetic tests of function in different organisms. *Genes & Develop.* 8: 133-146.
- Markowitz, S. et al., (1995) Inactivation of the type II TGF- β receptor in colon cancer cells with microsatellite instability. *Science* 268: 1336-1338.
- Massagué, J. et al., (1994) The TGF- β family and its composite receptors. *Trends in Cell Biology* 4: 172-178.
- Pierce, D.F. et al., (1995) Mammary tumor suppression by transforming growth factor β 1 transgene expression. *PNAS* 92: 4254-4258.
- Staebling-Hampton, K., A. Laughon, and F. M. Hoffmann, (1995). A *Drosophila* protein related to the human zinc finger transcription factor PRDII/MBPI/HIV-EP1 is required for *dpp* signaling. *Development* 121: 3393-3403.