

AD \_\_\_\_\_

Award Number: DAMD17-99-1-9068

TITLE: Development of a Novel Ligand Binding Assay for Estrogen  
Receptor

PRINCIPAL INVESTIGATOR: Chi-Kong Arthur Chung, Ph.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine  
Houston, Texas 77030

REPORT DATE: April 2001

TYPE OF REPORT: Annual Summary

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.

20010620 217

|   |   |  |  |                               |
|---|---|--|--|-------------------------------|
| <b>REPORT DOCUMENTATION PAGE</b>  |   |  | <i>Form Approved</i><br><b>OMB No. 074-0188</b>                                  |                               |
| 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 this 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   |   |  |  |                               |
| <b>1. AGENCY USE ONLY (Leave blank)</b>   |   | <b>2. REPORT DATE</b><br>April 2001                            | <b>3. REPORT TYPE AND DATES COVERED</b><br>Annual Summary (1 Apr 00 - 31 Mar 01) |                               |
| <b>4. TITLE AND SUBTITLE</b><br>Development of a Novel Ligand Binding Assay for Estrogen Receptor   |   |  | <b>5. FUNDING NUMBERS</b><br>DAMD17-99-1-9068                                    |                               |
| <b>6. AUTHOR(S)</b><br>Chi-Kong Arthur Chung, Ph.D.   |   |  |  |                               |
| <b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b><br>Baylor College of Medicine<br>Houston, Texas 77030<br><br>E-Mail : <a href="mailto:cchung@bcm.tmc.edu">cchung@bcm.tmc.edu</a>  |   |  | <b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>                                  |                               |
| <b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b><br>U.S. Army Medical Research and Materiel Command<br>Fort Detrick, Maryland 21702-5012  |   |  | <b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>                          |                               |
| <b>11. SUPPLEMENTARY NOTES</b><br>This report contains colored photos   |   |  |  |                               |
| <b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b><br>Approved for Public Release; Distribution Unlimited  |   |  |  | <b>12b. DISTRIBUTION CODE</b> |
| <b>13. ABSTRACT (Maximum 200 Words)</b><br>Nuclear receptors undergo conformational changes when they bind their cognate ligands. It should be possible to monitor these changes in vivo using resonance energy transfer between fluorphores. The existence of inherently fluorescent proteins such as the variants of jellyfish green fluorescent protein (GFP) suggests that this problem may be approached by making fusions of these peoteins to nuclear receptors. We set out to study this problem using the estrogen receptor (ER), a nuclear receptor known to undergo a conformational change upon ligand binding. We have proposed to generate a novel intrinsic ligand binding assay for the estrogen receptor based on ligand dependent conformational changes detected by fluorescence resonance energy transfer (FRET) between complimentary fluorescent proteins. We are in the process of cloning double and single chimeras of the estrogen receptor and the various fluorescent proteins into mammalian CMV expression vectors. We have extended the number of chimeras that we are generating because of the advent of new fluorescent proteins now available from Clontech, which include cyan, yellow and red fluorescent protein vectors. These new fluorescent proteins are more optimal for FRET than the original blue and green variants. |   |  |  |                               |
| <b>14. SUBJECT TERMS</b><br>Breast Cancer   |   |  | <b>15. NUMBER OF PAGES</b><br>8  |                               |
|   |   |  | <b>16. PRICE CODE</b>  |                               |
| <b>17. SECURITY CLASSIFICATION OF REPORT</b><br>Unclassified  | <b>18. SECURITY CLASSIFICATION OF THIS PAGE</b><br>Unclassified | <b>19. SECURITY CLASSIFICATION OF ABSTRACT</b><br>Unclassified | <b>20. LIMITATION OF ABSTRACT</b><br>Unlimited                                   |                               |

## FOREWORD

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

N/A Where copyrighted material is quoted, permission has been obtained to use such material.

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

N/A 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.

X 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).

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

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

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

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

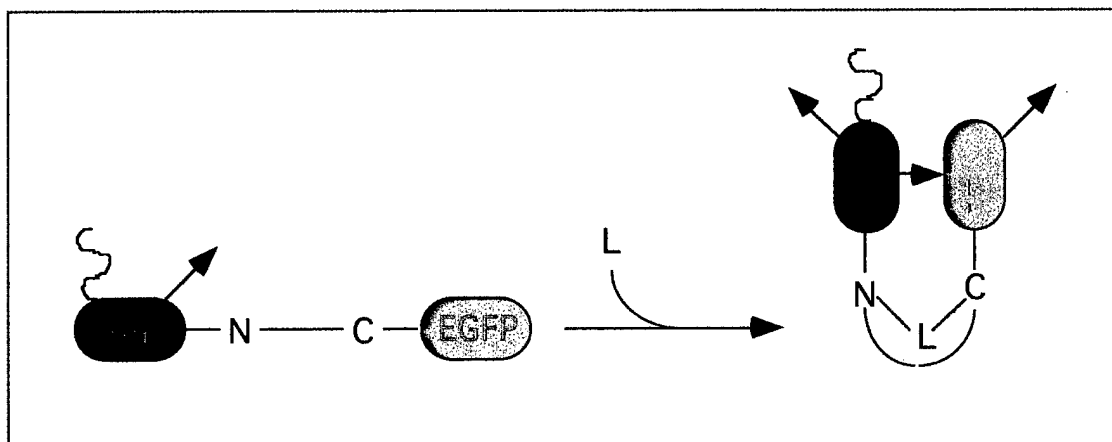
Arthur Chung 4/27/01  
PI - Signature Date

## Table of Contents

|                                   |     |
|-----------------------------------|-----|
| Cover.....                        | 1   |
| SF 298.....                       | 2   |
| Introduction.....                 | 5   |
| Body.....                         | 6   |
| Key Research Accomplishments..... | 8   |
| Reportable Outcomes.....          | 8   |
| Conclusions.....                  | N/A |
| References.....                   | N/A |
| Appendices.....                   | N/A |

## Introduction

Nuclear receptors undergo conformational changes when they bind ligands. It should be possible to monitor these changes *in vivo* using energy transfer between fluorophores. The existence of inherently fluorescent proteins such as the variants of jellyfish green fluorescent protein (GFP) suggests that this problem may be approached by making fusions of these proteins to nuclear receptors. We set out to study this problem using the estrogen receptor (ER), a nuclear receptor known to undergo a conformational change upon ligand binding. The proposed assay we have set out to develop is shown in Fig. 1



**Figure 1:** Ligand dependent steroid receptor assay based on FRET detection of conformational changes in the receptor upon hormone binding.

## **Summary of Progress 2001**

### **Training:**

I have gained much needed training in many areas of molecular biology including subcloning, protein expression, transfection of mammalian cell lines, and reporter assays. In addition, I am gaining biochemical training using hormone binding assays.

### **Technical Objective 1:**

#### **Task 1:**

I initially proposed to create estrogen receptor (ER) chimeras with blue fluorescent protein (BFP) and green fluorescent protein (GFP) to generate a novel ligand binding assay based on fluorescence resonance energy transfer (FRET) between the two fluorescent reporters (Figure 1). In addition, we proposed last year to generate single and double receptor chimeras with cyan and yellow fluorescent proteins as well as receptor chimeras with the new coral red fluorescent protein. We have generated all of these receptor single and double fluorescent chimeras with complimentary fluorescent proteins. We have functionally tested all of these receptor chimeras in hormone binding and transcription assays. All of the jellyfish fluorescent protein receptor chimeras bind hormone with an affinities equivalent to that of wild type receptor. In addition all of these chimeras were able to transactivate, in a ligand dependent manner, reporter gene expression in transient transfection assays in HeLa cells. However, the transactivation levels were lower than that observed with wild type receptors, suggesting that the fluorescent protein moities may be disrupting the normal interactions of these receptors somewhat. However, these receptor chimeras were functional in that they bound ligand and activated gene expression. In contrast the red fluorescent protein receptor chimeras were inactive, both in hormone binding and transactivation assays. When we visualized these chimeras within the cells we observed they formed large inactive cytoplasmic aggregates. The red fluorescent protein receptor chimeras have to be re-engineered to alter the linker region to see if that will restore the functionality of the chimeras.

We went on to test the functional fluorescent protein receptor chimeras in FRET assays. We were unable to detect either ligand-dependent or ligand-independent FRET in transfected cells using confocal fluorescent microscopy. The fluorescent protein moieties may be disrupting the normal dimerization of the N-terminal domain with the ligand binding domain; thus the fluorescent protein partners would be too far apart to engage in FRET.

**Task 2:**

To be initiated.

**Key Research Accomplishments:**

Generation of receptor fluorescent protein single and double chimeras.

**Reportable Outcomes:**

None