

AD _____

Award Number: DAMD17-99-1-9068

TITLE: Development of Novel Ligand Binding Assay for Estrogen Receptor

PRINCIPAL INVESTIGATOR: Arthur Chung, Ph.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine
Houston, Texas 77030

REPORT DATE: April 2000

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.

DTIC QUALITY INSPECTED 3

20010108 166

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

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 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

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE April 2000	3. REPORT TYPE AND DATES COVERED Annual Summary (1 Apr 99 - 31 Mar 00)	
4. TITLE AND SUBTITLE Development of Novel Ligand Binding Assay for Estrogen Receptor			5. FUNDING NUMBERS DAMD17-99-1-9068	
6. AUTHOR(S) Arthur Chung, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Baylor College of Medicine Houston, Texas 77030 E-MAIL: cchung@bcm.tmc.edu			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 This report contains colored photographs				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) Nuclear receptors undergo conformational changes when they bind their cognate ligands. It should be possible to monitor these changes <i>in vivo</i> using resonance 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. 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.				
14. SUBJECT TERMS Breast Cancer			15. NUMBER OF PAGES 9	
			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	

FOREWORD

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

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

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

___ 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).

N/A 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 5/11/00

PI - Signature Date

Table of contents

Section	Page number
Front cover	1
SF 298	2
Foreword	3
Table of contents	4
Introduction	5
Body	6-8
Appendix	9

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.

Summary of Progress

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.

Accomplishments:

Technical Objective 1:

Task 1:

Initially I 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. I am in the process of subcloning the GFP ER BFP chimeras into a CMV expression vector for our cell based studies. However, since this proposal was submitted several other fluorescent proteins became available that have greater efficacy for FRET than GFP and BFP. Other jellyfish fluorescent protein vectors became available from Clontech that are mutants of the original GFP and thus have different spectral properties. They include the yellow and cyan fluorescent proteins (YFP and CFP, respectively), which have better complimentary excitation and emission peaks than do BFP and GFP, allowing clearer distinction of FRET (Fig. 2A and B). In addition, this past year Clontech has commercialized some new coral fluorescent proteins, one of which emits in the red part of the spectra and is thus called red fluorescent. RFP will form a good FRET partner with either GFP and CFP, as their emission peaks overlap with the excitation peak of RFP (Fig. 2A and B)

Thus, I wanted to take advantage of these new fluorescent reagents, which will increase the likely of successfully establishing ligand dependent FRET. I have commenced generating a new set of ER chimeras. Double chimeras with a FP at the N-terminus and the complimentary FP at the C-terminus will be created for the cis

assay and single chimeras, which will allow mixing and matching, are being made to establish the assay in trans as well. The following chimeras are being generated:

G:ER:R	C:ER:R	C:ER:Y	
R:ER:G	R:ER:C	Y:ER:C	
G:ER	C:ER	R:ER	Y:ER
ER:G	ER:C	ER:R	ER:Y

Task 2:

To be initiated.

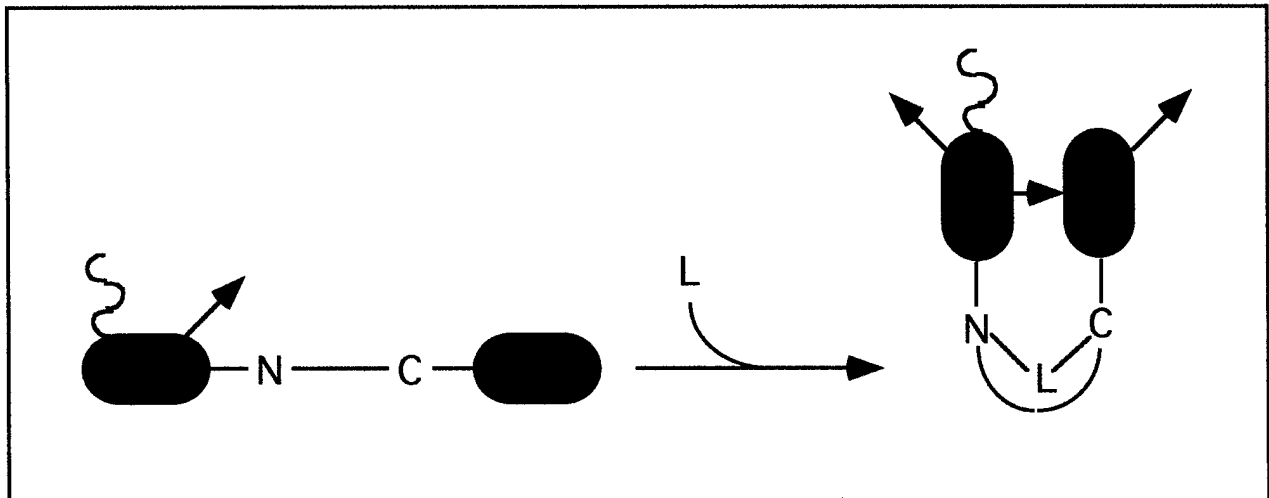


Figure 1: Ligand dependent steroid receptor assay based on FRET detection of conformational changes in the receptor upon hormone binding.

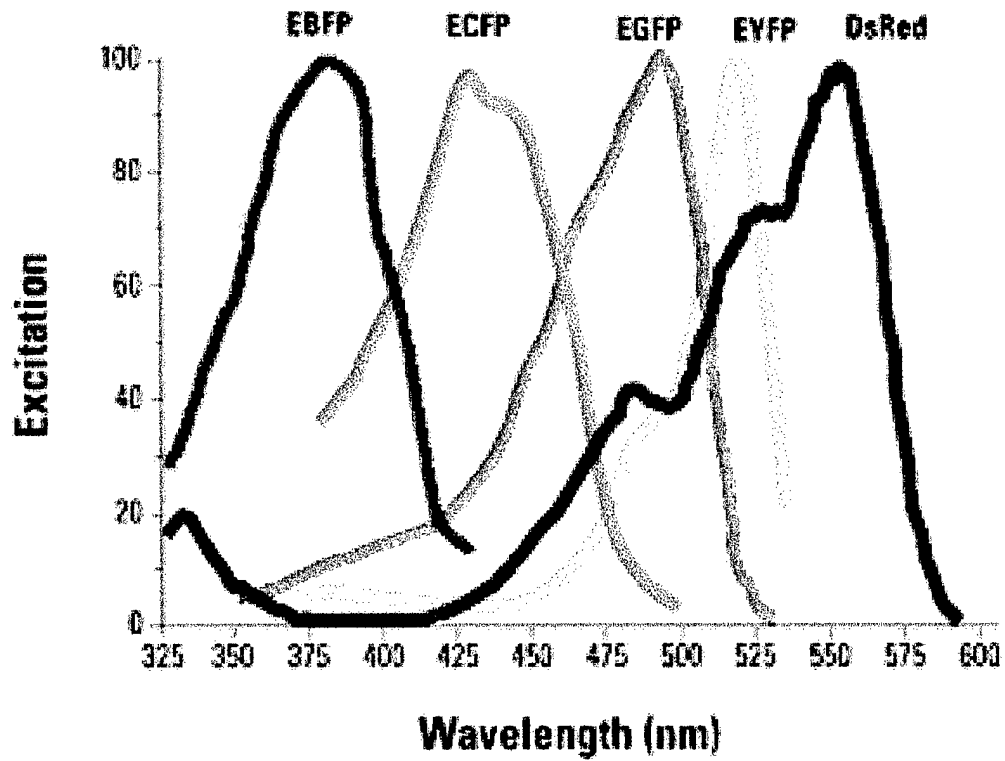
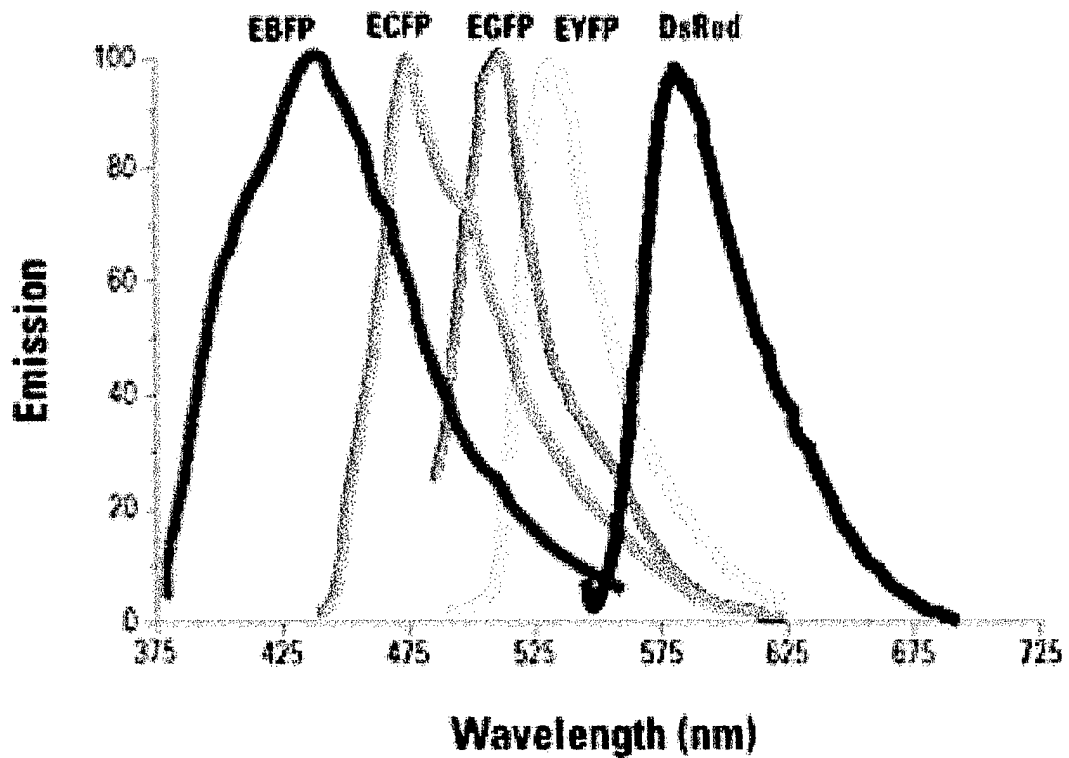
A**Excitation****B****Emission**

Figure 2: Excitation (A) and emission (B) spectra for the fluorescent proteins

Appendix

Key Research Accomplishments:

- Subcloning of ER fluorescent protein single and double chimeras.

Reportable Outcomes:

- **Research disclosure applied for.**