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FOREWORD

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Arthur Chung 4/27/01
PI - Signature Date

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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

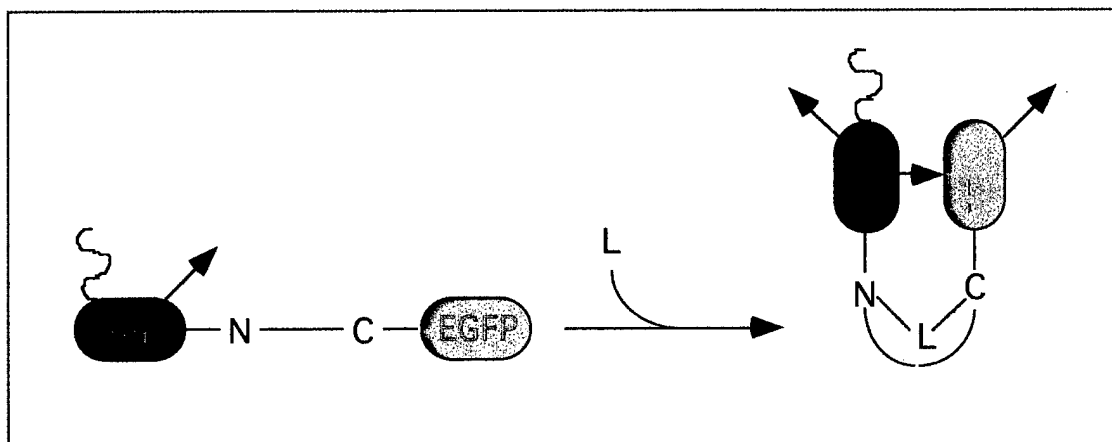


Figure 1: Ligand dependent steroid receptor assay based on FRET detection of conformational changes in the receptor upon hormone binding.

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