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Estrogens promote growth of certain breast endometrial cancers. Selective estrogen receptor modulators (SERMs), ligands that block the activity of estrogen in only selective tissues, allow to control the growth of these cancers while avoiding the many unwanted side effects associated with the use of antiestrogens. The goal of our work is to identify the mechanisms that control the activity of SERMs and to develop efficient high-throughput strategies for the identification of novel SERMs.
SERMs exert their activities by binding to the two estrogen receptors, Era and ERb. Ligand binding changes the position of a-helix 12 (H12) in the ER ligand-binding domain and regulates the interaction of ERs with cellular coactivators and corepressors. Previously, we identified that the F-domain, a receptor-specific domain that follows H12, contributes to coactivator binding and specificity of Era but not ERb. We have continued these studies by analyzing the role of the F-domain in SERM-and corepressor binding and in modulating the transcriptional response to hormone. Moreover, we have identified and optimized a strategy to introduce fluorescence labels into H12 or the F-domain. These fluorescent receptors will allow us to monitor the dynamics and structural reorganization of ER upon SERM binding.

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Introduction

Estrogens promote the growth of particular tissues, and are involved in the cause of breast and endometrial cancers. Antiestrogens block the activity of estrogens and play important roles in the treatment of these cancers. However, they have many unwanted side effects such as increased risk for osteoporosis and heart diseases. By blocking the activity of estrogens in some but not in all tissues, selective estrogen receptor modulators (SERMs) provide a powerful alternative to antiestrogens. The goal of our work is to identify the mechanisms that control the activity of SERMs and to develop efficient high-throughput strategies for their identification.

Like natural estrogens, SERMs exert their activities by binding to the two estrogen receptors, ERα and ERβ, which are ligand-regulated transcription factors. Upon ligand binding, the position of α–helix 12 (H12) in the ligand-binding domain (LBD) of ERα and ERβ changes in a ligand-specific manner and regulates the interaction of these receptors with cellular corepressors and coactivators. The current hypothesis is that the cell-specific activity of SERMs is caused by cellular differences in the repertoir of coactivators and corepressors that recognize particular, SERM-induced receptor conformations.

As part of our efforts to identify receptor-specific structural elements involved in the ligand-dependent repositioning of H12 and in the recruitment of corepressors and coactivators, previously we showed that the F-domain, a receptor-specific domain that follows H12, regulates the affinity of ERα but not of ERβ for different coactivator interaction motifs (NR-boxes). Thus, this domain is likely involved in determining the response of ERα to particular SERMs, presumably by modulating the ligand-induced repositioning of H12. We have continued these studies by analyzing the contribution of the F-domain of ERα and ERβ to SERMs and corepressor binding. Moreover, we have investigated the role of the F-domain for regulating the transcriptional activity of ERα.

The second goal of our work is to monitor the dynamics and structural reorganization of ERα and ERβ upon SERM binding or heterodimerization. In our previous report we identified a protein splicing approach as the most efficient strategy to introduce fluorescent labels into H12 or the F-domain. In this approach a fluorescent-labeled peptide is ligated to the C-terminus of the receptor LBD with the help of the yeast protein intein. While our first attempt to fuse the progesterone receptor (PR) LBD to a peptide that represents the F-domain has been quite successful , the repeat of this experiment with a fluorescent-labeled F-domain peptide was hampered by the low yield and solubility of this peptide. However, recently a variation of this approach has been pubblished that circumvents most of the difficultieswe encountered in our initial approach. This publication also demonstrates that it is feasibility to detect changes in the mobility of H12 with the help of fluorescence anisotropy. Our present focus is to refine the intein system to enable the fluorescent labeling of ERβ.

Research Accomplishments

Our revised "STATEMENT OF WORK" (08/10/01) contains three specific aims:

1. Monitor the location and dynamic of α-helix 12 of ER in the absence and presence of various estrogens and develop a high throughput screen for the identification of new potential SERMs
2. Analyze the role of the ER F-domain in the ligand-dependent relocation of α-helix 12
3. Probe for α-helix 12-dependent structural changes in ERα and ERβ homo- and heterodimers

Due to experimental problems and personnel changes (see below), we are still focusing entirely on aims 1 and 2. Since the results obtained in aim 2 are important for aim 1, we will begin with our accomplishments for aim 2.

Aim 2. Analyze the role of the ER F-domain in the ligand-dependent relocation of α-helix 12

In addition to very different N-terminal domains, ERα and ERβ are characterized by very diverse F-domains. These domains extend the C-terminus of the receptor LBD and play important roles in the ligand interpretation of steroid receptors. Mutations in the F-domain have

been identified that enable steroid receptors to activate transcription in the presence of antagonists [Montano et al., 1996; Nichols et al., 1998]. The recently solved structures of the progesterone and glucocorticoid receptors demonstrate that in the presence of agonists the F-domain is linked to the ligand binding domain via a β-strand [Williams and Sigler, 1998; Bledsoe et al., 2002]. This suggests that the F-domain might restrict the mobility of H12 and modulate the ligand-induced relocation of this helix. Moreover, since the the F-domain is located close to the coactivator and corepressor interaction sites, it is likely that the F-domain influences binding and selectivity of the ER LBD to these coregulators. Thus, the F-domain appears to be an important factor in the ligand-dependent activation of ERs. However, since the ER F-domain seems to impede crystallization, all available ER structures miss this important domain. The goal of the following experiments is to evaluate the role of the F-domain for the ability of ER to interact with ligands and coactivators and to investigate the influence of the F-domain on the ligand-dependent relocation of H12.

Aim 2a: Characterization of ERα/β±F domain

Hormone binding of ERα/β LBD±F domain - As outlined in our previous report, we have cloned and purified the LBDs of ERα, ERα-F, ERβ, and ERβ-F. Quantitative hormone binding studies revealed that in case of ERβ the absence of the F-domain does not change 17-ß estradiol (E2) binding, whereas removal of the ERα F-domain increases the affinity for E2 by a factor 2. We have continued these studies by measuring the binding of these proteins to 4 OH-tamoxifen (4OH-Tam). Similar to the results obtained for E2, the absence of the F-domain did not change the affinity of ERβfor 4OH-Tam. However, contrary to the binding of E2, the absence of the ERα F-domain resulted in a 5-fold decrease in the affinity for 4OH-Tam. This result demonstrates that the F-domain of ERα, but not that of ERβ, modulates ligand binding in a ligand-dependent manner.

Cofactor binding of ERα/β LBD±F domain - The transcriptional activity of ER depends on its interaction with coactivators and corepressors. Most coactivators, such as the p160 coactivator GRIP1, have multiple nuclear receptor interaction sites, called NR-boxes, which differ in their affinity for different receptors (Darimont et al., 1998). Previously we reported that ERα displays a higher affinity for the GRIP1 NR-boxes 1 and 2 than for NR-box 3, whereas ERβ does not discriminate between these NR-boxes. Deletion of the F-domain increased binding of ERα to all NR-boxes while abolishing the selectivity for particular NR-boxes. Removal of the ERβ F-domain had no obvious consequence for the binding of GRIP1. These results showed that the F-domain of ERα, but not that of ERβ, contributes to the affinity and selectivity of coactivators.

We have continued these studies by analyzing the ability of ERα±F to bind the corepressor NCoR in the presence of 4OH-Tam. Because tissues from mice that do not express NCoR are unable to inhibit the activity of ER in the presence of partial antagonists, the ability of a ligand to support NCoR binding appears to be directly linked to its ability to act as a SERM (Jepsen et al. 2000). Similar to coactivators, the interaction of NCoR with nuclear receptors also depends on conserved amphipathic α-helices that interact with the hydrophobic groove in the receptor LBD (Hu and Lazar, 1999; Nagy et al., 1999; Perissi et al., 1999). To study the interaction of ER±F with NCoR, we have cloned and expressed two NCoR fragments containing these amphipathic motifs (2365-2239; 2453-2057) as fusions with an N-terminal glutathione-S-transferase (GST)-tag and a C-terminal His_6-tag. After purification of these fusion proteins by Co^{2+}-affinity chromatography, we analyzed their binding to *in vitro* translated, S^{35}-labeled ERα and ERα-F in the presence of saturated levels of 4OH-Tam. Although binding of ERα to these NCoR fragments was weak, in the absence of the F-domain binding was consistently up to 3-fold higher than in the presence of the F-domain. Thus, the F-domain of ERα inhibits binding of both, coactivators as well as corepressors. We are in the process of completing these studies by analyzing binding of ERβ±F to these NCoR fragments.

Transcriptional activity of ERα/β ±F domain - To determine whether the observed changes in coactivator and corepressor binding lead to changes in the transcriptional activity of ERs, we utilized a reporter-based activity assay in transiently transfected CV1 cells to monitor the transcriptional activity of ERα and ERβ in the absence and presence of the F-domain. As

reported before, the presence or absence of the F-domain did not change the transcriptional activity of ERβ, whereas in the absence of the F-domain the efficacy of the transcriptional activity of ERα increased 2-fold (Fig. 1A). In the mean time we have demonstrated by Western blot analysis that the difference in the hormone responsiveness of ERα and ERα-F is not caused by differences in the expression levels of these proteins.

We have continued these studies by analyzing the transcriptional response of ERα±F to increasing concentrations of the coactivator GRIP1. To be able to understand the contributions of the individual NR-boxes of GRIP1, we have performed these experiments with GRIP1 derivatives that contain different combinations of NR-boxes. Surprisingly, contrary to the differential binding of the GRIP1 NR-boxes by ERα, the absence or presence of the F-domain did not affect the response of ERα to GRIP1 independently of the nature of the available NR-boxes (Fig. 1B, C). Thus, it is possible that the increased transcriptional activity of ERα in the absence of the F-domain is independent of the observed changes in p160 coactivator binding. To address this question we will investigate whether the transcriptional activity of ERα-F responds to the presence of an isolated GRIP1 nuclear receptor interaction domain (NID), which functions as a dominant negative inhibitor for the recruitment of p160 coactivators. Moreover, we are in the process of completing the analysis of F-domain dependent changes in the transcriptional response of ER by studying the functional interaction of ERβ with GRIP1, of ERα and β with NCoR, and by monitoring the cell-dependent response of ERα and β to 4OH-Tam.

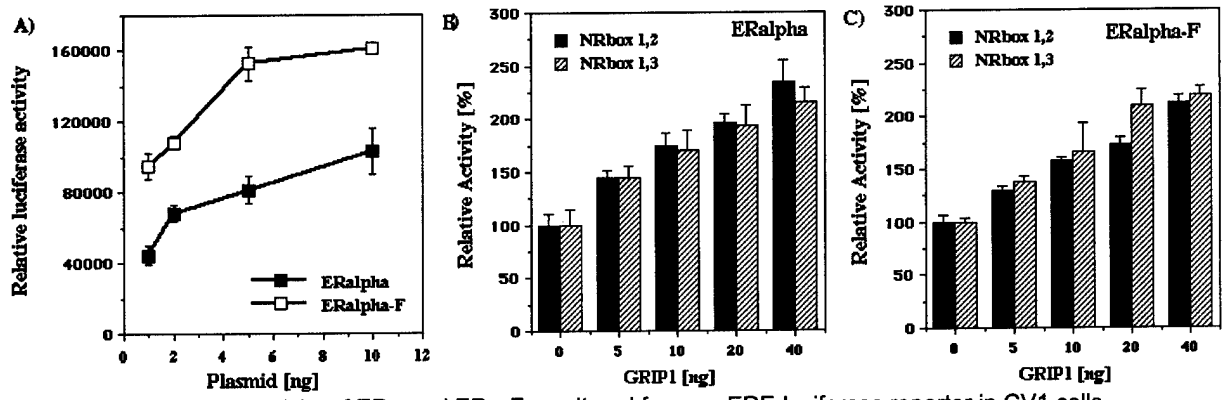

Fig. 1 A) Transcriptional activity of ERα and ERα-F monitored from an ERE-luciferase reporter in CV1 cells. B and C) Response of the transcriptional activity of ERα and ERα-F in the presence of increasing concentrations of GRIP1 NRbox1,2 and GRIP1 NRbox 1,3. All assays have been performed in the presence of saturating concentrations of 17ß estradiol (10 nM).

A note about the personnel situation - In Fall 2002 Dr. Christian Pullen, who initiated these studies, returned to Germany to accept a position in a pharmaceutical company. Since then these studies have been continued by Dr. Margarita Lib-Mygkov, a new postdoctoral fellow in our lab, who is just gaining expertise with these experimental techniques.

Aim 2b: Monitoring the ligand-dependent localization of the F-domain

A possible explanation for the differences in the roles of the ERα and ERβ F-domains might be that the F-domain of ERα is linked back to the ER LBD core and controls the ligand-dependent localization of H12, whereas the F-domain of ERβ is a flexible, solvent exposed extension of H12, which does not influence the movement of H12. Upon labeling the F-domains with a fluorophore, these structural differences could be monitored using time resolved fluorescence anisotropy.

Expression and purification of fluorescent-labeled PR LBD - As explained in the previous report, as a pilot study we have attempted the fluorescent labeling of the progesterone receptor (PR) F-domain using a protein splicing approach. In this system, the yeast protein intein is genetically fused to the PR LBD. Triggered by free sulphorhydryl groups intein catalyzes a transesteration reaction that can be used to link an *in vitro* synthesized and fluorescent-labeled F-domain peptide to the PR LBD. In preliminary studies we found that the PR LBD: intein protein expresses in *E. coli* largely as insoluble aggregates. Neither reducing

the incubation temperature during expression nor the addition of progesterone increased the solublity of the PR LBD: intein fusion protein.

Although most of the expressed PR LBD: intein fusion is insoluble, 0.1-0.5 mg/liter was soluble and bound to chitin beads (the intein domain is fused to a chitin binding domain). As mentioned in the previous report, incubation of 2 mg chitin-bound PR LBD-Intein with a 10-fold molar excess of F-domain peptide ((NH2-CGMVKPLLFHKK-COOH; synthesized by our Biotech core facility) resulted in 200 µg of more than 95% pure PR LBD-F-domain protein. This was a very encouraging result. To continue these studies, with the help of Molecular Probes (Eugene) we synthesized a fluorescein labeled version of this F-domain peptide. With respect to the structural considerations outlined in the previous progress report, to allow coupling of the fluorophore we exchanged alanine 922 by cysteine (C*). The resulting 12 amino acid long F-domain peptide (NH2-CGMVKC*LLFHKK-COOH) was purified by reverse phase chromatography. Unfortunately, the yield of the labeled peptide was more than 10-fold less than that of the unlabeled F-domain peptide; moreover the labeled peptide was much less soluble. These two unfortunate circumstances reduced the yield of the splicing reaction, and in several attempts we have been unable to obtain more than 50 µg of labeled PR out of 2 mg chitin-bound PR LBD: Intein fusion protein. To separate the unbound peptide from the labeled PR LBD, the chitin eluate needs to be fractionated by gelfiltration. This strategy requires that we concentrate the labeled protein. However, every concentration attempt failed because the protein bound unspecifically to the concentrators. Thus, in order to make this approach workable we need to find a way to increase the yield of coupling the fluorophore to the PR LBD.

A possible solution to our problem - A recently published study by Kallenberger et al. (2003) contains a possible solution to our problems. This study investigates the dynamic properties of H12 in the ER and PR-related peroxisome proliferator-activated receptor γ (PPARγ) by measuring the mobility of a fluorophore coupled to the C-terminus of H12 used fluorescence anisotropy. To ensure specific labeling of the C terminus, the authors are using a similar intein-dependent protein splicing system like the one we have been attempting to establish. However, most importantly, instead of coupling a fluorescent labeled peptide, Kallenberger et al. use cysteine-fluorescein to couple the fluorophore directly to the C terminus the PPARγ LBD. This approach circumvents the problems induced by the low yield and solubility of the fluorescent labeled peptide and we have begun to revise our experimental strategy accordingly. Moreover, this study demonstrates that it is feasible to detect ligand-dependent changes in the mobility of helix 12 using fluorescence anisotropy..

A note about the personnel situation - These studies have been performed by Josh Goodley, a graduate student, with help from our technician Lawrence Getubig. Unfortunately, after his comprehensive exam Josh decided to move to a less biophysical field. Thus, this project is presently continued by rotation students.

Aim 1. Monitor the location and dynamic of α-helix 12 of ER in the absence and presence of various estrogens and develop a high throughput screen for the identification of new potential SERMs

The biological activity of an ER ligand is determined by the cofactors that are recruited by the ligand-bound receptor. Since the recruitment of these cofactors by ER is regulated by ligand-induced structural changes in the receptor, monitoring these structural changes by fluorescence anisotropy, fluorescence resonance energy transfer or pyrene excimer fluorescence are powerful strategies to characterize the potential actions of ligands and to identify ligands with new activity profiles.

In aim 2 we demonstrated that ERα and ERβ have different biochemical features and mechanisms to regulate the recruitment of coactivators. Although it will be ultimately very interesting to compare the structural changes in ERα and ERβ upon binding to particular ligands, we decided to initially focus on ERβ, mainly because this receptor is more soluble. Moreover, since the F-domain of ERβ does not contribute to ligand- and coactivator-binding, the fluorescence studies can be performed with a C-terminally truncated form of the ERβ LBD for which structural information is available.

In agreement with the revised "STATEMENT OF WORK" (08/10/01) the majority of the experiments outlined in the aim 1a and 1b have been completed during the first year of this grant (see previous report). During the last year, we have mainly focused on aims 1c-e. Our present approach is to establish an ERβ: intein system to label H12 of the ERβ LBD according to the strategy described by Kallenberger et al. (2003).

Aim 1c-e: Introduction of fluorescence labels in the ER LBD

Selection of sites to be labeled - In the last three years structures of ERβ bound to ICI 164,384, Raloxifene, Genistein, (R,R)-5,11-cis-Diethyl-5,6,11,12-Tetrahydrochrysene-2,8-Diol (DTCD) and Triazine have been published (Pike et al., 2001; Pike et al, 1999; Shiau et al., 2002; Henke et al., 2002). Unfortunately, in the structure of ERβ bound to the antiestrogen ICI 164,384 no electron density has been identified for H12. In the remaining four structures the extension and position of H12 vary in a ligand-dependent manner (Fig. 2). Because our goal is to monitor the ligand-dependent movement of H12, the fluorophore has to be introduced as closly as possible to the C-terminal end of this helix. Based on the available structures we decided to cleave the C-terminus of ERβ at the following positions: alanine 497, leucine 500, and arginine 501. Because the previously characterized ERβ-F constructed extended to cysteine 503, we also constructed a ERβ version that terminates at cysteine 503 (Fig. 3). As part of aim 2 we already have shown that ERβ cleaved at position 503 binds 17ß estradiol, 4OH-tamoxifen, GRIP1 and NCoR with similar affinity than wild type ERβ.

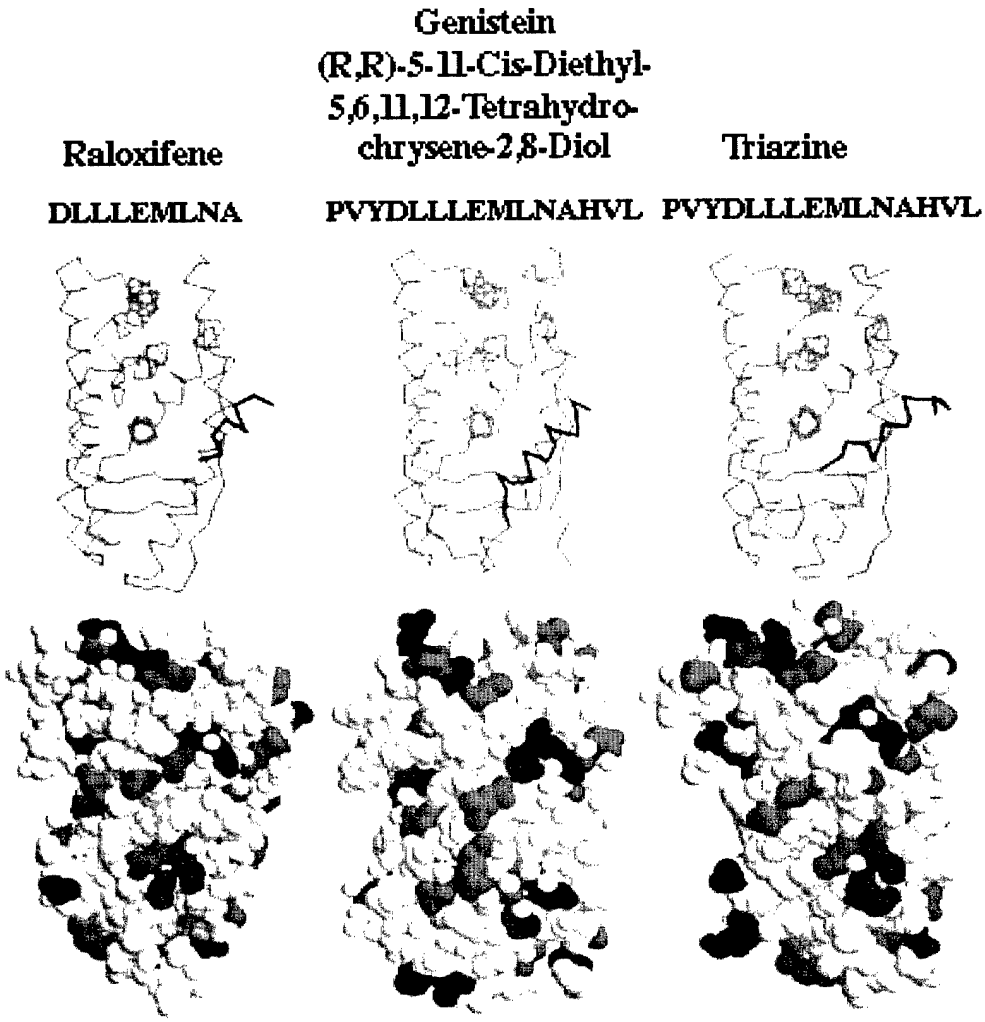

Fig. 2 Structures of ERβ LBD bound to raloxifene, genistein, DTCD and Triazine (Pike et al, 1999; Shiau et al., 2002; Henke et al., 2002). In the backbone presentations H12 is labeled black and for each of these structures the sequence of H12 is shown above the picture. The green residue in the space filled representation markes the C-terminus of the ERβ LBD. Solvent exposed positively charged residues are shown in blue, negatively charged residues are red.

Construction of ERβ LBD: intein fusion proteins - The ERβ LBD fragments 258-497, 258-500, 258-501 and 258-503 were amplified by PCR and, with the help of restriction sites encoded in the primers, cloned into the NdeI/ SmaI sites of the vector pTYB2. All clones were verified by sequence analysis. The junction sequences or labeled C-termini of the corresponding ERβ LBD proteins are shown in (Tab. 1).

Due to the construction of the pTYB2 expression vectors, the spliced cysteine residue in the protein: intein linkage is always preceded by a glycine residue. We are concerned that this design will increase the H12 independent rotation of the fluorophore, which could impair the detection of the H12 movement. To test the influence of this glycine residue on the dynamics of the fluorophore, we mutated the cloning sites of pTYB2 to allow the construction of the ERβ 258-503 (G) variants ERβ 258-501 (P) and ERβ 258-501 (Tab. 1). Expression and purification of these proteins are in progress.

ERβ construct	ERβ LBD: intein fusion	Fluorescent labeled ERβ LBD
ERβ 258-497(G)	ERβ - DLLLEMLNA**GC** - **Intein**	ERβ - DLLLEMLNA**GC***
ERβ 258-500 (G)	ERβ - DLLLEMLNAHVL**GC** - **Intein**	ERβ - DLLLEMLNAHVL**GC***
ERβ 258-503 (G)	ERβ - DLLLEMLNAHVLRGC - **Intein**	ERβ - DLLLEMLNAHVLRGC*
ERβ 258-501 (P)	ERβ - DLLLEMLNAHVLRPC - **Intein**	ERβ - DLLLEMLNAHVLR**PC***
ERβ 258-501	ERβ - DLLLEMLNAHVLRC - **Intein**	ERβ - DLLLEMLNAHVLR**C***

Tab. 1 ERβ: intein junction sequences and labeled ERβ C-termini of the ERβ variants 258-497, 258-500, 258-501 and 258-503. Heterologous sequences not present in ERβ are in bold. "*" symbolizes the fluorescence label.

Fluorophore selection and synthesis - We decided to use fluorescein as fluorescence label because (1) fluorescein has been already successfully used to monitor the mobility of H12 (Kallenberger et al., 2003), and (2) fluorescein is negatively charged. As shown in Fig. 2, different ligands change the location of H12 and expose the C-terminus of H12 environments that differ in their charge distribution. Thus, a charged fluorophore has the potential to be sensitive to these changes and might facilitate the detection of differences in the location or mobility of H12.

The cysteine-fluorescein used by Kallenberger et al. (2003) is being synthesized for us by Molecular Probes (Eugene). As an alternative strategy we linked fluorescein to 2-mercaptoethylamine that, like cysteine, also contains a free SH-group and is able to induce the cleavage reaction. We are in the process of purifying these compounds by reverse phase chromatography.

A note about the personnel situation - These studies have been conducted by B. Darimont and C. Beyer during Summer 2003 and will be continued by B. Darimont with help from our technician, L. Getubig.

Key Research Accomplishments

Aim 2. Analyze the role of the ER Fdomain in the ligand-dependent relocation of H12

ad Aim 2a:

- *Hormone binding of ERα and ERβ ± F-domains expressed in bacterial and mammalian expression systems:* Previously we showed that removal of the F-domain did not affect binding of 17ß-estradiol (E2) by ERβ and increased binding of E2 by ERα 2-fold. We now showed that removal of the F-domain also has no effect on the binding of 4OH-tamoxifen (4OH-Tam) by ERβ, but led to 5-fold decrease in the affinity of ERα for 4OH-Tam. These results demonstrate that the F-domain of ERα modulates ligand binding in a ligand-dependent manner, whereas the F-domain of ERβ does not contribute to ligand binding.
- *Corepressor-binding of ERα in the absence or presence of the F-domain:* In the absence of the F-domain binding of ERα to NCoR was up to 3-fold higher than in the presence of the F-domain. Thus, the F-domain of ERα inhibits binding of both, coactivators as well as corepressors..
- *Transcriptional activity of ERα and ERβ ± F-domains*:: Based on reporter assays using transiently transfected CV1 cells removal of the F-domain resulted in a 2-fold increase in the transcriptional activity of ERα. We have demonstrated by Western blot analysis that this activity difference is not caused by differences in the expression levels of ERα and ERα-F. Contrary to the differential binding of the GRIP1 NR-boxes by ERα, the absence or presence of the F-domain did not affect the response of ERα to GRIP1, independently of the nature of the available NR-boxes. Thus, it is possible that the increased transcriptional activity of ERα-F is independent of the observed changes in p160 coactivator binding.

These results demonstrate that the F-domains of ERα and ERβ differ in their contribution to ligand, coactivator and corepressor binding. Contrary to ERβ, the F-domain of ERα is important for coactivator selectivity and needs to be included in the structural studies proposed in aim 1.

ad Aim 2b:

- *Expression and purification of PR LBD: intein*: Neither reducing the incubation temperature nor the addition of progesterone increased the solublity of the PR LBD: intein fusion protein.
- *Synthesis, purification and coupling of a fluorescent labeled F-domain peptide to PR LBD*: With the help of Molecular Probes (Eugene), we synthesized and purified an12 amino acid long F-domain peptide (NH2-CGMVKC*LLFHKK-COOH) that contained fluorescin coupled to a cysteine residue (C*) that replaces the PR residue A922. Unfortunately, the yield of the labeled peptide was more than 10-fold less than that of the unlabeled F-domain peptide, which we have used before. Moreover the labeled peptide was much less soluble than the unlabeled F-domain peptide. These two unfortunate circumstances reduced the yield of the splicing reaction. In several attempts we have been unable to obtain more than 50 µg of labeled PR out of 2 mg chitin-bound PR LBD: Intein fusion protein and lost most of this protein during the following purification steps. Thus, in order to make this approach workable we need to find a way to increase the yield of coupling the fluorophore to the PR LBD.

In spite to the encountered difficulties, intein-catalyzed protein splicing appears to be the most promising experimental strategy to introduce a fluorophore into precise positions in the F-domain or H12. A recently published study by Kallenberger et al. (2003) describes a successful application of this strategy for studying the dynamic movement of H12 of PPARγ. Instead of a fluorescein labeled peptide used in our approach, in this study cysteine-fluorescein is connected to the C-terminus of the receptor. This strategy avoids the difficulties caused by the low yield and solubility of the fluorescent labeled peptide. We are in the process of refining our experimental approach accordingly.

Aim 1. Monitor the location and dynamic of the ER H12 in the absence and presence of various estrogens and develop a high throughput screen for the identification of new potential SERMs

Our results in aim 2 indicate that the structural analysis of ERβ is likely to be easier than that of ERα (higher solubility, no contribution of the F-domain to ligand- and cofactor-binding). Hence, our focus has been to label the ERβ LBD in the absence of the F-domain with the help of the intein-based fluorescence labeling system used by Kellenberger et al. (2003).

ad Aim 1c-e:

- *Selection of the cleavage sites* - With the help of available X-ray structures of ERβ bound to various ligands we selected four cleavage sites for the splicing reaction (A497, L500, R501, C503).
- *Construction of ERβ LBD: intein fusion proteins* - We completed the construction of the corresponding ERβ LBD: intein expression vectors. Moreover, using site directed mutagenesis we constructed variants of some of these plasmids. These plasmids will enable us to evaluate whether non-homologous residues introduced by the cloning of ERβ LBD: intein affect the mobility of H12.
- *Fluorophore selection and synthesis* - Based on structural considerations we decided to use fluorescein for the C-terminal labeling of the ERβ LBDs. The cysteine-fluorescein used by Kallenberger et al. (2003) is being synthesized for us by Molecular Probes (Eugene). As an alternative strategy we linked fluorescein to 2-mercaptoethylamine that, like cysteine, also contains a free SH-group to induce the cleavage reaction.

Reportable Outcomes

Abstracts/Presentation:

C. Pullen, J. Goodley, M. Lib-Myagkov, C. Beyer, B. Darimont
Novel strategies for the identification and characterization of selective estrogen receptor modulators
Annual Retreat of the Institute of Molecular Biology, University of Oregon, Silverfalls, Oregon, September 23/24, 2003

Employment:

In Fall 2002, Christian Pullen has accepted a scientist position at a pharmaceutical company in Germany.

Experience/Training:

Undergraduate research experience: Corinna Beyer
Research Associate research experience: Galina Kouzmitscheva, Lawrence Getubig
Postdoctoral training: Christian Pullen, Margarita Lib-Myagkov
Doctoral training: Joshua Goodley

Conclusions

While the characterization of ligand-bound ER LBDs by X-ray crystallography gave many interesting insights into the mechanisms of ligand binding and ligand interpretation, these structures do not provide a coherent explanation for the tissue- and receptor isotype-specific activity displayed by many ligands. There is increasing evidence that other receptor domains, such as the F-domain modulate these activities. Moreover, receptors bound to ligands such as SERMs, which display both agonistic and antagonistic activities, might differ from receptors bound to pure agonists or antagonists in terms of the dynamics rather than the nature of the ligand-induced structural changes. Our research provides one of the first attempts to monitor the dynamics of structural changes in the ER LBD upon ligand binding. These studies will not only give novel insights into the actions of SERMs but also provide a powerful strategy for the identification of novel SERMs that might improve the treatment of breast cancer.

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Appendices

Change in personnel:

Dr. Christian Pullen, who returned to Germany last fall, has been replaced by Dr. Margarita Lib-Myagkov (see enclosed CV).

Joshua Goodley has left this program in June 2003 and has not been replaced yet.

Biographical Information

ame, first name
Lib-Myagkov Margarita

ıship status: U.S. citizen _X_ Permanent alien resident ___ (attach notarized evidence as described in Instructions)

Education

Institution/location	Degree/month and year conferred	Field of study
Moscow Nursing School. of Academy of Science, Russia	1987-1990, A.A. (RN)	Nursing
Moscow Medical Academy, Russia	incompleted due to immigration to USA	Medicine
University of California , Los Angeles, CA, USA	1993-1997, B.S.	Immunology & Molecular Genetics
University of Oregon, Eugene, OR	1998-2002, Ph.D.	Molecular Biology
University of Oregon, Eugene, OR	06/2002-present, Postdoc. Fellow	Molecular Biology

Honors and Awards

Graduated Summa Cum Laude (Diploma "with Honor"),Moscow Nursing school of Academy of Science, Moscow, Russia
University of Oregon Graduate Teaching Fellowship
Invited speaker at the conference "Mitochondria 2001", San Diego, CA
Invited speaker at the conference 'Mitochondrial Pathology", Moscow, Russia

Training/Appointments

Nurse, Moscow Pediatric Institute, Oncology Unit, Moscow, Russia
Medical Student , Moscow Medical Academy (Department of Medicine), Moscow, Russia (incompleted due to immigration to USA)
Medical Surgical Technician, Cedars-Sinai Medical Center, Los Angeles, CA
Clinical Interpreter, Cedars-Sinai Medical Center, Los Angeles, CA
Laboratory Assistant, Department of Neuroscience ,UCLA, Los Angeles, CA
Teaching Assistant, Department of Biology, University of Oregon, Eugene, OR
Research Assistant (Graduate student- RA Capaldi), Department of Biology and Institute of Molecular Biology, University of Oregon, Eugene, OR
Postdoctoral Fellow (BD Darimont), Institute of Molecular Biology, University of Oregon, Eugene, OR

Publications

M.Y. Lib, M.F. Marusich, R.A. Capaldi (2001) Analysis of Assembly of Pyruvate Dehydrogenase Complex in Normal Human Fibroblasts and PDH Deficient Cell Lines Using Anti-PDH Monoclonal Antibodies. Abstracts. Mitochondria 2001 Meeting. Mitochondrion 1:87-116 (94)

Margarita Y. Lib, Ruth M. Brown, Garry K. Brown, Michael F. Marusich and Roderick A. Capaldi (2002) Detection Of Pyruvate Dehydrogenase E1alpha Subunit Deficiencies In Females By Immunohistochemical Demonstration Of Mosaicism In Cultured Fibroblasts. Journal of Histochemistry and Cytochemistry 50:877-885