Molecular details of the BRCA1 interaction with estrogen receptor alpha (ERα)

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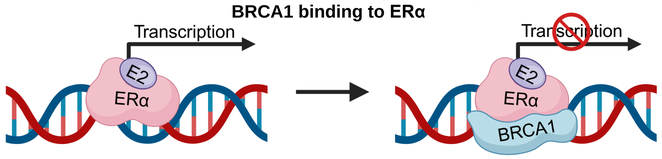
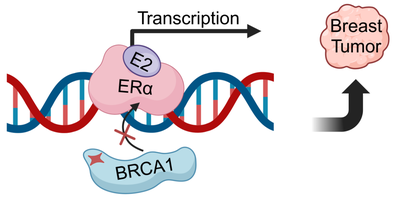
Introduction

Transcriptional activity is promoted by estrogen (E2) binding to ERα, and inhibited by BRCA1 binding to ERα

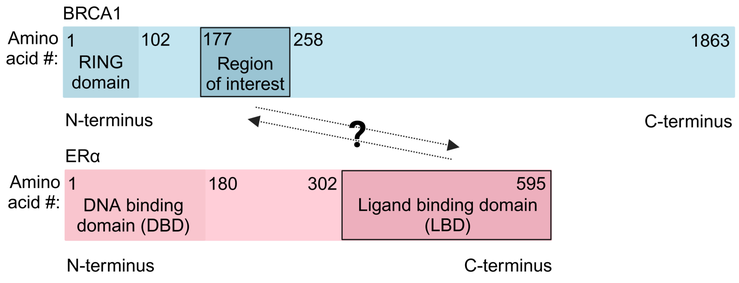


BRCA1 mutations in cancer cells are associated with increased ERα activity



Above: Estrogen receptor alpha (ERα) is a nuclear receptor that binds to DNA and induces transcription of estrogen-responsive genes. Breast cancer type 1 susceptibility protein (BRCA1) is a tumor suppressor protein found in all cells and is essential to many cellular functions such as DNA repair, initiation of apoptosis, and regulation of gene transcription. In the estrogen response pathway, BRCA1 regulates the transcription of estrogen-responsive genes turned on by ERα. If a mutation were to occur that interrupted the ability for BRCA1 to mediate genes transcription by ERα, a higher risk of breast, ovarian, and prostate cancer could result [1].

ERα ligand binding domain (LBD) and amino acids 177-258 of BRCA1 are theorized to physically interact

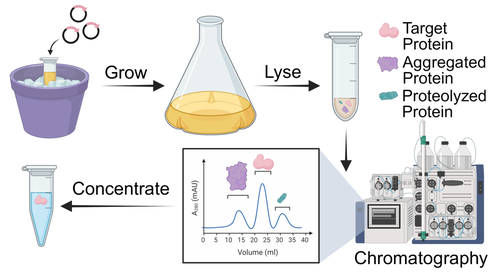


Above: BRCA1 is present in all cells, yet its inactivation is associated primarily with the tissue-specific malignant tumor of the breast. Due to the tissue-specificity of the ERα protein and its known association with the BRCA1 protein [2, 3, 4, 5, 6], it is postulated that the ERα-BRCA1 interaction is central to the reason mutations in BRCA1 are more closely associated with breast cancer than any other cancer. Specifically, the residues between 1 and 258 of BRCA1 are associated with the ubiquitination of ERα ligand binding domain (LBD), with the residues between 177 and 258 being important for the level of ubiquitination [3]. This study utilizes a shorter construct of BRCA1 in an effort to narrow down the region involved in the direct physical interaction with ERα LBD, indicated above by the darker blue and darker pink colors, respectively. Although amino acids 177-258 of BRCA1 have been linked to enhancing the ubiquitination of ERα LBD [3], no previous studies have investigated the binding interaction of these shortened protein constructs, nor have they looked at the effect of estrogen on this interaction.

Objectives

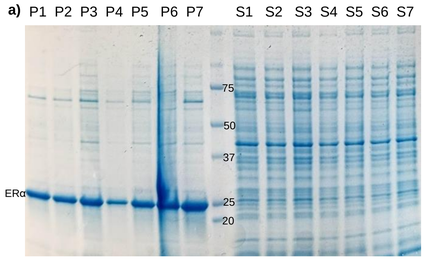
(1) Synthesize the ERα and BRCA1 protein constructs with minimal proteolysis and aggregation. **(2)** Use fluorescence emission spectroscopy to analyze ERα-estrogen binding. **(3)** Study the quenching of ERα ± estrogen by iodide with Stern-Volmer plots. **(4)** Use a fluorescence emission recovery assay and isothermal titration calorimetry (ITC) to verify the BRCA1-ERα interaction.

A conventional protein purification protocol was used



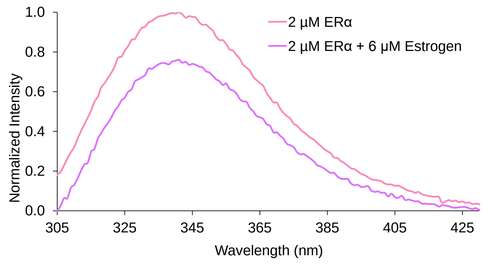
Recombinant protein expression is done in *E. coli* by transforming the desired plasmid, growing bacterial colonies in a nutrient-rich media, inducing protein expression, lysing the cells via sonication, centrifuging the solution to isolate the soluble protein-containing layer, separating proteins from cellular debris via metal affinity chromatography, isolating the protein of interest by size exclusion chromatography, and concentrating the protein.

Improved yield of soluble ERα with certain conditions



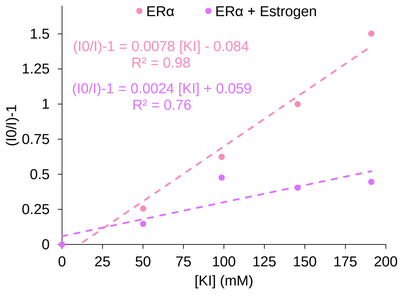
Small-Scale Expression and Solubility Assay of ERα LBD. a) Samples were taken of the pellet (P) and supernatant (S) of the centrifuged lysate for each condition and run on an SDS-PAGE. **b)** ImageJ software analysis was utilized to determine the fraction of soluble ERα. Condition 4 (changing the OD600 of the *E. coli* at cooling before induction from 0.4 to 0.2) and condition 6 (adding the reducing agent DTT into the cell lysis buffer prior to sonication) result in 53% and 37% of the protein in the lysate supernatant (soluble) instead of the lysate pellet (insoluble), respectively.

Estrogen (E2) decreases fluorescence intensity of ERα



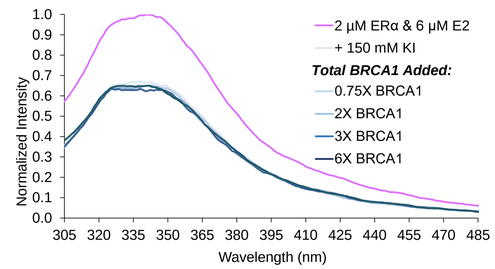
Fluorescence Emission Spectra of ERα LBD ± Estrogen. The average emission spectra is shown of eleven samples of 2 μM ERα with and without 6 μM estrogen. Tryptophan residues in the protein were excited with a wavelength of 294 nm. There is a 24% decrease in intensity upon addition of estrogen, indicating a change in protein conformation upon ERα-estrogen binding.

Unique ERα quenching dependent on E2 presence



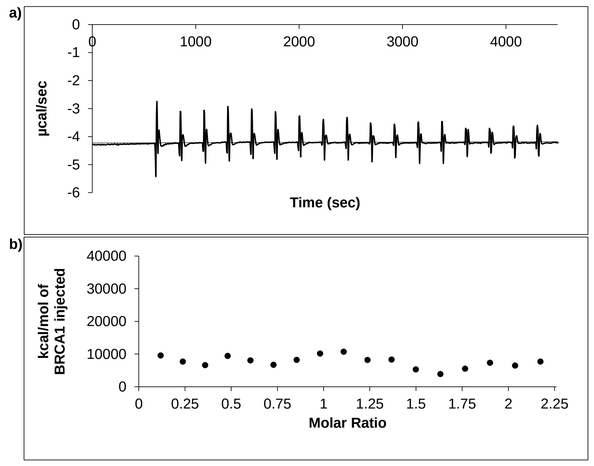
Stern-Volmer Plots for the Iodide Quenching of ERα ± Estrogen. The fluorescence emission spectra of estrogen-bound and unbound ERα were taken upon incremental addition of potassium iodide, and their peak intensities contrasted with the maximum unquenched intensity were plotted as a function of increasing concentration of the iodide quencher. This yielded a K_{SV} of 0.0078 mM^{-1} for ERα and 0.0024 mM^{-1} for ERα bound to estrogen, indicating that iodide is a more effective ERα tryptophan quencher in the absence of its ligand.

Fluorescence assay indicates no BRCA1-ERα binding



Fluorescence Emission Recovery Assay for Estrogen-Bound ERα. Following the quenching of ERα emission signal using iodide, no recovery of fluorescence intensity is visualized upon BRCA1 addition, indicating a lack of protein-protein interaction. If an interaction was present, BRCA1 would displace the iodide as it bound to ERα, resulting in an increase in emission upon addition of BRCA1.

No binding confirmed by isothermal titration calorimetry



Isothermal Titration Calorimetry (ITC) of BRCA1 into ERα-Estrogen. a) The raw data for sequential 2-μL injections of 300 μM BRCA1 into 25 μM ERα and 75 μM estrogen solution at 25°C over 1 hour. **b)** The plot of the heat evolved (kcal) per mole of BRCA1 added, against the molar ratio of BRCA1 to ERα. No binding curve was obtained by titrating BRCA1 into ERα in the conditions tested, demonstrating a lack of an interaction between the two proteins.

Conclusions

(1) Reducing the bacterial concentration at which cell cultures are cooled and adding the reducing agent DTT results in higher yields of soluble ERα. **(2)** Estrogen binding to ERα LBD decreases fluorescence emission intensity by 24%. **(3)** The estrogen-binding site of ERα is in close proximity to the tryptophan residues of the protein. **(4)** The BRCA1 construct containing residues 177-258 is unable to bind to the ligand binding domain of ERα in the presence of estrogen. In future studies, a longer BRCA1 construct should be utilized to study the BRCA1-ERα interaction.

References and Funding

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