

**Title of Student\_Conference\_Travel\_Award POSTER:**

Nuclear Export of the Thyroid Hormone Receptor

**Statement of research experience:**

Your statement of research experience should describe: the research projects in which you have participated, indicating the faculty advisor(s) on the project(s) and the length of your involvement; your role in the research you will be presenting at the conference; and a list of any other publications or previous presentations.

Since the spring semester of 2005, I have been investigating the nuclear export of the thyroid hormone receptor (TR). The aim of this project is to determine whether nuclear export of TR-a occurs by a calreticulin (CRT) -mediated pathway, as opposed to being mediated by CRM-1 as is v-ErbA, an oncogenic variant of TR. CRT has been proposed to play a role in steroid hormone receptor nuclear export, although its role remains controversial. These experiments contribute to the research being conducted in Dr. Allison's lab on the nucleocytoplasmic shuttling of TR.

My involvement in the project has spanned over three semesters and has included the synthesis of purified GFP-TR, GST-CRT, and His-CRM-1. I have conducted several in vitro digitonin permeabilization assays which suggest that TR is rapidly exported by GST-CRT from the nucleus but not by His-CRM-1. Also, I have conducted controls that indicate that GST-CRT remains localized in the nucleus in the absence of exportins. These findings were submitted in an abstract and accepted for a poster presentation at the American Society of Biochemistry and Molecular Biology Annual Conference in San Francisco from April 1-5, 2006.

**A copy of the submitted abstract.** (Please be sure to include all Authors; copy and paste from original application or re-type exactly as submitted)

(Authors' names) ....

**Thyroid Hormone Receptor Nuclear Export**

Most shuttling proteins exit the nucleus via a CRM-1 mediated process. A role has been proposed for calreticulin (CRT) in mediating steroid receptor nuclear export, although this is debated. We recently showed that the thyroid hormone receptor (TR), shuttles rapidly between the nucleus and cytoplasm. Nuclear export of TR is not blocked by leptomycin B, a specific inhibitor of CRM1, indicating that TR export is CRM1-independent. In contrast to TR, its oncogenic homolog v-ErbA follows a CRM1-mediated pathway. To determine whether nuclear export of TR occurs by a CRT-mediated pathway, in vitro digitonin permeabilization assays were used to assess export requirements. HeLa cells were transfected with a GFP-TR expression construct. Twenty-four hours after transfection, the cells were permeabilized with digitonin, and incubated in either recombinant GST-CRT or His-CRM-1 in export buffer. Cells were fixed at time points between 0 to 40 min and the distribution of TR was visualized by fluorescence microscopy. Our data show that GFP-TR remains localized in the permeabilized cell nucleus over time in the presence of rabbit reticulocyte lysate, export buffer alone, or CRM-1. However, in the presence of CRT, after 10 min GFP-TR no longer localizes in the nucleus and undergoes nuclear export. These findings suggest that TR follows a CRT-mediated export pathway. Understanding the mechanism for TR nuclear export contributes to understanding the normal cellular response to thyroid hormone, and also provides important insight into the mode of action of oncogenic variants of TR. This research was funded in part by NIH #DK058028-02 and by a HHMI grant through the Undergraduate Biological Sciences Education Program to William & Mary.