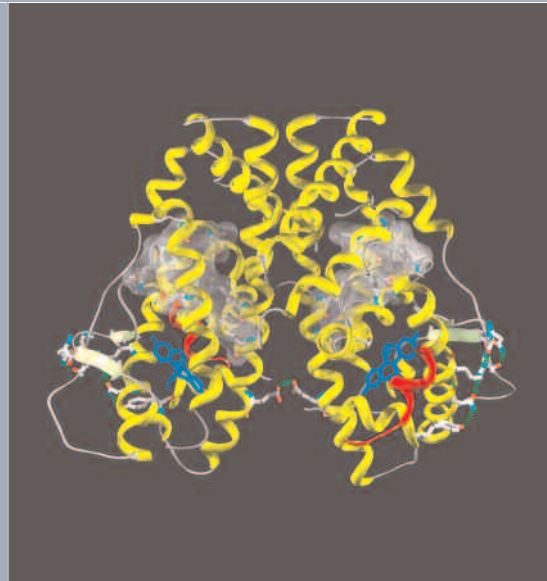


Cancer Biology

Activation of the estrogen receptor- α (ER- α). Ribbon diagram shows ER- α (yellow) bound to a peptide of the Grip1 coactivator (red) and to the ER- α agonist tetrahydrocrysene (blue). ER- α has well-known roles in the progression and treatment of breast and uterine cancer, whereas ER- β contributes to resistance to prostate and colon cancer. This structure defines features that are required for tetrahydrocrysene to act as an ER- α agonist, but as an antagonist of ER- β , and it reveals the mechanism of ligand-selective signaling. Work and image done in the laboratory of Kendall W. Nettles, Ph.D.



**DEPARTMENT OF
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Chairman's Overview

The Department of Cancer Biology was launched this year to recruit faculty to the Florida campus who have close ties with cancer researchers at the California campus. Broadly, the goals of our research programs are to understand the molecular pathogenesis of cancer. Our focus is on signaling pathways directed by oncogenes and tumor suppressors that control cell division, growth, survival, and differentiation, as well as those that modify the response to therapeutics. Members in our department use a battery of state-of-the-art technologies for target discovery and validation, and have developed preclinical models to evaluate the efficacy of new leads in cancer prevention and therapeutics. Our investigators are primarily interested in understanding the molecular underpinnings of all the major human cancers, but also have an interest in pediatric oncology, the interplay between cancer and the immune system, and the relationships between aging and cancer. One of the many strengths of Scripps Research is its access to high-throughput technologies, which enable investigators to explore potential leads very quickly using both genetic and small-molecule screens. Collaborations with the major cancer centers in the state of Florida, along with cancer researchers at the California campus, further enable us to convert leads into translational and clinical studies.

INVESTIGATORS' REPORTS

Role of Ubiquitin-Mediated Proteolysis in Irreversible Transitions During the Cell Cycle

N.G. Ayad, A. Smith, D. Harme, S. Simanski

We are interested in the cell biological basis of irreversible transitions that occur during the eukaryotic cell cycle. Ubiquitin-mediated proteolytic pathways ensure that irreversibility is achieved by targeting specific inhibitors of these transitions for proteasomal degradation. One of the most important ubiquitin E3 ligases is the anaphase-promoting complex (APC). The APC is active during G₁ and in fully differentiated cells. Furthermore, our recent studies indicate that the APC is required to initiate differentiation of neuronal precursors. This finding is especially important because it suggests that the APC is controlling an essential step in cell-cycle exit or differentiation, a control that is both biologically and medically relevant.

Although we understand a great deal about the role of the APC during the cell cycle, its role in initiating exit from the cycle is virtually unknown. We are uncovering the mechanism of APC activation and the proteins turned over via the APC during differentiation. For these studies, we are using both PC12 cells and the primary cerebellar granule cell system to probe the role of the APC during differentiation. In collaboration with J. Hogenesch, Scripps Florida, we are using cell-based screening technologies to identify novel activators and inhibitors of the APC. For this research, we have developed a high-throughput luciferase-based measure of APC activity. In this method the N terminus of the APC substrate cyclin B is fused with luciferase, so an increase in luciferase corresponds to increased levels of cyclin B, a finding that indicates lower levels of APC activity. We are continuing our screening of 15,000 human cDNAs to identify novel APC regulators. Identification of these regulators most likely will illuminate both temporal and spatial control of APC activity during development.

In addition to identifying novel regulators and substrates of the APC, we have concentrated on one of the known APC substrates, the cytosolic protein trigger of mitotic entry 1. This protein is required for degradation of the mitosis-inhibitory kinase *wee1* and entry

into mitosis in both frogs and humans. We are determining the intracellular location of *wee1* degradation in humans and the role of phosphorylation on *wee1* degradation. In collaboration with J. Busby, Scripps Florida, we are using mass spectrometry to identify *wee1* phosphorylation sites. This multidisciplinary approach will provide greater insight into proteolysis, the cell cycle, and neurogenesis and may be useful in cancer research and nerve regeneration studies.

PUBLICATIONS

Ayad, N.G., Rankin, S., Ooi, D., Rape, M., Kirschner, M.W. Identification of ubiquitin ligase substrates by in vitro expression cloning. *Methods Enzymol.* 399:404, 2005.

Molecular Mechanisms of cAMP-Mediated Transcription

M.D. Conkright, B.A. Mercer, A.L. Amelio, M.A. Morris

A variety of biological functions depend on the cAMP signaling cascade, including long-term memory, survival of beta cells, glucose metabolism, cardiomyopathy, and proliferation of chondrocytes. We study the molecular mechanisms involved in the conversion of these signals into transcriptional events. Increases in cellular levels of cAMP stimulate the expression of numerous genes by liberating the catalytic subunits of protein kinase A, which phosphorylates the transcription factor cAMP-responsive element binding protein (CREB). Phosphorylation of CREB promotes the recruitment of the coactivators CREB-binding protein/p300 and the initiation of transcription.

The diversity of biological functions associated with CREB and the cAMP pathway will be impossible to fully understand until all of the components involved in the pathway have been identified and characterized. Currently, we are using high-throughput cell-based screening technologies, including cDNA expression libraries, small interfering RNA libraries, and small-molecule libraries, to identify all of the proteins that make up and regulate the cAMP pathway. Using this technology, we identified proteins called transducers of regulated CREB activity, a novel family of CREB coactivators. Ascertaining the factors involved in the cAMP signaling pathway will be paramount in delineating why the biological function of CREB differs so drastically between tissues.

Suppression of NF- κ B by the Estrogen Receptor

K.W. Nettles, J. Bruning, J. Janjic

Our overall goal is to improve the treatment of inflammatory diseases through a novel pathway by which the estrogen receptor reduces the action of a key transcription factor, NF- κ B. Suppression of NF- κ B by the estrogen receptor is critical for maintaining the protective effects of estrogens in inflammatory bowel disease, sepsis, arthritis, atherosclerosis, and lipid metabolism, highlighting the broad importance of this pathway.

Several mechanisms have been proposed to explain cross talk between the estrogen receptor and NF- κ B, including inhibition of DNA binding by NF- κ B, competition for limiting transcriptional coactivators, and the formation of a direct inhibitory complex. Our preliminary data indicate that the estrogen receptor is recruited to the NF- κ B response element of the gene for monocyte chemoattractant protein 1 in the cell line MCF7, which is positive for the estrogen receptor, and that the coactivator CREB-binding protein is a bridging factor between the estrogen receptor and NF- κ B.

We have also developed a technique that greatly speeds our ability to obtain x-ray crystal structures of the estrogen receptor, allowing us to obtain structural information on entire classes of receptor-ligand complexes in a short time. We are using this approach to define the chemical and structural features that determine NF- κ B-selective signaling through the estrogen receptor.

PUBLICATIONS

Nettles, K.W., Greene, G.L. Ligand control of coregulator recruitment to nuclear receptors. *Annu. Rev. Physiol.* 67:309, 2005.