

**Regulation of FSH Target Gene Expression in Granulosa Cells.** Mary Hunzicker-Dunn, Department of Cell and Molecular Biology, Northwestern University Feinberg School of Medicine, Chicago IL 60611.

Follicle-stimulating hormone (FSH) stimulates the growth and differentiation of ovarian follicles. We have investigated the signaling pathways by which FSH directs activation of a select group of target genes in rat granulosa cells (GCs) cultured under serum-free conditions. Under these conditions, rat GCs differentiate in response to FSH but do not proliferate unless activin is also present. Activation of signaling pathways is monitored by western blotting of total GC extracts using phospho-specific antibodies. Induction of target gene expression is monitored either by promoter-reporter assays following transient transfections or by mRNA or protein analysis of specific target genes. The requirement for specific protein kinases is evaluated using specific pharmacological inhibitors. The requirement for the function of specific transcription factors is evaluated by infecting GCs with adenoviral constructs of constitutively active or dominant negative mutants versus infection with empty adenoviral vectors. Our results show that FSH plus activin signal to induce expression of cyclin D2 and GC proliferation, as well as to promote GC differentiation, via cAMP, phosphatidylinositol-3 kinase (PI3-K), Akt, and Smad2/3 in part by directing the phosphorylation and consequent inactivation of FOXO1, a Forkhead bOX-containing transcription factor in the O subfamily. A second consequence of PI-3K/Akt activation in FSH-treated GCs is increased translation and activation the transcription factor Hypoxia-inducible factor (Hif)-1 $\alpha$  downstream of the protein kinase mammalian target of rapamycin, leading to transcriptional activation of Hif-1 targets such as vascular endothelial growth factor (VEGF). The collaboration of various these signaling pathways to regulate FSH target gene expression will be discussed. We conclude that a complex combination of signaling pathways and regulated transcription factors is required for FSH to induce GC differentiation and proliferation. Supported by P01 HD 21921.