PKA and GAB2 play central roles in the FSH signaling pathway to PI3K and AKT in ovarian granulosa cells

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**Maturation of cells within the ovarian follicle of mammals is necessary for fertility. Follicles are restrained at an immature stage until stimulated by FSH from pituitary gonadotropes. FSH acts through its G protein-coupled receptor located on granulosa cells (GCs) within the immature follicle to inhibit programmed cell death (apoptosis), promote GC proliferation, produce steroid and protein hormones and growth factors, induce ligand receptors and intracellular signaling intermediates, and induce formation of a fluid-filled follicular antrum. This program of maturation results in a preovulatory-stage follicle competent to receive the surge of luteinizing hormone that promotes ovulation and terminal differentiation of follicle cells.**

**FSH-dependent activation of the PI3K/protein kinase B (AKT) signaling pathway in immature GCs is necessary for not only induction of transcriptional regulators, such as hypoxia inducible factor-1α, but also, activation of key downstream FSH target genes, such as those genes encoding the luteinizing hormone/choriogonadotropin receptor, hydroxy-Δ-5-steroid dehydrogenase, β3- and steroid Δ-isomerase cluster, aromatase, and inhibin-α (1–4). However, the mechanism by which FSH activates PI3K/AKT signaling in GCs is not known. We used a serum-free, primary rat ovarian GC model to investigate how FSH signals to activate PI3K/AKT. Our results show that cAMP-dependent protein kinase (PKA) mediates the actions of FSH to promote the phosphorylation of insulin receptor substrate-1 (IRS-1) on a canonical binding site for the regulatory subunit of the PI3K heterodimer.**

**As described in Fig. P1, our results show that PKA mediates the actions of FSH and initiates signals to multiple targets to activate PI3K/AKT. This conclusion is based on the ability of a PIK inhibitor that functions as a pseudosubstrate for the catalytic subunit of PKA to abrogate FSH-stimulated phosphorylation of AKT on both its proximal Thr308 and the distal Ser473 residues that are required for full activation of AKT. Activation of PI3K/AKT through PKA in GCs contrasts with the more common G protein-coupled receptor pathway in other cell types, where Gβγ-subunits bind to and directly activate the p110 catalytic subunit of PI3K (5). We showed that PKA in GCs indirectly promotes the phosphorylation of IRS-1 on Tyr308, a canonical binding site for the 85-kDa regulatory subunit of the** PI3K heterodimer. FSH-stimulated IRS-1 phosphorylation in GCs was confirmed by Western blotting both with an anti–p-IRS-1(Tyr308) antibody and a pan anti-pTyr antibody after IRS-1 immunoprecipitation from GC extracts. Our results indicate that the adaptor growth factor receptor bound protein 2-associated binding protein 2 (GAB2) is central to the regulation of PI3K/AKT by PKA. GAB2 forms a complex with IRS-1, the PI3K regulatory and catalytic subunit heterodimer, and the type I PKA holoenzyme. In the absence of FSH, GAB2 is phosphorylated on Tyr452, a canonical binding site for the 85-kDa regulatory subunit of PI3K. We hypothesize that Tyr452 on GAB2 serves as a docking site for PI3K in the absence of FSH. FSH through PKA stimulates both dephosphorylation of GAB2(Tyr452) and phosphorylation of GAB2 on Ser159; moreover, GAB2 is a direct PKA target. GAB2 also functions as an A-kinase anchoring protein, directly binding the type I regulatory subunit of PKA. Peptides that competitively disrupt the anchoring of PKA regulatory subunits to A-kinase anchoring proteins significantly reduce FSH-stimulated phosphorylation of AKT on Ser473. GAB2, thus, seems to coordinate signals from the FSH-stimulated rise in cAMP that lead to the activation of PI3K/AKT. Consistent with this hypothesis, overexpression of GAB2 enhances FSH-stimulated AKT phosphorylation.

**The ability of PKA to commandeer both IRS-1 and GAB2, two adaptors that normally integrate receptor and nonreceptor tyrosine kinase signaling into the PI3K/AKT pathway, reveals a route for PKA to regulate the maturation of follicles.** The

**Fig. P1. Model of the signaling pathway by which FSH promotes AKT activation in GCs. FSH binding to and activation of the FSH G protein-coupled receptor leads to activation of the stimulatory G protein (Gs), activation of adenyl cyclase (AC), generation of cAMP, and activation of type I PKA (R1,C). PKA directly (unbroken line) or indirectly (broken line) promotes dephosphorylation (slashed P) of GAB2(Y452) and phosphorylation (P) of GAB2(Ser159) and IRS-1(Tyr308). Phosphorylation of IRS-1 on Tyr308 provides the binding site for the p85 βγ-subunit of PI3K to allosterically activate the p110 catalytic subunit, resulting in phosphorylation-dependent activation of AKT(Thr308)(Ser473) that promotes GC proliferation and differentiation.**
mechanism by which FSH, through its G protein-coupled receptor, signals in GCs to activate PI3K/AKT is important to the field of ovarian follicular maturation and thus, fertility. Failure of FSH to activate PI3K/AKT in immature GCs would lead to infertility. We believe that it is reasonable to conclude that this pathway may also contribute to tumorigenesis in nonovarian cells, particularly those cells that ectopically express G protein-coupled receptors and signal through PKA. Because PKA signaling replicates conditions that promote increased cell proliferation, inhibit apoptosis, and increase hypoxia inducible factor-1 activity and ultimately, angiogenesis, extracellular ligands, such as FSH or luteinizing hormone, that activate PKA should be considered potentially tumorigenic.