Systems Investigation Reveals an Unexpected LH-mediated Signal Transducer of Steroidogenesis in Leydig Cells

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Trophic hormone regulation of gonadal and adrenal steroidogenesis elicits acute responses, initiating the production and release of steroid hormones within minutes. The discovery of the steroidogenic acute regulatory protein (STAR) as an essential mediator of this process has focused subsequent studies on signaling and transcriptional responses around the established understanding of STAR. However, recent findings from our laboratory demonstrate that STAR is not a protein kinase A (PKA)-regulated phosphoprotein, and that it functions alone within the mitochondrial intermembrane space as a cholesterol shuttle, challenging the basis of several key interpretations made in this field. Consequently, we decided to reconstruct *de novo,* the cellular impacts of luteinizing hormone (LH) exposure using precision nuclear run-on sequencing (PRO-seq) to uncover the identity of transcriptional regulators and predict upstream signaling mediators that form the basis of a steroidogenic response.

We performed PRO-seq using MA-10^{Slip5} cells at baseline and after a 15-minute LH exposure, a timepoint that results in a 2.3-fold increase in STAR mRNA and a 6.9-fold increase in progesterone production. From this dataset, we generated a high-resolution map capturing all active transcriptional regulatory sites (enhancers and promoters) associated with steroidogenesis across the mouse genome by using the dREG and tfTarget pipelines. This analysis highlighted differences in expressed transcriptional regulatory elements (dTREs), identifying 508 dTREs responsible for LH-mediated effects, corresponding to 336 protein coding genes. We employed a Hidden Markov model within RSAT for de novo motif discovery, identifying putative transcription factors associated with these dTREs. The analysis revealed three motif families with a normalized correlation greater than 0.85, responsive to LH: the ETS-domain family (including Erf, Elk, and Gapba), the NF-Y family (Nyfa and Nfyc), and the SP/KLF family (Sp3, Sp6, Sp8, Sp9, Klf2, Klf6, Klf13, Klf14). Intriguingly, despite the frequent implication of CREB in prior signaling studies, it was not identified in our current analysis.

By analyzing the target genes identified by dTREs using Ingenuity Pathway Analysis®, we unraveled the primary mechanisms activated by LH. These include activation of ER stress, Rho signaling, MAPK signaling, mTOR signaling, and EGF signaling. Additionally, our findings highlight pathways that support mitochondrial protein import, COPI-mediated anterograde transport, endocytosis, cholesterol biosynthesis, and transcriptional activation of gene expression by Srebp (sterol regulatory element-binding protein). Contrary to the current understanding, we found that cAMP-mediated signaling was downregulated following LH exposure.

Given its proximity to LH receptor responses at the plasma membrane interface, we investigated RhoA as a potential signal transducer in LH-induced steroidogenesis. We utilized an inducible, constitutively active form of RhoA (RhoA.Q63L) in MA-10^{Slip5} cells and observed a full steroidogenic response without LH stimulation, confirming RhoA as a central mediator of LH signaling. Induction of RhoA.Q63L resulted in progesterone production levels that were 4-fold higher than those induced by LH alone. This finding challenges the prevailing model of LH signal transduction, which is thought to occur via LH-GPCR activation of adenyl cyclase and subsequent protein kinase A (PKA) activity, and suggests that a reevaluation of this model is warranted.

In summary, this study provides a detailed analysis of LH signaling impact in Leydig cells. The original mechanisms of transcriptional regulation and signaling uncovered by our research, while unexpected, are unprecedented and critically important for advancing our understanding of steroidogenesis.