Intercellular communication between the luteinizing hormone receptor and the NPR2 guanylyl cyclase in mouse ovarian follicles

Valentina Baena, Corie M. Owen, Siu-Pok Yee, Giulia Vigone, Jeremy R. Egbert, and Laurinda A. Jaffe
Department of Cell Biology, University of Connecticut Health Center, Farmington, CT 06030 USA

UCONN HEALTH

Background

In mammalian preovulatory follicles, meiotic arrest is maintained by cyclic GMP (cGMP) that diffuses into the oocyte through gap junctions (1). The cGMP is generated by the guanylyl cyclase natriuretic peptide receptor 2 (NPR2). Meiotic resumption is triggered when luteinizing hormone (LH) activates a receptor (LHR) to initiate a pathway that dephosphorylates and inactivates NPR2.

Localization of these signaling molecules has been investigated by binding of antisense mRNA probes and of radioactively labelled ligands (2-6). However, these methods have low resolution, and due to the low expression of the LHR and NPR2, antibodies suitable for immunolocalization are not available. To overcome this problem, we used CRISPR/Cas9 genome editing to generate mice with a 9-amino acid HA tag attached to the N-termini of the LHR and NPR2. Using an HA-tag antibody, we investigated the localization of these proteins in preovulatory follicles, with higher resolution than possible with previous methods.

Validation of Genetic Modifications

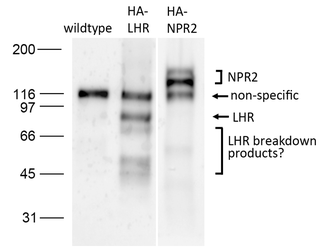
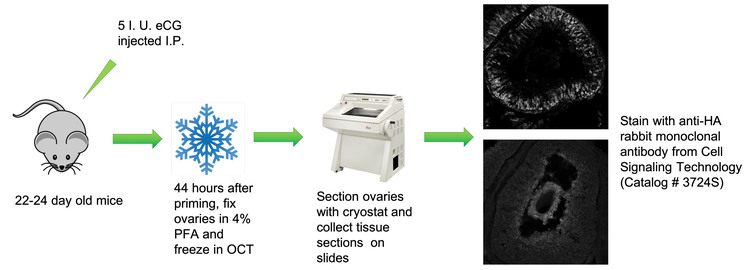


Figure 1. Western blots show that follicles from mice modified to express HA-tagged LHR or NPR2 contain proteins that are detected by an HA tag antibody, at the expected molecular weights for HA-LHR and HA-NPR2 (75 kDa and 120-130 kDa, respectively). A non-specific band at 116 kDa is present in all samples including wildtype.

Methods for Immunofluorescence



Localization of HA-LHR

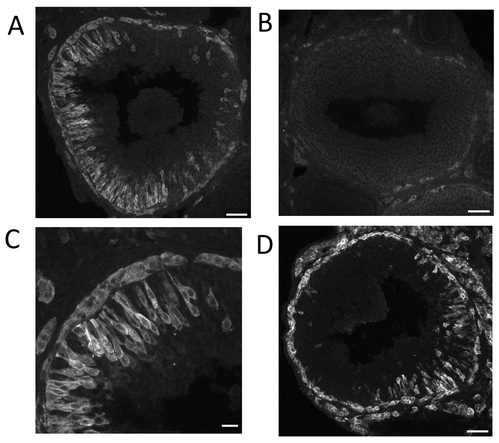


Figure 2. A, B. Expression of HA-LHR seen in 24 day old HA-LHR (A) or wildtype (B) ovaries (scale bar = 50 µm). HA-LHR is localized in the outer layers of mural granulosa cells and is expressed heterogeneously in these cells. Some staining is also seen in the theca. **C.** HA-LHR is in flask shaped granulosa cells that extend long projections to the basal lamina from cell bodies several layers deep (scale bar = 25 µm). **D**. Expression of HA-LHR in naturally cycling adult mice is similar to prepubertal females. Female mice were induced into estrus using the Whitten effect and ovaries were collected ~28 hours after exposure (scale bar = 50 µm).

Localization of HA-LHR by immunogold labelling and electron microscopy

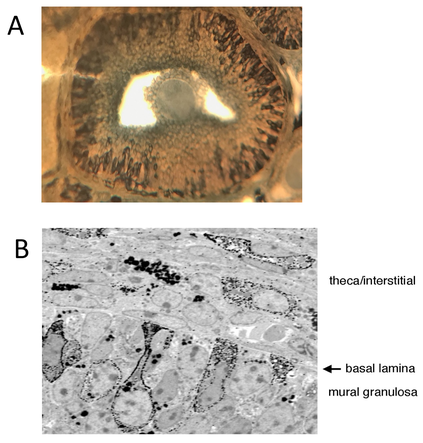


Figure 3. A. Light microscope view of a 50 µm thick vibratome section permeabilized with 0.1% triton and labelled with immunogold. The vibratome section was then embedded and sectioned for electron microscopy. **B.** 60 nm section of HA-LHR ovary stained with immunogold. Small black dots label cells expressing HA-LHR.

Quantitation of cells expressing LHR

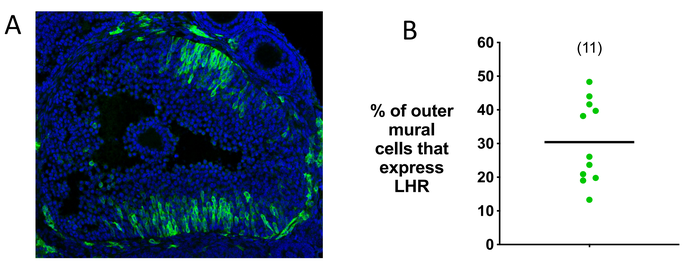


Figure 4. A. 24 day old HA-LHR mice were injected with eCG to stimulate LH receptor expression. 10 µm thick cryosections were stained for HA-LHR (green) and counterstained with 0.5 µg/mL DAPI to label nuclei. **B.** Percent of outer mural cells expressing the LHR. Cells were manually counted in equatorial sections of HA-LHR ovaries. In preovulatory follicles, the fraction of cells expressing the LHR ranges from ~10 to 50%.

Localization of HA-NPR2

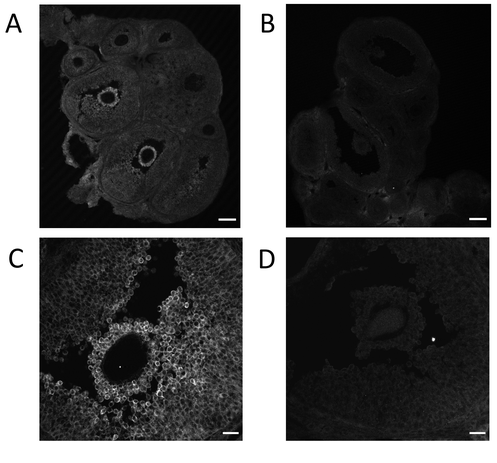


Figure 5. A, B. Expression of HA-NPR2 seen in 24 day old HA-NPR2 (A) or wildtype (B) ovaries. HA-NPR2 is present throughout the follicle, although it is more concentrated in the cumulus cells (scale bar = 100 µm). **C, D.** Higher magnification of HA-NPR2 seen in 24 day old HA-NPR2 (C) or wildtype (D) ovaries (scale bar = 25 µm).

Approximate percentage of total NPR2 in LHR-expressing cells

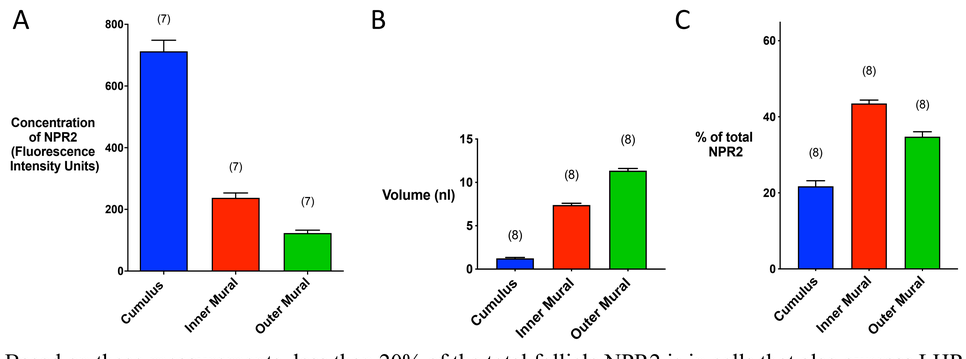


Figure 6. A. Relative concentrations of HA-NPR2 in cumulus, inner mural, and outer mural cells based on relative fluorescence intensities after background subtraction. **B.** Approximate volumes of cumulus, inner mural, and outer mural regions determined by counting the number of cells in each region in equatorial sections. Total granulosa cell volume is ~20 nl. Volumes were calculated assuming volumes are proportional to the numbers of cells. **C.** Percent of total NPR2 content in cumulus, inner mural, and outer mural regions of the follicle.

Based on these measurements, less than 20% of the total follicle NPR2 is in cells that also express LHR. This estimate was made by multiplying the range of values for the percent of outer mural cells that express LHR (~10-50% based on graph in Figure 4B) by the percentage of NPR2 that is in outer mural cells (~35%, Figure 6C). This calculation relies on our findings that essentially all LHR is in the outer mural cells (Figure 2-4) and that the NPR2 concentration is similar in all of the outer mural cells (Figure 5).

Conclusions

Less than 20% of the NPR2 is in the same cells that express the LHR. Therefore, to explain how LH signaling decreases cGMP in the follicle from a few µM to ~100 nM (1), the LHR must inactivate NPR2 in neighboring cells that do not express LHR. If only the NPR2 in the LHR-expressing cells was inactivated, this large cGMP decrease would not occur.

How does the signal that inactivates NPR2 pass from the subset of outer mural granulosa cells that express LHR to the neighboring cells that do not express LHR?

Proposed Mechanisms:

1. cAMP generated in LHR-expressing cells diffuses through gap junctions to activate a phosphatase that dephosphorylates and inactivates NPR2 in neighboring cells.
2. EGFR ligands released from LHR-expressing cells diffuse extracellularly to act on EGF receptors on neighboring cells, and EGFR signaling activates a phosphatase that dephosphorylates and inactivates NPR2 in these cells.

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