Contrasting Changes in cAMP Levels in Mouse Ovarian Follicles and Oocytes in Response to LH: Insights from Mice Expressing the cAMPFIRE-M Sensor

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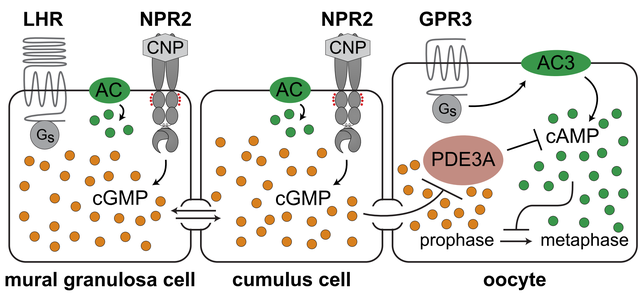
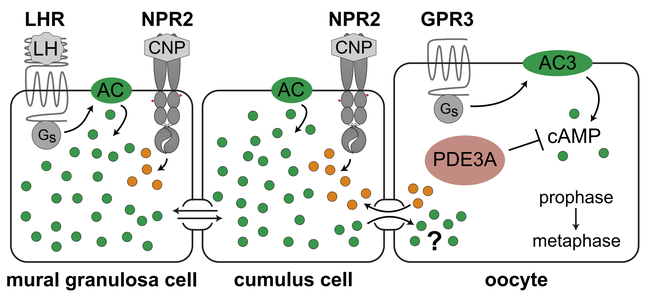
Background

Before the LH surge, oocytes within mammalian preovulatory follicles exhibit high cAMP levels that maintain meiotic arrest[1]. In the surrounding somatic cells of the follicle, high levels of cGMP diffuse through gap junctions into the oocyte to maintain the elevated cAMP[2]. LH rapidly elevates cAMP in the granulosa cells, which spreads through gap junctions to the cumulus cells[3]. High cAMP in the granulosa cells increases PKA signaling, which inactivates the NPR2 guanylyl cyclase[4], rapidly lowering cGMP levels in the somatic cells and oocyte[5]. This allows oocyte cAMP levels to fall within 1 hour after LH[2]. The dynamics of how the cAMP *increase* in somatic cells may impact the simultaneous *decrease* in oocyte cAMP levels remain unknown, and have been difficult to study with previous generations of genetically-encoded cAMP sensors[3]. Here, we generated mice that ubiquitously express a recently-described FRET sensor for cAMP, called cAMPFIRE-M[6], with increased sensitivity and dynamic range compared to previous sensors.

How oocyte meiotic arrest is maintained



How oocyte meiosis resumes



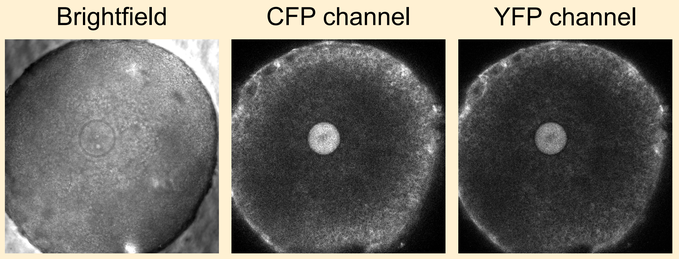
Questions

1) Does the LH-induced cAMP increase in somatic cells spread to the oocyte?

2) If so, what are its temporal dynamics and is it dependent on gap junctions?

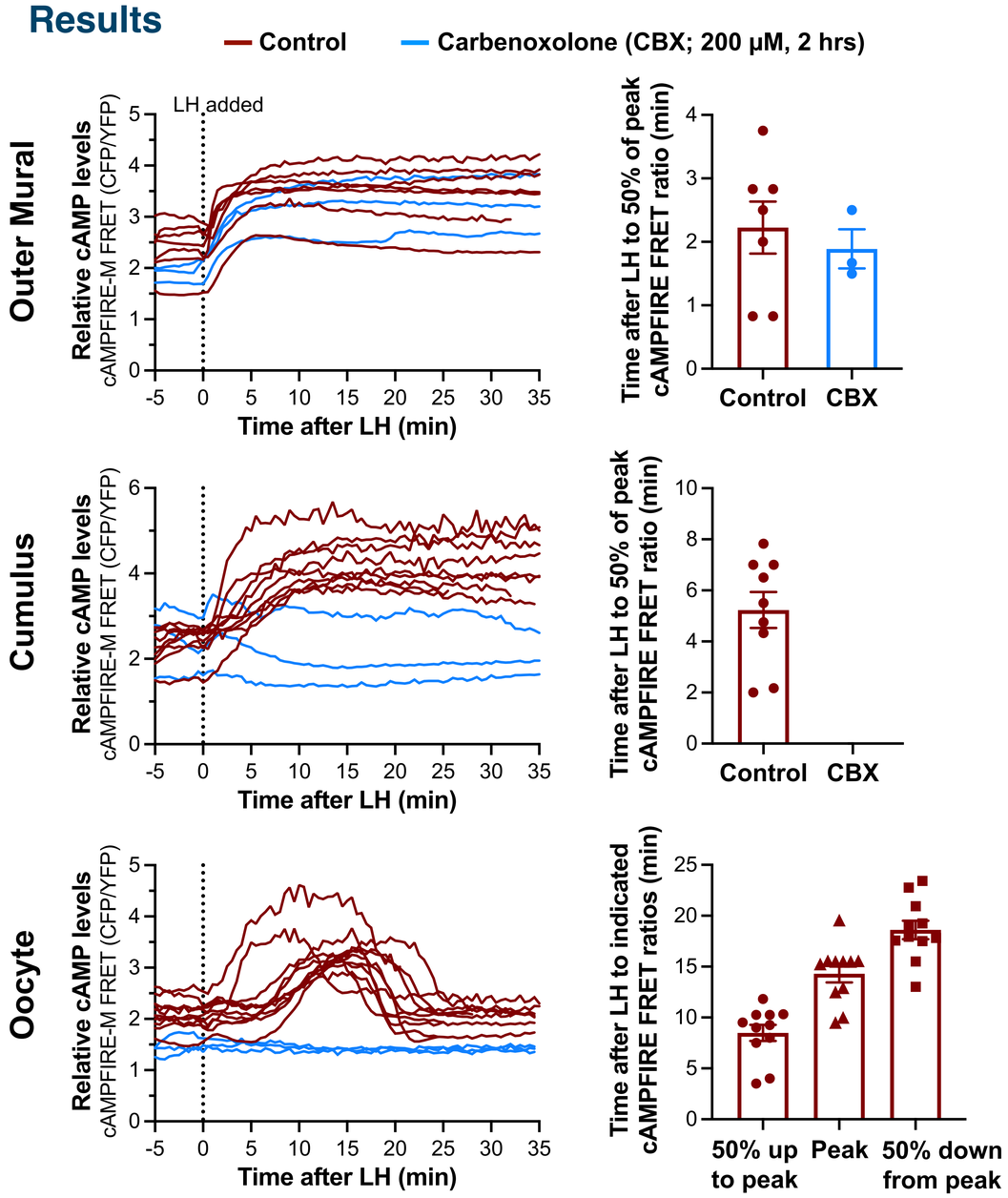
Approach

- **Measure relative cAMP levels using a new FRET sensor, cAMPFIRE-M**
  - **Sensor is endogenously expressed in mural granulosa and cumulus cells**
  - **Oocyte expression achieved by microinjecting cAMPFIRE-M mRNA**



Isolated follicle from a mouse endogenously expressing cAMPFIRE-M in somatic cells, ~16 hours after microinjection of cAMPFIRE-M mRNA into the oocyte.

Results



Conclusions

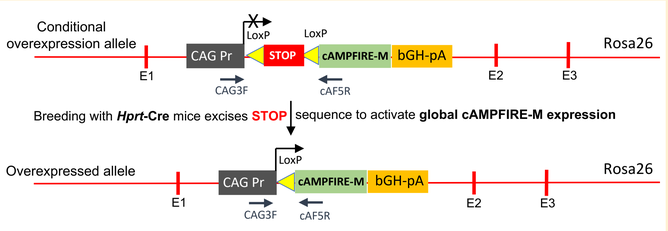
- We report the successful generation of a mouse line that globally expresses an improved FRET sensor for cAMP called cAMPFIRE-M[6].
- Microinjection of cAMPFIRE-M mRNA is required to quantify cAMP changes in the oocyte.
- As previously reported[3], LH signaling causes a rapid increase in cAMP in outer mural granulosa cells that is **not** dependent on gap junction communication.
- We also confirm a previous report[3] that the slower LH-induced increase in cAMP levels in cumulus cells **does** depend on gap junction communication.
- For the first time, we have shown that **LH causes a transient *increase* in oocyte cAMP.** This is mediated by elevated cAMP levels in the somatic cells diffusing through gap junctions into the oocyte.
- The timing of the oocyte cAMP increase is relatively consistent, peaking around 15 minutes after LH treatment, and decreasing at about the same rate.
- The oocyte cAMP increase represents a barrier to meiotic resumption that must be overcome.

Future Directions

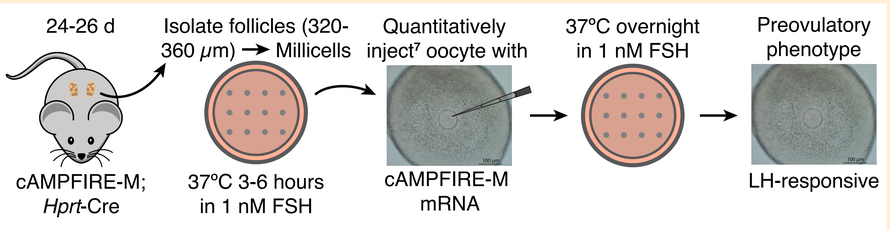
- Determine the mechanism by which oocyte cAMP falls after the transient increase.
  - Entirely due to an increase in cAMP hydrolysis following the decrease in oocyte cGMP?
  - Also mediated by a decrease in gap junction permeability?
- Investigate the kinetics of the oocyte cAMP decrease at later time points after LH.

Methods

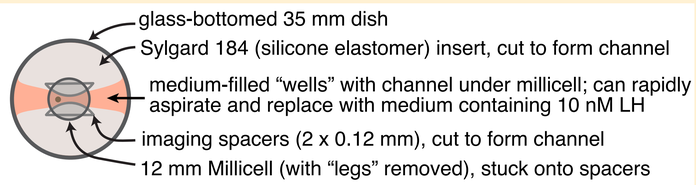
Generation of cAMPFIRE-M[6]-LSL;*Hprt*-Cre mouse line



Mouse follicle culture, oocyte injection



Imaging



Imaged on a Zeiss 980 confocal with 25X or 20X/0.8 NA objective

References

1. Jaffe and Egbert. 2017. *Annu Rev Physiol* **79**: 237-260
2. Norris et al. 2009. *Development* **136**: 1869-1878
3. Lyga et al. 2016. *Endocrinology* **157**: 1613-1621
4. Egbert et al. 2021. *Biol Reprod* **104**: 939-941
5. Shuhaibar et al. 2015. *PNAS* **112**: 5527-5532
6. Massengill et al. 2022. *Nat Methods* **19**: 1461-1471
7. Jaffe et al. 2009. *Methods Mol Biol.* **518**: 157-173

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