Regulation of Gap Junction Communication is Required for the Normal Timing of the Oocyte cAMP Changes that Initiate Meiotic Resumption

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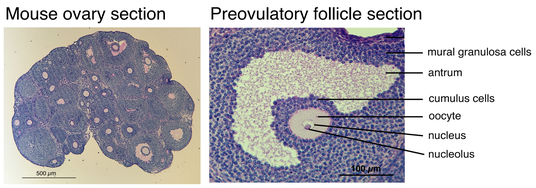
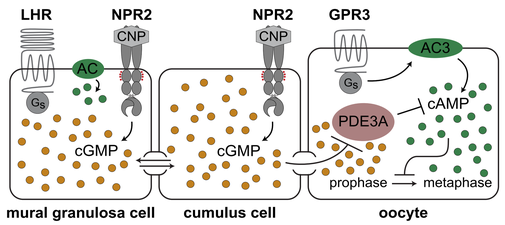
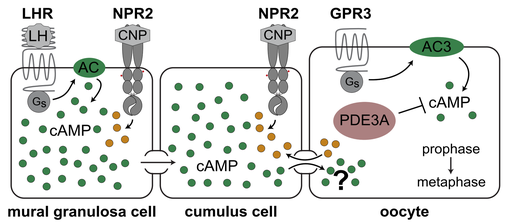
Background



How oocyte meiotic arrest is maintained[1,2]

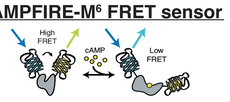


How oocyte meiosis resumes in response to LH[3,4,5]

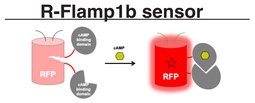


Methods

cAMPFIRE-M[6] FRET sensor



R-Flamp1b sensor



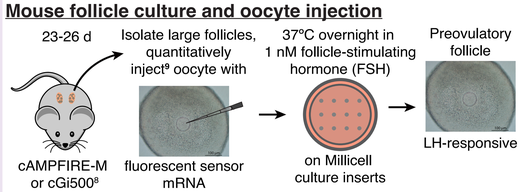
Generation of cAMPFIRE-M-lox-STOP-lox mouse line

- Created a CAG-driven conditional (LSL) overexpression allele.
- Inserted into Rosa26 locus using CRISPR/Cas9 tools.
- Bred with *Hprt*-Cre mice for global cAMPFIRE-M expression (lower in oocyte, so inject mRNA into oocyte).

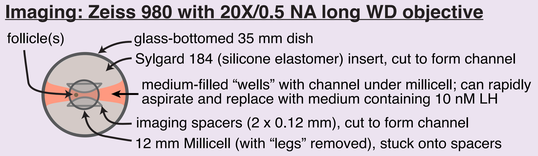
R-Flamp1b

- Unpublished red cAMP sensor similiar to the green sensor G-Flamp1[7].

Mouse follicle culture and oocyte injection



Imaging: Zeiss 980 with 20X/0.5 NA long WD objective



Ibidi stage top incubator maintained 37 °C, 5% CO_2, humidified air

Question #1

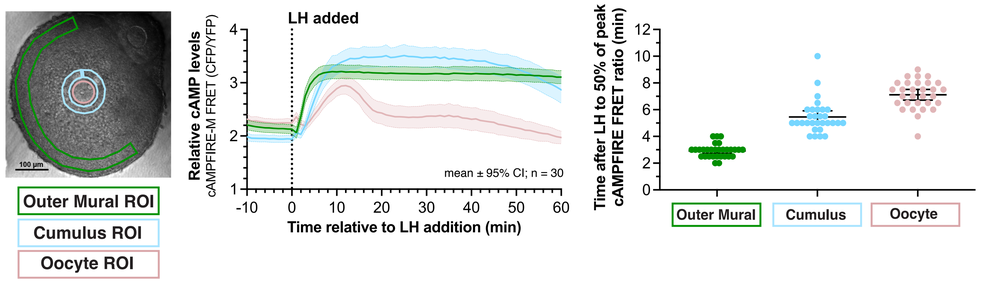
A) Does the LH-induced cAMP increase in somatic cells spread to the oocyte?

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

Approach

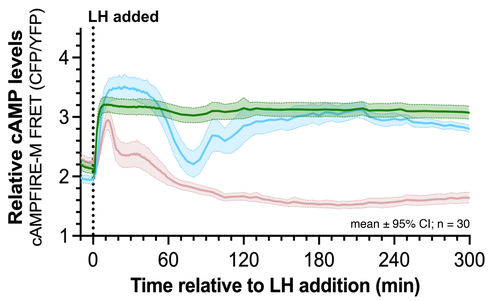
Isolated follicle from a mouse endogenously expressing cAMPFIRE-M in somatic cells, ~19 hours after microinjection of cAMPFIRE-M mRNA into the oocyte.

Results: LH-induced cAMP elevation progresses inward, causing a transient oocyte cAMP increase which later falls below baseline.

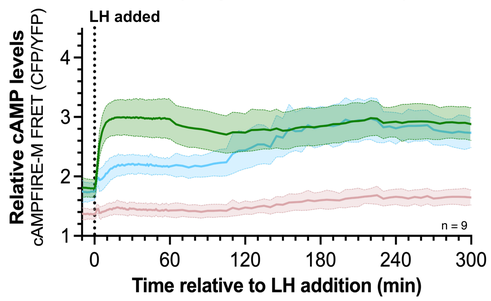


Results: Blocking gap junctions with carbenoxolone (CBX) prevented the LH-induced cAMP increase in the oocyte. Conversely, preventing the LH-induced closure of gap junctions[10] with the EGFR inhibitor AG1478 delayed both the oocyte cAMP decrease and nuclear envelope breakdown (NEBD, indicating meitoic resuption) by ~3 hours.

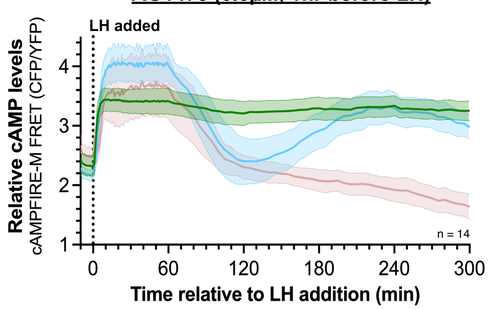
Control



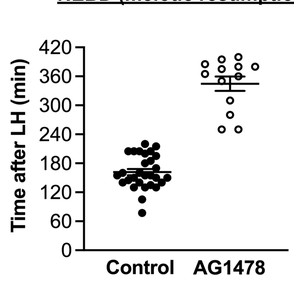
CBX (200µM, 2hrs before LH)



AG1478 (0.5µM, 1hr before LH)



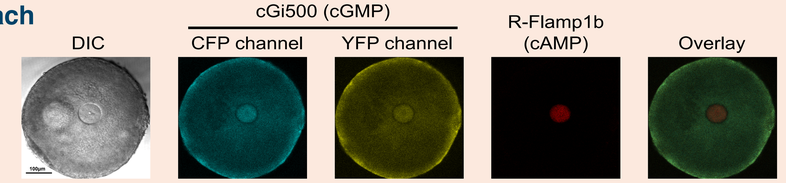
NEBD (meiotic resumption)



Question #2

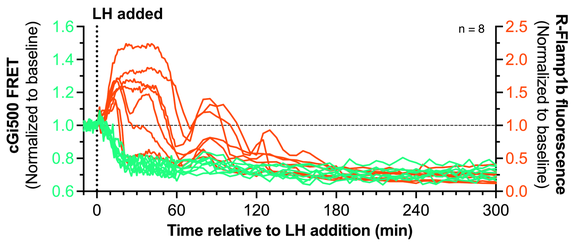
How do the kinetics of the LH-induced decreases in cGMP and cAMP compare?

Approach



Isolated follicle from a mouse endogenously expressing cGi500 in somatic cells, ~19 hours after microinjection of both cGi500 mRNA and R-Flamp1b mRNA into the oocyte.

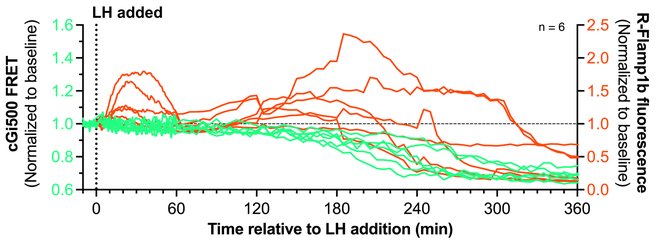
WT



Results: In WT follicles, the LH-induced cGMP decrease precedes the cAMP decrease.

Npr2-7E/7E[11] (phosphomimetic mutations)

Mice in which NPR2 cannot be inactivated by dephosphorylation.



Results: Persistent elevation of cGMP prevents oocyte cAMP from falling below baseline. Eventually, other mechanisms cause a decline in oocyte cGMP, allowing cAMP to subsequently decrease.

Conclusions

- We generated a mouse line globally expressing an improved cAMP FRET sensor, cAMPFIRE-M.
- LH causes a transient increase in oocyte cAMP mediated by elevated cAMP levels in the somatic cells diffusing through gap junctions into the oocyte. Thus, signaling to cause meiotic resumption creates a transient barrier to meiotic resumption that must be overcome.
- LH-induced gap junction closure is essential for the normal time course of the fall in oocyte cAMP that initiates meiotic resumption.
- Though the fall in oocyte cGMP is required for oocyte cAMP to fall, other factors (like gap junction closure) also influence the timing of the cAMP decrease.

Future Directions

- Determine the mechanisms that result in differing cAMP dynamics between the oocyte and cumulus.
- Investigate FSH-induced cyclic nucleotide dynamics in smaller follicles and oocytes.

References and Funding

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