Signaling between the LH Receptor and the NPR2 Guanylyl Cyclase in Mouse and Rat Preovulatory Follicles

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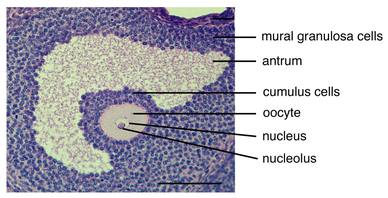
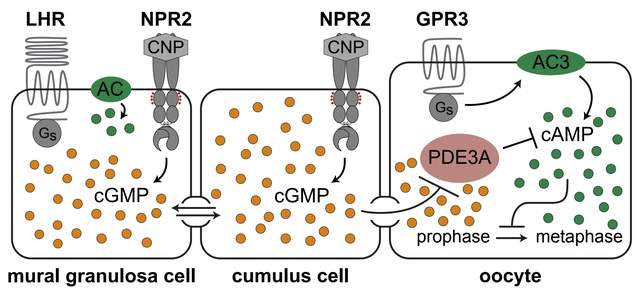
Background

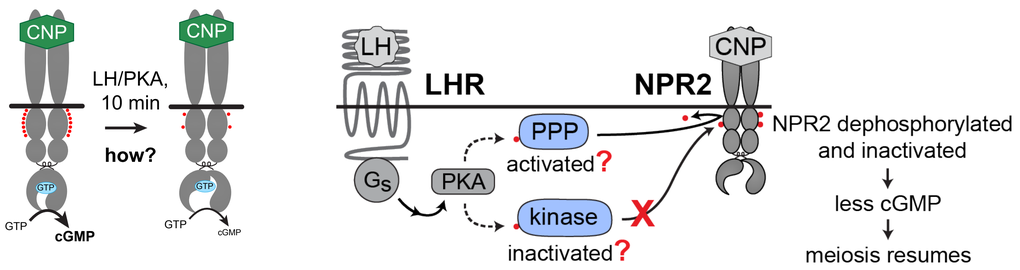
Preovulatory follicle structure



How oocyte meiotic arrest is maintained[1,2]



LH signaling dephosphorylates and inactivates NPR2, leading to meiotic resumption[2-5]



Questions

To cause NPR2 dephosphorylation and inactivation, does LH signaling:

- **Activate one or more phosphatases?**
- **Inactivate one or more kinases? Or both?**

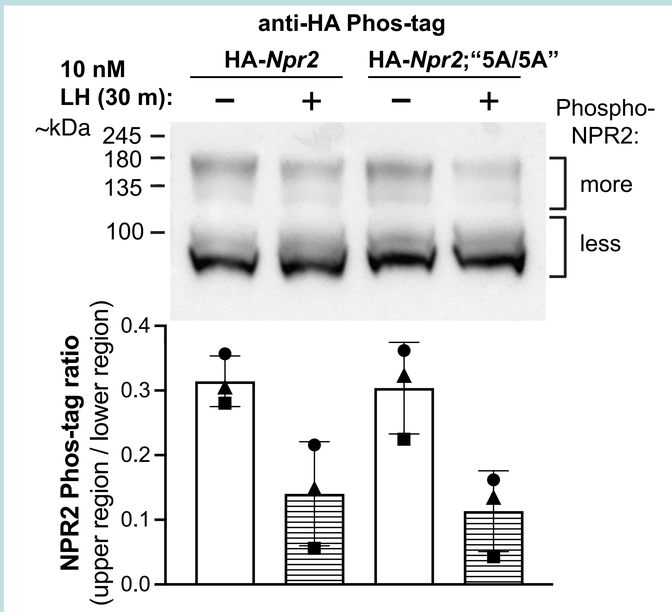
Results

1) A phospho-mass spec screen performed on rat follicles revealed 5 LH-regulated phosphosites on two candidate PPP-family regulatory proteins[6]:

Gene name	Amino acid	#	Phosphopeptide intensity (mass spec) (mean ± SEM) Control (PBS)	300 nM oLH	Fold change
PPP2R5D	**S**	**53**	16.20 ± 0.03	19.49 ± 0.27	**10.4**
PPP1R12A	**S**	**507**	16.42 ± 0.06	18.57 ± 0.59	**4.7**
PPP2R5D	**S**	**566**	19.94 ± 0.32	21.90 ± 0.35	**4.1**
PPP2R5A	S	38	16.45 ± 0.13	17.92 ± 0.07	2.9
PPP1R9A	S	185	14.20 ± 0.07	15.36 ± 0.14	2.4
PPP2R5C	S	497	19.03 ± 0.18	20.08 ± 0.31	2.2
PPP2R5D	**S**	**81***	22.39 ± 0.12	23.15 ± 0.15	**1.8**
PPP2R5D	**S**	**82***	22.12 ± 0.11	22.84 ± 0.17	**1.7**

Table 1: PPP-family phosphatase subunits that are significantly more phosphorylated in rat follicles after a 30 min LH treatment. *indicates marginally significant FDR q-values (0.041 and 0.046, respectively).

2) Mutation of these 5 sites to alanine ("5A/5A" mice) does not prevent NPR2 dephosphorylation by LH:



3) GSK3A/B is a candidate kinase that is constantly active unless inhibited by phosphorylation[7]:

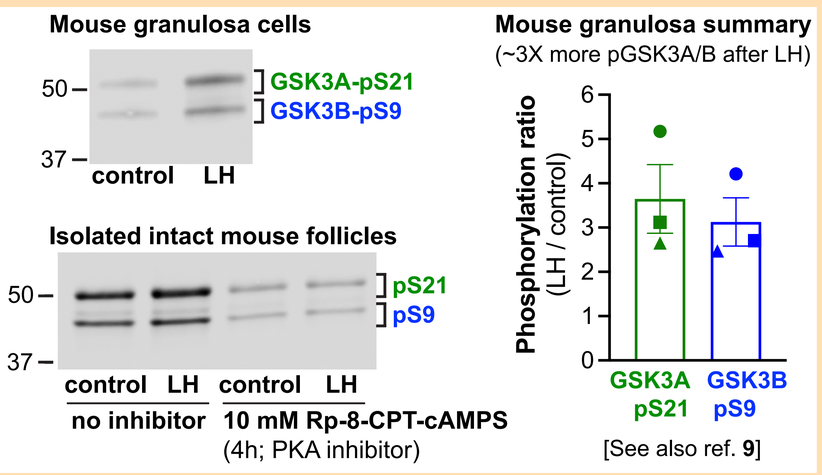
S/T_1-X-X-X-pS/pT_2

GSK3 consensus sequence. Note that the number of X amino acids can vary from 3. Generally, a priming phosphorylation on S/T_2 is required for GSK3 to act on S/T_1.

KGAGSRLTLSLRGSSYGSLMTAHGK
513 516 518 523 526 529

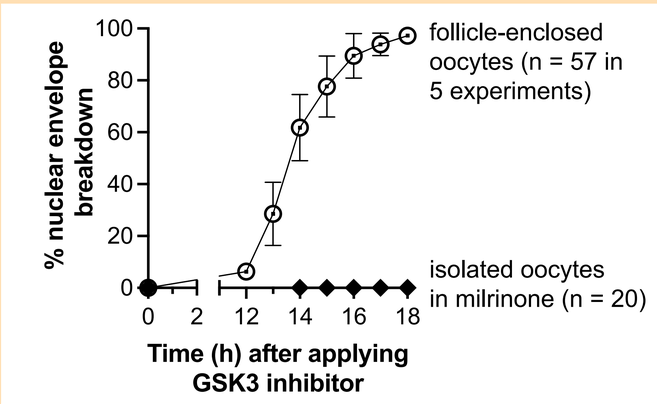
There are six known NPR2 regulatory phosphosites.[8] Five are putative **GSK3** sites if T529 is primed by a different kinase.

4) GSK3A/B is phosphorylated by LH/PKA signaling:

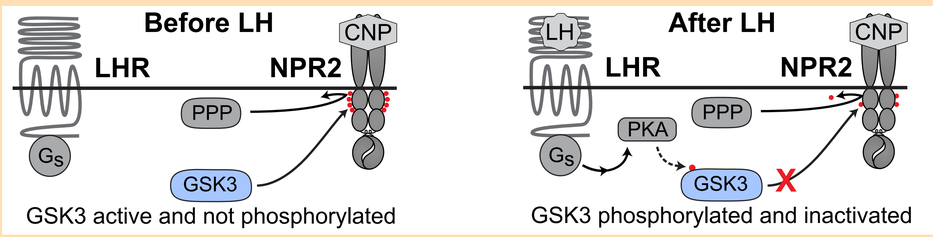


5) GSK3A/B inhibitor CHIR99021 (10 μM, 16+ h) causes NPR2 dephosphorylation in isolated intact mouse follicles:

6) GSK3A/B inhibitor causes NEBD in oocytes within intact follicles, but not isolated oocytes:

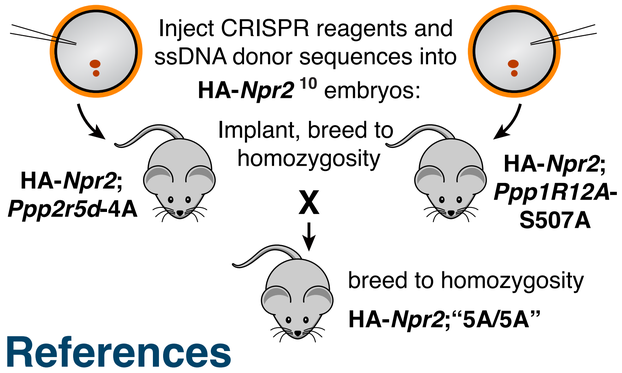


7) Current hypothesis for LH-NPR2 signaling:

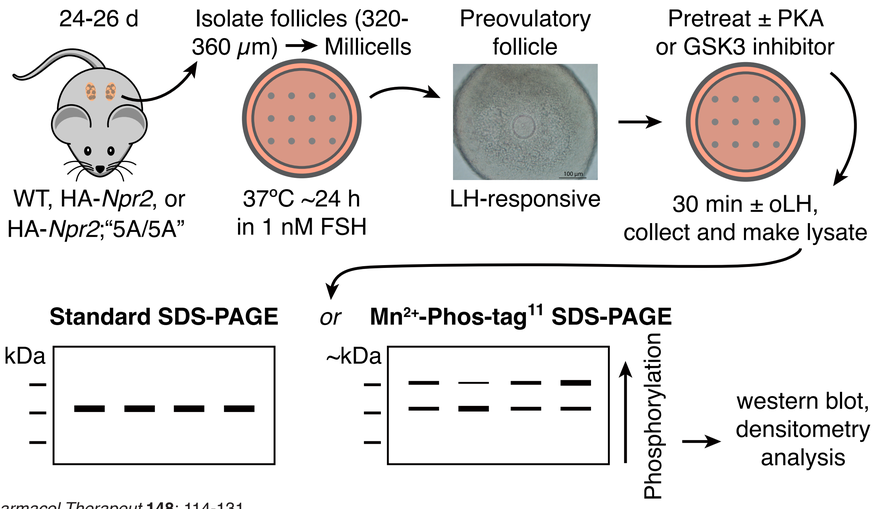


Methods

Generation of HA-*Npr2*;"5A/5A" mouse line, where serines 53/81/82/566 of PPP2R5D and S507 of PPP1R12A are mutated to alanine



Mouse follicle culture, western blot



Conclusions

- LH-induced NPR2 dephosphorylation may not be mediated by changes in PPP-family phosphatase activity.
- GSK3A/B is a candidate kinase for NPR2 regulatory sites.
- LH/PKA signaling phosphorylates inhibitory sites on GSK3A/B.
- An inhibitor of GSK3 causes NPR2 dephosphorylation and NEBD.

Future Directions

- Use GSK3A (global);GSK3B (granulosa-specific) knockout mice to test whether GSK3 is required to maintain NPR2 phosphorylation.
- Use GSK3A-S21A/S21A;GSK3B-S9A/S9A[12] mutant mice to test whether GSK3A/B phosphorylation is required for LH-induced NPR2 dephosphorylation and meiotic resumption.

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