

RNA sequencing analysis reveals the GnRH induced citrullinome

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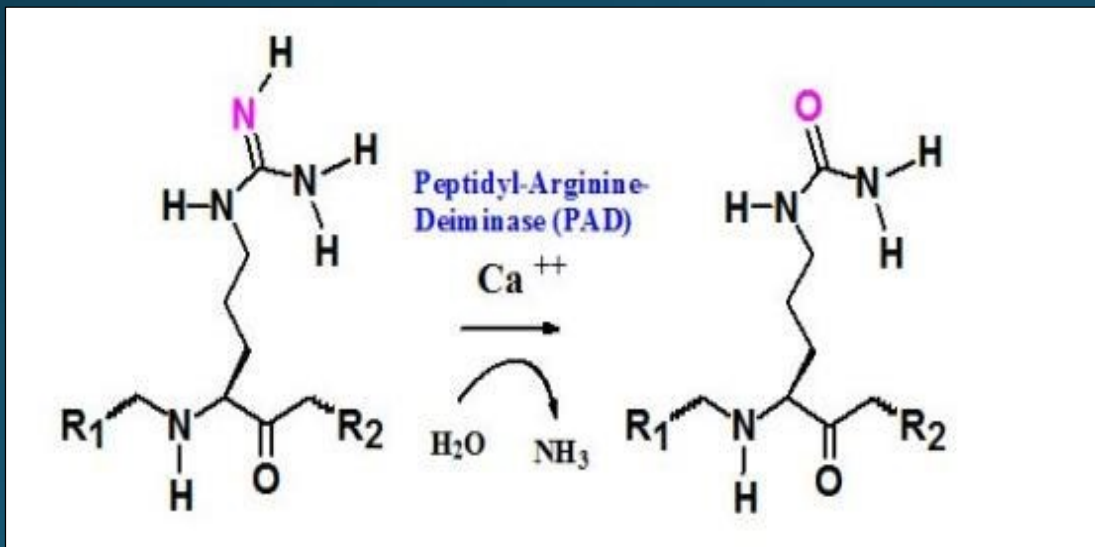
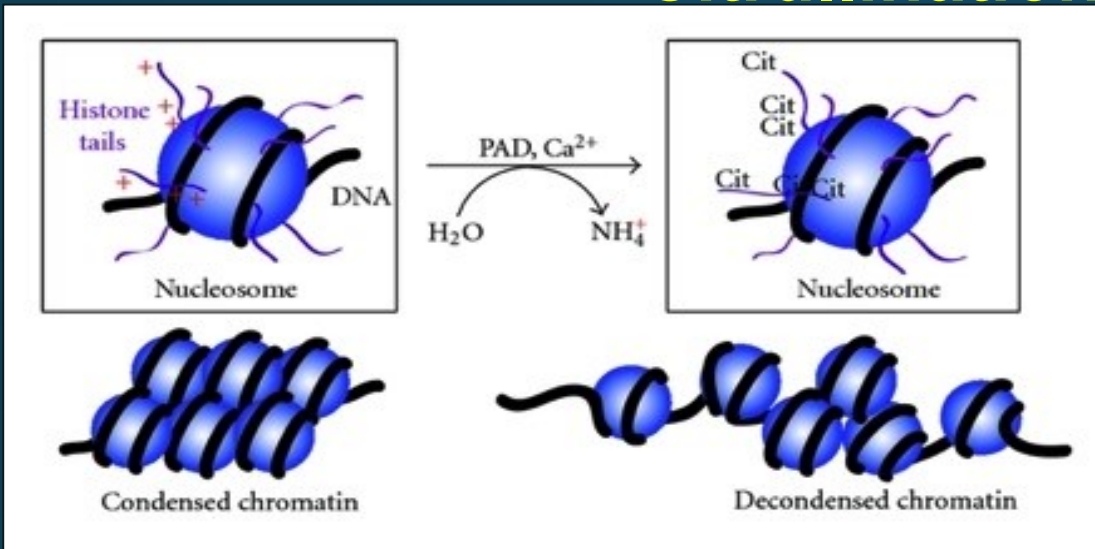
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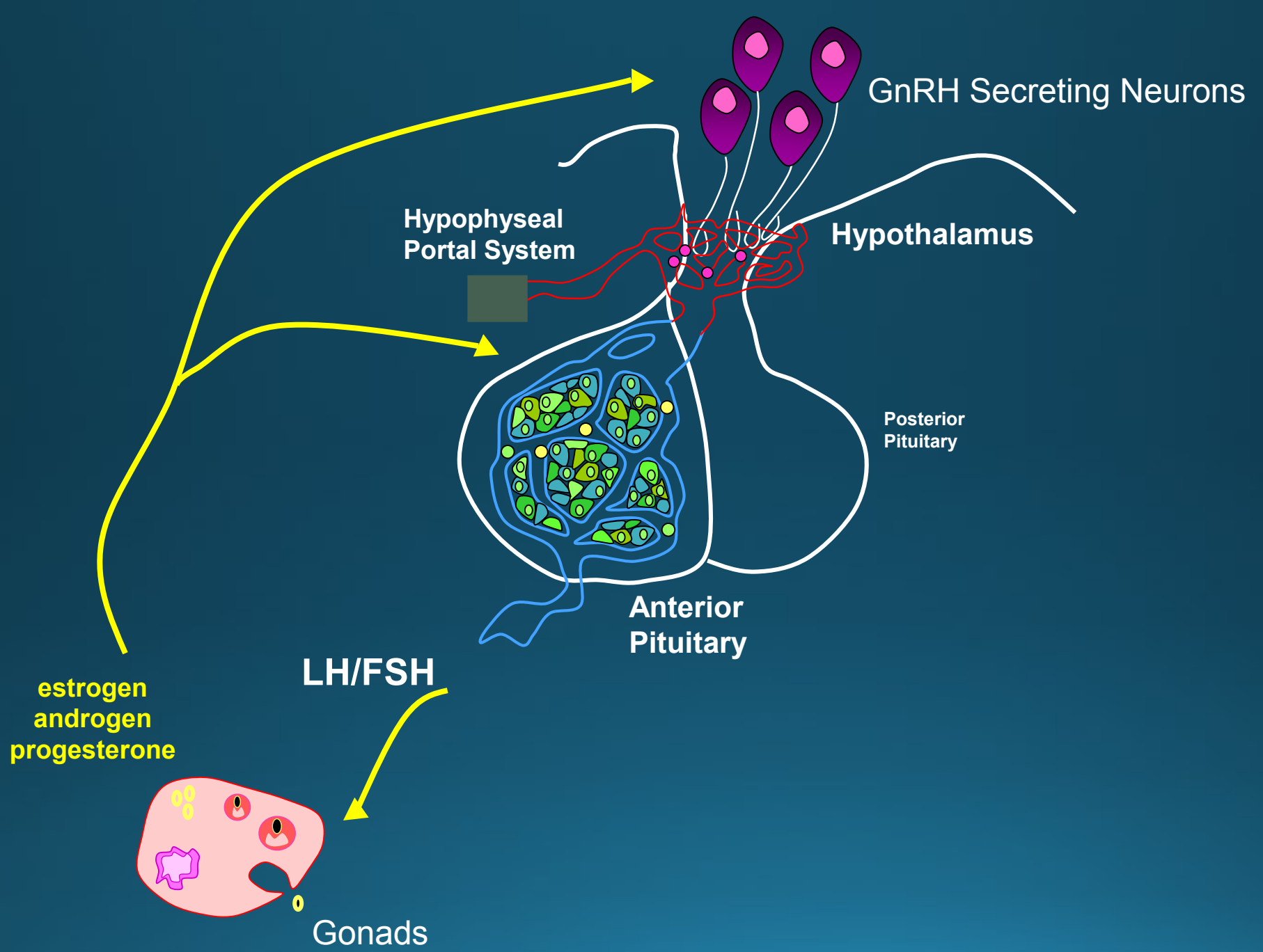
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Peptidyl-Arginine-Deiminases (PADs) Catalyze Citrullination



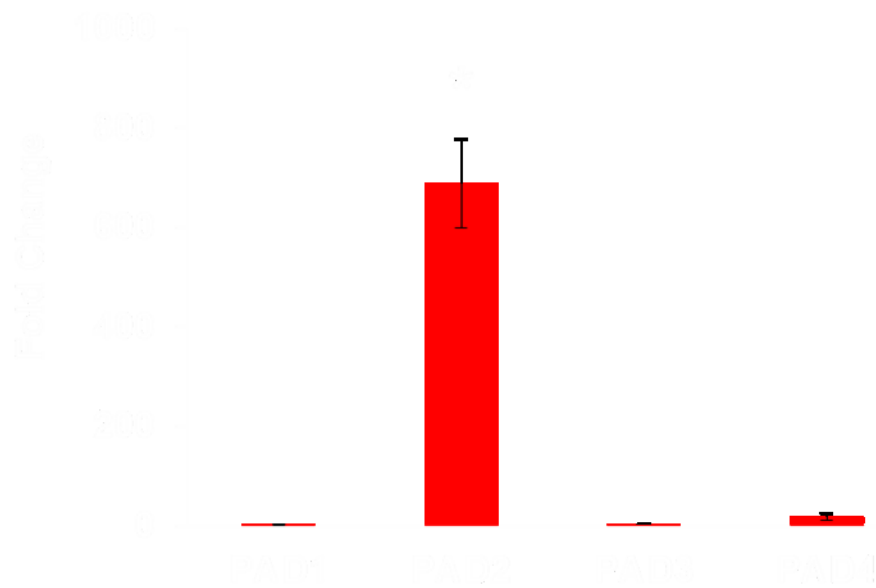
5 types of PAD enzymes

- PAD1: uterus, epidermis
- PAD2: brain, skeletal muscle, female reproductive tissues
- PAD3: hair follicle, epidermis, mammary gland
- PAD4: immune cells, ovaries, breast
- PAD6: germ cells

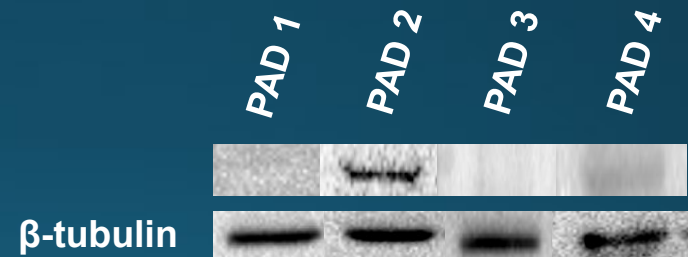


L β T2 Cells Express High Levels of PAD2

PAD1- 4 mRNA expression



PAD1- 4 protein expression

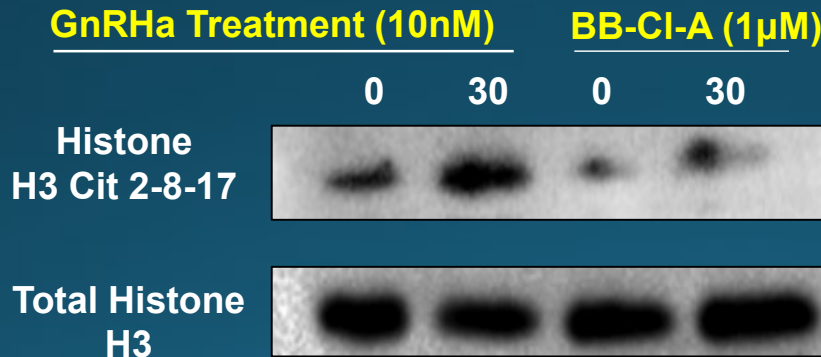
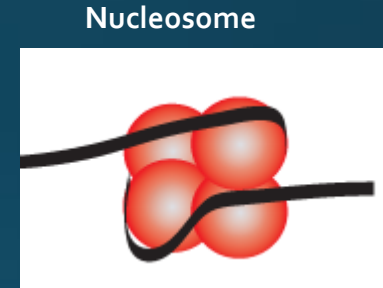


Central Questions:

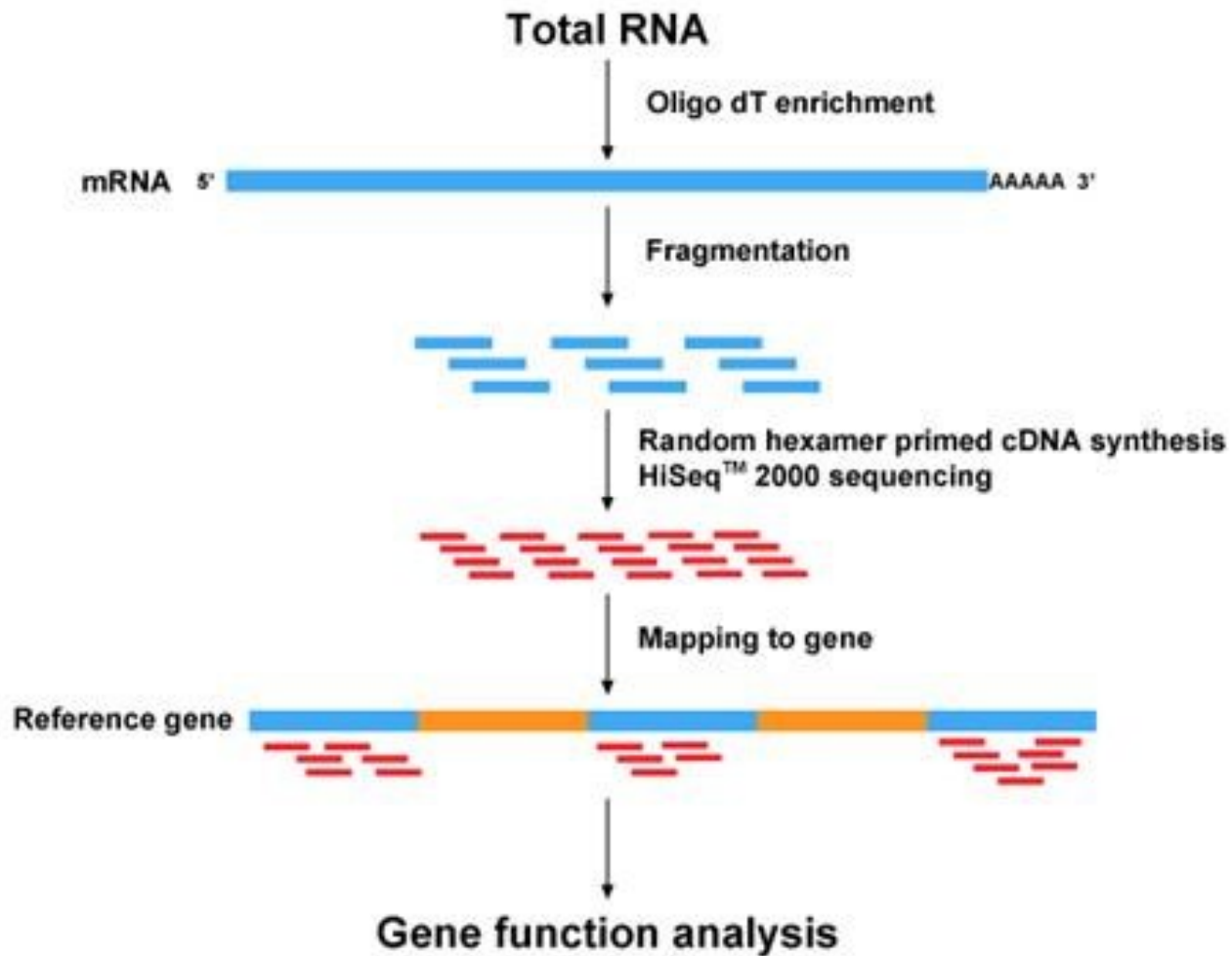
Does GnRH mediate PAD-catalyzed histone citrullination to regulate gonadotropin gene expression?

What are the genes that are differentially regulated between GnRH and GnRH+BBCIA treatment?

Inhibition of Histone Citrullination Reduces LH β mRNA Expression



RNA-Seq



RNA-Seq: Gene Analysis

- GnRH vs GnRH + BB-CIA
- Divide data into meaningful subsets
 - Fold changes > 2
 - Control Means > 5
 - Control Means > 5 and Fold Changes > 2
- Choose genes with largest fold changes



Why study this?

- **Characterize a completely novel mechanism stimulated by GnRH that is critical for gonadotrope function**
- **Positively contribute to develop treatments for PAD related diseases**
- **Inhibiting PAD function in gonadotropes may represent an innovative approach to regulate fertility and contraception**

Conclusions

- PAD2 is highly expressed in the L β T2 cell line and in gonadotropes.
- GnRH induces PAD2 protein expression
- GnRH increases histone citrullination of arginine residues 2, 8, and 17 on histone H3 tails.
- BB-CI-Amidine disrupts GnRH α induced histone citrullination

Future directions

- Perform RNA seq to identify the full cohort of genes regulated by PAD catalyzed histone citrullination.
- Examine the role of estrogen in PAD2 mediated citrullination.