Transcriptional Regulation of Pituitary POMC Is Conserved at the Vertebrate Extremes Despite Great Promoter Sequence Divergence

Variation In the Promoter Region of POMC
- Promoters
- Enhancers
- cis-regulatory elements
- No visible conservation in the 5'-flanking regions between any of the vertebrates

Subjects
- Green Spotted Pufferfish
- Mouse

Transgenes
- 8 transgenes were constructed and placed in the mouse genome
  - 1– Entire POMCα including promoter region
  - 2-5– Contain different lengths of the POMCα 5'-flanking region and the chicken β-globin minimal promoter (to help properly initiate transcription) upstream of a lacZ reporter gene
  - 6– missing pituitary POMC conserved element (PPCE)
  - 7– PP1 enhancer
  - 8– both missing PP1, but 7 has PPCE and 8 has a mutated PPCE

Does It Work?
- First take entire POMCα gene and 5'- and 3'-flanking regions and insert into the mouse genome– does not work
- Make transgenes– 2 and 3 give similar results (~1kb 5'-flanking POMCα DNA) plus (2) or minus (3) exon 1, chicken β-globin minimal promoter, and lacZ reporter gene upstream of mouse POMC
- X-Gal staining shows that lacZ product is formed

Hypothalamic-Pituitary-Adrenal Axis
- POMC gives rise to the precursor of ACTH
Discussion- Part I

- Enhancers evolve more slowly, so we know that they must be important
- No visible similarity between fish and mice, but they found that there must be a similarity because the upstream sequences of the green pufferfish still allowed the mouse gene to be expressed
- Even though there are mutations between the species, the enhancer still keeps its function

Is It Still Involved in the HPA axis?

- Glucocorticoids (like cortisol) normally inhibit POMC expression in mice whereas CRH activates it
- Cut half of the adrenal gland out of mouse (reduces cortisol production)
- To test effect of CRH, use AtT20 cells with transgene 3 vs. mouse Pomc cells—same results
- Dexamethasone does not inhibit POMCα like it does to mouse POMC

Part II

- Green puffer fish enhancer works on mice, now they go to find if it still affected by the HPA axis, which it is
- CRH and cortisol affect the enhancer similarly between fish and mice. When they used dexamethasone on the fish enhancer it did not inhibit its function, where as in the mouse it does
- So even though the sequences are different the final effect of POMC is conserved

Where is it? What is it?

- Using a computer program, Footprinter 2.1, find a short concensus sequence of 10 bp with 4 identical bp between humans and fish
- 2 copies in most vertebrates, Tetraodon has them adjacent to each other: pitutary POMC conserved element (PPCE)

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Is It the Right Sequence?

- Generate transgenes 4 and 5– 4 contains PPCE, 5 does not
- AtT20 cells showed a 68% decrease in lacZ expression when they contained transgene 5
- 19 of 21 transgenic mice containing transgene 5 didn’t produce any product
**Part III**

- Made sure the PPCE was the true enhancer instead of some other element by deleting it and mutating it which resulted in reduced function of the PPCE
- Used trans-gene 5 (PPCE was deleted) and created 21 transgenic mice: 19 did not express POMC at all, 2 produced it in only a couple of cells

**Same Function in All Vertebrates?**

- Construct transgenes 6, 7, and 8
- In 7, replace the mouse PP1 (proximal copy of the promoter) with a mutated PPCE
- In 8, replace the mouse PP1 with normal PPCE
- The unaltered PPCE not only served the same function in mice, but it even increased the rate of transcription of Pomc

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**Questions??**

- Do you think enough testing was done in order to make sure the PPCE was the only enhancer affecting transcription of the POMC gene?
- The PPCE has traveled through 500 million years of evolution and still serves the same function in fish as it does in mice. What other genes might have been so highly conserved?