Developing STAT5A Fluorescent Protein Fusions to Study the Effects of JAK-STAT Signaling on Pancreatic Beta Cell Proliferation

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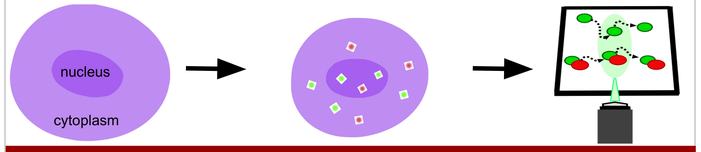
Abstract

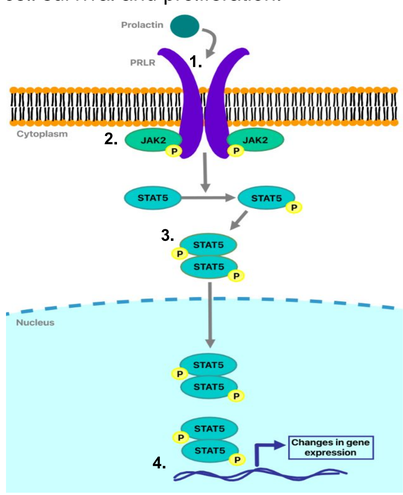
The **prolactin receptor (PRLR)** is an important component of the **JAK-STAT pathway**, because its signaling gives **pancreatic beta cells (PBCs)** a way to adapt to and manage metabolic stress, which could be used to later develop a **diabetes treatment**. **Prolactin** is one of the hormones that activates the pathway, as well as **estrogen** and **placental lactogen**. **Confocal fluorescence microscopy** played an essential role in making conclusions from this project. We inserted the transcription factor **STAT5A** into the **pcDNA3** vector through **genetic engineering**. By fusing the **STAT5A** gene inserted into the **pcDNA3** vector with genes encoding **fluorescent proteins**, we were able to get a clear view of the exact location of the **STAT5A** in the cells they were transfected into. Through **confocal fluorescence microscopy**, we found that there was cytoplasmic and nuclear expression of **STAT5A**. In addition, we also studied cells transfected with both **STAT5A** and the **PRLR**, and there was co-localization of **STAT5A** and the **PRLR**.



JAK-STAT Pathway

The JAK-STAT pathway plays a very important role in this project, because it is responsible for mediating gene expression linked to beta cell survival and proliferation.

1. Prolactin (PRL) binds to the prolactin receptor (PRLR).
2. PRLR dimerizes and JAK2 activates STAT5 monomers.
3. Activated STAT5 dimerize and translocate to the nucleus.
4. STAT5 regulates gene expression as a transcription factor.



PRLR signaling leads to rodent PBC population increase

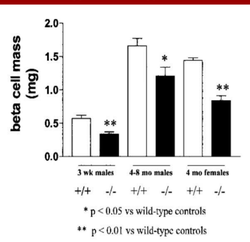
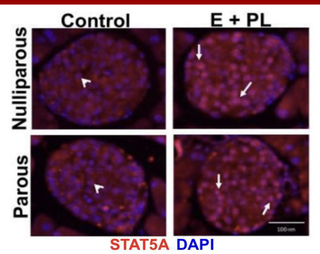


FIG. 2. Effects of PRLR deficiency on pancreatic β-cell mass. Values represent the means ± SE. The number of experimental samples is shown *in parentheses within the bars*.

Beta cell mass significantly decreases when the gene for the PRLR is knocked out.

Freemark et al., Endocrinology 2002



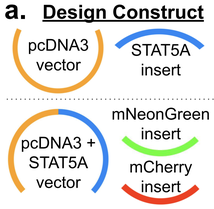
Higher levels of STAT5A in the nuclei of pancreatic islets after treatment with hormones that activate the JAK-STAT pathway.

Millette et al., Biomolecules 2022

Methods: Creating STAT5A fluorescent protein fusions to image

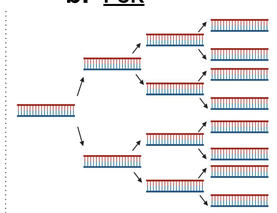
1. Molecular Cloning

a. Design Construct



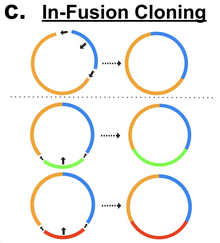
Determine constructs for the project before beginning lab work

b. PCR



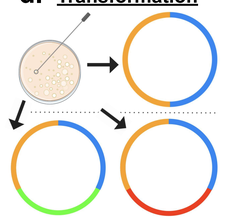
Amplification of insert to make millions of copies

c. In-Fusion Cloning



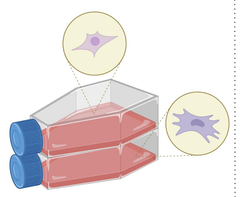
Insertion of insert into vector

d. Transformation



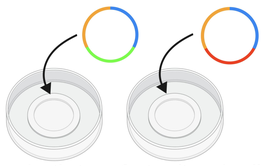
Bacteria transformation to isolate positive clones

2. Cell Culture



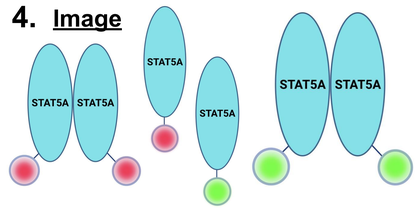
Culture HEK293T and U2OS cells

3. Transfect



Transiently transfect cultured cells with pcDNA3-mNeonGreen-STAT5A and pcDNA3-mCherry-STAT5A expression constructs

4. Image

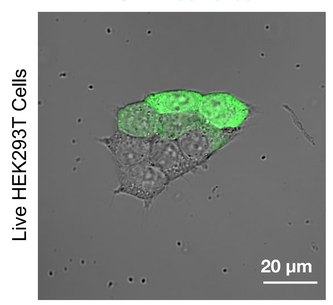


Perform confocal fluorescence microscopy to study STAT5A and PRLR

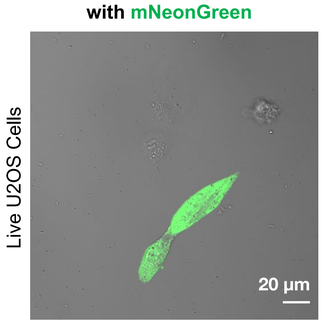
Results: Validation of cytoplasmic and nuclear STAT5A expression.

Image STAT5A with fluorescent proteins to study their location in live cells.

STAT5A detection with mNeonGreen



STAT5A detection with mNeonGreen



STAT5A detection with mCherry

mNeonGreen

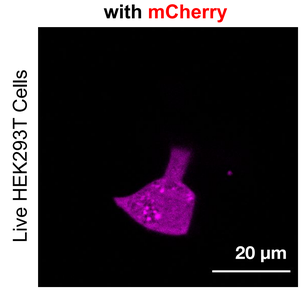
mCherry

- Transient transfection into live HEK293T and U2OS cells
- Cytoplasmic and nuclear expression of STAT5A

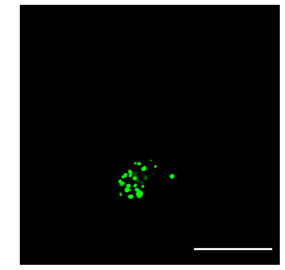
Results: Co-localization of STAT5A and PRLR

Image STAT5A and PRLR with fluorescent proteins to study their location in live cells.

STAT5A detection with mCherry

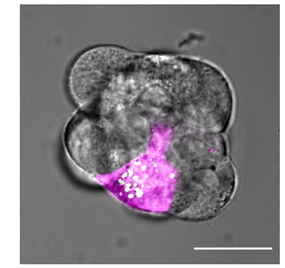


STAT5A detection with mNeonGreen

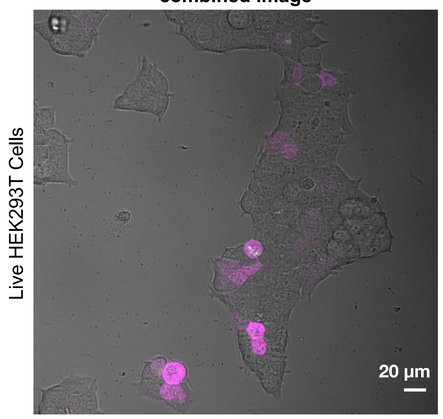


- Transient transfection into live HEK293T and U2OS cells
- Co-localization of STAT5A and PRLR

STAT5A and PRLR combined image



STAT5A and PRLR combined image



mNeonGreen

mCherry

Future Research

- How does STAT5A expression change when we add the PRLR, in addition to prolactin and placental lactogen?
- Our contributions to the field of beta cell signaling can be used by other researchers to develop diabetes treatments.

CONTACT US

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