Comparative Genomic Analysis of the FKBP51 Promoter in New World and Old World Primates, *Saimiri boliviensis, Aotus nancymaae* and *Homo sapiens*

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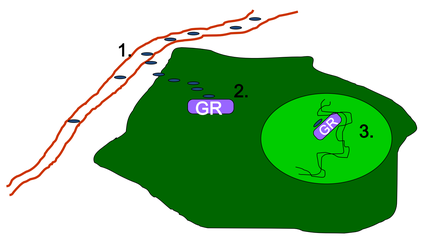
Introduction

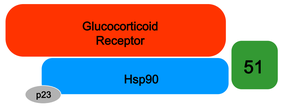
Glucocorticord Receptor Function

1. Glucocorticords are hormones released by the adrenal gland into the bloodstream.
2. Glucocorticords diffuse into cells & combine with the glucocorticoid receptor to activate the receptor.
3. Activated receptors interact with DNA to cause gene expression.



FKBP51(51) is a glucocorticoid receptor-associated protein.

FKBP51 affects glucocorticoid receptor function by <u>inhibiting</u> hormone binding to the receptor.



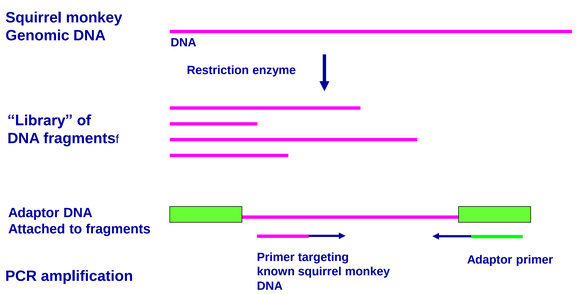
<u>Low affinity</u> binding to cortisol ⟷ Poor responsiveness

FKBP51 levels are **very high** in New World primates and relatively **low** in Old World primates such as humans. New World primates have high levels of cortisol in their blood, probably to compensate for poor responsiveness of the glucocorticoid receptor, due in part to high FKBP51 levels.

The focus of the research is to:

- Determine the New World primate squirrel monkey (*Saimairi boliviensis)* and owl monkey (*Aotus nancymaae*) FKBP51 promoter sequences.
- Compare New World and Old World (human) primate sequences.
- Look for DNA sequences that may explain differential expression of FKBP51 in New World versus Old World primates.

Genome Walking and PCR



PCR was performed using a primer targeting an "adaptor" DNA sequence and a primer targeting known New World primate sequence

The PCR products contained a small amount of known sequence and a large amount of **previously unknown** sequence

Gel Electrophoresis and gel extraction were used to analyze the products

Results

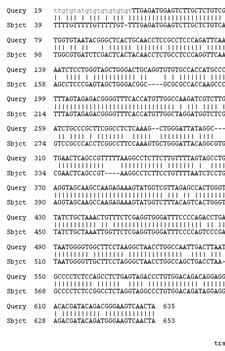
Squirrel Monkey

Human

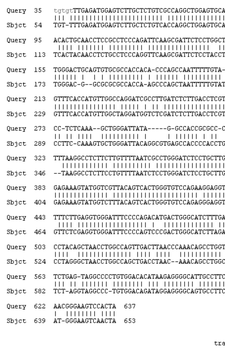
Owl Monkey

Comparison of Squirrel Monkey, Human and Owl Monkey FKBP51 Promoter Sequences

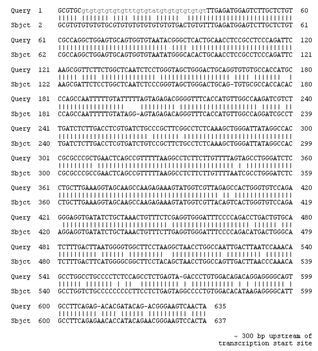
Sm vs H
85% Identity
5% gaps



Om vs H
84% Identity
6% gaps

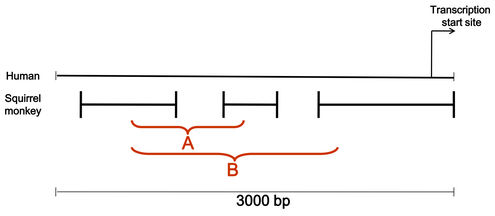


Sm vs OM
91% Identity
<1% gaps



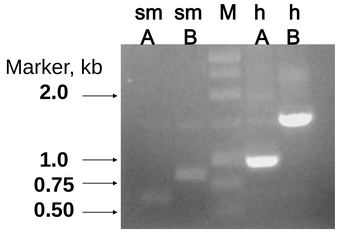
Confirmation of repetitive sequences in the Human FKBP51 promoter

Schematic of Squirrel Monkey and Human FKBP51 Promoter Sequences



Predicted sizes of corresponding FKBP51 promoter regions

region	sm	h
A	0.60 kb	0.90 kb
B	0.80 kb	1.50 kb



A comparison of the sm51 promoter revealed that the h51 promoter contains two repetitive sequences of ~ 300 bp each. These repeats are **absent** in the sm51 promoter.

Repetitive sequences in the h51 promoter increase the size of the promoter relative to the sm51 promoter. This is reflected in the difference in sizes of PCR products spanning the regions represented by A and B in the schematic.

Conclusions

The New World primate (sm and om) FKBP51 promoters exhibit greater sequence similarity than the Old World (human) and New World primate FKBP51 promoters.

Our current data suggests that the sm and om51 promoters are each missing repetitive sequences that are present in the human promoter.

The inclusion of 2 repetitive sequences in the h51 promoter increases the promoter length approximately 700 bp relative to the sm51 promoter.

Future Directions

The promoter sequence from 300 bp upstream of the transcription start site (-300) to the transcription start site (+1) is important for basal expression of the FKBP51 gene.

The DNA sequence of this region has been determined for the sm and h51 promoters. This region of the om51 promoter will be determined and compared to the sm and h51 promoter sequences.

Once the complete om51 promoter sequence is obtained, the sm and om51 promoters will be cloned into a reporter vector to study their activity (ability to cause gene expression) relative to the h51 promoter.

Acknowledgments

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References

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