Insights in promoter transactivation activity of CBX2

Dirk Hart [1], Anna Biason-Lauber [1]

[1] Endocrinology division, Section of Medicine, University of Fribourg, Fribourg, Switzerland

Correspondence: dirk.hart@unifr.ch

Background

Sexual differentiation is critical for reproduction in nearly all metazoan. Defects in any of the genes involved in either testicular or ovarian development can result in differences of sex development (DSD). CBX2 is a chromatin modifier that plays an important role in sexual development and its disorders, and exists in two isoforms. The promoter of these variants is unknown, however there are hints of differential expression by the isoforms in different cell lines and tissues.

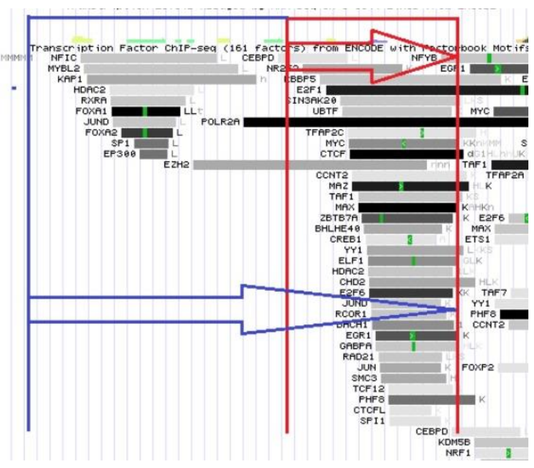
Objective

To characterize the differential regulation of CBX2 transcription in applicable cell lines.

Methodology

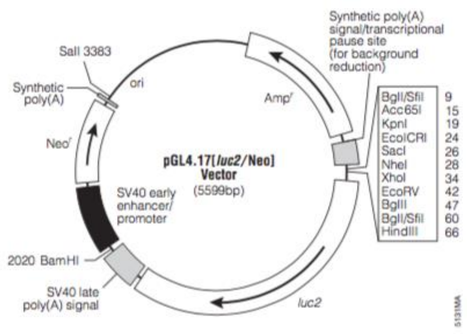
i) Cell culture & cloning:

To locate candidate CBX2 promoter regions, primer sequences were designed targeting transcription.



Predicted transcription binding sites assayed from ENCODE

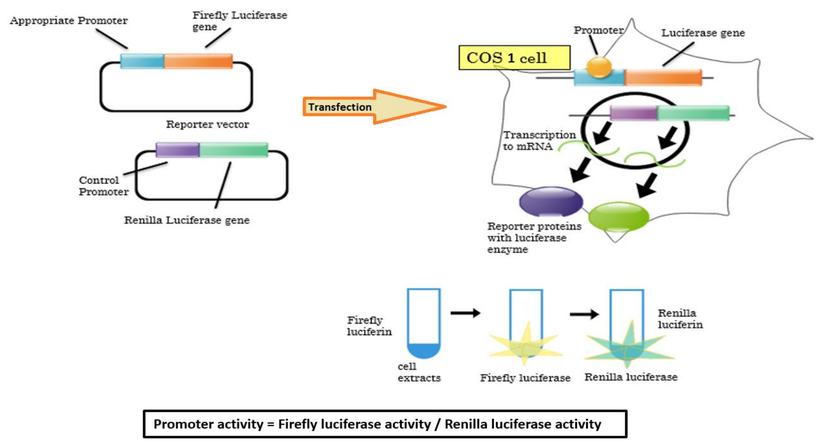
Amplified DNA fragments were cloned as reporter inserts into the pGL4.17 Vector which lacks a promoter, requires expression of SV40 T antigen, and encodes the luciferase reporter gene luc2.



Promega Corporation, Madison, USA

ii) Dual luciferase system:

Custom promoter constructs were transfected in COS-1 cells (SV40 transformed cell type), and subsequently in HeLa & HEK293T cells to determine the regulation of CBX2 transactivation activity.



Promoter activity = Firefly luciferase activity / Renilla luciferase activity

Reporter activity was established by performing a dual-reporter assay measuring Firefly and *Renilla* luciferases.

Results

Utilizing the dual-reporter assay system, we identified an optimal candidate CBX2 promoter construct that exhibited a 3.6 normalized fold change in activity when compared to a negative control ($p<0.0074$). (Fig.1)

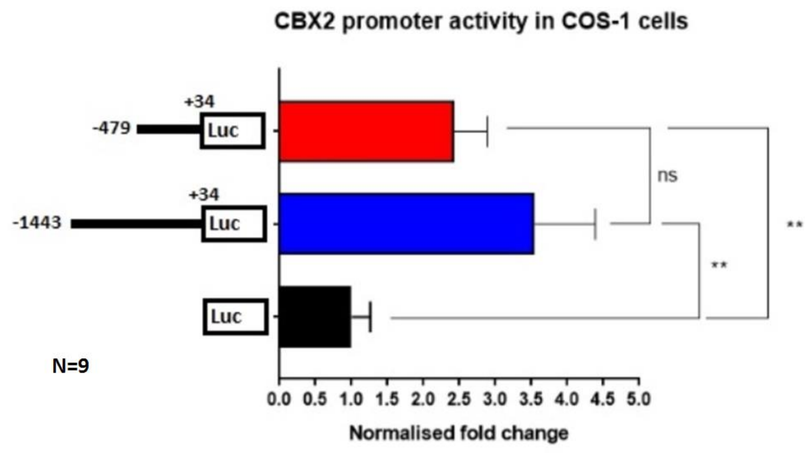


Figure 1. CBX2 promoter activity in COS-1 cells. Promoter activity of CBX2 constructs is expressed as relative to an empty vector (Luc). Error bars represent the mean ± SD of three independent experiments.

Subsequently, the selected CBX2 promoter construct exhibited significant transactivation potential in the HeLa cell line (3.11 normalized fold change) ($p<0.0038$). (Fig.2)

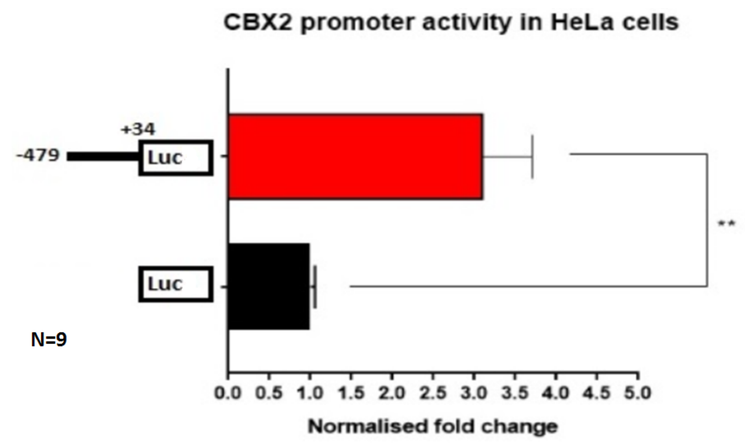


Figure 2. CBX2 promoter activity in HeLa cells. Promoter activity of CBX2 constructs is expressed as relative to an empty vector (Luc). Error bars represent the mean ± SD of three independent experiments.

The CBX2 promoter did not exhibit significant transactivation in the HEK293T cell line (1.5 normalized fold change) ($p=0.0748$). (Fig.3)

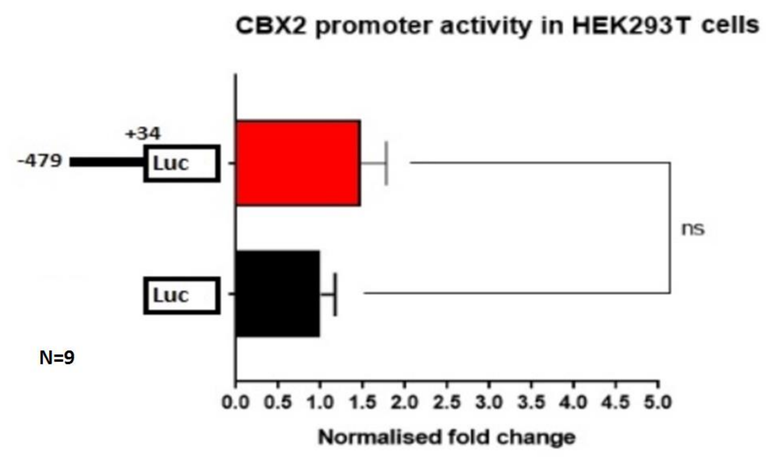


Figure 3. CBX2 promoter activity in HEK293 cells. Promoter activity of CBX2 constructs is expressed as relative to an empty vector (Luc). Error bars represent the mean ± SD of three independent experiments.

Conclusions

- **Preliminary results indicate our promoter construct may be applied to characterize differential transactivation of CBX2 in cell models recapitulating ovaries, testis and adrenal cells, and thereby elucidate its functional role as transactivator, distinct from its known function as chromatin-modifier.**
- **Further study of the impact of CBX2 activation and suppression may shed light on potential pathological mechanisms involved in DSD, and ultimately its diagnosis and management.**

References

1. Biason-Lauber A, Konrad D, Meyer M et al. Ovaries and female phenotype in a girl with 46, XY karyotype and mutations in the CBX2 gene. *American Journal of Human Genetics.* 2009; **23**(1): 658-663.

2. Ohnesorg T, Vilain E, Sinclair AH. The Genetics of Disorders of Sex Development in Humans. *Sexual development: genetics, molecular biology, evolution, endocrinology, embryology, and pathology of sex determination and differentiation.* 2014.

3. Biason-Lauber A. Control of sex development. *Best practice, research. Clinical endocrinology, metabolism.* 2010; **24**(2): 163-186.

Acknowledgements

Project supervisor:
Prof. Anna Biason-Lauber

Endocrinology lab: Dr. Daniel Rodriguez Gutierrez
Dr. Patrick Sproll
Ivan Domènech Mercadé
Anne Kolly
Maya Corminboeuf

FNSNF
FONDS NATIONAL SUISSE
SCHWEIZERISCHER NATIONALFONDS
FONDO NAZIONALE SVIZZERO
SWISS NATIONAL SCIENCE FOUNDATION