SCIENTIST SPOTLIGHT

ARIMA GENOMICS

CELEBRATING SCIENCE & SCIENTISTS

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RESEARCH SNAPSHOT

Research Area	Gene regulation
Species/Sample Type	Mouse embryonic stem cells E14Tg2a
Arima Product	Arima-HiC+ Kit
Application/Workflow	Arima-HiChIP

DEFINING THE RELATIVE AND COMBINED CONTRIBUTION OF CTCF AND CTCFL TO GENOMIC REGULATION

CTCF is highly involved in organizing chromatin into topologically-associated domain (TAD) structures. Its paralogue, CTCFL (CTCF-like), is implicated in cancer due to aberrant expression. This study examines the relationship between the two genes, how their relationship contributes to chromosome organization and transcription, and how these processes may be dysregulated in cancer. The Arima-HiC kit revealed the impact of CTCF and fusion proteins on chromatin organization.

Nishana M., et al. Genome Biology. 2020. doi: 10.1186/s13059-020-02024-0

RESEARCH QUESTION:

How do CTCF and CTCFL regulate chromosome organization and transcription?

HOW DID ARIMA GENOMICS MAKE A DIFFERENCE?

"The robustness and reliability of the Arima-HiC+ kit makes it an excellent tool for understanding gene regulation. We love working with Arima and have always had great, collaborative interactions with the team."

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EXPERIMENT OVERVIEW

Cell lines and DNA constructs

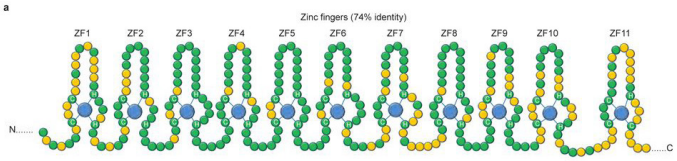
- Construction of vector for cloning transgenic, doxycycline-inducible expression of CTCFL
- Construction of vector for CTCF and CTCFL with the terminals swapped

Gene targeting

- Induction of auxin-inducible degradation of CTCF and doxycycline-induced expression
- Western blotting
- RNase A treatment
- Immunoprecipitation
- Flow cytometric analysis
- Microscopy
- ChIPmentation
- RNA-Seq
- Arima-HiC

Quantification, statistical analysis, and downstream analysis

- Data processing and quality control
- Annotation of ChIP peak sets and motif analysis
- Compartments, TADs, and boundaries
    - o Compartment analysis
    - o Domain boundary insulation scores
    - o Loop analysis



Schematic representation of the similarities and differences between CTCF and CTCFL. Figure adapted from Marshall et al. The DNA binding domain of both proteins is composed of 11 zinc fingers. ZFs 1-10 and ZF11 belong to the C_2H_2 and C_2HC class of ZFs, respectively. Shared and different amino acids in CTCF and CTCFL are shown in green and yellow, respectively. Blue circles indicate zinc ions and histidines and cysteines that form coordinate bonds with zinc are marked.

ACCOMPLISHMENTS / RESULTS / FUTURE DIRECTIONS

Similarities in zinc finger formation between CTCF and CTCFL allow the two proteins to compete for binding sites. Each protein exhibited a preference for certain types of binding sites, influenced by C/N terminal domains. RNA-binding regions of the zinc fingers also contribute to CTCF binding and 3D chromatin organization. In the absence of CTCF, CTCFL cannot rescue deficits in chromatin folding due to its inability to interact with cohesin. CTCFL also activates cancer testes antigens (CTA) and other components of cancer-relevant signaling pathways. This study sheds light on zinc finger and C/N terminus involvement in chromatin organization and presented CTCF and CTCFL inducible expression as a useful tool for studying this topic. The CTCF/CTCFL complementation system provides a useful too to to study the interaction between these two proteins. Future work will investigate the cofactors regulating these important proteins.

1. Marshall AD, Bailey CG, Rasko JE. CTCF and BORIS in genome regulation and cancer. Curr Opin Genet Dev. 2014;24:8–15.