Inter-chromosomal Interactions of Noncoding RNA Loci Visualized by CRISPR-Display

Introduction: **Nuclear architecture plays a critical role in gene regulation**. Evidence suggests that the genome is organized into distinct regions of local intra-chromosomal, such as promoters and enhancers, and broader inter-chromosomal interactions[1]. Despite the importance of these interactions on human disease such as the limb deformations of brachlydactly and developmental disorder like polymicrogyria, the dynamics and mechanism of formation of these chromosomal interactions are still largely uncharacterized.

Noncoding RNA has been implicated in establishing and maintaining nuclear architecture. For example, the long noncoding RNA *XIST* is capable of inducing large scale silencing, compaction, and periphery localization of the inactive X chromosome in female cells[2]. Of particular interest to this study is recent work indicating that **lncRNAs can mediate inter-chromosomal interactions** in mammalian cells. For example, the lncRNA *CISTR-ACT* regulates Sox9, resulting in chromosome 12 (the location of the *CISTR-ACT* locus) in spatial proximity to chromosome X (the location of the Sox9 locus).[3] Additionally, the lncRNA *FIRRE* is required for interactions between metabolism-associated genes on several chromosomes[4]. These examples suggest that lncRNA might play a broader role in establishing inter-chromosomal interactions that remains unexplored.

In this proposal, I will interrogate the role on lncRNA mediated inter-chromosomal interactions using a novel live-cell imaging technique. **I hypothesize that certain lncRNA loci form stable inter-chromosomal interactions with genomic loci in spatial proximity.**

Method: **CRISPR-display (CRISP-disp)[6],** a recent technique published by the Rinn lab, overcomes many limitations of the techniques currently used to probe chromosome interactions Fluorescence In Situ Hybridization (FISH) and Chromosome Conformation Capture technologies (3C to HiC). Both FISH and HiC require non-living fixed cells, which inherently limits the ability to study dynamic cellular processes. Additionally, both have limited spatial resolution as FISH projects the 3D nucleus into a 2D image and HiC is performed on bulk cell populations. In contrast, we have shown that CRISP-disp can be used for **3D live-cell imaging of a single genomic locus[6]**. For this application, inactive dCas9 targets an extended guide RNA (gRNA) containing a RNA-recognition loop to a locus of interest. The corresponding RNA-binding motif fused to a fluorophore is then recruited to the locus for imaging (Figure 1). This system is **modular** and can be used to independently target multiple genomic loci through the use of orthogonal RNA-recognition loops and RNA-binding protein pairs[6]. Preliminary data in the Rinn lab demonstrates that CRISP-disp can be used to visualize the long-range intra-chromosomal interactions of the *XIST* locus in live cells. The independently labeled *XIST* locus and distal regions on the X chromosome co-localize on the condensed inactive X chromosome, but not on the active X chromosome in female cells. This validates that CRISP-disp can be used to study how lncRNA loci contribute to chromosomal interactions.

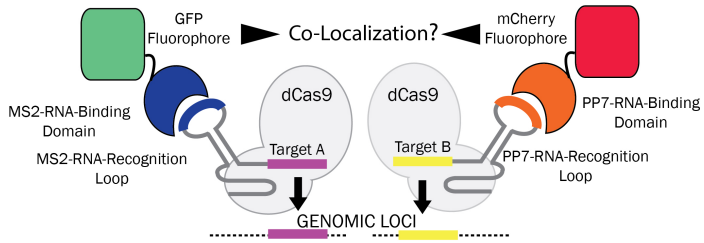

Figure 1: CRISPR-display Schematic. dCas9 (light gray) escorts a fluorophore (green or red) to a targeted genomic loci (purple or yellow) through an RNA-protein interaction (blue or orange) encoded in an extended guide RNA molecule (dark gray).

AIM1: **To determine the stability of inter-chromosomal interactions in live cells,** I will visualize the inter-chromosomal interactions mediated by the lncRNA *CISTR-ACT* during

interphase. I will design orthogonal CRISP-disp sgRNAs to the *CISTR-ACT* locus on chromosome 12 and to the region on the X chromosome with which it has been genetically and biochemically associated[3]. To evaluate the stability of the interaction, I will calculate the half-life of the interaction during time-lapse imaging. If the interaction is stable, I expect co-localization of the loci during imaging. If the interaction is transient, I expect the loci to move in and out of contact with one another. I will perform analogous experiments for *FIRRE*. Combined, this work will elucidate the dynamics of established inter-chromosomal interactions mediated by lncRNAs.

AIM2: **To explore the mechanism of lncRNA mediated inter-chromosomal interactions,** I will visualize the formation of inter-chromosomal interactions. To test this, I will replace the endogenous *FIRRE* promoter with a doxycycline inducible promoter in hESCs, a cell line that endogenously expresses *FIRRE* and forms *FIRRE*-mediated inter-chromosomal interactions[4]. I will use CRISP-disp live cell imaging targeting the *FIRRE* locus and previously identified inter-chromosomal interactions[4] to visualize the formation of these interactions upon induction of *FIRRE* lncRNA. In un-induced cells with no *FIRRE* expression, I expect that the *FIRRE* locus will be close to but not interacting with its inter-chromosomal partners. Upon *FIRRE* expression, I expect that the *FIRRE* locus will form interactions with its inter-chromosomal partners in an order correlated with their distance from the locus before induction. If the *FIRRE* locus does not follow this proximity-space approach, it would suggest that other factors might be required. I would then screen potential protein factors, beginning with the known nuclear architecture proteins hnRNPU and CTCF, to better understand how these interactions are mediated. This work would provide the first microscopy data showing how a lncRNA locus can exploit pre-existing spatial organization of chromosomes to form specific inter-chromosomal contacts.

AIM3: **To identify novel lncRNA mediated inter-chromosomal interactions,** I will find and validate inter-chromosomal contacts identified by publically available HiC data[5]. To identify these candidate lncRNAs, I will search for lncRNA loci with high inter-chromosomal contact frequencies in publically available HiC data[5] in collaboration with computational biologists in my lab. I will then visualize the top five loci and their inter-chromosomal interactions with CRISP-disp to confirm or rebut the existence of these interactions *in vivo*. After validating an inter-chromosomal interaction, I will knock out the associated lncRNA by inserting a transcription terminator. If the inter-chromosomal interaction is perturbed, this indicates that it is in part mediated by the lncRNA itself. Overall, this aim will elucidate the extent to which lncRNA loci contribute to inter-chromosomal interactions and will identify future lncRNA interactions to be studied. Additionally, this will provide the first spatial context to the large amount of sequence data in HiC experiments. If no interactions can be validated, this would suggest that we should re-evaluate our confidence in the widely used HiC technique.

Intellectual Merit and Broader Impact: This proposal represents the first demonstration of 4D live-cell imaging of inter-chromosomal interactions and dynamics. It paves the way for numerous follow up studies to further our understanding of how nuclear architecture is established and how it contributes to gene expression. Furthermore, I will use my research as an opportunity to continue teaching and mentoring the next generation of scientists both in an out of a research lab environment.

References: [1] Gorkin et al. Cell, 2014 [2] LncRNA Review [3] Maas et al. J. Clinical Investigation, 2012 [4] Hacisuleyman et al. Nature Structure and Molecular Biology, 2012 [5] Lieberman-Aiden et al. Science, 2009 [6] Shechner et al. Nature Methods, 2015.