Nima Ghorashi

Remodeling Chromatin Structure in Heart Failure

Nima Ghorashi, BS; Douglas J. Chapski, BS; Todd H. Kimball, BS; Elaheh Karbassi, PhD; Emma Monte, PhD; Thomas M. Vondriska, PhD; Manuel Rosa-Garrido, PhD.

Department of Anesthesiology and Perioperative Medicine; Department of Medicine; Department of Physiology; Department of Molecular, Cellular and Developmental Biology, UCLA, Los Angeles, CA.

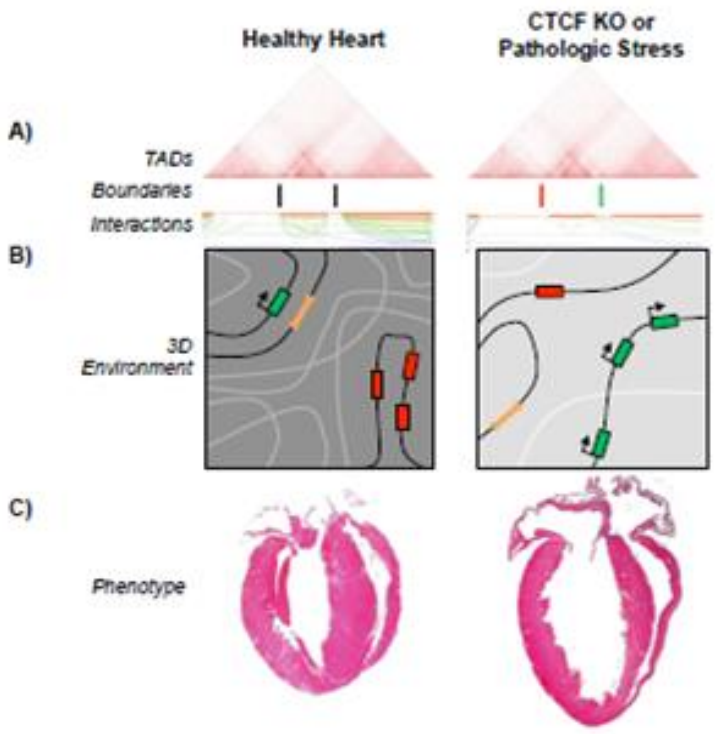

Figure 1. Connection between chromatin structure and cardiac phenotype. A) *Top*, HIC data models detected interactions between loci along the genome. These interactions can be grouped into individual TADs based on determined boundaries. Boundaries are 50kb long regions established on the basis of computer analysis where statistically determined significant interactions tend not to be found between regions of different TADs. There is a significant decrease of interactions seen between the control of a healthy heart and CTCF KO or pathologic stress models. B) *Middle*, Representation of the hypothesis that three-dimensional organization of the genome is not conserved between models resulting in loss of promotor-enhancer interactions and chromatin loop dynamics. C) *Bottom*, Three-dimensional reorganization of the genome represented by changes in gene expression patterns between models is seen to correlate with cardiac diseased phenotype.

Cardiac hypertrophy is a response to pressure or volume stress-overload on the heart leading to an increase in cell size without a change in the number of cells in the heart[1,2]. This diseased state seen in cardiac physiology presents adaptive changes both in gene expression and large-scale transcriptional modifications by means of DNA methylation, histone modifications, and chromatin remodelers[3]. The three-dimensional organization of a genome plays an essential role in regulating cardiac gene expression (Figure 1A,B); however, three-dimensional configuration of the genome and its role in heart disease had previously been undetermined. CTCF, a chromatin binding protein, plays a critical role in modulating both genome architecture and maintaining genome accessibility across cell types. Additionally, our data show CTCF depletion was sufficient enough to alter cardiac phenotype and induce heart failure in mice (Figure 1C). These adaptive changes in chromatin states had been known to play an essential role in the reprogramming of cardiac genes under physiological stress; however, it was not well understood how these changes in both gene expression patterns and chromatin remodeling are coordinated in the onset of heart disease.

To better understand this relationship, we examined endogenous genome structure by chromosome conformation capture (Hi-C) in concert with measurements of gene expression using RNA-seq. Hi-C structural data, RNA-seq translational data, and CHiP-seq CTCF binding sites provided insight as to how changes in chromatin structure mediate gene expression. Topologically associated domains (TADs) are highly conserved regions of chromatin compartments with high levels of interaction within a TAD region and little or no interaction between other TAD regions. TAD boundary elements are DNA or epigenetic elements that are defined to be localized between two TADs where inter-chromosomal interactions are prevented or minimized. It is well known that these boundaries are enriched in different proteins such as CTCF and cohesins. Our findings demonstrate that weakening of TAD boundaries in pressure-overload-induced heart disease or CTCF KO experiments correlate with the structural reorganization of chromatin. Furthermore, analysis of our data suggests heart failure reduces the number of intra-TAD interactions influencing the detected changes in gene expression. Our data suggests there is a relationship between chromatin organization, gene expression, and phenotypic states[4]. However, Hi-C does not allow for the examination of specific loci of the genome. On the other hand, chromosome imaging techniques allows for the detection of chromatin organization involving specific loci of genes along the genome within TADs, drawing a connection between physical interactions on chromatin to organ-level disease phenotype[5]. High-definition fluorescent *in situ* hybridization (HD-FISH), a method relying on fluorophore-labeled DNA to localize specific genes or regions on a chromosome within the nucleus, allows for the detection of spatial reorganization of chromatin modeled by Hi-C data and detected changes in gene expression[6,7]. We hypothesize that

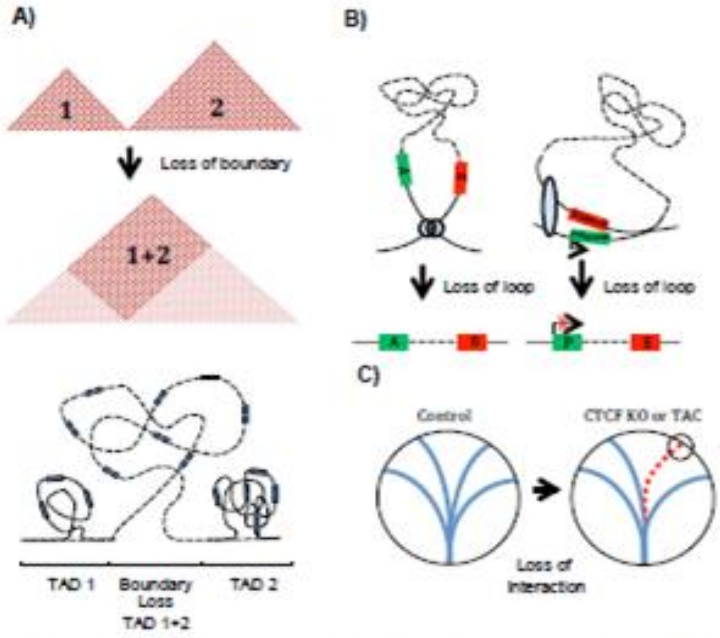

Figure 2. Conditions of interest regarding reorganizational patterns of the genome. A) *Top*, Contact matrix showing two TADs (TAD 1 and TAD 2) in one condition, that merge into one TAD (TAD 1+2) upon loss of TAD boundary in another condition. *Bottom*, Illustration depicts loss of TAD boundary, in which two TADs from one condition (black lines) merge into one larger TAD (dashed lines). B) Top, Chromatin loop coordinated by a loop mediator (LM) favoring the co-regulation of Gene A and Gene B. Bottom, Loss of loop resulting in a loss in 3D proximity and a loss of co-regulation between Gene A and Gene B (left panel). Chromatin loop allowing the interaction enhancer-promoter. Bottom, Loss of regulatory loop resulting in a loss in proximity between enhancer and promoter inducing the transcriptional inactivity of the gene (right panel). C) Control condition showing interactions within a chromosome (Left panel). Diseased condition showing a loss of interaction between two specific regions within a chromosome (red-dotted line, right panel).

changes in the expression of cardiac-specific genes during heart failure is mediated by the reorganization in global chromatin structure. We expect to detect changes in the three-dimensional distribution of the genes within the nucleus due to changes in gene expression revealed from our data. HD-FISH will be used to confirm our bioinformatics results comparing a healthy heart, pathologic pressure overload via transverse aortic constriction surgery (TAC), and CTCF depleted chromatin models in adult isolated cardiomyocytes. We will focus on chosen loci of interest from bioinformatics analysis orchestrating three possible modifications to chromatin structure between mouse model conditions (Figure 2): A) Changes in TAD dynamics, B) changes in chromatin looping and C) changes in intrachromosomal interactions between two specific genomic regions.

References

1. Frey, N. *et al.* Hypertrophy of the heart: A new therapeutic target? *Circulation.*;109:1580-1589 (2014)

2. Ahuja P., Sdek P. & MacLellan W. R. Cardiac myocyte cell cycle control in development, disease, and regeneration. *Physiol Rev* 87, 521–44 (2007).

3. Rosa-Garrido, M *et al.* Regulation of chromatin structure in the cardiovascular system. *Circulation Journal* (2013).

4. Rosa-Garrido, M *et al.* High Resolution Mapping of Chromatin Conformation in Cardiac Myocytes Reveals Structural Remodeling of the Epigenome in Heart Failure. *Circulation Journal* (2017).

5. Schmitt, D. Anthony, *et al.* Genome-wide mapping and analysis of chromosome architecture. *Nature Reviews Molecular Cell Biology,* 17, 743-755 (2016).

6. Bienko, M. *et al.* A versatile genome-scale PCR-based pipeline for high-definition DNA FISH. *Nature* Vol. 10 No. 2, 122-124 (2013).

7. Solovei I, Cremer M., 3D-FISH on cultured cells combined with immunostaining. *Methods Mol Biol* 659: 117–126 (2010).