Structural Reorganization of Cardiac Transcription Factories Mediates Transcriptional Changes in Response to Stress

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The heart's response to stress entails precise gene expression changes to affect the metabolic and structural features of the cardiomyocyte. The changes in gene expression are mediated by structural alterations in the packaging of the genome. However, the manner in which the three-dimensional architecture of the genome is established is unknown. In non-cardiac cells, genes that are actively transcribed are thought to reside in transcriptionally permissive compartments called transcription factories. The structural principles for achieving cardiac-specific transcription are not understood. We sought to understand the functional nature of cardiac transcription factories: whether they are stable structures (to which genes move in and out of) or are transiently formed around genes in response to cardiac stimuli. Using 5-fluorouridine incorporation into nascent RNA, we quantified changes in RNA polymerase II-mediated transcription in cardiomyocytes upon hypertrophic stress. Furthermore, we characterized the spatial distribution of transcription factories, marked by RNA polymerase II, from adult mice subjected to pressure overload. Using super-resolution microscopy, our analyses revealed reorganization of RNA polymerase II, evidenced by a significant increase in the distance between clusters (130nm in sham to 132.5nm in failing hearts, p=0.02) and a 38% increase in cluster intensity in failing hearts. To understand regulation of cardiac gene expression, we used DNA fluorescence *in situ* hybridization to map the nuclear position of the gene for SERCA2a (atp2a2), which is down regulated in disease. In failing hearts, we measured increased association of atp2a2 with the nuclear envelope (0/159 loci in sham to 11/278 loci in failure) and increased colocalization with heterochromatin (53/160 loci in sham versus 139/290 loci in failure), providing a structural mechanism for the decrease in SERCA2a expression. In contrast, atp2a2 positioning in the liver remained unaffected, with the majority of loci colocalizing with heterochromatin. These findings show that RNA polymerase II is redistributed to affect transcriptional programming and characterize for the first time the structural rearrangements in chromatin that underpin cardiac pathology.

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