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TBX5 and NuRD Divide the Heart

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Abstract

In this issue of *Developmental Cell*, Waldron et al. (2016) identify an interaction between a master regulator of heart development, TBX5, and the NuRD complex and describe how mutations affecting the interaction may contribute to congenital heart disease. Furthermore, these interactions may have contributed to the evolution of cardiac septation.

The transcription factor TBX5 is a key regulator of heart development, with known roles in cardiac septation, conduction system development, and differentiation of cardiomyocytes (Mori and Bruneau, 2004). In humans, *TBX5* mutations are associated with Holt-Oram syndrome, an autosomal-dominant disease marked by hand and heart malformations, with the most common heart malformations being atrial septal defects (ASDs) and ventricular septal defects (VSDs) (Mori and Bruneau, 2004). Disease-associated mutations are predominantly within the DNA-binding T-box domain of TBX5, disturbing DNA binding and protein-protein interactions. For missense mutations outside the T-box, mechanistic insight is often lacking.

In this issue of *Developmental Cell*, Waldron et al. (2016) explore the in vivo TBX5 interactome, using affinity-purified TBX5 complexes expressed at physiological levels from adult mouse cardiac nuclei. Through mass spectrometry analysis, they identified 40 proteins that form a single interconnected network of transcription-associated genes. Among these were multiple complexes that play roles in RNAPol II-dependent gene transcription, as well as six components of the nucleosome remodeling and histone deacetylase (NuRD) complex: CHD4, MTA1, HDAC2, GATAD2a, GATAD2b, and RBBP4. The broadly expressed NuRD protein complex has both chromatin remodeling activity and histone deacetylase activity and is generally associated with repression of target gene transcription (Denslow and Wade, 2007). To further characterize the interaction, Waldron et al. used in vitro protein interaction assays and show that TBX5 directly interacts with CHD4, an ATPase-dependent chromatin-remodeling subunit of the NuRD complex. Deletion analyses showed that a 100-amino-acid domain adjacent to the T-box within TBX5 is required for the interaction, and this region was dubbed the NuRD interaction domain (NID). Based on 3D structural modeling, this

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domain is predicted to form a small coil-to-helix region that may support interaction with the NuRD complex.

To examine the significance of the interaction during heart development, the authors generated *Tbx5;Mta1* compound heterozygous mice and studied the hearts of E13.5 embryos. No cardiac defects were observed in *Mta1^{+/-}* pups, and *Tbx5^{+/-}* hearts displayed ASD and VSD with incomplete penetrance, as described previously (Mori and Bruneau, 2004). In contrast, septal defects were observed in all compound heterozygous embryos examined, suggesting that *Tbx5* functionally interacts with the NuRD complex in cardiac septation. To identify TBX5 targets, the authors performed transcriptome analysis of wild-type and *Tbx5* null hearts—and subsequent intersection of these data with available TBX5 ChIP-Seq studies in atrial-like cells—which led to identification of both upregulated and downregulated direct target genes, providing further support for a direct role of TBX5 in repression, which was subsequently validated using in vitro enhancer-reporter studies.

Interestingly, Waldron et al. identified five known Holt-Oram syndrome-associated *TBX5* missense mutations within the NID. When tested in vitro, these disease-associated mutant TBX5 proteins did not interact with CHD4, supporting the significance of TBX5-NuRD interactions for heart development and disease. However, mutations in NID may also interfere with proper folding of the T-box domain or with other protein-protein interactions. More support for a potential developmental role of the interaction came from phylogenetic analysis, which showed that a key residue required for TBX5-NuRD interaction is conserved in frogs, birds, and mammals—species with an atrial septum—but not in fish, which have a single atrium. The authors furthermore show that frog TBX5 interacts with CHD4. Notably, mutation of a key lysine residue of the NID (K266) into arginine, which is the aligned amino acid in the fish ortholog, abolishes TBX5 interaction with CHD4. Therefore, the TBX5-NuRD interaction may be significant for the evolution of atrial septation.

The identification of a TBX5-NuRD interaction adds support for previous observations that suggest requirements for NuRD complex function in heart development. Cardiac-specific ablation of the histone deacetylase subunits of the NuRD complex, HDAC1 and HDAC2, revealed a requirement for these factors in regulation of cardiomyocyte proliferation (Montgomery et al., 2007). More recently, interaction between the NuRD complex subunit MTA1 and the transcription factor FOG-2 was shown to be required for cardiac septation (Garnatz et al., 2014). In addition, the transcription factor *Sall4* interacts with the NuRD complex in embryonic stem cells and regulates ventricular septation through complex interactions with TBX5 (Koshiba-Takeuchi et al., 2006). From the foregoing, FOG-2, *SALL4*, and TBX5 may either cooperatively recruit the NuRD complex or compete for interaction with the NuRD complex to regulate cardiac septation. Previous work has also identified interactions between members of the TBX1 subfamily (TBX15, TBX18, and TBX20) and components of the NuRD complex in a Groucho-dependent manner (Farin et al., 2007; Kaltenbrun et al., 2013). However, the TBX1 subfamily interacts with the NuRD complex via a conserved domain not found within TBX5. In addition, TBX5 interacts with other transcription factors and chromatin remodeling complexes to regulate gene expression during heart development (Boogerd et al., 2009) (Figure 1). However, mechanisms by which TBX5 represses gene expression are largely unexplored. The present study thus provides

insight into mechanisms by which TBX5 represses gene transcription. It will be interesting to know whether genes upregulated in *Tbx5;Mta1* mutants contribute to the observed phenotypes. As the authors note, future studies will be required to differentiate between NuRD-dependent and -independent TBX5-mediated gene repression. Mouse models that harbor Holt-Oram syndrome-associated mutations in the NID might be one way to start to address these questions. Other questions that arise are whether TBX5 can also interact directly with other subunits of the NuRD complex, such as CHD3, and what the specific roles of individual NuRD complex components are during cardiogenesis. This will enable a better understanding of the full spectrum of NuRD complex function in heart development.

The four-chambered heart has evolved from a single layered tube with peristaltic contractility (Moorman and Christoffels, 2003). Division of the common atrium into right- and left-sided chambers, as observed in amphibians, birds, and mammals, represents an evolutionary milestone required for separation of oxygenated and deoxygenated blood. In amphibians, two atrial chambers exist, separated by a septum, connecting to a single ventricle. In fish, the heart is a single atrium connected to a single ventricle. The observation that a functional NID arose simultaneously with the advent of atrial septation gives insight into an exciting additional role for TBX5 in cardiac septation. Further support for this theory might be found in the lungfish, which already has an atrial septum comparable to the amphibian condition (Moorman and Christoffels, 2003). One wonders: Could the introduction of a functional NID in zebrafish TBX5 be sufficient to induce atrial septation? Furthermore, do interactions with the NuRD complex contribute to TBX5's known role in ventricular septation? These and other experiments should provide insight into the evolution of septation and the contribution of TBX5 and NuRD to the process.

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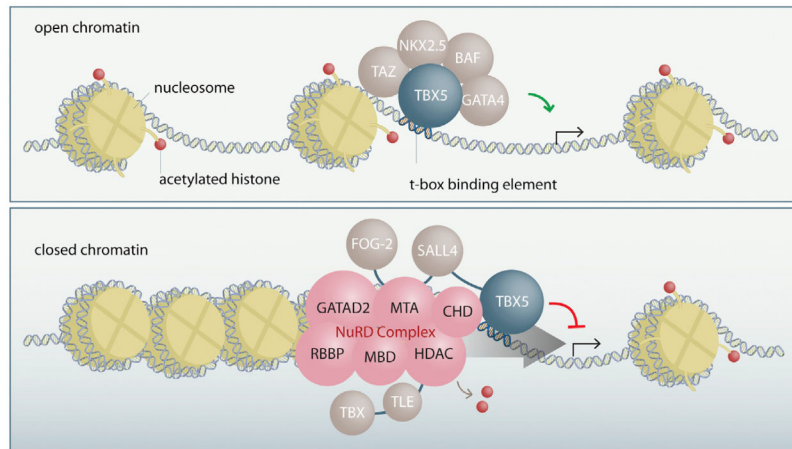


Figure 1. TBX5 Recruits the NuRD Complex to Repress Target Gene Expression

Active enhancers and transcriptionally active genes are marked by open chromatin through histone modifications, such as acetylation of histone tails. TBX5 interacts with the BAF chromatin remodeling complex and other transcription factors to activate expression of target genes (for review, see Boogerd et al., 2009). The model presented by Waldron et al. (2016) predicts that TBX5 binds the NuRD complex via interactions with CHD4 and recruits it to regulatory regions containing T-box binding elements. Additional cardiac transcription factors FOG-2 and SALL4, and T-box factors TBX15, TBX18, and TBX20 (TBX), also interact with distinct components of the NuRD complex. The NuRD complex deacetylates histones and remodels chromatin to a transcriptionally inactive state, repressing gene expression.