TBX5: A Key Regulator of Heart Development

J.D. Steimle and **I.P. Moskowitz**[1]

University of Chicago, Chicago, IL, United States

Abstract

TBX5 is a member of the T-box transcription factor family and is primarily known for its role in cardiac and forelimb development. Human patients with dominant mutations in *TBX5* are characterized by Holt–Oram syndrome, and show defects of the cardiac septa, cardiac conduction system, and the anterior forelimb. The range of cardiac defects associated with *TBX5* mutations in humans suggests multiple roles for the transcription factor in cardiac development and function. Animal models demonstrate similar defects and have provided a useful platform for investigating the roles of *TBX5* during embryonic development. During early cardiac development, *TBX5* appears to act primarily as a transcriptional activator of genes associated with cardiomyocyte maturation and upstream of morphological signals for septation. During later cardiac development, *TBX5* is required for patterning of the cardiac conduction system and maintenance of mature cardiomyocyte function. A comprehensive understanding of the integral roles of *TBX5* throughout cardiac development and adult life will be critical for understanding human cardiac morphology and function.

1. *TBX5,* A MEMBER OF THE T-BOX FAMILY OF TRANSCRIPTION FACTORS

Ever since the association of *TBX5* mutations with Holt–Oram syndrome (Basson et al., 1997; Li et al., 1997), the *TBX5* gene has been a source of study, particularly with respect to cardiac and limb development. This review covers progress since those initial reports, published two decades ago.

The T-box family of transcription factors share a common T-box DNA-binding domain (Bollag et al., 1994) and are named after the founding member, *T*, which encodes the transcription factor Brachyury (Herrmann, Labeit, Poustka, King, & Lehrach, 1990; Pflugfelder, Roth, & Poeck, 1992). The T-box domain is approximately 170–200 amino acids in length (Agulnik, Bollag, & Silver, 1995; Agulnik et al., 1996; Bollag et al., 1994; Papaioannou, 2014), binds DNA directly (Pflugfelder et al., 1992), and is required for transcriptional activity (Kispert, Koschorz, & Herrmann, 1995). All members of the T-box gene family appear to bind the DNA consensus motif AGGTGHBA (Conlon, Fairclough, Price, Casey, & Smith, 2001; He, Kong, Ma, & Pu, 2011; Jolma et al., 2013; Kispert et al., 1995; Mathelier et al., 2016; Waldron et al., 2016; Wilson & Conlon, 2002). While some members, such as *T* and *EOMES*, are only able to bind palindromic sequences as dimers (Conlon et al., 2001; Ghosh et al., 2001; Kispert et al., 1995; Muller & Herrmann, 1997),

[1]Corresponding author: imoskowitz@peds.bsd.uchicago.edu.

others, including *TBX2*, *TBX3*, and *TBX5*, can bind individual motifs as monomers (Bruneau et al., 2001; Carreira, Dexter, Yavuzer, Easty, & Goding, 1998; Ghosh et al., 2001; He, Wen, Campbell, Wu, & Rao, 1999).

In the human genome, there are 17 coding genes that fall within five subfamilies of the T-box family (Agulnik et al., 1996; Papaioannou & Silver, 1998), most of which show sequence similarity between vertebrates and invertebrates (Agulnik et al., 1995, 1996; Papaioannou & Silver, 1998; Pflugfelder et al., 1992; Ruvinsky, Silver, & Gibson-Brown, 2000; Wilson & Conlon, 2002). The human genes *TBX2*, *TBX3*, *TBX4*, and *TBX5* belong to one subfamily, and are homologous to the Drosophila gene *omb* (Agulnik et al., 1996). These four genes likely originated through tandem duplication of the ancestral gene through unequal crossover to form the ancestral *TBX2/3* and *TBX4/5* (Agulnik et al., 1996; Ruvinsky et al., 2000), followed by cluster duplication prior to the divergence of bony fish and tetrapods approximately 400 million years ago (Agulnik et al., 1996; Ruvinsky & Silver, 1997). This second duplication event generated one cluster containing *TBX2* and *TBX4* and a second containing *TBX3* and *TBX5*, which are located on human chromosomes 17 and 12, respectively (Agulnik et al., 1996; Ruvinsky & Silver, 1997).

The T-box family gene *TBX5* encodes a 518-amino acid protein with a 180-amino acid T-box domain located between amino acid residues 56 and 236 (Basson et al., 1997; Li et al., 1997). TBX5 contains two nuclear localization sequences (NLS): NLS1 located within the T-box domain (amino acids 78–90), and NLS2 located outside the T-box domain on the C-terminal end (325–340) (Collavoli et al., 2003; Zaragoza et al., 2004). While each NLS is sufficient to drive nuclear localization, they appear to work cooperatively (Collavoli et al., 2003). As a transcription factor, TBX5 also contains a transactivation domain located from amino acids 339–379 with functional requirement of amino acids 349–351 (Zaragoza et al., 2004). The sequence of amino acids 152–160 has been proposed to act as a nuclear export signal through the CRM1 export pathway by which TBX5 subcellular localization can be regulated through binding with the PDLIM7 protein (Camarata et al., 2006, 2010; Kulisz & Simon, 2008); however, this theory remains controversial as the crystal structure of TBX5 suggests this domain would be located on the inside of the protein and inaccessible without major protein rearrangements (Stirnimann, Ptchelkine, Grimm, & Muller, 2010). Aside from these domains, TBX5 also contains several other protein–protein interaction domains, discussed in detail later.

In addition to the best-described isoform, sometimes referred to as *TBX5a* (Georges, Nemer, Morin, Lefebvre, & Nemer, 2008), there have been four additional isoforms described in the literature (Georges et al., 2008; Yamak et al., 2015). These isoforms are derived from alternative splicing within the *TBX5* locus and result in proteins of varying lengths, including either an N-terminal or a C-terminal truncated form as well as forms with varying C-terminal modifications (Georges et al., 2008; Yamak et al., 2015). Interestingly, all described isoforms retain the T-box domain (Yamak et al., 2015). The alternative isoforms show differential expression and activity, including antagonism of TBX5a, and further investigation into these isoforms will be important for understanding the roles of *TBX5* in human development and health (Yamak et al., 2015).

2. *TBX5* EXPRESSION

The broad spatiotemporal expression domains of *TBX5* during development appear to be generally conserved throughout vertebrate evolution and consist of the heart, forelimb, and retina (Bruneau et al., 1999; Chapman et al., 1996; Gibson-Brown et al., 1996; Gibson-Brown, Agulnik, Silver, & Papaioannou, 1998; Horb & Thomsen, 1999; Showell, Christine, Mandel, & Conlon, 2006; Takabatake, Takabatake, & Takeshima, 2000); however, some tissue-specific expression differences occur between species. First, we will examine the expression domains of *TBX5* in the common tetrapod models as well as humans, with the greatest emphasis on subcardiac domains, and then we will discuss the regulation of *TBX5* gene expression based on evidence from mouse and human studies.

2.1 *TBX5* Expression Domains in the Embryonic and Adult Heart

The cardiac expression patterns of *TBX5* in human, mouse, chick, and frog are very similar. In human hearts, *TBX5* is expressed in the epicardium, myocardium, and endocardium of embryonic and adult hearts (Hatcher, Goldstein, Mah, Delia, & Basson, 2000). Human *TBX5* is expressed in the free walls and septa of all four chambers during development; however, atrial expression is much greater than ventricular, as seen in mouse and chicken (Hatcher et al., 2000). *TBX5* is expressed in the embryonic atrioventricular (AV) node, and is excluded from the AV valves (Hatcher et al., 2000). *TBX5* is expressed throughout the epicardium, but not in the endocardium of the left ventricle (Hatcher et al., 2000). Much like in animal models, *TBX5* expression is absent from the developing outflow tract of the heart (Hatcher et al., 2000). In human adults, *TBX5* expression is highest in the atrial appendages, followed by the lungs, left ventricle, and esophagus (GTEx Consortium, 2013; Mele et al., 2015).

In mice, *Tbx5* becomes abundantly expressed around E8.0 throughout the cardiac crescent (Bruneau et al., 1999), and this expression becomes restricted to the posterior portion of the forming heart tube, corresponding to the sinus venosa and future atria, between E8.25 and E8.5 (Bruneau et al., 1999; Chapman et al., 1996). At E9.0, *Tbx5* expression expands throughout the future left ventricle (Bruneau et al., 1999). Additionally, atrial expression of *Tbx5* is stronger than in the left ventricle, and in the ventricular free wall it is higher than in the trabeculae (Bruneau et al., 1999). *Tbx5* is also expressed in the right ventricular trabeculae, but not free wall (Bruneau et al., 1999). Expression of *Tbx5* in the left ventricle and atria is maintained throughout embryonic development (Chapman et al., 1996). During maturation of the mouse heart, like humans, *Tbx5* is expressed in and colocalizes with markers of the cardiac conduction system, including the AV bundle and bundle branch (Moskowitz et al., 2004). Genetic inducible fate mapping demonstrated that left ventricular *Tbx5* expression arises from the first heart field, specified prior to morphogenesis of the heart, whereas atrial and atrial septum *Tbx5* expression arises from *Mef2cAHF*[+] second heart field domain, suggesting potential independent roles of *Tbx5* in the first and second heart fields during development (Devine, Wythe, George, Koshiba-Takeuchi, & Bruneau, 2014).

In cardiac development of the chick, *Tbx5* expression is first detected throughout the entire bilateral cardiac primordia (Bruneau et al., 1999). This expression is maintained following

fusion of the heart tube along the entire rostrocaudal length (Gibson-Brown et al., 1998), but adopts an anterior-to-posterior gradient shortly after (Bruneau et al., 1999). Although there appears to be a gradient to the expression, *Tbx5* is expressed throughout the whole heart during cardiac looping (Gibson-Brown et al., 1998). After looping is complete, *Tbx5* expression remains in the entire heart except the outflow tract, and this is the only major difference between mouse and chick heart expression. However, as cardiac development and maturation proceed, expression of *Tbx5* is restricted from the right ventricle, similar to the expression pattern in the mouse embryo (Bruneau et al., 1999).

In *Xenopus*, the earliest expression domains of *tbx5* are in two lateral stripes, corresponding to the cardiac primordia, on either side of the embryo and continue to be expressed in the migrating precardiac mesoderm (Horb & Thomsen, 1999; Showell et al., 2006). Similar to chick cardiogenesis, after fusion at the midline and formation of the early heart tube, *tbx5* is expressed throughout most of the cardiac tissue including the sinus venosus/inflow tract of the heart (Horb & Thomsen, 1999; Showell et al., 2006). As development continues, *tbx5* expression is lost from the most anterior structure, the bulbus cordis, and is strongly detected in the posterior regions of the heart, while it is also maintained in the ventricle (Horb & Thomsen, 1999; Showell et al., 2006). *tbx5* is expressed robustly in both the endocardium and myocardium and is detected in the epicardium (Horb & Thomsen, 1999).

2.2 Extracardiac *TBX5* Expression

In addition to the cardiac expression domains, *TBX5* is expressed in many noncardiac tissues. Perhaps the best-studied expression domain outside of the heart is that of the developing forelimb. *Tbx5* is expressed in the lateral plate mesoderm giving rise to the forelimb starting at E8.8 of mouse embryonic development (Gibson-Brown et al., 1996) and is robustly expressed in the forelimb bud at E9.5 (Chapman et al., 1996; Gibson-Brown et al., 1996). Expression throughout the developing limb is maintained until E11.5, when it then becomes restricted to the proximal portion of the forelimb (Gibson-Brown et al., 1996). *Tbx5* is also expressed in the periochondrium of the forelimb at E13.5 (Gibson-Brown et al., 1996).

Outside of the heart and forelimb, *TBX5* expression has been reported in several notable domains during development. The earliest reported expression domain for *Tbx5* during mouse development is in the allantois at E7.5 where it is transiently coexpressed with *Tbx4* (Chapman et al., 1996). Expression of *Tbx5* in the allantois has been suggested to be a mammalian-specific trait, as transcription of *Tbx5* is never observed in the allantois of chick embryos (Gibson-Brown et al., 1998). *Tbx5* is also expressed in the optic vesicle and the neural retina of the developing eye in mouse, chick, and *Xenopus* where it is coexpressed with the other members of the *omb* family of T-box genes (Chapman et al., 1996; Gibson-Brown et al., 1998; Horb & Thomsen, 1999; Showell et al., 2006). Additionally, *Tbx5* is expressed in the mesenchyme of the mandibular arch, the trachea, and the lung, as well as the body wall of the thorax (Chapman et al., 1996; Gibson-Brown et al., 1998). In both mouse and chicken, expression of *Tbx5* has been reported in the genital papilla (Chapman et al., 1996; Gibson-Brown et al., 1998). Specific to avian development, *Tbx5* expression is observed in the notochord during midembryonic development (Gibson-Brown et al., 1998).

2.3 Transcriptional Regulation of *TBX5*

The mechanisms governing spatiotemporal regulation of *TBX5* have begun to be addressed by defining *cis*-regulatory elements driving *TBX5* expression in the embryo and adult. Preliminary investigations have identified several elements that drive distinct spatial domains during development; however, this area is ripe for future investigations. Tiling experiments have identified three enhancers associated with in vivo expression of *Tbx5* in the mammalian heart (Minguillon et al., 2012; Smemo et al., 2012). The first enhancer, corresponding to hg19 chr12:114,463,712–114,464,080, drives expression of a reporter construct in both ventricular and atrial myocardium of E11.5 mouse hearts (Smemo et al., 2012). The second enhancer, hg19 chr12:114,701,207–114,704,691, drives expression in the posterior portion of the heart, including the ventricles, interventricular septum, and AV canal (Smemo et al., 2012). Additionally, this second enhancer contains a low-frequency SNP that abrogates the enhancer's ability to drive expression (Smemo et al., 2012). While this SNP was predicted to disrupt a TAL1 binding site, *Tal1* is not expressed in the myocardium, suggesting other members of the basic helix–loop–helix E-box-binding transcription factors may be driving expression of this enhancer (Smemo et al., 2012). The third *Tbx5* enhancer, hg19 chr12:114,853,271–114,858,238, is sufficient to drive expression in the ventricles, interventricular septum, and AV canal as well as the atria (Smemo et al., 2012).

In addition to the identified cardiac enhancers, there have been two additional enhancers identified that regulate limb expression. The first is located within intron 2 of *Tbx5* and drives expression within the lateral plate mesoderm of the forelimb, but not the heart (Minguillon et al., 2012). This forelimb enhancer is regulated in part through Hox4/5 genes, expressed in the region of the lateral plate mesoderm that gives rise to the forelimb, and has been proposed as the mechanism by which forelimb *Tbx5* expression is positionally defined along the anterior–posterior body axis (Minguillon et al., 2012). The second forelimb enhancer identified is known as CNS12 and is located approximately 120 kbp downstream of the *Tbx5* coding region (Adachi, Robinson, Goolsbee, & Shubin, 2016). The CNS12 enhancer drives expression in the lateral plate mesoderm and is sufficient to drive *Tbx5* expression for forelimb formation (Adachi et al., 2016).

Taken together, *Tbx5* expression in the developing heart and forelimb appears to be driven by distinct *cis*-regulatory elements, although the factors and transcriptional complexes that control the expression of these enhancers have yet to be uncovered.

Recent insight into the regulation of *Tbx5* expression has come from investigations of the local three-dimensional architecture of the *Tbx5* locus and its neighboring genes: *Rbm19*, *Tbx3*, and *Med13l* (Jin et al., 2013; van Weerd et al., 2014). Circular chromosome conformation capture with sequencing (4C-seq) data from the viewpoints of the *Tbx3* and *Tbx5* promoters, and the CTCF binding site between the two loci, suggests that the loci are in contact and that putative *cis*-regulatory elements for each gene are located almost exclusively within their own loci with CTCF sites acting as a regulatory barrier (Fig. 1) (van Weerd et al., 2014). Additionally, 4C-seq from the viewpoint of *Rbm19*, the nearest gene 3′ of *Tbx5*, suggests partially overlapping regulatory elements (van Weerd et al., 2014). Together, this suggests that most of the *cis*-regulatory information for *Tbx5* expression is

located in the 375 kbp region between the CTCF sites demarcating the boundaries of the *Tbx3*/*Tbx5* and the *Tbx5*/*Rbm19* loci (Fig. 1) (van Weerd et al., 2014).

In addition to transcriptional regulation, *Tbx5* has also been shown to be regulated through microRNA-dependent mechanisms (Wang et al., 2014). In a screen of candidate human microRNAs, MiR-10a and MiR-10b were shown to bind to the 3′-UTR of the *Tbx5* and inhibit its translation (Wang et al., 2014). More recently, it has been shown that regulation through these two microRNAs may play a role in adult conduction defects and pathological remodeling in disease-state hearts (Torrado et al., 2015).

3. *TBX5* HAPLOINSUFFICIENCY: HOLT–ORAM SYNDROME

Holt–Oram syndrome is an autosomal dominant disorder caused primarily by dominant mutations in *TBX5*. Holt–Oram syndrome is a clinical diagnosis that includes completely penetrant, variably expressed upper-limb malformations including preaxial radial ray anomalies and congenital heart defects, typically septal and/or conduction defects (Holt & Oram, 1960). Although sometimes difficult to detect, upper-limb malformations are fully penetrant, while structural cardiac defects occur in 76% of patients with Holt–Oram syndrome (Basson et al., 1994, 1999; Holt & Oram, 1960; Newbury-Ecob, Leanage, Raeburn, & Young, 1996). Holt–Oram syndrome affects 1 in 100,000–135,000 live births in European populations (Barisic et al., 2014; Elek, Vitez, & Czeizel, 1991), although defects can occur in any population (Al-Qattan & Abou Al-Shaar, 2015; Ekure, Okoromah, Briggs, & Ajenifuja, 2004; Kimura, Kikuchi, Ichinoi, & Kure, 2015; Najjar, Mardini, Tabbaa, & Nyhan, 1988). Holt–Oram syndrome exhibits classic Mendelian inheritance for a dominant trait (Basson et al., 1997, 1994; Holt & Oram, 1960; McDermott et al., 2005), and the risk of nonaffected parents with an affected proband giving rise to a second child with a de novo pathogenic mutation is the same as the average population (McDermott, Fong, & Basson, 1993). Manifestations of upper-limb defects can include single or combinatorial abnormalities of the hand and digits, bones of the lower arm, humerus, or shoulder girdle (Basson et al., 1994; Newbury-Ecob et al., 1996). Defects of the hand and digits must include defects of the thumb for the Holt–Oram diagnosis, while defects of the lower arm are associated primarily with the radius (Basson et al., 1994; Holt & Oram, 1960; McDermott et al., 2005; Newbury-Ecob et al., 1996). Structural abnormalities of the heart can include secundum-type atrial septal defects, primum-type atrial septal defects, and/or ventricular septal defects (Basson et al., 1994; Holt & Oram, 1960; Newbury-Ecob et al., 1996). Conduction system defects manifest as long PR interval, AV block, bundle branch block, bradycardia, sick sinus syndrome, and atrial fibrillation (Basson et al., 1994; Holt & Oram, 1960; Newbury-Ecob et al., 1996), and these conduction defects can occur in the absence of overt structural defects (Basson et al., 1994; Newbury-Ecob et al., 1996). Holt–Oram syndrome is not associated with defects of the lower limb, postaxial upper limb, gastrointestinal system, genitourinary, or nervous system, which if present suggest an alternative diagnosis (Basson et al., 1994; Debeer, Race, Gewillig, Devriendt, & Frijns, 2007; Holt & Oram, 1960; McDermott et al., 2005; Newbury-Ecob et al., 1996).

Current evidence supports a model in which Holt–Oram syndrome is caused by *TBX5* haploinsufficiency (Basson et al., 1997; Li et al., 1997) with genetic abnormalities

associated with *TBX5* coding or splice regulatory sequences underlying approximately 70% of patients meeting strict clinical diagnoses (Debeer et al., 2007; McDermott et al., 2005). In the latest collection of the Human Gene Mutation Database, there have been 103 reported mutations in coding, splicing, or regulatory sequences of *TBX5*, which result in Holt–Oram syndrome or other cardiac defects (Stenson et al., 2014), recently reviewed in Yamak et al. (2015). Additionally, there have been 44 pathologic point mutations reported in the coding region of *TBX5* (Stenson et al., 2014). Similar to the eight reported gross deletions (Stenson et al., 2014), some of these are nonsense mutations resulting in highly truncated proteins that are thought to act as null alleles (Basson et al., 1997; Fan et al., 2003; Gruenauer-Kloevekorn & Froster, 2003; McDermott et al., 2005). Missense mutations have been reported throughout much of the T-box domain, typically resulting in transcriptional decrements (Basson et al., 1999; Boogerd et al., 2010; McDermott et al., 2005; Postma et al., 2008). There are also several reported mutations that result in missplicing or alternative splicing (Basson et al., 1999; Borozdin et al., 2006; Cross et al., 2000; Heinritz et al., 2005; McDermott et al., 2005; Vianna, Miura, Pereira, & Jatene, 2011). Interestingly, duplications of *TBX5* are pathogenic, resulting in atypical Holt–Oram syndrome (Kimura et al., 2015; Patel, Silcock, McMullan, Brueton, & Cox, 2012). Additionally, a patient with a homozygous, single-base-pair mutation within a *cis*-regulatory element controlling *TBX5* displayed decreased *TBX5* expression and non-syndromic congenital heart disease, raising the possibility that the etiology of some of the remaining 30% of Holt–Oram patients may result from *cis*-regulatory element mutations (Smemo et al., 2012).

While all cases of Holt–Oram syndrome result in both cardiac and forelimb defects, several cases have been reported in which defects in either the heart (Basson et al., 1997; Brassington et al., 2003; Li et al., 1997; Yang et al., 2000) or the limb (Brassington et al., 2003; Li et al., 1997; Yang et al., 2000) appear more severe in one tissue than the other, suggesting a potential tissue-specific role for distinct domains of the protein (Isphording, Leylek, Yeung, Mischel, & Simon, 2004). While the underlying mechanisms of these biased defects have not been identified, some proposed models include differences in binding partners and binding motif recognition (Basson et al., 1999; Camarata et al., 2006; Garg et al., 2003; Isphording et al., 2004; Krause et al., 2004).

4. ANIMAL MODEL OF HOLT–ORAM SYNDROME

Investigations into the role of *TBX5* in cardiac development have been undertaken in most major animal model systems: mouse, chick, frog, and zebrafish. Each system provides a unique set of tools for investigating the role of *TBX5* in the developmental etiology of Holt–Oram syndrome. The most well-characterized model of Holt–Oram syndrome is the mouse heterozygous for a *Tbx5* knockout allele. The *Tbx5*^{tm1Jse} mouse allele contains loxP sites surrounding exon 3, which encodes a portion of the T-box DNA-binding domain, and upon Cre-mediated recombination, will generate truncated *Tbx5* transcripts (Bruneau et al., 2001). Germline deletion of exon 3 generates the *Tbx5*$^{tm1.1Jse}$ mouse (Bruneau et al., 2001). Heterozygous *Tbx5*$^{tm1.1Jse}$ mice exhibit the characteristic haploinsufficient phenotype of Holt–Oram syndrome, including anterior defects of the forelimb, septal defects of the heart, and defects of cardiac conduction (Bruneau et al., 2001; Moskowitz et al., 2004). The mouse animal model has provided a robust system in which to study Holt–Oram syndrome in vivo

and will continue to provide a platform by which to study the disease and the role of *Tbx5* in cardiac and limb development.

5. *TBX5* IN CARDIAC MORPHOLOGIC DEVELOPMENT

The morphologic cardiac defects associated with Holt–Oram syndrome are most commonly malformations of the septa dividing the left and right sides of the heart (Basson et al., 1994; Holt & Oram, 1960; McDermott et al., 2005; Newbury-Ecob et al., 1996). From our current understanding, the ontogeny of the septa dividing the ventricular and atrial chambers is quite different (Anderson, Webb, Brown, Lamers, & Moorman, 2003), and yet defects in both arise from haploinsufficiency of *Tbx5* (Basson et al., 1997; Bruneau et al., 1999, 2001; Hoffmann et al., 2014; Koshiba-Takeuchi et al., 2009; Takeuchi et al., 2003; Xie et al., 2012).

5.1 Ventricular Septum

The morphology of the ventricular septum depends heavily on the localization of *Tbx5* expression during development. Both the left and the right ventricles contribute equally toward the formation of the interventricular septum, suggesting that a balance of left and right contributions may underlie development of the septum (Franco et al., 2006). During development of the ventricular chambers, *Tbx5* is unilaterally expressed on the left side, including the left side of the ventricular septum (Bruneau et al., 1999, 2001; Takeuchi et al., 2003). Overexpression of *Tbx5* bilaterally results in malformation of the ventricular chambers and absence of the ventricular septum (Koshiba-Takeuchi et al., 2009; Liberatore, Searcy-Schrick, & Yutzey, 2000; Takeuchi et al., 2003). However, it remains unclear whether *Tbx5*-dependent transcriptional regulation alone controls ventricular septum formation (Franco et al., 2006; Koshiba-Takeuchi et al., 2009; Takeuchi et al., 2003). Two additional T-box family genes, *Tbx18* and *Tbx20*, are unilaterally expressed in the left and right ventricles, respectively (Franco et al., 2006; Takeuchi et al., 2003). The boundary between *Tbx5*-positive, *Tbx20*-negative, and *Tbx5*-negative, *Tbx20*-positive myocardium appears to demarcate the location of ventricular septation and shifts in the expression levels can result in ventricular septum abnormalities; however, the exact mechanism by which the interface between *Tbx5* and the other T-box family genes instructs formation of the ventricular septum remains unclear (Koshiba-Takeuchi et al., 2009; Takeuchi et al., 2003).

5.2 Atrial Septum

Haploinsufficiency of *TBX5* in Holt–Oram patients results in atrial septal defects in approximately half of cases (Bruneau et al., 1999), and *Tbx5* haploinsufficient mice exhibit atrial septal defects approximately 40% of the time (Bruneau et al., 2001). The atrial septum is derived from the second heart field contributing to the inflow tract of the heart (Goddeeris et al., 2008; Hoffmann, Peterson, Friedland-Little, Anderson, & Moskowitz, 2009; Mommersteeg et al., 2006; Snarr, Wirrig, Phelps, Trusk, & Wessels, 2007; Wessels et al., 2000). *Sonic hedgehog* (*Shh*), secreted from the pulmonary endoderm, signals through GLI-dependent transcription factors and is essential for atrial septation (Goddeeris et al., 2008; Hoffmann et al., 2009, 2014; Xie et al., 2012). *Gli1* genetically interacts with *Tbx5* in the second heart field to coactivate downstream targets, including *Osr1* and *Foxf1* (Goddeeris et

al., 2008; Hoffmann et al., 2009, 2014; Xie et al., 2012). Furthermore, deletion of one or both copies of *Tbx5* from *Shh*-receiving cells results in primum-type atrial septal defects, which can be rescued through constitutive activation of hedgehog signaling (Xie et al., 2012). This supports a model in which *Tbx5* acts upstream of active *Shh* signaling in the second heart field and that both *Tbx5* and activating GLI factors coregulate transcription at the top of a hierarchy of atrial septation genes (Hoffmann et al., 2014; Xie et al., 2012).

6. *TBX5* IN CARDIAC CONDUCTION SYSTEM DEVELOPMENT

The cardiac conduction system is a highly specialized network of cardiomyocytes within the heart that generate and transmit electrical impulses throughout the heart to coordinate contraction. A majority of Holt–Oram syndrome patients present with conduction system abnormalities (Basson et al., 1994; Holt & Oram, 1960; Newbury-Ecob et al., 1996). Evidence suggests that *Tbx5* plays three key roles in the cardiac conduction system: specification of the conduction system during development, regulation of the conduction system transcriptome, and maintenance of conduction system identity in the adult (Arnolds et al., 2012; Moskowitz et al., 2007, 2004). *Tbx5* and *Nkx2-5* genetically interact to specify the ventricular cardiac conduction system in a ventricular subdomain with the highest expression of both factors (Moskowitz et al., 2004; Thomas et al., 2001). Furthermore, *Tbx5* and *Nkx2-5* are required to coregulate the transcriptional repressor *Id2*, which is required for proper formation and function of the conduction system (Moskowitz et al., 2007). Although the *Tbx5*-dependent transcriptome in the conduction system has not been well characterized to date, *Tbx5* is required for the regulation of critical conduction system ion channels, *Gja5* and *Scn5a*, and removal of *Tbx5* from the adult ventricular conduction system results in loss of these ion channels and altered ventricular conduction system function (Arnolds et al., 2012; Bruneau et al., 2001; Moskowitz et al., 2004; van den Boogaard et al., 2014).

7. HOMOZYGOUS *TBX5* MUTATIONS REVEAL NOVEL ROLES OF TBX5

While much attention has been focused on understanding *Tbx5* haploinsufficiency as it relates to human disease, homozygous deletion of *Tbx5* in animal models reveals novel requirements for *Tbx5* not uncovered by haploinsufficiency.

7.1 Complete Loss of Mammalian *Tbx5*

As *Tbx5* haploinsufficiency is associated with the phenotype of Holt–Oram syndrome in mice and *Tbx5* null embryos die in utero around E10.5, most studies examining the role of *Tbx5* in cardiac morphogenesis and transcription have focused on the *Tbx5* null heterozygote. In contrast, the null state provides the opportunity to understand critical roles of *Tbx5* not observed in heterozygotes (Agarwal et al., 2003; Bruneau et al., 2001; Hoffmann et al., 2014; Luna-Zurita et al., 2016; Mori et al., 2006; Moskowitz et al., 2004; Rallis et al., 2003; Xie et al., 2012). In the mouse, germline deletion of both copies of *Tbx5*, *Tbx5$^{tm1.1Jse/tm1.1Jse}$*, results in embryonic lethality by E10.5 (Bruneau et al., 2001). These animals exhibit a grossly abnormal, linear heart tube (Bruneau et al., 2001) and complete absence of the forelimb buds (Agarwal et al., 2003; Bruneau et al., 2001). The complete absence of the forelimb buds indicates a role for *Tbx5* in limb bud initiation, shown to be downstream of fibroblast growth factor signaling (Agarwal et al., 2003; Hasson, Del Buono,

& Logan, 2007; Rallis et al., 2003). Similar to the germline homozygous null embryos, homozygous hypomorphs for *Tbx5* show embryonic lethality prior to E11.5, with hypoplastic left ventricles and sinoatrial structures (Mori et al., 2006). Distinctly, unlike the *Tbx5* homozygous null phenotype, homozygous hypomorphs still undergo heart looping and rudimentary formation of the left and right atrial chambers (Mori et al., 2006). These observations indicate important roles for *Tbx5* at sequential stages of cardiac development, although the distinctions between these roles have yet to be elucidated.

7.2 Zebrafish *tbx5a/tbx5b*

The *heartstrings* mutation is the first published mutation of *tbx5a* in zebrafish and was found as part of a screen for recessive lethal mutations affecting cardiac function (Garrity, Childs, & Fishman, 2002). The *tbx5a/heartstrings* mutants or morpholino knockdown of *tbx5a* recapitulate some aspects of Holt–Oram syndrome including forelimb defects and conduction defects (Ahn, Kourakis, Rohde, Silver, & Ho, 2002; Garrity et al., 2002). The hearts of these animals appear to develop normally during early cardiac development, only later displaying defects starting with the failure of heart looping and subsequent deterioration of chamber myocardium and heart failure (Ahn et al., 2002; Garrity et al., 2002).

More recently, a second copy of *tbx5* (*tbx5b*) was found in the genome of zebrafish (Albalat, Baquero, & Minguillon, 2010). While *tbx5a* is expressed in the eye, heart, and forelimb during early development, *tbx5b* is only expressed robustly in the eye and heart (Albalat et al., 2010), suggesting possible redundant functions for the two *tbx5* genes during early heart and eye development. Using morpholinos, *tbx5a*, *tbx5b*, or *tbx5a/tbx5b* double knockdowns all result in the heartstrings phenotype, i.e., normal heart tube formation, bradycardia, and progressive deterioration/heart failure (Garrity et al., 2002; Parrie, Renfrew, Wal, Mueller, & Garrity, 2013). Different downstream targets have been identified for *tbx5a* and *tbx5b* (Parrie et al., 2013). While *tbx5b* knockdown does not result in patterning defects of chamber formation or sinus venosus seen in either the *tbx5a* mutants or knockdown experiments, *tbx5b* knockdown results in abnormal expansion of two morphogenesis markers, *hand2* and *vcana*, similar to *tbx5a* (Garrity et al., 2002; Parrie et al., 2013). However, known direct targets of mammalian *TBX5* or zebrafish *tbx5a*, such as *bmp4*, *nppa*, *tbx2b*, and *hey2* (Bruneau et al., 2001; Camarata et al., 2010; Chi et al., 2008; Plageman & Yutzey, 2004; Puskaric et al., 2010), were not disrupted with *tbx5b* knockdown, and neither *tbx5a* overexpression nor *tbx5b* overexpression rescues the reciprocal knockdown, suggesting the role of *tbx5b* is different than that of *tbx5a* (Parrie et al., 2013). The apparent differences in *tbx5a* and *tbx5b* function suggest that following gene duplication there may have been evolutionarily beneficial subfunctionalization of the two copies.

8. THE TBX5 GENE REGULATORY NETWORK

As a T-box transcription factor, the primary role of TBX5 is thought to be the regulation of target gene transcription. Historically, TBX5 has thought to act a positive regulator of transcriptional activity; however, recent evidence suggests that TBX5 may have a role in

both transcriptional activation and repression (Fig. 2). In this section, we will explore what is known about both roles.

8.1 Positive Transcriptional Activation by TBX5 and Cofactors: Cardiomyocyte Specific Factors, Chromatin Modification, and Maturation

TBX5 has long been known to act as a positive regulator of transcription in heart development and cardiomyocyte maturation (Bruneau et al., 2001; Goetz, Brown, & Conlon, 2006; Hiroi et al., 2001; Moskowitz et al., 2004). Some of the earliest identified direct targets of *TBX5* were *NPPA* (encoding ANF) and *GJA5* (encoding cx40), both of which are expressed in differentiating cardiomyocytes during development and are markers of cardiac chamber differentiation (Bruneau et al., 2001; Christoffels et al., 2000; Delorme et al., 1997; Hiroi et al., 2001). *Gja5* and *Nppa* are both highly sensitive markers of TBX5 activity with a nearly complete loss of *Gja5* in *Tbx5*$^{tm1.1Jse/+}$ embryos and a graded response of *Nppa* across a *Tbx5* allelic series (Bruneau et al., 2001; Mori et al., 2006).

The first identified interaction partner of TBX5 was the *tinman* transcription factor NKX2-5 (Hiroi et al., 2001). Identified by a classic yeast two-hybrid screen, TBX5 and NKX2-5 interact through the highly conserved C-terminus of NKX2-5, relying on four key amino acids in an α-helix, P139, D140, R150, and Q151 (Bruneau et al., 2001; Hiroi et al., 2001; Luna-Zurita et al., 2016). The interaction between TBX5 and NKX2-5 allows the proteins to synergistically activate targets such as *Nppa* through tandem transcription factor binding motifs (Bruneau et al., 2001; Hiroi et al., 2001). This interaction of TBX5 and NKX2-5 at tandem binding motifs induces bending in the DNA for transcriptional activity, a molecular mechanism by which synergistic activities of TBX5 and NKX2-5 interactions are determined at specific *cis*-regulatory elements (Luna-Zurita et al., 2016). Furthermore, the interaction between TBX5 and NKX2-5 is required to maintain fidelity in transcription factor binding throughout the genome, and the absence of either factor allows for inappropriate localization and activation of noncardiac genes by the other (Luna-Zurita et al., 2016). To this point, several sites within TBX5 are important for the synergistic activation of *Nppa*, which can be abrogated by HOS mutation in the α-helix mentioned earlier as well as G80R and R237W (Boogerd et al., 2010; Garg et al., 2003; Hiroi et al., 2001).

Besides NKX2-5, TBX5 also shows direct interaction with other major cardiac transcription factors, including GATA4 (Garg et al., 2003; Maitra et al., 2009), GATA6 (Maitra et al., 2009), TBX20 (Brown et al., 2005), MEF2C (Ghosh et al., 2009), and Myocardin (Wang, Cao, Wang, & Wang, 2011). The interaction between TBX5 and GATA4 was diminished by *GATA4* mutations causing nonsyndromic congenital heart defects (Garg et al., 2003), as well as by *TBX5* Holt–Oram mutations causing heart defects but not those causing only limb defects (Boogerd et al., 2010; Garg et al., 2003; Luna-Zurita et al., 2016). Both GATA4-TBX5 and MEF2C-TBX5 interactions are required for synergistic activation of α-cardiac myosin heavy chain encoded by *MYH6* (Ghosh et al., 2009; Maitra et al., 2009), though GATA6-TBX5 protein interactions are not, suggesting that TBX5 interaction partners generate tissue- and context-specific gene expression (Maitra et al., 2009).

TBX5 as a transcription factor appears to act as part of a multifactor transcriptional complex for the activation and maintenance of cardiac lineage genes; however, the ability of transcription factors to regulate gene targets requires the ability of the factors to bind open chromatin. Addition of *Gata4*, *Mef2c*, and *Tbx5* to fibroblasts is sufficient to drive reprogramming toward a cardiomyocyte fate (Ieda et al., 2010; Qian et al., 2012). This ability to reprogram cells suggests that this core set of transcription factors may drive chromatin accessibility. In support of this supposition, it has been shown that TBX5 interacts with Baf60c and Brg1, encoded by *Smarcd3* and *Smarca4*, respectively, members of the SWI/SNF family of proteins involved in chromatin remodeling to drive mesodermal cells to cardiomyocyte fate in vitro and in vivo (Lickert et al., 2004; Takeuchi & Bruneau, 2009; Takeuchi et al., 2011). Furthermore, in *Tbx5* haploinsufficient mice, there is a loss of chromatin remodeling complexes at the promoters of *Tbx5*-dependent cardiac genes (Takeuchi et al., 2011). For another T-box family member, T-bet, encoded by *Tbx21*, it has been previously shown that T-bet, the Brg1 chromatin-remodeling complex, and H3K27 demethylases physically interact (Miller, Mohn, & Weinmann, 2010), suggesting potential shared mechanisms with *Tbx5* in chromatin transitions seen in cardiac development (Wamstad et al., 2012).

8.2 TBX5-Mediated Repression: Inhibition of Noncardiomyocyte Fate Through Chromatin Remodeling

In addition to its positive role driving cardiac gene regulatory networks, recent genomic studies indicate that TBX5 acts as a direct transcriptional repressor during cardiac development where it is required for inhibition of inappropriate gene expression (Lewandowski et al., 2014; Waldron et al., 2016). While transcriptional repression by T-box factors in cardiac development and function has been well documented in the cases of TBX2 (Carreira et al., 1998; Christoffels et al., 2004) and TBX3 (He et al., 1999; Hoogaars et al., 2007, 2004; Lingbeek, Jacobs, & van Lohuizen, 2002), and it has been shown that TBX20 has roles in both transcriptional repression and activation (Kaltenbrun et al., 2013; Sakabe et al., 2012; Stennard et al., 2005), only recently has a repressive role for TBX5 been elucidated. Waldron et al. (2016) demonstrated that *Tbx5* inhibits noncardiac gene regulatory programs, including neuronal networks during early cardiac development. Through biochemical and genetic interaction studies, it was shown that TBX5 protein interacts with the nucleosome remodeling and deacetylase (NuRD) complex during embryonic development (Waldron et al., 2016). Similar to TBX20, the TBX5–NuRD interaction complex acts as an inhibitory mechanism by which TBX5 is able to repress noncardiogenic gene expression in the heart (Kaltenbrun et al., 2013; Waldron et al., 2016). TBX5 physically interacts with the NuRD complex through an evolutionarily conserved α-helix domain located from amino acids 255–264 and disruption of this domain can result in Holt–Oram syndrome (e.g., S261C; Brassington et al., 2003; Waldron et al., 2016). Using cardiomyocytes derived from murine embryonic stem cell differentiation, Luna-Zurita et al. (2016) demonstrated that TBX5 imparts specificity in cardiac transcription factor complexes by preventing off-target binding of other cardiac transcription factors. These findings suggest that the dual roles of T-box factors (i.e., TBX5 and TBX20 repression of noncardiomyocyte fate) may be a more common theme in cardiac development than previously thought.

8.3 Direct Targets of TBX5 Regulation

As a member of the T-box family of transcription factors, TBX5 regulates transcription through direct interaction with DNA. In recent years, multiple groups have turned to chromatin immunoprecipitation with sequencing (ChIP-sequencing) to identify direct targets of TBX5 binding and regulation (He et al., 2011; Luna-Zurita et al., 2016). The first ChIP-seq dataset was generated in the HL-1 atrial cardiomyocyte cell line using an overexpression construct of biotinylated TBX5, which identified over 56 k binding sites within the genome (He et al., 2011). The second set of data was generated using ChIP-exo technology in the context of mouse ES cell differentiation in both cardiac progenitors and cardiomyocytes, resulting in approximately 5 and 9 k bindings sites, respectively (Luna-Zurita et al., 2016). While these datasets share many of the same locations, each also identifies many unique sites, suggesting that binding site information will need to be generated in each *Tbx5* expression context in order to understand the direct *Tbx5* transcriptome. For example, whereas approximately 60% and 40% sites identified in the cardiac progenitor and cardiomyocyte mouse ES cell datasets are shared, only 4% of sites identified in the HL-1 dataset are shared with the mouse ES cell datasets. It is currently unclear to what degree the HL-1 dataset overestimates and the mouse embryonic stem cell datasets underestimate the total number of relevant binding sites, or whether both datasets overestimate functional binding events, HL-1 to a greater extent. Inclusion of additional markers, such as open chromatin, histone marks, and known TBX5-binding partners may allow the broad utilization of current datasets for identification of truly functional TBX5-binding sites.

To date, functionally confirmed direct targets of *TBX5* are almost exclusively in genes implicated in cardiac proliferation, maturation, and function, including *Nppa*, *Gja5*, and *Scn5a* (Arnolds et al., 2012; Bruneau et al., 2001; Goetz et al., 2006; Hatcher et al., 2001; Hiroi et al., 2001; Mori et al., 2006; Moskowitz et al., 2007, 2004; Puskaric et al., 2010; Xie et al., 2012). Interestingly, the direct targets mediating the morphogenesis requirement for *TBX5* remain unknown. While some candidate target genes may act prior to morphological changes (Hoffmann et al., 2014; Xie et al., 2012), no direct mediators of morphology have been uncovered. *TBX5* may therefore indirectly regulate these processes. With advances in genome-wide technology, understanding the basis by which *TBX5* regulates morphological change will be key to understanding the role of *TBX5* in cardiac development.

9. CONCLUDING REMARKS

The requirement of *TBX5* for normal human cardiac structure was identified over 20 years ago (Basson et al., 1997; Li et al., 1997); however, surprisingly, the mechanistic role of *TBX5* in cardiac development remains unclear. Little is known about the essential downstream targets of TBX5-mediated transcription in the context of cardiac development. Similarly, the complex temporal and spatial gene expression of *TBX5* has been mapped throughout development and into adult life; however, the *cis*-regulatory architecture governing this expression is just beginning to be described. From a biochemical perspective, significant strides have been made in recent years to understand how TBX5 activates gene expression. However, the mechanisms by which TBX5 and its cofactors are targeted to specific loci, the temporal recruitment of TBX5 and its cofactors, the interplay between

TBX5 and it cotranscriptional partners, and the mechanisms distinguishing active and repressive TBX5 activity are just recently coming into focus, and provide opportunities for exciting mechanistic studies. These areas of investigation will contribute to a broader understanding of the mechanisms underlying the requirement for TBX5 in cardiac morphogenesis and more generally the transcriptional control of metazoan development.

References

Adachi N, Robinson M, Goolsbee A, Shubin NH. Regulatory evolution of Tbx5 and the origin of paired appendages. Proceedings of the National Academy of Sciences of the United States of America. 2016; 113(36):10115–10120. [PubMed: 27503876]

Agarwal P, Wylie JN, Galceran J, Arkhitko O, Li C, Deng C, et al. *Tbx*5 is essential for forelimb bud initiation following patterning of the limb field in the mouse embryo. Development. 2003; 130(3): 623–633. [PubMed: 12490567]

Agulnik SI, Bollag RJ, Silver LM. Conservation of the T-box gene family from Mus musculus to Caenorhabditis elegans. Genomics. 1995; 25(1):214–219. [PubMed: 7774921]

Agulnik SI, Garvey N, Hancock S, Ruvinsky I, Chapman DL, Agulnik I, et al. Evolution of mouse T-box genes by tandem duplication and cluster dispersion. Genetics. 1996; 144(1):249–254. [PubMed: 8878690]

Ahn DG, Kourakis MJ, Rohde LA, Silver LM, Ho RK. T-box gene *tbx*5 is essential for formation of the pectoral limb bud. Nature. 2002; 417(6890):754–758. [PubMed: 12066188]

Albalat R, Baquero M, Minguillon C. Identification and characterisation of the developmental expression pattern of *tbx*5*b*, a novel *tbx*5 gene in zebrafish. Gene Expression Patterns. 2010; 10(1): 24–30. [PubMed: 19925885]

Al-Qattan MM, Abou Al-Shaar H. A novel missense mutation in the *TBX*5 gene in a Saudi infant with Holt-Oram syndrome. Saudi Medical Journal. 2015; 36(8):980–982. [PubMed: 26219450]

Anderson RH, Webb S, Brown NA, Lamers W, Moorman A. Development of the heart: (2) Septation of the atriums and ventricles. Heart. 2003; 89(8):949–958. [PubMed: 12860885]

Arnolds DE, Liu F, Fahrenbach JP, Kim GH, Schillinger KJ, Smemo S, et al. TBX5 drives *Scn*5*a* expression to regulate cardiac conduction system function. Journal of Clinical Investigation. 2012; 122(7):2509–2518. [PubMed: 22728936]

Barisic I, Boban L, Greenlees R, Garne E, Wellesley D, Calzolari E, et al. Holt Oram syndrome: A registry-based study in Europe. Orphanet Journal of Rare Diseases. 2014; 9:156. [PubMed: 25344219]

Basson CT, Bachinsky DR, Lin RC, Levi T, Elkins JA, Soults J, et al. Mutations in human *TBX*5 [corrected] cause limb and cardiac malformation in Holt-Oram syndrome. Nature Genetics. 1997; 15(1):30–35. [PubMed: 8988165]

Basson CT, Cowley GS, Solomon SD, Weissman B, Poznanski AK, Traill TA, et al. The clinical and genetic spectrum of the Holt-Oram syndrome (heart-hand syndrome). New England Journal of Medicine. 1994; 330(13):885–891. [PubMed: 8114858]

Basson CT, Huang T, Lin RC, Bachinsky DR, Weremowicz S, Vaglio A, et al. Different *TBX*5 interactions in heart and limb defined by Holt-Oram syndrome mutations. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96(6):2919–2924. [PubMed: 10077612]

Bollag RJ, Siegfried Z, Cebra-Thomas JA, Garvey N, Davison EM, Silver LM. An ancient family of embryonically expressed mouse genes sharing a conserved protein motif with the T locus. Nature Genetics. 1994; 7(3):383–389. [PubMed: 7920656]

Boogerd CJ, Dooijes D, Ilgun A, Mathijssen IB, Hordijk R, van de Laar IM, et al. Functional analysis of novel *TBX*5 T-box mutations associated with Holt-Oram syndrome. Cardiovascular Research. 2010; 88(1):130–139. [PubMed: 20519243]

Borozdin W, Bravo Ferrer Acosta AM, Bamshad MJ, Botzenhart EM, Froster UG, Lemke J, et al. Expanding the spectrum of *TBX*5 mutations in Holt-Oram syndrome: Detection of two intragenic

deletions by quantitative real time PCR, and report of eight novel point mutations. Human Mutation. 2006; 27(9):975–976.

Brassington AM, Sung SS, Toydemir RM, Le T, Roeder AD, Rutherford AE, et al. Expressivity of Holt-Oram syndrome is not predicted by TBX5 genotype. American Journal of Human Genetics. 2003; 73(1):74–85. [PubMed: 12789647]

Brown DD, Martz SN, Binder O, Goetz SC, Price BM, Smith JC, et al. *Tbx*5 and *Tbx*20 act synergistically to control vertebrate heart morphogenesis. Development. 2005; 132(3):553–563. [PubMed: 15634698]

Bruneau BG, Logan M, Davis N, Levi T, Tabin CJ, Seidman JG, et al. Chamber-specific cardiac expression of *Tbx*5 and heart defects in Holt-Oram syndrome. Developmental Biology. 1999; 211(1):100–108. [PubMed: 10373308]

Bruneau BG, Nemer G, Schmitt JP, Charron F, Robitaille L, Caron S, et al. A murine model of Holt-Oram syndrome defines roles of the T-box transcription factor *Tbx*5 in cardiogenesis and disease. Cell. 2001; 106(6):709–721. [PubMed: 11572777]

Camarata T, Bimber B, Kulisz A, Chew TL, Yeung J, Simon HG. LMP4 regulates Tbx5 protein subcellular localization and activity. The Journal of Cell Biology. 2006; 174(3):339–348. [PubMed: 16880269]

Camarata T, Krcmery J, Snyder D, Park S, Topczewski J, Simon HG. Pdlim7 (LMP4) regulation of Tbx5 specifies zebrafish heart atrioventricular boundary and valve formation. Developmental Biology. 2010; 337(2):233–245. [PubMed: 19895804]

Carreira S, Dexter TJ, Yavuzer U, Easty DJ, Goding CR. Brachyury-related transcription factor *Tbx*2 and repression of the melanocyte-specific TRP-1 promoter. Molecular and Cellular Biology. 1998; 18(9):5099–5108. [PubMed: 9710594]

Chapman DL, Garvey N, Hancock S, Alexiou M, Agulnik SI, Gibson-Brown JJ, et al. Expression of the T-box family genes, *Tbx*1-*Tbx*5, during early mouse development. Developmental Dynamics. 1996; 206(4):379–390. [PubMed: 8853987]

Chi NC, Shaw RM, De Val S, Kang G, Jan LY, Black BL, et al. Foxn4 directly regulates *tbx*2*b* expression and atrioventricular canal formation. Genes & Development. 2008; 22(6):734–739. [PubMed: 18347092]

Christoffels VM, Habets PE, Franco D, Campione M, de Jong F, Lamers WH, et al. Chamber formation and morphogenesis in the developing mammalian heart. Developmental Biology. 2000; 223(2):266–278. [PubMed: 10882515]

Christoffels VM, Hoogaars WM, Tessari A, Clout DE, Moorman AF, Campione M. T-box transcription factor Tbx2 represses differentiation and formation of the cardiac chambers. Developmental Dynamics. 2004; 229(4):763–770. [PubMed: 15042700]

Collavoli A, Hatcher CJ, He J, Okin D, Deo R, Basson CT. TBX5 nuclear localization is mediated by dual cooperative intramolecular signals. Journal of Molecular and Cellular Cardiology. 2003; 35(10):1191–1195. [PubMed: 14519429]

Conlon FL, Fairclough L, Price BM, Casey ES, Smith JC. Determinants of T box protein specificity. Development. 2001; 128(19):3749–3758. [PubMed: 11585801]

Cross SJ, Ching YH, Li QY, Armstrong-Buisseret L, Spranger S, Lyonnet S, et al. The mutation spectrum in Holt-Oram syndrome. Journal of Medical Genetics. 2000; 37(10):785–787. [PubMed: 11183182]

Debeer P, Race V, Gewillig M, Devriendt K, Frijns JP. Novel *TBX*5 mutations in patients with Holt-Oram syndrome. Clinical Orthopaedics and Related Research. 2007; 462:20–26. [PubMed: 17534187]

Delorme B, Dahl E, Jarry-Guichard T, Briand JP, Willecke K, Gros D, et al. Expression pattern of connexin gene products at the early developmental stages of the mouse cardiovascular system. Circulation Research. 1997; 81(3):423–437. [PubMed: 9285645]

Devine WP, Wythe JD, George M, Koshiba-Takeuchi K, Bruneau BG. Early patterning and specification of cardiac progenitors in gastrulating mesoderm. eLife. 2014; 3:1–23.

Ekure EN, Okoromah CN, Briggs E, Ajenifuja OA. Holt-Oram syndrome with hypoplastic left heart syndrome in an African child. The Nigerian Postgraduate Medical Journal. 2004; 11(3):190–192. [PubMed: 15505648]

Elek C, Vitez M, Czeizel E. Holt-Oram syndrome. Orvosi Hetilap. 1991; 132(2):73–74. 77–78. [PubMed: 1996196]

Fan C, Duhagon MA, Oberti C, Chen S, Hiroi Y, Komuro I, et al. Novel TBX5 mutations and molecular mechanism for Holt-Oram syndrome. Journal of Medical Genetics. 2003; 40(3):e29. [PubMed: 12624158]

Franco D, Meilhac SM, Christoffels VM, Kispert A, Buckingham M, Kelly RG. Left and right ventricular contributions to the formation of the interventricular septum in the mouse heart. Developmental Biology. 2006; 294(2):366–375. [PubMed: 16677630]

Garg V, Kathiriya IS, Barnes R, Schluterman MK, King IN, Butler CA, et al. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. Nature. 2003; 424(6947):443–447. [PubMed: 12845333]

Garrity DM, Childs S, Fishman MC. The heartstrings mutation in zebrafish causes heart/fin Tbx5 deficiency syndrome. Development. 2002; 129(19):4635–4645. [PubMed: 12223419]

Georges R, Nemer G, Morin M, Lefebvre C, Nemer M. Distinct expression and function of alternatively spliced Tbx5 isoforms in cell growth and differentiation. Molecular and Cellular Biology. 2008; 28(12):4052–4067. [PubMed: 18391012]

Ghosh TK, Packham EA, Bonser AJ, Robinson TE, Cross SJ, Brook JD. Characterization of the TBX5 binding site and analysis of mutations that cause Holt-Oram syndrome. Human Molecular Genetics. 2001; 10(18):1983–1994. [PubMed: 11555635]

Ghosh TK, Song FF, Packham EA, Buxton S, Robinson TE, Ronksley J, et al. Physical interaction between TBX5 and MEF2C is required for early heart development. Molecular and Cellular Biology. 2009; 29(8):2205–2218. [PubMed: 19204083]

Gibson-Brown JJ, Agulnik SI, Chapman DL, Alexiou M, Garvey N, Silver LM, et al. Evidence of a role for T-box genes in the evolution of limb morphogenesis and the specification of forelimb/hindlimb identity. Mechanisms of Development. 1996; 56(1–2):93–101. [PubMed: 8798150]

Gibson-Brown JJ, Agulnik SI, Silver LM, Papaioannou VE. Expression of T-box genes Tbx2-Tbx5 during chick organogenesis. Mechanisms of Development. 1998; 74(1–2):165–169. [PubMed: 9651516]

Goddeeris MM, Rho S, Petiet A, Davenport CL, Johnson GA, Meyers EN, et al. Intracardiac septation requires hedgehog-dependent cellular contributions from outside the heart. Development. 2008; 135(10):1887–1895. [PubMed: 18441277]

Goetz SC, Brown DD, Conlon FL. TBX5 is required for embryonic cardiac cell cycle progression. Development. 2006; 133(13):2575–2584. [PubMed: 16728474]

Gruenauer-Kloevekorn C, Froster UG. Holt-Oram syndrome: A new mutation in the TBX5 gene in two unrelated families. Annales de G en etique. 2003; 46(1):19–23.

GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. Nature Genetics. 2013; 45(6):580–585. [PubMed: 23715323]

Hasson P, Del Buono J, Logan MP. Tbx5 is dispensable for forelimb outgrowth. Development. 2007; 134(1):85–92. [PubMed: 17138667]

Hatcher CJ, Goldstein MM, Mah CS, Delia CS, Basson CT. Identification and localization of TBX5 transcription factor during human cardiac morphogenesis. Developmental Dynamics. 2000; 219(1):90–95. [PubMed: 10974675]

Hatcher CJ, Kim MS, Mah CS, Goldstein MM, Wong B, Mikawa T, et al. TBX5 transcription factor regulates cell proliferation during cardiogenesis. Developmental Biology. 2001; 230(2):177–188. [PubMed: 11161571]

He A, Kong SW, Ma Q, Pu WT. Co-occupancy by multiple cardiac transcription factors identifies transcriptional enhancers active in heart. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(14):5632–5637. [PubMed: 21415370]

He M, Wen L, Campbell CE, Wu JY, Rao Y. Transcription repression by Xenopus ET and its human ortholog TBX3, a gene involved in ulnar-mammary syndrome. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96(18):10212–10217. [PubMed: 10468588]

Heinritz W, Moschik A, Kujat A, Spranger S, Heilbronner H, Demuth S, et al. Identification of new mutations in the TBX5 gene in patients with Holt-Oram syndrome. Heart. 2005; 91(3):383–384. [PubMed: 15710732]

Herrmann BG, Labeit S, Poustka A, King TR, Lehrach H. Cloning of the T gene required in mesoderm formation in the mouse. Nature. 1990; 343(6259):617–622. [PubMed: 2154694]

Hiroi Y, Kudoh S, Monzen K, Ikeda Y, Yazaki Y, Nagai R, et al. Tbx5 associates with Nkx2-5 and synergistically promotes cardiomyocyte differentiation. Nature Genetics. 2001; 28(3):276–280. [PubMed: 11431700]

Hoffmann AD, Peterson MA, Friedland-Little JM, Anderson SA, Moskowitz IP. Sonic hedgehog is required in pulmonary endoderm for atrial septation. Development. 2009; 136(10):1761–1770. [PubMed: 19369393]

Hoffmann AD, Yang XH, Burnicka-Turek O, Bosman JD, Ren X, Steimle JD, et al. Foxf genes integrate tbx5 and hedgehog pathways in the second heart field for cardiac septation. PLoS Genetics. 2014; 10(10):e1004604. [PubMed: 25356765]

Holt M, Oram S. Familial heart disease with skeletal malformations. British Heart Journal. 1960; 22:236–242. [PubMed: 14402857]

Hoogaars WM, Engel A, Brons JF, Verkerk AO, de Lange FJ, Wong LY, et al. Tbx3 controls the sinoatrial node gene program and imposes pacemaker function on the atria. Genes & Development. 2007; 21(9):1098–1112. [PubMed: 17473172]

Hoogaars WM, Tessari A, Moorman AF, de Boer PA, Hagoort J, Soufan AT, et al. The transcriptional repressor Tbx3 delineates the developing central conduction system of the heart. Cardiovascular Research. 2004; 62(3):489–499. [PubMed: 15158141]

Horb ME, Thomsen GH. Tbx5 is essential for heart development. Development. 1999; 126(8):1739–1751. [PubMed: 10079235]

Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell. 2010; 142(3):375–386. [PubMed: 20691899]

Isphording D, Leylek AM, Yeung J, Mischel A, Simon HG. T-box genes and congenital heart/limb malformations. Clinical Genetics. 2004; 66(4):253–264. [PubMed: 15355425]

Jin F, Li Y, Dixon JR, Selvaraj S, Ye Z, Lee AY, et al. A high-resolution map of the three-dimensional chromatin interactome in human cells. Nature. 2013; 503(7475):290–294. [PubMed: 24141950]

Jolma A, Yan J, Whitington T, Toivonen J, Nitta KR, Rastas P, et al. DNA-binding specificities of human transcription factors. Cell. 2013; 152(1–2):327–339. [PubMed: 23332764]

Kaltenbrun E, Greco TM, Slagle CE, Kennedy LM, Li T, Cristea IM, et al. A Gro/TLE-NuRD corepressor complex facilitates Tbx20-dependent transcriptional repression. Journal of Proteome Research. 2013; 12(12):5395–5409. [PubMed: 24024827]

Kimura M, Kikuchi A, Ichinoi N, Kure S. Novel TBX5 duplication in a Japanese family with Holt-Oram syndrome. Pediatric Cardiology. 2015; 36(1):244–247. [PubMed: 25274398]

Kispert A, Koschorz B, Herrmann BG. The T protein encoded by Brachyury is a tissue-specific transcription factor. EMBO Journal. 1995; 14(19):4763–4772. [PubMed: 7588606]

Koshiba-Takeuchi K, Mori AD, Kaynak BL, Cebra-Thomas J, Sukonnik T, Georges RO, et al. Reptilian heart development and the molecular basis of cardiac chamber evolution. Nature. 2009; 461(7260):95–98. [PubMed: 19727199]

Krause A, Zacharias W, Camarata T, Linkhart B, Law E, Lischke A, et al. *Tbx*5 and *Tbx*4 transcription factors interact with a new chicken PDZ-LIM protein in limb and heart development. Developmental Biology. 2004; 273(1):106–120. [PubMed: 15302601]

Kulisz A, Simon HG. An evolutionarily conserved nuclear export signal facilitates cytoplasmic localization of the Tbx5 transcription factor. Molecular and Cellular Biology. 2008; 28(5):1553–1564. [PubMed: 18160705]

Lewandowski SL, Janardhan HP, Smee KM, Bachman M, Sun Z, Lazar MA, et al. Histone deacetylase 3 modulates Tbx5 activity to regulate early cardiogenesis. Human Molecular Genetics. 2014; 23(14):3801–3809. [PubMed: 24565863]

Li QY, Newbury-Ecob RA, Terrett JA, Wilson DI, Curtis AR, Yi CH, et al. Holt-Oram syndrome is caused by mutations in TBX5, a member of the Brachyury (T) gene family. Nature Genetics. 1997; 15(1):21–29. [PubMed: 8988164]

Liberatore CM, Searcy-Schrick RD, Yutzey KE. Ventricular expression of tbx5 inhibits normal heart chamber development. Developmental Biology. 2000; 223(1):169–180. [PubMed: 10864469]

Lickert H, Takeuchi JK, Von Both I, Walls JR, McAuliffe F, Adamson SL, et al. Baf60c is essential for function of BAF chromatin remodelling complexes in heart development. Nature. 2004; 432(7013):107–112. [PubMed: 15525990]

Lingbeek ME, Jacobs JJ, van Lohuizen M. The T-box repressors *TBX*2 and *TBX*3 specifically regulate the tumor suppressor gene p14ARF via a variant T-site in the initiator. Journal of Biological Chemistry. 2002; 277(29):26120–26127. [PubMed: 12000749]

Luna-Zurita L, Stirnimann CU, Glatt S, Kaynak BL, Thomas S, Baudin F, et al. Complex interdependence regulates heterotypic transcription factor distribution and coordinates cardiogenesis. Cell. 2016; 164(5):999–1014. [PubMed: 26875865]

Maitra M, Schluterman MK, Nichols HA, Richardson JA, Lo CW, Srivastava D, et al. Interaction of *Gata*4 and *Gata*6 with *Tbx*5 is critical for normal cardiac development. Developmental Biology. 2009; 326(2):368–377. [PubMed: 19084512]

Mathelier A, Fornes O, Arenillas DJ, Chen CY, Denay G, Lee J, et al. JASPAR 2016: A major expansion and update of the open-access database of transcription factor binding profiles. Nucleic Acids Research. 2016; 44(D1):D110–D115. [PubMed: 26531826]

McDermott DA, Bressan MC, He J, Lee JS, Aftimos S, Brueckner M, et al. TBX5 genetic testing validates strict clinical criteria for Holt-Oram syndrome. Pediatric Research. 2005; 58(5):981–986. [PubMed: 16183809]

McDermott, DA., Fong, JC., Basson, CT. Holt-Oram syndrome. In: Pagon, RA.Adam, MP.Ardinger, HH.Wallace, SE.Amemiya, A.Bean, LJH., et al., editors. GeneReviews(R). Seattle, WA: University of Seattle; 1993.

Mele M, Ferreira PG, Reverter F, DeLuca DS, Monlong J, Sammeth M, et al. Human genomics. The human transcriptome across tissues and individuals. Science. 2015; 348(6235):660–665. [PubMed: 25954002]

Miller SA, Mohn SE, Weinmann AS. Jmjd3 and UTX play a demethylase-independent role in chromatin remodeling to regulate T-box family member-dependent gene expression. Molecular Cell. 2010; 40(4):594–605. [PubMed: 21095589]

Minguillon C, Nishimoto S, Wood S, Vendrell E, Gibson-Brown JJ, Logan MP. Hox genes regulate the onset of *Tbx*5 expression in the forelimb. Development. 2012; 139(17):3180–3188. [PubMed: 22872086]

Mommersteeg MT, Soufan AT, de Lange FJ, van den Hoff MJ, Anderson RH, Christoffels VM, et al. Two distinct pools of mesenchyme contribute to the development of the atrial septum. Circulation Research. 2006; 99(4):351–353. [PubMed: 16873717]

Mori AD, Zhu Y, Vahora I, Nieman B, Koshiba-Takeuchi K, Davidson L, et al. *Tbx*5-dependent rheostatic control of cardiac gene expression and morphogenesis. Developmental Biology. 2006; 297(2):566–586. [PubMed: 16870172]

Moskowitz IP, Kim JB, Moore ML, Wolf CM, Peterson MA, Shendure J, et al. A molecular pathway including *Id*2, *Tbx*5, and *Nkx*2–5 required for cardiac conduction system development. Cell. 2007; 129(7):1365–1376. [PubMed: 17604724]

Moskowitz IP, Pizard A, Patel VV, Bruneau BG, Kim JB, Kupershmidt S, et al. The T-Box transcription factor *Tbx*5 is required for the patterning and maturation of the murine cardiac conduction system. Development. 2004; 131(16):4107–4116. [PubMed: 15289437]

Muller CW, Herrmann BG. Crystallographic structure of the T domain-DNA complex of the Brachyury transcription factor. Nature. 1997; 389(6653):884–888. [PubMed: 9349824]

Najjar H, Mardini M, Tabbaa R, Nyhan WL. Variability of the Holt-Oram syndrome in Saudi individuals. American Journal of Medical Genetics. 1988; 29(4):851–855. [PubMed: 3400729]

Newbury-Ecob RA, Leanage R, Raeburn JA, Young ID. Holt-Oram syndrome: A clinical genetic study. Journal of Medical Genetics. 1996; 33(4):300–307. [PubMed: 8730285]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Papaioannou VE. The T-box gene family: Emerging roles in development, stem cells and cancer. Development. 2014; 141(20):3819–3833. [PubMed: 25294936]

Papaioannou VE, Silver LM. The T-box gene family. Bioessays. 1998; 20(1):9–19. [PubMed: 9504043]

Parrie LE, Renfrew EM, Wal AV, Mueller RL, Garrity DM. Zebrafish *tbx*5 paralogs demonstrate independent essential requirements in cardiac and pectoral fin development. Developmental Dynamics. 2013; 242(5):485–502. [PubMed: 23441045]

Patel C, Silcock L, McMullan D, Brueton L, Cox H. TBX5 intragenic duplication: A family with an atypical Holt-Oram syndrome phenotype. European Journal of Human Genetics. 2012; 20(8):863–869. [PubMed: 22333898]

Pflugfelder GO, Roth H, Poeck B. A homology domain shared between Drosophila optomotor-blind and mouse Brachyury is involved in DNA binding. Biochemical and Biophysical Research Communications. 1992; 186(2):918–925. [PubMed: 1497674]

Plageman TF Jr, Yutzey KE. Differential expression and function of *Tbx*5 and *Tbx*20 in cardiac development. Journal of Biological Chemistry. 2004; 279(18):19026–19034. [PubMed: 14978031]

Postma AV, van de Meerakker JB, Mathijssen IB, Barnett P, Christoffels VM, Ilgun A, et al. A gain-of-function *TBX*5 mutation is associated with atypical Holt-Oram syndrome and paroxysmal atrial fibrillation. Circulation Research. 2008; 102(11):1433–1442. [PubMed: 18451335]

Puskaric S, Schmitteckert S, Mori AD, Glaser A, Schneider KU, Bruneau BG, et al. Shox2 mediates Tbx5 activity by regulating Bmp4 in the pacemaker region of the developing heart. Human Molecular Genetics. 2010; 19(23):4625–4633. [PubMed: 20858598]

Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, et al. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. Nature. 2012; 485(7400):593–598. [PubMed: 22522929]

Rallis C, Bruneau BG, Del Buono J, Seidman CE, Seidman JG, Nissim S, et al. *Tbx*5 is required for forelimb bud formation and continued outgrowth. Development. 2003; 130(12):2741–2751. [PubMed: 12736217]

Ruvinsky I, Silver LM. Newly identified paralogous groups on mouse chromosomes 5 and 11 reveal the age of a T-box cluster duplication. Genomics. 1997; 40(2):262–266. [PubMed: 9119393]

Ruvinsky I, Silver LM, Gibson-Brown JJ. Phylogenetic analysis of T-Box genes demonstrates the importance of amphioxus for understanding evolution of the vertebrate genome. Genetics. 2000; 156(3):1249–1257. [PubMed: 11063699]

Sakabe NJ, Aneas I, Shen T, Shokri L, Park SY, Bulyk ML, et al. Dual transcriptional activator and repressor roles of TBX20 regulate adult cardiac structure and function. Human Molecular Genetics. 2012; 21(10):2194–2204. [PubMed: 22328084]

Showell C, Christine KS, Mandel EM, Conlon FL. Developmental expression patterns of *Tbx*1, *Tbx*2, *Tbx*5, and *Tbx*20 in Xenopus tropicalis. Developmental Dynamics. 2006; 235(6):1623–1630. [PubMed: 16477648]

Smemo S, Campos LC, Moskowitz IP, Krieger JE, Pereira AC, Nobrega MA. Regulatory variation in a *TBX*5 enhancer leads to isolated congenital heart disease. Human Molecular Genetics. 2012; 21(14):3255–3263. [PubMed: 22543974]

Snarr BS, Wirrig EE, Phelps AL, Trusk TC, Wessels A. A spatiotemporal evaluation of the contribution of the dorsal mesenchymal protrusion to cardiac development. Developmental Dynamics. 2007; 236(5):1287–1294. [PubMed: 17265457]

Stennard FA, Costa MW, Lai D, Biben C, Furtado MB, Solloway MJ, et al. Murine T-box transcription factor Tbx20 acts as a repressor during heart development, and is essential for adult heart integrity, function and adaptation. Development. 2005; 132(10):2451–2462. [PubMed: 15843414]

Stenson PD, Mort M, Ball EV, Shaw K, Phillips A, Cooper DN. The Human Gene Mutation Database: Building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. Human Genetics. 2014; 133(1):1–9. [PubMed: 24077912]

Stirnimann CU, Ptchelkine D, Grimm C, Muller CW. Structural basis of TBX5-DNA recognition: The T-box domain in its DNA-bound and -unbound form. Journal of Molecular Biology. 2010; 400(1):71–81. [PubMed: 20450920]

Takabatake Y, Takabatake T, Takeshima K. Conserved and divergent expression of T-box genes Tbx2-Tbx5 in Xenopus. Mechanisms of Development. 2000; 91(1–2):433–437. [PubMed: 10704879]

Takeuchi JK, Bruneau BG. Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. Nature. 2009; 459(7247):708–711. [PubMed: 19396158]

Takeuchi JK, Lou X, Alexander JM, Sugizaki H, Delgado-Olguin P, Holloway AK, et al. Chromatin remodelling complex dosage modulates transcription factor function in heart development. Nature Communications. 2011; 2:187.

Takeuchi JK, Ohgi M, Koshiba-Takeuchi K, Shiratori H, Sakaki I, Ogura K, et al. *Tbx*5 specifies the left/right ventricles and ventricular septum position during cardiogenesis. Development. 2003; 130(24):5953–5964. [PubMed: 14573514]

Thomas PS, Kasahara H, Edmonson AM, Izumo S, Yacoub MH, Barton PJ, et al. Elevated expression of Nkx-2.5 in developing myocardial conduction cells. Anatomical Record. 2001; 263(3):307–313. [PubMed: 11455540]

Torrado M, Franco D, Lozano-Velasco E, Hernandez-Torres F, Calvino R, Aldama G, et al. A microRNA-transcription factor blueprint for early atrial arrhythmogenic remodeling. BioMed Research International. 2015; 2015:263151. [PubMed: 26221584]

van den Boogaard M, Smemo S, Burnicka-Turek O, Arnolds DE, van de Werken HJ, Klous P, et al. A common genetic variant within *SCN*10*A* modulates cardiac *SCN*5*A* expression. Journal of Clinical Investigation. 2014; 124(4):1844–1852. [PubMed: 24642470]

van Weerd JH, Badi I, van den Boogaard M, Stefanovic S, van de Werken HJ, Gomez-Velazquez M, et al. A large permissive regulatory domain exclusively controls Tbx3 expression in the cardiac conduction system. Circulation Research. 2014; 115(4):432–441. [PubMed: 24963028]

Vianna CB, Miura N, Pereira AC, Jatene MB. Holt-Oram syndrome: Novel *TBX*5 mutation and associated anomalous right coronary artery. Cardiology in the Young. 2011; 21(3):351–353. [PubMed: 21272410]

Waldron L, Steimle JD, Greco TM, Gomez NC, Dorr KM, Kweon J, et al. The cardiac TBX5 interactome reveals a chromatin remodeling network essential for cardiac septation. Developmental Cell. 2016; 36(3):262–275. [PubMed: 26859351]

Wamstad JA, Alexander JM, Truty RM, Shrikumar A, Li F, Eilertson KE, et al. Dynamic and coordinated epigenetic regulation of developmental transitions in the cardiac lineage. Cell. 2012; 151(1):206–220. [PubMed: 22981692]

Wang C, Cao D, Wang Q, Wang DZ. Synergistic activation of cardiac genes by myocardin and Tbx5. PloS One. 2011; 6(8):e24242. [PubMed: 21897873]

Wang F, Yang XY, Zhao JY, Yu LW, Zhang P, Duan WY, et al. miR-10a and miR-10b target the 3′-untranslated region of TBX5 to repress its expression. Pediatric Cardiology. 2014; 35(6):1072–1079. [PubMed: 24714979]

Wessels A, Anderson RH, Markwald RR, Webb S, Brown NA, Viragh S, et al. Atrial development in the human heart: An immunohistochemical study with emphasis on the role of mesenchymal tissues. Anatomical Record. 2000; 259(3):288–300. [PubMed: 10861362]

Wilson V, Conlon FL. The T-box family. Genome Biology. 2002; 3(6):1–7. REVIEWS3008.

Xie L, Hoffmann AD, Burnicka-Turek O, Friedland-Little JM, Zhang K, Moskowitz IP. *Tbx*5-hedgehog molecular networks are essential in the second heart field for atrial septation. Developmental Cell. 2012; 23(2):280–291. [PubMed: 22898775]

Yamak A, Georges RO, Sheikh-Hassani M, Morin M, Komati H, Nemer M. Novel exons in the *tbx*5 gene locus generate protein isoforms with distinct expression domains and function. Journal of Biological Chemistry. 2015; 290(11):6844–6856. [PubMed: 25623069]

Yang J, Hu D, Xia J, Yang Y, Ying B, Hu J, et al. Three novel *TBX*5 mutations in Chinese patients with Holt-Oram syndrome. American Journal of Medical Genetics. 2000; 92(4):237–240. [PubMed: 10842287]

Author Manuscript

Author Manuscript
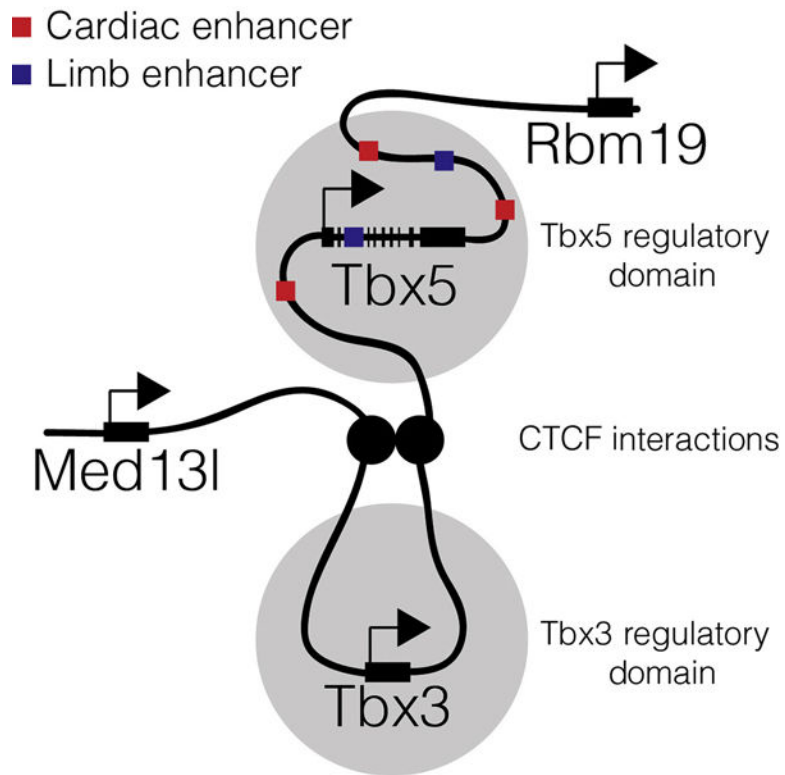
Author Manuscript

Author Manuscript

Zaragoza MV, Lewis LE, Sun G, Wang E, Li L, Said-Salman I, et al. Identification of the TBX5 transactivating domain and the nuclear localization signal. Gene. 2004; 330:9–18. [PubMed: 15087119]
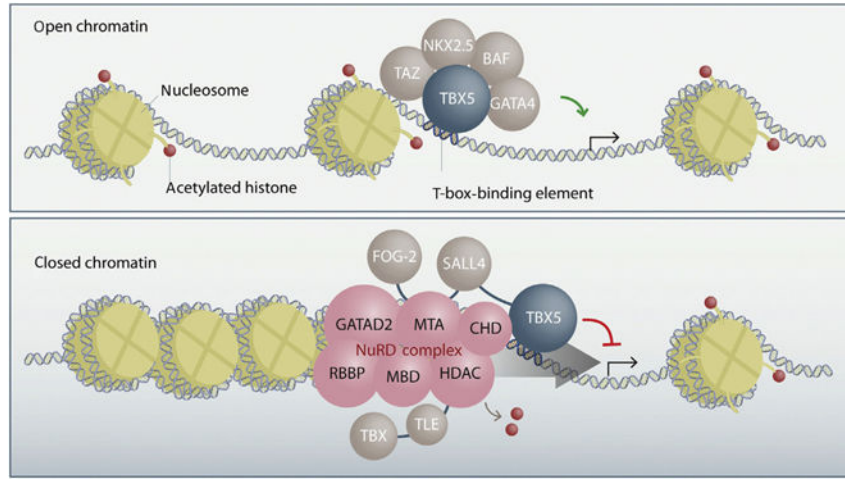
Fig. 1.

Representation of *Tbx5* regulatory domain. Chromatin capture techniques suggest
interactions between the promoter of Tbx5 and cis-regulatory elements are limited to a 375-
kbp region demarcated by functional CTCF sites located between *Rbm19* and *Tbx5* and
Tbx5 and *Tbx3* (van Weerd et al., 2014).

Fig. 2.

Transcriptional regulation by TBX5. TBX5, through its interactions with other cardiac transcription factors, such as GATA4 and NKX2-5, and the BAF chromatin-remodeling complex drive active transcription of target cardiac genes in regions of open chromatin (*top panel*). TBX5, through its interactions with the NuRD complex and other transcriptional repressors, such as SALL4, remodel chromatin to a closed state, which represses gene expression of noncardiac genes (*bottom panel*). *Reprinted from Boogerd, C. J., & Evans, S. M. (2016). TBX5 and NuRD divide the heart.* Developmental Cell, *36(3), 242–244, with permission from Elsevier.*