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Commentary

Heart or hand? Unmasking the basis for specific Holt-Oram phenotypes

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> . . . the hearts of old gave hands;
> But our new heraldry is hands, not hearts.
> —William Shakespeare, *Othello*

A paramount challenge in developmental biology and genetics is to provide explicit molecular explanations for the complex processes that together comprise morphogenesis—not merely the activation of tissue-specific differentiation programs, but also broader phenomena, such as positional information along multiple axes, orchestrated cell movements, and other aspects of pattern formation. In vertebrates, steps toward the formation of a mature, multichambered heart include the induction of cardiac precursors in lateral plate mesoderm, their migration to the ventral midline to form a linear heart tube, rightward looping (the first overt left-right asymmetry in the embryo), and subsequent developmentally regulated events, including chamber specialization, septation, and irreversible cell cycle exit (1). Many genes affecting cardiac organogenesis have been identified in recent years by saturation mutagenesis in flies and fish, fortuitous insertions in mice, and targeted deletions in embryonic stem cells. Congenital cardiac malformations are the most common form of heart disease in childhood, occurring in ≈1% of live births and perhaps 10% of stillbirths (2, 3), yet a genetic basis for such defects in humans is understood only rarely. The manuscript by Basson *et al.* in this issue of the *Proceedings* (4) provides surprising new insight into the variable phenotypes in Holt-Oram syndrome, a disorder of heart and forelimb development caused by mutations of the T-box transcription factor, TBX5.

The *Brachyury* (T) gene, the prototype for this family, was identified early in this century as a mutation affecting primitive streak and notochord differentiation in mice, resulting in a short tail phenotype when heterozygous and loss of posterior mesoderm formation (trunk and tail) when homozygous (5–7). Positional cloning of the *Brachyury* gene, isolation of *Brachyury* orthologues in diverse other vertebrates, the similar phenotype (*no tail*) in zebrafish lacking the gene, localization of *Brachyury* protein to the cell nucleus, identification of a palindromic *Brachyury* binding site, and proof that this protein indeed can function as a transcription factor together comprise a remarkable set of advances toward the transcriptional mechanisms underlying mesoderm formation (5–7). Direct evidence for *Brachyury* function is illustrated by its ability to trigger ectopic mesoderm formation when over-expressed in *Xenopus* embryos (8) and to disrupt mesoderm formation when engineered as a dominant-negative protein in *Xenopus* or zebrafish, causing axial phenotypes like those in *Brachyury* mutant mice (9). The *Drosophila* protein *optomer blind* (*omb*) was the first paralogue to be found, with similarity to the N-terminal T domain for DNA-binding but departing from *Brachyury* outside this domain. In mammals, the T-box family presently encompasses at least 10 members, 5 of which are expressed in the developing limbs including TBX5 (10).

Two years ago, TBX5 was identified as a gene for Holt-Oram syndrome (HOS1, OMIM 142900), a human disorder of forelimb and heart development with autosomal dominant inheritance that is seen in 1 per 100,000 live births (refs. 11 and 12; http://www.ncbi.nlm.nih.gov/omim). The skeletal involvement varies but is typically bilateral, with the left side and radial (thumb) defects predominating. Defects of the interatrial and interventricular septa are the characteristic cardiac findings, although other cardiac structural abnormalities and cardiac conduction system disease also occur (refs. 11 and 12; http://www.ncbi.nlm.nih.gov/omim). Linkage studies had mapped a Holt-Oram syndrome gene to chromosome 12q2, genetic and physical mapping refined the locus to an ≈1-centimorgan critical region, and exon trapping revealed TBX5 as an expressed sequence from this region, which otherwise contained no known genes (11, 12). In human embryos, TBX5 was abundant in the early heart tube at 26 days of gestation and later was expressed in the forelimbs and other sites (12). Most common among the initially identified mutations were premature stop codons and frame-shift mutations, which are expected to function as null alleles (11, 12), with one missense mutation in the carboxyl terminus of the predicted DNA-binding T domain (Arg237Gln) (4, 11).

The current article by Basson *et al*. reports significant new information—a second family with this exact missense mutation, a second mutation affecting this residue (Arg237Trp), and a second residue as the site for a mutation (Gly80Arg)—information that is made intriguing by suggestive structure–function correlations. Gly80 resides near the amino terminus of the T-box: Based on the crystallographic structure of *Xenopus Brachyury* protein bound to a target DNA palindrome (13), the arginine substitution is predicted to alter interaction with the major groove. The C-terminal helix, by contrast, mediates sequence-specific recognition in the minor groove, and the substitution of glutamine or tryptophan for Arg237 therefore is also expected to alter interactions of TBX5 with DNA. What elevates the present study beyond "just" an astute extrapolation from the *Xbra* crystal structure is the remarkable concordance of Holt-Oram phenotypes with these specific missense mutations, despite the varigated nature of this syndrome overall. For null alleles, composite cardiac defects and severe skeletal malformations both were frequent (57% and 65%, respectively). For Gly80Arg, cardiac phenotypes were severe, but the skeletal involvement was mild; for Arg237Gln and Arg237Trp, a reciprocal pattern was seen, with severe skeletal malformations but only rare composite cardiac defects. Thus, preferential involvement of heart versus limb occurs predictably as the consequence of which residue is altered in the DNA-binding T domain. The authors offer the logical speculation that their findings may point to different target genes for TBX5 in these two developmental pathways, perhaps involving different protein–protein interactions. Such

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a model accounts for the diverse, cell type-specific functions driven by certain other transcription factors, including the MADS (MCM1, agamous, deficiens, serum response factor) box proteins, serum response factor itself (14, 15), and myocyte enhancer factor 2 (16, 17). In these instances, the domains for binding DNA also confer essential protein-binding interactions; however, whether this is also true for the T-box is unknown.

A wealth of information implicates T-box proteins in organogenesis, including recent evidence that TBX3 is the cause of ulnary-mammary syndrome in humans (18) and that Tbx6 is required for posterior paraxial mesoderm (19). Despite these advances, the identification of genes that T-box proteins regulate directly has been elusive. One such target, the homeobox gene, *Bix1*, recently was isolated by using a combination of hormone-inducible *Brachyury* protein and subtractive hybridization (20), a strategy that might well be applied to other T-box proteins, including TBX5, or to comparisons between wild-type and mutant TBX5. Insights reported in the present study should give impetus to the quest for target genes controlled by TBX5 as the necessary next step to map the pathways governed by this protein in heart and hand morphogenesis. One further challenge is to pinpoint which cell lineages are affected as the primary consequence, an issue that could be addressed by conditional deletions in mice (21–23).

Other heart-hand syndromes exist that do not map to chromosome 12q2 (24). The concurrence of cardiac and limb malformations may be even more general than supposed, with positional correlations seen between specific malformations within the spectrum of possible defects (25). This has been taken to suggest the likelihood of clinical mutations that fall elsewhere in these developmental cascades controlling the creation of hands and hearts (25).

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