

## Fending for a Braveheart

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**Recent articles by Klattenhoff *et al* (2013) and Grote *et al* (2013) identify long non-coding RNAs, or lncRNAs, important for specifying the cardiac lineage. Depletion of a lncRNA, aptly named *Braveheart*, resulted in loss of beating cardiomyocytes during embryonic stem (ES) cell differentiation and failure to activate a key network of cardiac transcription factors. Immunoprecipitation of the protein complex associated with *Braveheart* revealed that the lncRNA physically interacts with epigenetic machinery that regulates cardiac gene expression. Similarly, a second lncRNA, *Fendrr*, also interacts with epigenetic regulators to promote proper cardiac gene expression and function *in vivo* in mice. These studies highlight the importance of lncRNAs during lineage commitment and provide a new layer of regulation involved in determining cardiac cell fate.**

In the last few years, scientific advances have changed the way we conceptualize the ‘dark matter’ of the genome: it is not junk DNA anymore. This is owing, in part, to deep sequencing technologies that expanded our view of the extent and complexity of the mammalian transcriptome. In 2012, the ENCODE project identified many novel and known non-coding RNAs (ncRNAs) and demonstrated that between 70–90% of the human genome is actively transcribed, although protein-coding genes account for only ~1% of the genomic sequences (Dunham *et al*, 2012).

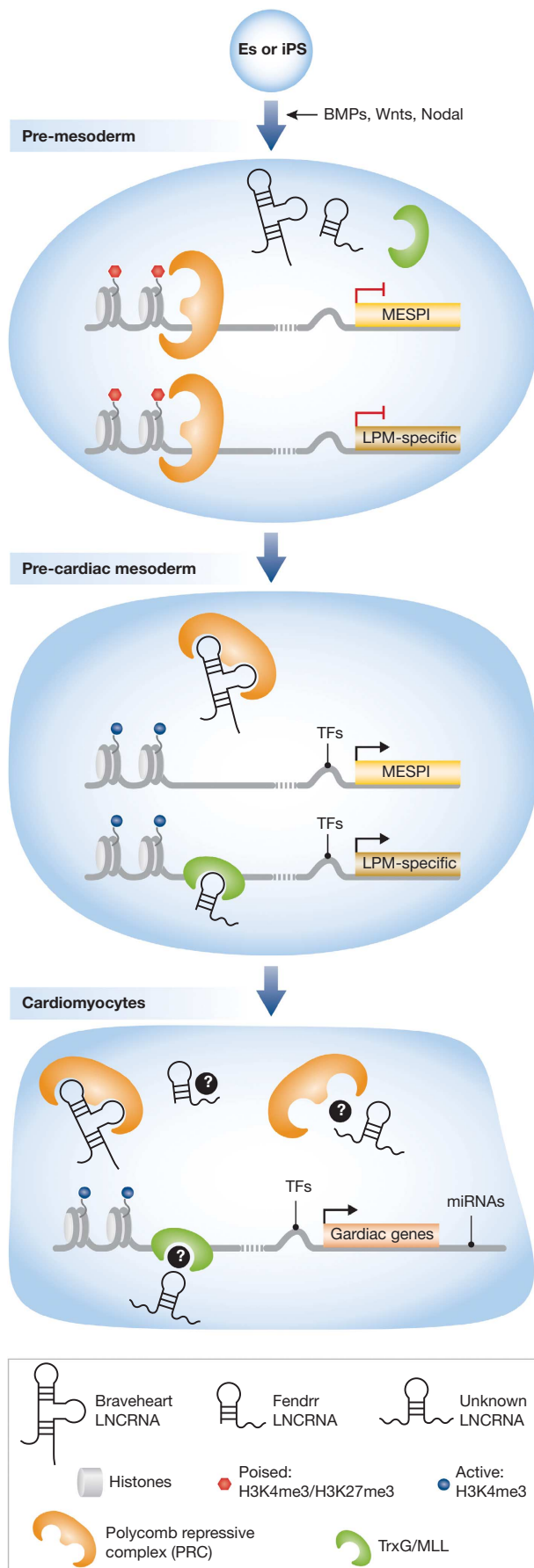
Based on their size and function, ncRNAs are subdivided into classes, including microRNAs (miRNAs), small interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), and lncRNAs, among others (Esteller, 2011). miRNAs were initially shown to be important for cardiac differentiation and cardiac development (Zhao *et al*, 2005), and have subsequently been implicated in myriad events related to cardiovascular development and responses to stress (Cordes and Srivastava, 2009). In many of these cases, miRNAs are embedded in the core transcriptional circuitry, regulated by key transcription factors, and function in positive and negative feedback loops to reinforce cellular decisions. However, the functions of other classes of ncRNAs in cardiovascular biology have been relatively unexplored.

In 1991, Willard and colleagues discovered X-inactive-specific transcript *Xist*, involved in X inactivation (Brown *et al*, 1992), representing the first-described mammalian

lncRNA. Twenty years of research on *Xist* is now being revisited as we begin to understand the thousands of newly discovered lncRNA transcripts. lncRNA transcripts are typically greater than ~200 nucleotides in length and are 5' capped and polyadenylated like most mRNAs. They have key roles in epigenetics, stem cell biology, cancer, and disease (Lee, 2012). Unlike miRNAs, lncRNAs generally lack strong evolutionary conservation. Like *Xist*, which is also poorly conserved, lncRNA sequences may be under less evolutionary constraint than protein-coding genes, allowing lncRNAs to evolve rapidly.

In one of the current papers, Klattenhoff *et al* identified a novel mouse-specific lncRNA transcript, *Braveheart* (*Bvht*), which is expressed in early mesodermal progenitors that give rise to the heart. Depletion of *Bvht* in mouse ES cells resulted in failure to activate key regulators of the cardiovascular programme, including the early mesodermal marker, *Mesp1*, which is important for emergence of the cardiac lineage (Lindsley *et al*, 2008). Consequently, mouse ES cells in which *Bvht* was knocked down did not differentiate into beating cardiomyocytes. The authors further showed that *Bvht* acts upstream of *Mesp1* by interacting with chromatin-modifying complexes that mediate transcriptional repression. Specifically, *Bvht* interacts directly with the polycomb-repressive complex 2 (PRC2), and this interaction is necessary for PRC2 to dissociate from key developmentally regulated promoters. Removal of PRC2 from promoters of regulators of specific cell fates, such as *Mesp1*, is important as ES cells adopt a differentiated state. In the absence of *Bvht*, trimethylation of histone H3 lysine 27 (repressive mark) at the promoters of important cardiac regulatory genes persisted, leading to a failure of cardiac gene expression. In addition, *Bvht* is abundantly expressed in the mouse neonatal heart, and its depletion in isolated neonatal cardiomyocytes resulted in the failure to maintain cardiac sarcomeric gene expression, such as  $\alpha$ - and  $\beta$ -myosin heavy chains. The *in vitro* data from Klattenhoff *et al* support an important role for *Bvht* in determining cardiac cell fate by regulating the epigenetic machinery required to control activation or repression of gene transcription. Whether *Bvht* is also required for cardiomyocyte differentiation *in vivo* during cardiac development awaits targeted deletion in mice.

The findings of Boyer and colleagues are similar to other reports of lncRNA interaction with PRC2 that regulate the



epigenetic signature and downstream gene transcription in cells (Lee, 2012). Shortly after the *Bvht* report, Grote *et al* (2013) described another mesoderm-enriched lncRNA, *Fendrr*, which is expressed in the lateral plate mesoderm that gives rise to the heart and body wall muscles. Deletion of *Fendrr* in mice resulted in embryonic lethality owing to impaired heart function and deficits in the body wall. In addition, the authors found that *Fendrr* interacts with components of PRC and the trithorax group/MLL (TrxG/MLL) to regulate the epigenetic state of mesodermal genes. Unlike *Bvht*, the interaction of *Fendrr* with TrxG/MLL promotes trimethylation of histone H3 lysine 4, an activating mark, at promoters of lateral plate mesoderm-specific genes (Grote *et al*, 2013). These two studies represent the first-described lncRNAs involved in cardiac differentiation and development of the heart, both by epigenetically regulating the cardiac gene network.

Dynamic regulation of cardiac gene expression is important for guiding cells into their proper cardiac lineage, including cardiomyocytes, endothelial cells, smooth muscle cells, and cardiac fibroblasts (Srivastava, 2006). Subtle disruptions in this regulation lead to congenital heart defects and disease (Srivastava, 2006). While many transcription factors are key regulators of the cardiovascular programme, the miRNA class of ncRNAs also are pivotal in cell fate, differentiation, or behaviour of each cell type (reviewed in (Cordes and Srivastava, 2009)) by negatively regulating numerous mRNAs and controlling dosage of cardiac networks. The findings by Klattenhoff *et al* and Grote *et al* demonstrate that lncRNAs provide yet another layer of genomic regulation in specific cell types (Figure 1). It will be interesting to determine if there are specific lncRNAs for other cardiovascular cell types that are important for their regulation in an analogous fashion. Furthermore, it will be important to determine if there is a lncRNA counterpart of *Bvht* in humans, and if not, whether this might help explain species-specific differences in cardiomyocyte biology.

It has become increasingly clear that knowledge of the critical regulators of the genome in individual cell types can be leveraged to control cell fate. Direct reprogramming of skin fibroblasts into induced-pluripotent stem cells by the core transcriptional machinery of ES cells has great promise for modelling human disease and potentially for cell transplantation therapies. To accurately recapitulate cardiac differentiation in a dish, it is important to gain a complete understanding of the layers of transcript regulation.

**Figure 1** Epigenetic regulation of cardiac gene expression by lncRNAs. Top, pluripotent cells (ES or iPS) are differentiated by the addition of growth factors and signals, including bone-morphogenetic proteins (BMPs), Wntless proteins (Wnt), and Nodal signals, into pre-mesodermal progenitors; in the pre-mesoderm stage, genes are poised by histone modifications and PRC binding at promoter regions; in the pre-cardiac mesoderm, transcription factors bind upstream of the *Mesp1* promoter, and the lncRNA, *Braveheart* (*Bvht*), binds to the PRC and removes it from the *Mesp1* promoter to activate the pre-cardiac mesoderm programme. Concurrently, the lncRNA, *Fendrr*, binds the activating complex, TrxG/MLL (shown above), and PRC (not shown) to allow activation of lateral plate mesoderm (LPM)-specific genes; in cardiomyocytes, TFs and miRNAs regulate cardiac-specific gene expression at the 5' and 3' ends, respectively. It remains in question whether *Bvht*, *Fendrr*, and possibly an unknown lncRNA may bind PRC and TrxG/MLL in cardiomyocytes to add further regulation.

More recently, direct reprogramming—reprogramming fibroblasts into an alternative mature cell type without first passing through a pluripotent state—represents an exciting new alternative approach for regenerative therapies (Ieda *et al*, 2010; Vierbuchen *et al*, 2010). In particular, the use of a combination of developmental transcription factors to reprogram fibroblasts to cardiomyocyte-like cells *in vitro* was first reported in 2010 (Ieda *et al*, 2010). The reprogramming process is even more effective in an *in vivo* setting after injury, harnessing the vast pool of endogenous cardiac fibroblasts in the adult heart (Qian *et al*, 2012; Song *et al*, 2012). Applying new knowledge of lncRNAs during differentiation may allow for even greater efficiency of cellular reprogramming for cardiac and other lineages. The pre-requisite for such approaches will be the development of thorough genetic blueprints of the cell types of interest, as was recently reported for the cardiomyocyte lineage (Paige *et al*, 2012; Wamstad *et al*, 2012).

As more lncRNAs are investigated at a functional level, many important questions will have to be addressed. Do lncRNAs contribute to the species diversity and complexity of higher organisms? How does lncRNA interaction with PRC or other epigenetic machinery result in specificity of gene

regulation? Do lncRNAs positively regulate transcription by interacting with activating complexes? What are the specific functions of sequences throughout the length of the lncRNA? What is the structure–function relationship of the transcript? The coming years will undoubtedly answer these and many other questions, and provide insight into the complex gene networks that control cell fate decisions. As Klattenhoff *et al* and Grote *et al* have begun to reveal, the answers are waiting to be discovered for those brave enough to delve into the ‘dark matter’ of our DNA.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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