The Yin and Yang of enhancer-like RNAs

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Long noncoding RNAs are emerging as a critical component of transcriptional activation. Over the past few years, there has been a number of examples where long noncoding RNAs expressed from distal sites exert their activating functions on neighbouring protein-coding genes. A new study in *The EMBO Journal* (Lu *et al*, 2013) now extends this paradigm by revealing a role for the transcription factor Yin Yang 1 (YY1) in the regulation of the expression of long noncoding RNAs (termed Yams) during myogenesis. Importantly, the authors demonstrate a functional role for Yam-1 in muscle differentiation through activation of a neighbouring microRNA-715 leading to downregulation of Wnt7b.

There is converging evidence that distal regulatory sites commonly known as ‘enhancers’ are transcribed in a stimulus-dependent manner as long noncoding RNAs (known as enhancer RNAs, eRNAs) and such eRNAs contribute to transcriptional activation of their neighbouring genes (De Santa *et al*, 2010; Kim *et al*, 2010; Hah *et al*, 2013; Lam *et al*, 2013; Li *et al*, 2013; Melo *et al*, 2013). Concomitantly, studies of long noncoding RNAs located in intergenic sites (termed lincRNAs, large intergenic noncoding RNAs) have uncovered a role for a class of lincRNAs (named noncoding RNA-activating, ncRNA-a) having enhancer-like functions (Orom *et al*, 2010; Wang *et al*, 2011). These activating lincRNAs are enriched with transcription factor binding sites at their promoters and display responsiveness to differentiating stimuli (Orom *et al*, 2010). While the degree of overlap between eRNAs and ncRNA-a is not clear at the present, recent studies have suggested a similar mechanism of action for the two classes of long noncoding RNAs through recruitment of Mediator–Cohesin complexes and promoting DNA looping (Kagey *et al*, 2010; Lai *et al*, 2013; Li *et al*, 2013). However, the transcription and chromatin regulatory factors underlying the expression and biogenesis of these activating long noncoding RNAs have not been illustrated.

The study by Lu *et al* (2013) examined the genome-wide occupancy of transcription factor and chromatin regulator, Yin Yang 1 (YY1), in proliferating C2C12 myoblasts (MBs) before and following their differentiation into myotubes (MTs) using low mitogen conditions. Interestingly, the authors found no significant overlap in the chromatin residence of YY1 following differentiation of the MBs into MTs. Additionally, while the authors did not find a significant overlap between YY1 and components of the polycomb repressive complex, Ezh2, binding sites, YY1 displayed a substantial (31.9%) co-occupancy with muscle master

regulator, MyoD, binding sites. Examination of publically available RNA sequencing data sets corresponding to proliferating MBs versus differentiating MTs indicated that a large number of YY1-regulated genes decreased their expression during MB to MT transition concomitant with diminished levels of YY1. These results suggested a role for YY1 as a positive regulator of these set of genes in MBs. Moreover, detailed analysis of YY1 binding sites revealed that while about 60% of YY1 occupied regions were close to the transcriptional start sites (TSS) of protein-coding genes, about a quarter of the binding sites mapped to intergenic sites distal to annotated TSS.

The occurrence of intergenic YY1 binding sites compelled the authors to examine the association of YY1 with lincRNA loci and to assess the possible regulation of lincRNA expression by YY1. Analysis of 475 intergenic YY1 binding sites led to the identification of 13.3% of binding events within 100 kb of previously annotated lincRNAs. The authors validated YY1 binding to four lincRNA loci using chromatin immunoprecipitation followed by real-time PCR. They found an enrichment of YY1 binding to these lincRNAs (named YY1-associated muscle lincRNAs or Yams) in MBs but not in MTs. Closer examination of one of these lincRNAs, Yam-1, revealed a YY1 binding site 687 bp upstream of its TSS. Importantly,

Figure 1 Long noncoding RNA, Yam-1, in enhancer looping and function. The role of long noncoding RNA, Yam-1, in the establishment or maintenance of enhancer promoter looping and activation of gene expression is depicted. Following Yam-1 expression, the loop is established to the promoter of microRNA-715 by interaction with the transcription factors and bridging factors such as the Mediator and Cohesin complexes.

while overexpression of YY1 increased Yam-1 expression, decreased YY1 concentrations using knockdown approaches resulted in decreased Yam-1 expression. These results suggested a role for YY1 as a positive regulator of Yam-1 expression in C2C12 cells. They found a similar positive regulation of Yam-1 by YY1 using primary myoblasts and following widely used muscle regeneration model in which cardiotoxin injection *in vivo* results in muscle injury precipitating a muscle regenerative process.

To assess whether Yam-1 plays a role during myogenesis, the authors examined Yam-1 expression during C2C12 differentiation. While they observed an initial increase in Yam-1 expression in proliferating MBs, its concentration gradually decreased during the course of myogenic differentiation. A similar decline in Yam-1 concentration was observed following differentiation of freshly isolated mouse primary myoblasts and during the muscle regeneration phase following cardiotoxin injection. Collectively, these results suggested that Yam-1 may play an inhibitory role in myogenic differentiation. To directly test this hypothesis, the authors depleted Yam-1 concentrations in C2C12 cells and examined the differentiation from MBs to MTs. Diminished Yam-1 concentrations led to increased numbers of myotube formation as well as increased expression of myogenic markers indicating an inhibitory role for Yam-1 in muscle differentiation. These results are consistent with the observations that YY1 similarly displays an inhibitory role in muscle differentiation and indeed Yam-1 depletion could rescue the inhibitory effect of YY1 on C2C12 differentiation. The authors extended these observations by demonstrating a similar inhibitory role for Yam-1 during muscle regeneration *in vivo*.

To assess the mechanism by which Yam-1 mediates its inhibitory role, the authors examined the possible regulation

of the neighbouring genes by Yam-1. Depletion of Yam-1 in C2C12 myoblasts resulted in a decreased expression of neighbouring microRNA, miR-715. Additionally, miR-715 displayed a similar expression profile to that of Yam-1 during C2C12 or primary muscle differentiation. It was concluded that YY1 positively regulates Yam-1 expression, which led to increased concentration of miR-715 during myogenesis. Finally, the authors propose a role for miR-715 in post-transcriptional silencing of Wnt7b leading to decreased MT formation. Further knockdown experiments revealed both positive and negative roles for additional Yams (Yam-2, Yam-3 and Yam-4) in muscle differentiation.

Taken together, these experiments define a foundation for analysis of the role of long noncoding RNAs in muscle differentiation. The authors point out an important regulatory role for the YY1 protein in positive regulation of a set of lincRNAs that may have enhancer-like functions. Their detailed functional analysis suggested an activating function for one of these lincRNAs, Yam-1, in regulation of a neighbouring microRNA gene. However, the precise molecular mechanism by which Yam-1 activates its neighbouring genes remains to be elucidated. While recent experiments have shed some light on the mechanisms by which long noncoding RNAs activate transcription (Figure 1), the precise RNA code(s) required for activating function remains to be discovered. In the future uncovering the long noncoding RNA sequence determinants or structural requirements (secondary or tertiary) that govern enhancer function will be of prime importance.

Conflict of interest

The author declares that he has no conflict of interest.

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