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RESEARCH HIGHLIGHT

A lincRNA switch for embryonic stem cell fate

Liuqing Yang[1], Chunru Lin[1], Michael G Rosenfeld[1, 2]

[1]*Howard Hughes Medical Institute;* [2]*Department of Medicine, Division of Endocrinology and Metabolism, University of California, San Diego School of Medicine, 9500 Gilman Drive, La Jolla, CA 92093-0648, USA*
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Cumulative evidences have demonstrated that most of the non-repetitive genome in higher organisms are actively transcribed and surprisingly only a small percentage (< 20%) of transcripts are associated with genes that encode proteins [1]. One of the emerging themes in the study of non-coding transcripts is large intergenic non-coding RNAs (lincRNAs), a class of large regulatory RNAs implicated in imprinting, dosage compensation, and transcriptional regulation [2]. In light of recent discoveries revealing the flexibility of lincRNAs and their abilities to act as modular scaffolds for protein–chromatin interactions and to form spatially compact arrays of complexes [3, 4], many would acknowledge that most lincRNAs act as sensors and integrators of a wide variety of regulated transcriptional responses and probably epigenetic events, which may have an impact on various human diseases. However, skeptics would suggest that multifocal polymerase entry has no functional significance, and present the challenge of determining whether there is a broad functional network and biological roles of lincRNAs that might regulate gene transcriptional programs involved in homeostasis and human diseases. An initial broad attack on this problem is provided by the report by Guttman *et al*. [5], which helps to shed further new light on the "doubts" by delineating a comprehensive network of lincRNAs in a specific cell type and their function as key regulators of global transcriptional programs controlling embryonic stem (ES) cell pluripotency and differentiation (Figure 1). Beyond their importance for our understanding of complexity of non-coding transcripts, these findings show that a previously unsubstantiated network of such influence, as lincRNA can synchronize the activities of different histone-modifying enzymes to regulate gene expression important to the development of life (Figure 1).

The research group, led by Eric Lander at Broad Institute, uncovered a chromatin "signature" for actively transcribed regions between known protein coding genes through a massive sequencing of ChIP data, and used this signature to identify > 3 500 unique forms of lincRNAs in 2009 [6]. Since then, the identification of mechanisms underlying their transcription, regulation, and potential functional roles has been challenging. Studies from several groups suggested a fundamental role of lincRNA in transcription regulation by orientating chromatin-modifying factors/complexes to specific locations in the genome [7, 8]. Now, Guttman *et al*. use genetic techniques to turn off and on the production of 100 specific lincRNAs in ES cells and find out that one of surprising functions of these transcripts is to coordinate and organize the assembly of chromatin modifying proteins in ES cells, thus permitting the fine tuning of gene expression pattern critical for pluripotent state. Evidence that lincRNAs bind diverse chromatin proteins in ES cells was inferred from an impressive set of computational and experimental data. These included (i) ES cell lincRNAs are globally associated with histone "reading", "writing" and "erasing" complexes; (ii) most of ES lincRNAs are strongly associated with multiple chromatin complexes; (iii) correlation of ES cell gene expression programs with lincRNA-protein interactions (> 40%). Guttman *et al*. thus further elucidate two important questions in the field: (i) how many distinct complexes are recruited to various ncRNAs, as reported for individual ncRNAs such as *ANRIL* [8]? (ii) what is the combinatorial "code" of multiple lincRNA-protein complexes that might be required for maintaining epigenetic memory of ES cells?

Given the widespread involvement of lincRNAs in gene repression and/or activation, acting as sensors of various regulatory signals [9], an immediate question concerns the way in which the functionality of lincRNAs is exerted in *cis* or in *trans*. In contrast to what was previously emphasized as a *cis* acting mode of linRNAs [10], Guttman *et al*. favor the notion that lincRNAs that they studied based largely on their high levels of expression seem to influence

Correspondence: Liuqing Yang[a], Michael G Rosenfeld[b]
[a]E-mail: liy001@ucsd.edu
[b]E-mail: mrosenfeld@ucsd.edu

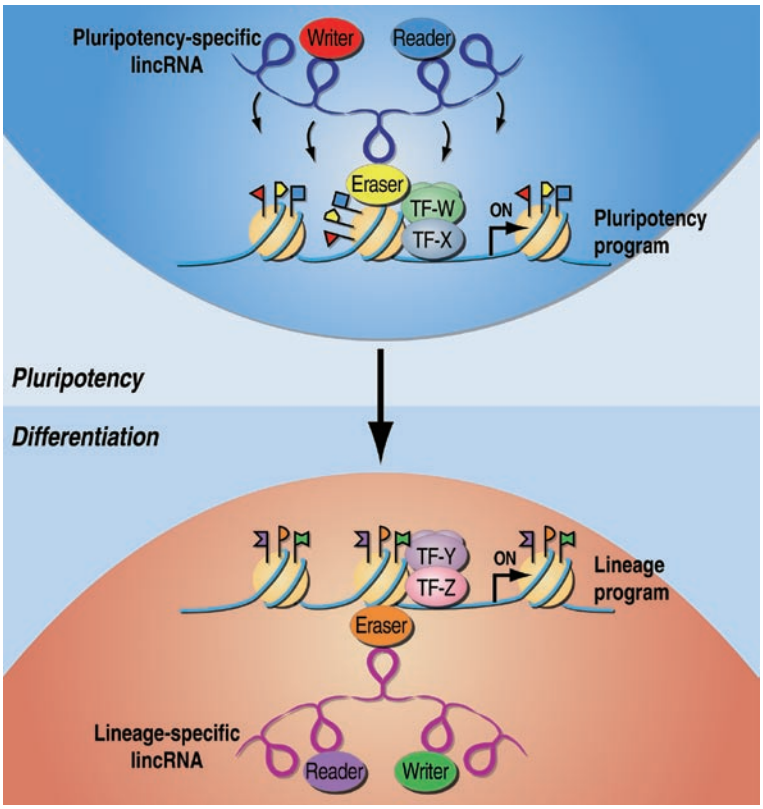

Figure 1 Interplay between cell-type-specific lincRNA-protein complexes determines ES cell transcription programs, which dictates pluripotency vs. differentiation states. Pluripotency- and lineage-specific lincRNAs serve as platforms for assembly of multiple chromatin-modifying complexes, thus licensing the "reading", "writing" and "erasing" of a variety of histone tail marks.

gene expression largely in *trans*. There are several possibilities for this apparent discrepancy. First, the *cis* and *trans* action of lincRNAs might be regulated by signal-induced nuclear architecture changes. Recent evidence suggests that dynamic three-dimensional genomic interactions in the nucleus, in addition to long-range intra- and inter-chromosomal interactions, exert critical roles in regulated gene expression and chromosomal translocations [11]. *Cis* and *trans* action of lincRNAs could be switched by signal-induced intra- and inter-chromosomal interactions. It would be intriguing to investigate whether pluripotency and differentiation gene expression in ES cells is associated with nuclear architecture changes and if this is the case, how ES lincRNAs are involved in *trans*. Second, same lincRNAs may function in *cis* or in *trans* depending on their subnuclear locations. It is now evident that the nucleus is a complex, dynamic "organelle" with functional subnuclear domains intimately linked to the genome allowing signaling and ultimately regulation of gene activity [12]. Considering that several non-coding transcripts have been localized to specific subnuclear structures, *e.g.*, nuclear speckles, paraspeckles [13], one might envision that potential relocation of lincRNAs between transcriptionally repressive and permissive environment could switch their action mode.

The findings from Guttman *et al*. enforce the growing evidence that lincRNAs exert important functional expression of the genome rather than biological noise, suggesting that the genome might encompass intricate lincRNA-based networks that are far more sophisticated than we might have expected. It seems likely that these networks have continued to be harnessed by development in a range of biological processes. From therapeutic perspective, the work of Guttman *et al*. may make it possible to overcome the challenge of coaxing stem cells into differentiation for therapeutic exploitation. For example, by inhibiting lincRNAs in specific combinations, stem cells could be transformed in specific ways, and this will advance the utility of stem cells for treatment of degenerative diseases.

The report by Guttmann *et al*. also reminds us that there are many more interesting questions to be answered. First, how do specific proteins interact with lincRNAs and how do these interactions regulate gene transcription? Because of RNA sequence and structural flexibility, it will be of interest to generalize the sequences and the structural motifs of lincRNAs that direct specific protein recognition. Second, it is very interesting that lincRNAs are mostly associated with chromatin-modifying factors. An immediate question is how lincRNA and chromatin-modifying protein interactions promote specialized functions. One possibility is that lincRNAs can allosterically regulate histone "readers", "writers" or "erasers", altering their ability to modulate "repressive" or "activating" epigenetic marks. Third, where are lincRNAs localized within three-dimensional space of nucleus? Analogous to the role of rRNAs in ribosome assembly, lincRNAs can exert functional roles in specific subnuclear organelles and play key roles in regulation of nuclear architecture. Finally, we need to build a catalogue of lincRNAs with common characteristics that will be used to identify and predict the functional features, complemented by experimental analyses in individual cases to determine the mechanisms by which lincRNAs are connected to

diseases. The development of next-generation sequencing and the demonstration of its utility in the identification of non-coding transcripts, intersected with existing molecular techniques from other fields, such as live cell RNA imaging, RNA-protein proteomics and RNA structural biology, suggests that it is likely that many of these questions will be answered in the not too distant future.

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