Noncoding RNA Scaffolds in Pluripotency
Tanmoy Mondal, Chandrasekhar Kanduri

LincRNAs Act in the Circuitry Controlling Pluripotency and Differentiation
Guttman et al

Human Long Non-Coding RNAs Promote Pluripotency and Neuronal Differentiation by Association With Chromatin Modifiers and Transcription Factors
Ng et al

The molecular circuitry that maintains pluripotency of mouse and human embryonic stem cells has been protein-centric. Two recent reports now add long noncoding RNAs as partners alongside the transcription factors in the maintenance of pluripotency.

Understanding of the biological pathways that maintain pluripotency is one of the most striking developments in modern biology and has the potential to revolutionize the field of regenerative medicine. In the recent past, these pathways have been successfully employed to turn the lineage-committed somatic cell into functional pluripotent stem cell. Until recently research in this area was restricted to protein factors whose role in different biological pathways have been relatively well characterized. Pervasive transcription across mammalian genomes generates thousands of long noncoding transcripts and have been shown to involve diverse biological functions that have impact on development and differentiation. These recent developments generated intense scientific interest in finding new RNAs and mechanisms that could have a potential role in maintaining pluripotency.

Previously, small and long RNAs, which are under the control of pluripotent transcription factors, have been implicated in the maintenance of embryonic stem cell (ESC) state and it has been shown that their downregulation promotes ESC differentiation. For example, it has been reported that miRNAs like mir-141, mir-200, and mir-145 regulate the

![Diagram](http://circres.ahajournals.org)

**Figure.** A, Models proposing the mode of action of pluripotent long noncoding RNAs. **B.** Long noncoding RNA mediated repression of differentiation-specific gene expression programs. Left panel shows long noncoding RNA-PRC2/CoREST complex mediated silencing of genes that promote differentiation in embryonic stem cells (ESCs). Right panel shows Sox2 guides long noncoding RNA-PRC2 complex to silence genes of non-neuronal lineages in neural stem cells. lincRNA indicates long intergenic noncoding RNA; TF, transcription factors.

ESC pluripotency program and that their expression is controlled by pluripotent transcription factors like c-Myc and Oct4.1,2 Recent evidence also implicates long noncoding RNAs (lncRNAs), range in size from 200 nt to several hundred kb long in the ESC pluripotency.3 Using custom-designed microarrays, a previous study identified a couple hundred lincRNAs, which are differentially expressed between ESCs and differentiated embryoid bodies. Some of these lincRNAs have been shown to interact with chromatin modifiers and regulate gene expression in cis by controlling local chromatin structure.4 Like small noncoding RNAs, some lincRNAs are also under control of the pluripotency factors like Oct4 and Nanog, and interestingly, these lincRNAs seem to activate the transcription of pluripotent transcription factors in a regulatory positive feedback loop.1 Though these reports point toward a functional role of lincRNA in maintaining pluripotency on a smaller scale, comprehensive investigations on the functional role of lincRNAs on a global perspective are lacking. Addressing this

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pluripotent transcription factors. More importantly, gene promoters are also enriched with binding sites for finding in the human ESCs (hESCs) that several lncRNA pluripotency associated transcription factors. This observa-
tion in cis, indicating that the lncRNAs characterized using active genes, in the complex molecular circuitry of ESCs. Previously, these authors have identified 226 lincRNAs that are differentially expressed in mouse ESCs. One hundred forty-seven of 226 lincRNAs were successfully downregulated using shRNA and their effects on global transcription were analyzed using genome-wide microarrays. The majority of the lincRNAs affect the global transcriptional profiles, and in each case the affected target genes range from 20 to 1000. The target genes are often located in trans, whereas only a few candidates were noted affecting target gene expression in cis, indicating that the lncRNAs characterized using active chromatin maps have gene targets preferentially located in trans. Another interesting observation that Guttmann et al noted is more than 75% of 227 ES cell specific lincRNAs have binding sites for at least 1 of 9 of the well-characterized pluripotency associated transcription factors. This observation is consistent with previous data and also with a recent finding in the human ESCs (hESCs) that several lncRNA gene promoters are also enriched with binding sites for pluripotent transcription factors. More importantly, knockdown of the pluripotency associated transcription factors using shRNA affected about 50% of the ES cell-specific lincRNAs. Collectively, these observations emphasize a strong and consistent link between pluripo-
tency and lncRNAs.

Next, Guttmann et al have addressed the functional role of lincRNAs in the pathways that maintain ESC state. The authors have characterized the lincRNA role in ESC state based on their effects on maintenance of pluripotency and repression of the lineage-specific gene expression programs in ESCs on their downregulation using shRNAs. To identify lincRNAs involved in the maintenance of pluripotency, the RNAs were downregulated in an ESC line expressing the luciferase gene under the endogenous Nanog promoter. By scoring the Nanog promoter activity in the loss of function experiments, they identified 26 lincRNAs with possible role in the maintenance of pluripotency. This indicates that lincRNAs could execute their functions via regulating key pluripotency factors like Nanog. This is not surprising considering the earlier observation that Oct4 transcription can be modulated by lncRNA (AK141205) in a regulatory positive feedback loop. Of note, this observation implicates lincRNAs operating upstream of the key pluripotency factors. Though the molecular mechanisms underlying the lincRNA mediated maintenance pluripotency are far from clear, the following models could explain the effects of lncRNA on pluripotency. (1) Association of the lincRNAs with active chromatin modifiers as documented by Guttmann et al, and also by earlier studies, indicate that lincRNAs may directly regulate pluripotency factors by recruiting the active chroma-
tin modifiers to their gene promoters (Figure A, left panel). (2) lincRNAs activate transcription factors, which in turn regulate the expression of the pluripotency factors (Figure A, middle panel). (3) Alternatively, downregulation of Nanog promoter could also occur as a consequence of exit from the pluripotency state on downregulation of lincRNAs. In the latter scenario, the lincRNA could be maintaining nonredundant pluripotency pathways in parallel to that of protein factor controlled pluripotency pathways.

Repression of lineage-specific differentiation programs is another important requisite in the maintenance of ESC state. Several protein factors including polycomb proteins have been implicated in the repression of lineage-specific differ-
etiation programs. To identify the lincRNAs that may have a role in the repression of genes responsible for differentia-
tion programs, the authors compared the gene expression data sets from the shRNA-mediated knockdown of the lincRNAs with the gene expression patterns obtained after induced differentiation of mouse ESCs into different lineages. These comparisons yielded nearly 30 lincRNAs whose downregu-
lation led to specific activation of gene expression programs that are critical for maintaining different lineages, suggesting that this subset of lincRNAs act as a repressor of the differentiation programs in mouse ESCs. However, their downregulation did not result in exit from the pluripotent state, indicating the existence of several redundant players in the repression of lineage-specific differentiation programs.

Guttmann et al also addressed the lincRNA–protein interac-
tions crucial for the maintenance of pluripotency and sup-
pression of lineage-specific differentiation programs. They found that about 30% of 227 ESC-specific lincRNAs interact with the chromatin modifiers capable of writing, reading, and erasing the chromatin marks, indicating that lincRNAs like protein counterparts can program chromatin landscapes across the genome, and this is consistent with several lines of previous evidence on the role of noncoding RNA in chroma-
tin organization. In the shRNA knockdown experiments of lincRNA and their associated chromatin modifiers, more than 40% of 74 ESC-specific lincRNAs had overlapping gene expression programs with their associated chromatin modifici-
ers, thus presenting a synergistic picture where lincRNA and protein partners act together on common gene targets.

In another interesting investigation, Stanton and col-
leagues addressed the functional role of lncRNA in the maintenance of pluripotency and differentiation programs using hESCs as a model system. They used a customized microarray system containing 6671 transcripts with at least 6 to 8 probes covering each transcript. Of the 6671 lncRNAs, 36 lncRNAs showed hESC-specific differential expression on arrays. Further validation by qPCR identified 3 lncRNAs with exclusive expression to hESCs. All 3 lncRNA promoters are occupied with binding sites for pluripotency factors like Oct4 or Nanog and downregulation of these factors affected the expression of the lncRNAs, indicating that these lncRNA molecules could be direct targets of pluripotency transcription factors. However, downregulation of pluripotent trans-
scription factors and upregulation of lineage-specific gene expression programs on downregulation of lncRNAs indicate that there is a tight interplay between lncRNA and pluripo-
tency factors, where lncRNA and pluripotency factors regu-
late each other in a positive feedback loop mechanism (Figure
A, right panel). From the data, it is difficult to conclude whether lncRNAs act upstream or downstream of pluripotent transcription factors. Interestingly, pluripotency factors (Sox2) not only regulate the transcriptional activity of lncRNAs but also interact with lncRNAs, indicating that lncRNAs and pluripotency factors together with PRC2 complex members could be involved in lineage-specific repression programs.

The authors have also characterized lncRNA required for neuronal differentiation and identified about 35 lincRNAs that showed significant expression in neurons in comparison to hESCs and neuroprogenitor cells. Functional characterization of 4 lincRNAs revealed that their expression is crucial for differentiation to neurons from neural stem cells and this act of lincRNAs in neuronal differentiation also involves interactions with nuclear proteins like SUZ12 and REST. lincRNA’s interaction with PRC2 in hESCs is required for maintaining pluripotency in ESCs through silencing lineage-specific differentiation genes (Figure B, left panel), whereas their interaction with PRC2 complex in neuronal stem cells is required to promote neuronal differentiation probably through repressing genes of nonneuronal lineages (Figure B, right panel). These contrasting observations highlight the cell type-specific plasticity of lincRNA functions with common protein interacting partners. We presume that this plasticity could be achieved in part through cell type-specific secondary structures of lincRNAs that could allosterically change the conformation of the interacting chromatin modifiers or transcription factors such that they could execute lineage-dependent gene expression programs.

**Conclusions and Outlook**

There have been several missing links in understanding how transcription factors alone can maintain ESC pluripotency and lineage-specific commitments of differentiating cells. Identification of genome-wide transcription encoding thousands of small noncoding RNAs and lincRNAs and uncovering of their role in differentiation and development has started providing more information on hitherto uncovered mechanisms involved in the maintenance of pluripotency and lineage commitment. Studies from Guttman et al and Ng et al along with previous studies have begun to provide insights into the intricate mechanisms that establish and maintain pluripotency. The common theme emerging from these studies is that there is a strong nexus between pluripotency factors and lincRNAs. Characterization of Oct4, Nanog, and Sox2 binding sites in the lincRNA promoter regions and the similarities in the lineage repression programs executed by lincRNAs and pluripotent transcription factors as indicated by Guttman et al suggest that lincRNAs take cues from pluripotency-specific transcription factors in maintaining pluripotency. On the other hand, their downregulation in lincRNA knockdown experiments suggest that lincRNA could act upstream of pluripotency factors. These studies have highlighted the intricate nature of the communication between pluripotent transcription factors and the so-called pluripotent lincRNAs and further studies in this direction would uncover the hidden communication between pluripotent transcription factors and pluripotent lincRNAs. It seems that pluripotency factors not only regulate the transcription of lincRNA promoters but also seem to interact with their encoded products. For example, interaction of the lincRNAs with both pluripotency factors like SOX2 and along with the chromatin repressor proteins like REST, indicate that Sox2 probably guide these lincRNAs to their target sites to mediate lineage-dependent repression function (Figure B, right panel).

Given a wide reach of lincRNAs in the execution of various biological functions, one can envisage lincRNA regulating biological pathways independent of pluripotency factors. This contention is supported by the observation that knockdown of several lincRNAs responsible for maintenance of pluripotency resulted in downregulation of several pluripotent factors. Future research in this direction will help us in understanding whether lincRNAs carryout a primary role or a supporting role to pluriprotein protein factors in the maintenance of pluripotency.

Deeper understanding of maintenance of pluripotency and various lineage-specific differentiation events hold a great promise for cell-based therapies in regenerative medicine. So far, no studies have been conducted to identify lincRNAs that are required for lineage-commitment of differentiating ES cells. Identification lincRNAs by Ng et al in neuronal differentiation is the first such investigation that could stimulate more studies in other differentiation events. Genome-wide association studies have identified a few hotspots encoding lincRNAs in cardiovascular diseases. An antisense RNA, CDKN2B-AS1, expressed from a gene desert region in 9p21 locus, is strongly associated with various cardiovascular diseases including coronary atherosclerosis. Likewise, MIAT lincRNA on human chromosome 22 with pluripotency functions is linked to heart disease like myocardial infarction. HESCs have been used as ex vivo source for cardiomyocytes for cell-based heart therapies. Given that the majority of lincRNAs interact with chromatin modifiers and their potential role in the maintenance of pluripotency and repression of lineage-specific gene expression programs, characterization of lincRNA involved in the cardiomyocyte differentiation would help significantly in improving cell-based therapies for cardiovascular disease.

Though the genome-wide association studies implicated several SNPs, mapping to gene desert and intergenic regions, in human diseases, only a few of them have been functionally characterized. With the availability of transcriptional landscapes across the human genome in normal and disease conditions, it is now possible to explore the functional link between disease-associated SNPs and lincRNA regulation and their significance to pathogenesis of various diseases, including cancer and cardiac diseases.

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**Disclosures**

None.
References
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