Epigenetic regulation of stem cell fate

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Received February 13, 2008; Revised April 18, 2008; Accepted May 2, 2008

Stem cell-based regenerative medicine holds great promise for repair of diseased tissue. Modern directions in the field of epigenetic research aimed to decipher the epigenetic signals that give stem cells their unique ability to self-renew and differentiate into different cell types. However, this research is only the tip of the iceberg when it comes to writing an ‘epigenetic instruction manual’ for the ramification of molecular details of cell commitment and differentiation. In this review, we discuss the impact of the epigenetic research on our understanding of stem cell biology.

INTRODUCTION

Recent analysis of specific epigenetic features of human and mouse stem cells has provided important insights into the unique properties of pluripotent and lineage-restricted stem cells. Epigenetic mechanisms include: modifications of the histones and incorporation of histone variants; changes in DNA methylation and Adenosine-5'-triphosphate-dependent chromatin remodeling; implementation of RNAi pathways and non-protein-coding RNAs (ncRNA). These mechanisms represent the final outcome in the transcriptional hierarchy mediated by transcriptional factors and are designated to alter the gene function without changes to the DNA sequence. Slight variations of these epigenetic components might result in slight changes of the transcriptional regulation pattern within the cell. By examining the abundance of modified histones, polycomb group (PcG) protein binding patterns, replication timing and chromatin accessibility, new studies have revealed that stem cells manage their status by multiple layers of molecular events designed to impose flexible but precise control over the expression of the important regulatory genes. This can be done by promoting the expression of pluripotency-associated factors, such as OCT4 and NANOG and transiently prohibiting activation of the genes that drive the cellular differentiation along specific differentiation pathways. Until recently, the analysis of stem cells and their lineages has largely focused on transcriptional regulation. Emerging evidence, however, suggests that genome undergoes major epigenetic alterations during mammalian development and embryonic stem cells (ESC) differentiation (1). Therefore, the molecular mechanisms that provide control of these epigenetic events are essential components of stem cell biology, indispensable for establishing and conveying gene expression patterns during cellular differentiation. These gene expression patterns not only heavily rely on the transcriptional factor networks (Fig. 1), but also on the properties of the chromatin within the cells [histone modification, histone variants, DNA methylation status, non-protein-coding RNAs (ncRNAs)], which represent ‘epigenetic make-up’ of the cell. Somatic cell nuclear transfer experiments demonstrate that reprogramming to a pluripotent state does require large-scale epigenetic changes (2,3).

In this review we will discuss the recent progress in stem cell research by looking at the data accumulated in the recent year throughout the prism of the basic epigenetic terms. We will address the following questions: (i) How is epigenetic involved in retaining stem cell potential? (ii) What underlies progenitor cell plasticity? (iii) How does a stem cell rapidly transition into a morphologically and molecularly distinct cell type? (iv) Is this process reversible?

STEM CELLS AND EPIGENETICS

The multipotency of stem cells is reduced over time due to progressive gene silencing. Genes active in earlier progenitors are gradually silenced at developmentally later stages, and subsets of cell type-specific genes are turned on. This progression is the result of selective expression of transcription factors (TFs) in concert with classical ‘corner stones’ of epigenetics – chromatin remodeling and chromatin modifications, DNA methylation of CpG dinucleotides (4,5). As a result of
these events compactization of the chromatin, its accessibility and positioning within specialized nuclear domains undergo dynamic changes. For example, it has been shown that heterochromatic markers, such as HP1 proteins, as well as heterochromatic histone modifications change their localization from dispersed and very dynamic in ESC to more concentrated distinct loci during cellular differentiation (6,7). This suggests that differentiation leads to the restructuring of the chromatin accompanied by the change in the global nuclear architecture, thus allowing the pluripotent nature of ESC genome to become more condensed, and, therefore, more transcriptionally restrained with maturation of the heterochromatin.

In mammalian cells, both DNA methylation and specific histone modification are involved in chromatin silencing. DNA methylation and histone modification are believed to be interdependent processes. Recent studies suggest that a combination of histone acetylation and DNA demethylation induces neuronal stem cells (NSC) to trans-differentiate into

Figure 1. Epigenetic response to extrinsic signals occurs through the transcriptional factors network.
hematopoietic cells (8). This observation allows speculating that alteration of the epigenetic properties of NSC results in greater NSC plasticity to cross the barrier of their commitment and differentiate along alternate lineages. Contrary to this, when mouse hematopoietic stem cells (HSC) were exposed in vitro to soluble factors known to induce neuronal differentiation of neural stem cells and ESC, only a low number of these HSC cells displayed some features of immature neural-like cells. However, in vivo or in vitro neural environments, they either died or gave rise to cells with a macrophage/microglial phenotype (9). This set of published data argues that there are no simplified algorithms for trans-differentiation events and main epigenetic principles of these events have yet to be uncovered. Where is the place for epigenetic study in stem cell biology?

TRANSCRIPTIONAL FACTORS NETWORKS AND EPIGENETICS

Batteries of TFs have been proposed to control stem cell self-renewal and lineage progression and are also a powerful mechanism for generating cell diversity (10). Progression along the lineage from stem cell to differentiated cell is characterized by striking morphological and functional changes at each stage of the lineage commitment. The sequential expression of TFs and other signaling molecules, which elicit cascades of gene expression, regulate each other and interact with epigenetic control factors to form large gene regulatory networks. The identification of the common target sites by chromatin immuno-precipitation (ChIP) assay together with genome-wide location analysis has suggested that OCT3/4, SOX2 and NANOG might form a regulatory network that maintains pluripotency and self-renewal in mouse and human ESC (11,12). In the emerging picture, a large number of auxiliary transcriptional factors have been implicated to play a role in ESC biology in addition to ‘master regulators’. These auxiliary factors include Stat3, Tbx3, FoxD3, Myc, p53 (13–19). Several attempts to create a TF’s gene regulatory network in ESCs in order to predict regulatory interactions (20) resulted in outlining the collaborative roles of these factors in gene repression or activation and revealed a surprising role of nuclear receptors (16,17,21). Then another crucial observation emerged from this type of research, with the discovery that the most important direct targets activated by the core regulatory network were themselves transcriptional regulators, the activities of which might extend the regulatory effects of the network to numerous secondary targets (22). How is this TF regulatory network connected with epigenetic phenomena?

Highly conservative non-coding elements and chromatin ‘bivalency’

Our understanding of the regulatory mechanisms for the development—specific TF’s network significantly benefit from the study of highly conservative non-coding elements (HCNE). These DNA sequence elements are highly concentrated near genes encoding developmentally important transcriptional factors (23) and have been shown to partially overlap with regions of ‘bivalent’ chromatin modifications (24). Classically, methylation of lysine 27 of histone H3 (metK27H3) is implicated in formation of the silent chromatin and transcriptional repression. Methylation of lysine 4 residue on histone H3 (metK4H3) defines active chromatin. Genome-wide ChIP analysis performed independently by several groups revealed that large areas of repressive histone modification of methylated lysine 27 of histone H3 (metK27H3) inter-mingle with smaller regions of permissive chromatin marked by methylated lysine 4 (metK4H3) at HCNE (24–26). These ‘bivalent’ domains allow important TF genes to be poised for rapid transcriptional activation upon differentiation. In the proposed model of the gene regulation through the ‘bivalent’ domains, the differentiation events shift the balance of the opposing chromatin modifications in the way that the silent genes will be enriched only for metK27H3 and the active genes will bear only euchromatic modification metK4H3. Interestingly enough, a significant number of ‘bivalent’ domains are enriched for binding sites for at least one of the three pluripotency associated factors, OCT4, NANOG and SOX2, which implies that their formation and/or maintenance might be regulated through these ‘master regulators’.

Chromatin ‘bivalency’ is posing an interesting hypothesis proposed to provide for molecular mechanisms involved in regulation of ESC differentiation and maintenance of pluripotency, however, it fails to explain epigenetic regulation of some loci during B-cell development (27). In this study, the intergenic cis-acting element in the mouse lambda5-VpreB1 locus does not show proposed ‘bivalency’. On the contrary, it is marked by histone H3 acetylation and histone H3 lysine 4 methylation at a discrete site in ESCs. The epigenetic modifications spread from this site toward the VpreB1 and lambda5 genes at later stages of B-cell development, and a large, active chromatin domain is established in pre-B cells when the genes are fully expressed. These results suggest that localized and unambiguous epigenetic marking is important for establishing the region of transcriptional competence for the lambda5 and VpreB1 genes as early as the pluripotent ESC stage thus by-passing the need for ‘bivalency’. Future investigation of the nature of ‘bivalent’ chromatin and chromatin modifying activities depositing these modifications will shed additional light on the functional meaning of this interesting observation.

Epigenetic memory and histone variants

In order to maintain the stable self-renewal of ESCs, the mechanisms that prevent their differentiation and promote their proliferation must be transmitted to their daughter cells, which imply the significance of the ‘epigenetic memory’ mechanisms in this process. This ‘epigenetic memory’ allows cells to maintain their identity, even when exposed to extracellular environments that induce formation of other cell fates. This is also important for maintaining stem cells over time and in preventing tumor formation. Historically cellular inheritance was explained by methylation of a promoter DNA, but recently published data argues in favor that DNA methylation is not the only mechanism elaborated by the cell to provide for epigenetic memory. Histone variants deposition into chromatin of actively transcribed genes (for example H3.3) can contribute to the cellular memory phenomena. Experiments performed by Ng and Gurdon in Xenopus laevis provide for the first...
documented evidence of the persistence of epigenetic memory of a transcriptionally active state and propose the role of histone variant H3.3 in this process (28). How is the epigenetic memory of the silent chromatin handled?

**An epigenetic modifier—the Polycomb-group complex**

A series of recent studies have revealed that in order to maintain pluripotency, mouse and human embryonic cells elaborate epigenetic mechanisms for a dynamic repression of genes regulating developmental pathways in such a way, that this repression can be maintained through cell division (20). One of the most intriguing candidates in the spotlight of current research is an epigenetic modifier Pcg proteins (29–33). PcG proteins were originally identified in Drosophila as repressors of Hox genes which, when mutated, resulted in posterior transformation of body segments (34). Polycomb repressive complex 2 (PRC2) acts to stabilize a repressive chromatin structure through the function of chroma-
tin modifiers such as enhancer of zeste (Ezh2), embryonic ectoderm development protein (Eed) and suppressor of zeste 12 (Suz12) – histone methyltransferases responsible for depositing tri-met K27H3 mark on the chromatin (25,35). Ezh2 has been shown to be required for maintaining the proper tri-met K27H3 marks in pluripotent epiblast cells (36,37), and is proposed to play a major role in maintenance of the stem cell state. By performing ChIP-on-CHIP analysis for Suz12 and Eed proteins in ESCs, Lee et al. (30) have demonstrated that genome-wide binding of these modifiers overlaps with chromosomal region of metK27H3 deposition within the highly evolutionary-conserved genomic segments in the vicinity of the transcriptionally silent genes. The 1800 genes identified as targets include the majority of the OCT3/4, Nanog and Sox2 repressed genes in human ESC from Boyer’s analysis (11), including Gata4 and Cdx2 regulators of differentiation. These results suggest that Prc2 could be potentially viewed as a component of ‘epigenetic memory’ strategy required for the ESC maintenance.

However, these results do not explain fully ESC models deficient for one of Prc2 component (e.g. Eed). Mutant ESCs demonstrate growth lost of K27H3 methylation but still retain their ability to self-renew and do maintain normal morphology (31,38). Surprisingly, despite the fact that several neuron-specific genes and Gata4 and Gata6 factors are transcriptionally up-regulated at the background of Eed deficiency, the mutant ESCs simply manifest the high level of spontaneous differentiation (31) and still are capable of producing all the three germ layers upon injection in blastocyst (22,38,39). Similar to this, Suz12−−/− ESCs do not demonstrate a full requirement for the Prc2 in maintenance of ESC pluripotency (30).

Many lines of evidence suggest that DNA methylation regulates the timing of differentiation and maintenance of the cell-type identity (40–42). Therefore, the cellular inheritance could be achieved by means of methylation on the DNA. The PcG proteins can directly control DNA methylation (43), thus providing another important role for PcG as connector between key epigenetic regulators. Future analysis of the DNA methylation status in polycomb deficient ESC models will extend our understanding of ‘epigenetic memory’ of a silent state and its link to pluripotency.

**Role of histone demethylases in embryonic stem cell epigenetics**

Epigenetic component is proven to be significant for the differentiation of pluripotent stem cells into specific tissue lineages. The exit from the self-renewing state is accompanied by changes in the covalent modifications of the histones, for example, an increase in the silencing-associated histone H3 Lys 9 dimethylation and trimethylation (Met2/Met3K9H3) marks on the chromatin and removal of MetK27H3. DNA sequence-specific factors can provide the ‘landing pad’ for the recruitment of specialized enzymatic machineries that either deposit (32) or remove the modification on the chromatin (for details in multitude of the histone modifications and substrate-specificity of enzymes responsible for their deposition see 44,45). While it has been known for a number of years that most of histone modifications are reversible, only recently it has been shown that methyl-groups could be enzymatically removed from lysine residues and enzymes that remove this modification have been identified (44,46–49). The debate as to whether the tri-met K27H3 can be actively removed has been recently settled by a series of papers (50–54) identifying two related jumonji-family proteins, Jmjd3 and Utx, which specifically demethylate tri-met K27H3. These demethylases are members of the mixed-lineage leukemia (MLL) protein complexes known to antagonize PcG-mediated gene silencing. Interestingly, Jmjd3 is a direct gene target of Silencing Mediator of Retinoic Acid and Thyroid hormone receptors (SMRT), which, through its interaction with retinoic acid receptors (RARs) represses Jmjd3 expression to maintain the neural stem cell state (55). RA treatment of neural progenitors resulted in up-regulation of Jmjd3 and a decrease in tri-met K27H3 levels on the promoter of the Dlx5 gene, a marker of differentiated neurons.

It has also been shown that the di-met K9H3 and tri-met K9H3 demethylase genes, Jmjda and Jmjd2c, are positively regulated by the ESC TF OCT4. Interestingly, Jmjda or Jmjd2c depletion leads to ESC’s differentiation, which is accompanied by a reduction in the expression of ESC-specific genes and an induction of lineage marker genes. Jmjda demethylates di-met K9H3 at the promoter regions of Tcf11, Tcfcp2l1, and Zfp57 positively regulates the expression of these pluripotency-associated genes. Jmjd2c acts as a positive regulator for Nanog, which encodes for a key TF for self-renewal in ESCs (56).

In general, histone demethylases are tightly integrated in the transcriptional factor regulatory network in ESCs (57,58). To give one example, the high-ranking gene Jarid2 is the target for seven core regulators of Es. Jarid2, also known as Jumonji (Jmj), is highly expressed in ESCs and down-regulated in the whole embryoid body, after which, during cellular differentiation events the regions where Jmj is expressed, expand gradually and the expression is detected in almost all adult tissues, although the intensities are different among cell types (58,59).

These observations suggest that histone demethylases link core TF network to the regulation of chromatin status during cellular differentiation.
EPIGENETICS IN CELL-FATE CONVERSION

The responsiveness of stem cells to extrinsic signals changes over time, and their developmental potential becomes more restricted due to changes in their internal state (60–62). There is growing evidence that epigenetic modifications are required for nuclear reprogramming and cell-fate conversion (63,64). Recent advances in epigenetics suggest that cell fates can be reset by the alteration of epigenetic marks on histones or DNA methylation, and that such converted cells are functional when transplanted in vivo.

Experimental data suggests that epigenetic modifications might permit the generation of new sources of neuronal SCs (stem cells) and neurons from non-neuronal SCs. It was demonstrated that the addition of either valproic acid, an histone deacetylases inhibitor, or 5-azacytidine (5-AzaC), a DNA methylation inhibitor, can convert bone marrow stromal cells to NSCs (65,66). In addition, very early evidence points out that chromatin remodeling factors such as ISW1 and Brd1 can reset the somatic nuclei transplanted into Xenopus eggs (67–69) and that the histone codes are modified during the nuclear reprogramming (70). Successful reversion of oligodendrocyte precursor cells to NSC-like cells is reported to be associated with the recruitment of Brm to the SOX2 promoter and with two modifications of histone H3, K4 methylation and K9 acetylation, at the promoter (71).

Several lines of evidence in recent research point to the fact that the terminal differentiation process could be reversible if the key regulator of the lineage restriction is identified. For instance, repressor element-1 transcription factor/neuron restrictive silencer factor transcriptional silencer is an important regulator of the restriction of the neuronal gene expression outside of central nervous system (CNS) (62,72–74). An interesting study demonstrates that the activation of neuronal genes that are blocked by REST is sufficient to induce C2C12 myoblasts to convert to functional neurons (75). Another example proves that cell-fate conversion can be successfully performed when the proper signaling pathways are engaged. Collas and co-workers (76) have established a new method for cell-fate conversion. They demonstrated that when 293T fibroblasts are cultured in a cell extract from either T cells or NTera2 (NT2) embryonic carcinoma cells, the cells are reprogrammed and express, respectively, either T-cell markers, including interleukin 2 (IL-2) and T-cell receptors, or a neuronal marker, neurofilament 200. Published work demonstrates that Brd1 is recruited to the IL-2 promoter, and histone H4 in the promoter adopts the active form during the reprogramming. Together, these findings suggest that cell fate as well as developmental marks can be reset on genomes by epigenetic alterations.

EPIGENETICS IN REPROGRAMMING OF DIFFERENTIATED SOMATIC CELLS AND INDUCED PLURIPOTENT STEM CELLS

Research focused on creation of patient and disease-specific stem cells faces ethical controversies that hinder the progress of human ESC research worldwide. Despite the obvious benefits in using such a system to understand disease mechanisms, to screen effective and safe drugs, and to treat patients of various diseases and injuries, the idea to use human embryos to obtain ESCs, represents a social and ethical challenge (77). One way to circumvent these issues is to induce pluripotent status in somatic cells by direct reprogramming (18,78–82). This field has experienced tremendous breakthroughs with successful reprogramming of differentiated human and mouse somatic cells into a pluripotent state (for review 77,83). Generation of the induced pluripotent stem (iPS) cells from mouse somatic cells and adult human dermal fibroblasts has been successfully achieved by transduction of four defined TFs. The ectopic expression of OCT4, SOX2, Klf4 and Myc genes to yield iPS cells has revealed that iPS cells were similar to human ESCs in morphology, proliferation, surface antigens, gene expression, and telomerase activity (79). They retained epigenetic signature of pluripotency and were capable of germline transmission. When mouse iPS cells were injected into mouse blastocysts, they contributed to all tissue types in the resulting adult mice, including sperm and oocytes (81,83).

It is important to mention, however, that human ESCs cells are different from mouse counterparts in many respects. Despite the differences between mouse and human ESCs, the published data demonstrate that the same four TFs induce iPS cells in both human and mouse. Surprisingly, these four factors, however, could not induce human iPS cells when fibroblasts were kept under the culture condition for mouse ESCs after retroviral transduction. Taking these results into consideration, one can suggest that although the fundamental transcriptional network governing pluripotency is common in humans and mice, the extrinsic factors and signals as well as an epigenetic strategy to execute these signals and maintain pluripotency are unique for each species. Interestingly, although OCT3/4 and SOX2 as core TFs are capable of determining pluripotency of ESCs, these factors cannot bind their target genes in differentiated cells due to differences in epigenetic make-up of the designated target chromatin, which includes DNA methylation and histone modifications. It has been suggested that that c-Myc and Klf4 modify chromatin structure so that OCT3/4 and SOX2 can bind to their targets (82). For instance, Klf4 as well as c-Myc interacts with p300 histone acetyltransferase and regulates gene transcription by modulating histone acetylation (82,84). An emerging theme from recent studies is that the regulation of higher-order chromatin structures by DNA methylation and histone modification is crucial for genome reprogramming during early embryogenesis and gametogenesis. These events can deviate a tissue-specific gene expression through the changes in the chromatin architecture within the nuclei, thus resulting in silencing of one portion of the genome and activating the other.

Although the studies describing the iPS cells are incredibly promising, it is still far-fetched from their direct applications for human therapies. It is reasonable to think that the increased amount of retroviral integration sites in iPS cells may increase the risk of tumorigenesis. In fact, it has been reported that 20% of mice derived from iPS cells developed tumors, which were attributable, at least in part, to reactivation of the c-Myc by retrovirus (81). Then another interesting question raised by the study of iPS cell lies in the phenomena that only a small
portion of somatic cells, both in mice and human, can be transduced to acquired iPS cell identity (78). Such low efficiency raises several possibilities. First, the origin of iPS cells may be undifferentiated stem or progenitor cells coexisting in fibroblast culture. Secondly, it is plausible to speculate that retroviral integration into some specific loci or epigenetic alterations may be required for an iPS cell induction. Thirdly, the origin of iPS cells may be undifferentiated stem or progenitor cells coexisting in fibroblast culture. These issues need to be investigated in the future studies.

SMALL NON-PROTEIN-CODING RIBONUCLEIC ACIDS AS EPIGENETIC REGULATORS OF NUCLEAR ARCHITECTURE

A new integrated global regulatory network is currently emerging based on the dynamic interplay of chromatin remodeling components, TFs, and small ncRNAs. These three mechanisms synergize to choreograph stem cell self-renewal and the generation of cell diversity. Mammalian cells harbor numerous small ncRNAs, including small nucleolar RNAs, (snoRNAs), microRNAs (miRNAs), short interfering RNAs (siRNAs) and small double-stranded RNAs, which regulate gene expression at many levels including chromatin architecture. Most show distinctive temporal- and tissue-specific expression patterns in different tissues, including embryonic (ESC) stem cells and the brain, and some are imprinted (reviewed in 85). This suggests the existence of an extensive regulatory network on the basis of RNA signaling.

Although only 1.2% of the human genome encodes protein, a large fraction of it is transcribed. Whole chromosome-tiling chip arrays have shown that the range of detectable ncRNAs in human cells is much greater than can be accounted for by mRNAs (86,87). Indeed, as much as 98% of the transcriptional output in humans and other mammals has been proposed to consist of ncRNA (88). Until recently, the non-coding RNA fraction was considered mainly useless with the exception of the common structural RNAs involved in protein synthesis, transport and splicing. The number of known functional ncRNA genes has risen dramatically in recent years and over 800 ncRNAs [excluding, transfer RNAs (tRNAs), ribosomal RNAs (rRNAs) and small nuclear RNAs (snRNAs)] (89,90).

Many miRNAs are specifically expressed during ESC differentiation (91,92) and embryogenesis (93), as well as during brain development (94,95), neuronal differentiation (96), and hematopoietic lineage differentiation (92,97). It has been shown that Dicer-deficient mouse ESC are defective in differentiation and centromeric silencing (98). These observations and the apparent inability to generate viable Dicer1-null ESC in vitro suggest a role for Dicer, and, by implication, ncRNAs, in maintaining stem cell populations during early mouse development.

There is also considerable evidence that small RNAs also regulate chromosome dynamics, chromatin modification and epigenetic memory, including imprinting, DNA methylation and transcriptional gene silencing (reviewed in 85). The RNAi pathway and non-coding RNAs have been shown to be central to the formation of silenced chromatin and chromosomal dynamics in animals, plants, fungi and protozoa and, by analogy, might be an important component of ESC epigenetic regulation.

The RNAi machinery affects silencing and heterochromatin formation, accompanied by reduction in histone H3 lysine-9 methylation and delocalization of the heterochromatin proteins HP1 and HP2. The localization of mammalian HP1 to heterochromatin involves its co-ordinate binding to methylated histone H3 and RNA, involving interactions in the hinge region between its chromodomains (99,100). Surprisingly, the chromodomains are present in many different types of chromatin-binding and chromatin-remodeling proteins, including the polycomb family, the histone methyltransferase and histone acetyltransferase families. It is also important to notice that RNA-interacting proteins are also components of the mammalian DNA methylation system (101,102).

These observations point to the possibility that the non-coding RNA ability to induce either gene silencing (103) or gene activation (104) could be an integral component of epigenetic mechanisms dedicated to orchestrate gene transcription programs in ESCs. Approximately 20% of 179 small RNA sequences cloned from mouse ESCs show similarity to tRNA or rRNA, which may act as small regulatory RNAs in some other yet unknown functional capacity (91). More than 50 unknown short non-coding RNAs were cloned from adult neural stem cells (105). One of these sequences is present in more than 60 copies in the mouse genome and has similarity to the NRSE/RE1 sequence, which is preferentially localized in promoter regions of neuron-specific genes. This RNA, which occurs in the nucleus as a small 20 nt dsRNA, controls the differentiation of adult neural stem cells and activates the transcription of genes containing NRSE/RE1 sequence, apparently mediated through dsRNA–protein interactions, rather than through the classic function of siRNA or miRNA aimed to regulate mRNA production on the post-transcriptional level (104).

While most non-protein coding regions within the genome have previously considered to be the ‘genomic junk’, the extent of non-coding RNA transcription and rapidly emerging evidence of regulatory networks controlled by these RNA have much broader horizons than previously anticipated. Recent evidence of ‘functional junk’ includes the example of a developmental-specific SINE B2 retrotransposon transcriptional unit providing the molecular boundary for separation of chromosomal domains of the differential transcriptional activity during cellular differentiation (106) in mammals. Assuming that the nuclear architecture dictates transcriptional outcome, it is plausible to speculate that specialized separation of genome into active or silent chromosomal domains changes when ESCs traverse from pluripotent state to differentiation. It should be a goal of future investigations to assess how the non-protein coding component of the genome participates in the restructuring of the nuclear compartments.

CONCLUSIONS

The cumulative research data briefly discussed in this review suggests that epigenetic players such as histone modifications, DNA methylation, the alteration of chromatin structure due to
chromatin remodeling, and non-coding RNAs represent another crucial mechanism, besides the transcriptional factor network, which is designed by nature for the regulation of gene expression and cellular differentiation. Elucidating epigenetic mechanisms promise to have important implications for advances in stem cell research and nuclear reprogramming and may offer novel targets to combat human disease potentially leading to new diagnostic and therapeutic avenues in medicine.

ACKNOWLEDGEMENTS

We apologize to our colleagues for omission of so many important research contributions due to space constraints of this review. We thank J. Hightower for figure preparation. M.G.R. is an investigator with Howard Hughes Medical Institute. We acknowledge Libbie Butler, Lab Administrative Assistant, Buck Institute for Age Research, for her assistance in proofreading and preparation of this manuscript.

Conflict of Interest statement. None declared.

FUNDING

The experiments from Michael’s laboratory cited in this review were funded by NIH.

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