REVIEW ARTICLE

Epigenetic regulation of open chromatin in pluripotent stem cells

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The recent progress in pluripotent stem cell research has opened new avenues of disease modeling, drug screening, and transplantation of patient-specific tissues unimaginable until a decade ago. The central mechanism underlying pluripotency is epigenetic gene regulation; the majority of cell signaling pathways, both extracellular and cytoplasmic, alter, eventually, the epigenetic status of their target genes during the process of activating or suppressing the genes to acquire or maintain pluripotency. It has long been thought that the chromatin of pluripotent stem cells is open globally to enable the timely activation of essentially all genes in the genome during differentiation into multiple lineages. The current article reviews descriptive observations and the epigenetic machinery relevant to what is supposed to be globally open chromatin in pluripotent stem cells, including microscopic appearance, permissive gene transcription, chromatin remodeling complexes, histone modifications, DNA methylation, noncoding RNAs, dynamic movement of chromatin proteins, nucleosome accessibility and positioning, and long-range chromosomal interactions. Detailed analyses of each element, however, have revealed that the globally open chromatin hypothesis is not necessarily supported by some of the critical experimental evidence, such as genomewide nucleosome accessibility and nucleosome positioning. Greater understanding of epigenetic gene regulation is expected to determine the true nature of the so-called globally open chromatin in pluripotent stem cells. (Translational Research 2014;■:1–10)

Abbreviations: 5hmc = 5-hydroxymethylcytosine; 5 mC = 5-methylcytosine; ATP = adenosine triphosphate; BAF = Brg/Brahma-associated factor; DHS = DNase I hypersensitivity site; ESC = embryonic stem cell; GFP = green fluorescent protein; LOCK = large, organized chromatin K9 modification; iPSC = induced pluripotent stem cell; miRNA = micro-RNA; mRNA = messenger RNA; lncRNA = long noncoding RNA; PRC = polycomb repressive complex; PSC = pluripotent stem cell; SWI/SNF = switching defective/sucrose nonfermenting; Tet = Ten-eleven Translocation

It is generally accepted that chromatin in pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced PSCs (iPSCs), is decondensed globally or "open" so the cells can express many genes readily as they become necessary for differentiation into many lineages. Although the idea of open chromatin in PSCs is not novel, the recent progress in PSC biology—in particular, the creation of iPSCs[1]—uncovered new

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Conflicts of Interest: All authors have read the journal's policy on the disclosure of potential conflicts of interest and have none to declare.

Submitted for publication December 20, 2013; revision submitted March 3, 2014; accepted for publication March 6, 2014.

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1931-5244/$ - see front matter

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http://dx.doi.org/10.1016/j.trsl.2014.03.004

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pieces of evidence that potentially explain mechanistic links between pluripotency and open chromatin. A major impetus for this progress has been various types of genomewide surveys, represented by the international effort of the Encyclopedia of DNA Elements, or ENCODE, project.[2-6] As of December 2013, this project had uncovered a wide spectrum of epigenetic modifications, including histone modifications, DNA methylation, transcription factor binding, chromatin accessibility, and long-range chromosomal interactions in 338 human cell types and 86 mouse cell types, including ESCs. In addition, the U.S. National Institutes of Health Roadmap Epigenomics Consortium has published genomewide analyses of histone modifications, DNA methylation, and chromatin accessibility with an emphasis on stem cells, including ESCs and iPSCs, and their differentiated derivatives.[7,8] Supported by the invention of sophisticated tools for chromatin analyses and bioinformatics, the explosive advance of this field is expected to continue at an accelerated pace. Because epigenetic regulation in PSCs has been discussed in many review articles,[9-17] this article focuses on epigenetic regulation directly relevant to globally open chromatin in PSCs. Methodological aspects of genomewide epigenetic studies have been reviewed elsewhere[18] and are not covered here.

CHROMATIN DECONDENSATION AT THE MICROSCOPIC LEVEL

The initial evidence for the presence of open chromatin in undifferentiated cells came from morphologic studies at the microscopic level. Long before mouse ESCs were created in 1981[19] and pluripotency became a major cell biologic research field, gradual, unidirectional, and global chromatin condensation was well documented with Wright stain during differentiation of hematopoietic cells.[20,21] Densely stained areas (heterochromatin) within the nucleus increase gradually and become coarse and granular during differentiation of cells of each hematopoietic lineage. This observation is even more conspicuous with transmission electron microscopy, which shows an increase of electron-dense material that is, initially, scattered in local patches and, eventually, occupies wide areas, sometimes more than half the nuclear space.[20-22] Electron microscopy also displays fine, evenly distributed granules in the ESC nucleus that become clustered more irregularly after differentiation.[23] Although the electron-dense materials represent poorly characterized aggregations of DNA, protein, and RNA, and their precise functions remain unknown, they are generally interpreted to represent condensed chromatin and to provide evidence that condensed chromatin increases during differentiation of undifferentiated cells, including PSCs.

In addition to the nucleuswide chromatin condensation, centromeres have served as a model structure that undergoes condensation during differentiation of PSCs. Round centromeric heterochromatin looks more diffuse in ESCs than in neural progenitor cells differentiated from the same ESCs when stained with an antibody against the heterochromatin protein HP1α, which is highly enriched in centromeres.[24] The total level of HP1α also increases during differentiation. Fluorescence *in situ* hybridization of the major satellite DNA sequence, 1 of the dominant DNA sequences of centromeres, also demonstrates more diffuse distribution in ESCs than in neural progenitor cells.[24]

PERMISSIVE TRANSCRIPTION

Another observation cited frequently as evidence for open chromatin in PSCs came from transcriptome studies comparing ESCs and differentiated cells.[23,25] ESCs contain twice as much total RNA and messenger RNA (mRNA), normalized to the amount of DNA, as neural progenitor cells.[23] Microarray studies indicated that although differentiated cells in general express 10%–20% of all mRNA species, ESCs express 30%–60% of all mRNA species.[25] The mRNAs expressed in ESCs include those of many tissue-specific genes that do not appear to be necessary for maintaining the undifferentiated state of ESCs. Although some of the tissue-specific genes are expressed at very low levels,[23] others are translated into proteins,[26] indicating they are not artifacts of detection by sensitive polymerase chain reaction. This permissive transcriptional environment is not limited to PSCs; hematopoietic stem cells also express nonhematopoietic genes, which are downregulated gradually during differentiation.[27,28]

Although these findings may fit the interpretation of uncontrolled leaky transcription resulting from open chromatin, definitive evidence is still missing. It is also possible the primary cause of the prevalent transcription may be as-yet-uncharacterized functions of the transcription machinery rather than the chromatin structure as a substrate for transcription. Chromatin immunoprecipitation and DNA microarray and genomewide nuclear runon experiments indicated that RNA polymerase II is paused at the promoters of 40%–50% of all the protein-coding genes without elongation of mRNA in ESCs, suggesting that postinitiation processes are the rate-limiting steps in transcription.[25,26] Although this high frequency of paused polymerase may look to be a promising explanation for the permissive expression in ESCs, similar results have in fact been obtained with differentiated cells, such as hepatocytes, B cells, and

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