Insights into the Establishment of Chromatin States in Pluripotent Cells from Studies of X Inactivation

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Abstract

Animal development entails the sequential and coordinated specialization of cells. During cell differentiation, transcription factors, cell signaling pathways, and chromatin-associated protein complexes cooperate in regulating the expression of a large number of genes. Here, we review the present understanding of the establishment of chromatin states by focusing on X chromosome inactivation (XCI) as a model for facultative heterochromatin formation in female embryonic cells. The inactive X chromosome is large enough to be investigated by biochemical and microscopy techniques. In addition, the ability to compare the inactivated chromatin to the active X in male cells enables us to differentiate events specific to gene silencing during XCI from gene regulatory effects from changing pathways in the same cell. Findings in XCI are useful as blueprints for investigation of the action of epigenetic pathways in differentiation and lineage commitment. We summarize recent studies that have identified factors that are critical for chromosome-wide gene repression in XCI, and we discuss their implications for epigenetic regulation in pluripotent cells of the early embryo.

Introduction

Initially, the cells of the mammalian embryo are totipotent. The potential to form the entire spectrum of cell types can be observed up to the 8-cell and 4-cell stage in mice and primates, respectively [1]. Thereafter, differential lineage fates are established, resulting in trophectoderm lineage (TE) and inner cell mass (ICM) cells of the blastocyst. Cells of the ICM are considered pluripotent as they form all tissues of the embryo. Pluripotent cells can be captured in embryonic stem (ES) cell cultures and provide a model for studying lineage development. Initially, a wide developmental potential gives rise to progressive diversification of cell types during differentiation into embryonic lineages. The molecular mechanisms for establishing chromatin configurations that guide and restrict the differentiation potential are beginning to be understood.

In pluripotent cells, gene expression is regulated by a network of transcription factors including Oct4, Sox2, Nanog, and Klf4. The latter factors have further been demonstrated to be sufficient for inducing a pluripotential developmental potential when introduced into somatic cells during induced stem cell reprogramming [2]. Notably, ES cells can compensate for the loss of epigenetic regulators that would otherwise compromise cell proliferation or survival of differentiated cells. Examples include mutations in DNA methyltransferases [3] or Polycomb group (PcG) protein genes [4] and suggest that pluripotent cell identity might rely more on stable transcriptional networks than on chromatin regulation. However, there is also evidence that epigenetic marks are present and functional in ES cells. DNA methylation of imprinted genes [5,6] and the *Elf5* gene promoter [7] are examples of epigenetic patterns that are maintained in ES cells. The transcription factor Elf5 is repressed in ES cells but active in the TE, where it contributes to the extraembryonic lineage transcriptional program.

The transcriptional dynamics have recently been analyzed in mouse and human preimplantation development at the single cell level [8–12]. Transcriptional

profiles in human development [9,10,12] change over developmental time and show major transitions at zygotic genome activation, when depletion of maternal transcripts is complete, and lineage segregation of the TE and pluripotent ICM into epiblast and primitive endoderm at day 5 [9]. These results are consistent with a specialization of gene expression as the cell fates become differentiated. In addition, some insight into chromatin regulation has been obtained from analyzing X chromosome dosage compensation by comparing gene expression between male and female embryos. In human preimplantation development, X chromosome dosage appears to be adjusted by dampening the expression of both X chromosomes in female embryos [9,13,14].

The situation is different in mouse development [8,11] and somatic cells. In mice, X chromosome inactivation (XCI) is initiated before the morula stage, and one X chromosome remains active, whereas the paternally inherited X chromosome of female cells is transcriptionally silenced [14]. In the cells of the developing epiblast, X inactivation is reversed again, and the paternal X chromosome loses its heterochromatic features [15]. In mouse embryos also, lineage segregation appears to be established earlier than in human embryos, which can be inferred from expression profiles [8,11]. Together, these data indicate differences in chromatin regulation between different mammalian species and between early embryonic cells and specialized differentiated cells. Mechanisms for establishing chromatin configurations are engaged throughout early embryogenesis and become critical as further developmental progression is disrupted by aberrant epigenetic patterns.

Establishment of Chromatin States during Random X Chromosome Inactivation

In the embryonic lineages of mouse and human, random inactivation of either the paternal or the maternal X chromosome is the underlying process of dosage compensation in female cells [16]. In mice, formation of an inactive X chromosome (Xi) is triggered by expression of the long noncoding RNA X-inactive specific transcript (*Xist*). *Xist* accumulates within the X chromosome territory and subsequently leads to the recruitment of factors for chromatin modification and gene silencing [17]. Random XCI can also be observed during the differentiation of mouse ES cells, which have been extensively used for studying the mechanism of forming facultative Xi heterochromatin. From these studies, a sequential process involving the localization of *Xist* to the Xi, formation of a repressive compartment, gene repression, and stable maintenance of the silent chromatin state has been deduced and is discussed in detail in the following section.

Xist RNA Association Induces Chromatin Changes on the Xi

The *Xist* gene produces a transcript, which is not exported from the nucleus and translated into protein but accumulates precisely within the chromosome territory of the Xi. *Xist* thereby does not strictly require X chromosomal sequences and has been observed to localize to autosomes, when it is expressed from transgenes [18,19]. However, a bias of *Xist* for X chromosomal sequences or sequences that are enriched in genomic repeats can be demonstrated when studying translocations involving the X chromosome [20]. The RNA and DNA binding protein scaffold attachment factor A (*SAF-A*, also called *hnRNPU*) is required for localizing *Xist* and is also enriched within the nuclear scaffold of the Xi [21,22]. Related proteins including hnRNPUL1 also appear to contribute to *Xist* localization in different cell types [23]. This indicates that overlapping functions of hnRNPU family members could mediate the localization of *Xist* through interactions with the nuclear scaffold. However, the details of this mechanism remain to be clarified.

After *Xist* accumulates within the chromosome territory, it induces chromatin modifications, recruitment of additional factors, and gene repression. Expression of *Xist* in pluripotent mouse ES cells has been shown to induce gene repression. This gene repression is reversible, and reactivation of the chromosome occurs if *Xist* is lost [19]. In contrast, *Xist* is not required for maintaining gene repression in differentiated cells, suggesting that other chromosomal modifications have been acquired by the Xi that allow it to autonomously maintain an inactive chromatin configuration [24]. The first change that has been observed within the forming Xi chromosome territory is a relative depletion of activating histone modifications including acetylated histone H3 lysine 4 and components of the transcription machinery including RNA polymerase II [25]. Subsequently, the nuclear volume that is occupied by *Xist* becomes enriched with proteins of the PcG. These include Eed, Suz12, the histone methyltransferase Ezh2, and the histone ubiquitinating enzyme Ring1b [15,25,26]. These histone-modifying activities result in the establishment of trimethylation of histone H3 lysine 27 (H3K27me3) and ubiquitination of histone H2A lysine 119 (H2AK119ub1) over the Xi. From kinetic studies, it appears that chromatin modifications precede gene repression [25]. The separation of gene repression from chromatin modifications is also supported by the observation of a specific mutated form of *Xist* that is defective in gene silencing but still can induce several of the chromatin changes characteristic for the Xi [25,26].

These findings have led to the proposal of a model for the initiation of gene repression. Chromatin on the