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Histone Chaperones Regulate Mammalian Gene Expression

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Abstract

Histone chaperones are fundamental molecules that aid in the synthesis, translocation, and exchange of histones across the barrier of cytoplasm to nucleus. Regulation in repair, replication, and nucleosome assembly constitute the widely associated functions of histone chaperones. Recently, they have been associated with transcriptional regulation. Different stages of mammalian development have been correlated to the expression of histone chaperones. From oocyte and sperm till the formation and development of zygote, different histone chaperones demonstrated distinct regulatory roles. Efficient models of studying mammalian development include differentiation of embryonic stem cells (ESCs) to different lineages. Both *in vitro* and *in vivo* differentiation of mammalian cells exhibit regulation by different subtypes of histone chaperones. Due to the ethical issues concerning the use of embryos for the derivation of ESCs, induced pluripotent stem cells (iPSCs) were derived from pre-existing differentiated cells by a phenomenon called cellular reprogramming. Cellular reprogramming is characterized by erasure of pre-existing epigenetic signature to a new modulated epigenome. Histone chaperones serve as either facilitator or barrier to reprogramming. Here, we will discuss how histone chaperones could regulate the gene expression pattern by regulating epigenetic modification during the complex process of mammalian development and reprogramming.

Keywords: histone chaperone, reprogramming, epigenetic, development, transcription factor, histone variant

1. Introduction

Nucleosomes comprising of histones and DNA could be considered as the basic unit of regulation of gene expression. Nucleosomes tightly regulate the transcriptional traffic while relaxing the structure of chromatin to bind the chromatin factors across the DNA. The marked

presence of euchromatin and heterochromatin determines the fate of gene expression as a result of presence or absence of the regulatory complex formed at different loci [1]. Thus, the assembly or disassembly of the nucleosome is a major contributory factor in the regulation of gene expression in mammalian cells. Histone chaperone are proteins in nature and regulates the nucleosomal function by the deposition or eviction of corresponding canonical histone subunits or non-canonical histone variants. Their function is not restricted to nucleosomal activity only. They regulate all sorts of histone metabolism throughout the life cycle of a mammalian cell. Histone deposition by histone chaperones may or may not be coupled to DNA replication. The entire dynamics associated with histone chaperones enable us to realize how they could regulate DNA replication, repair, transcription and finally the genomic integrity of the cells (**Figure 1**).

The fundamental unit of chromatin is nucleosome. Nucleosome is composed of histone octamer with each two units of histone H2A, H2B, H3, H4 wrapped around by 147 bp of DNA. A linker DNA, along with other histone subunit H1, connects one nucleosome to the other and forms a beaded chain like structure in the nucleus. The degree of compactness of these nucleosomes determines the fate of gene expression in mammalian cells. Along with the canonical histone units of H2A, H2B, H3.1, H3.2 and H4, replacement histone variants have evolved which are essentially regulatory in nature including H3.3, CENP-A, H2A.Z and deposited in a replication-independent manner unlike the canonical ones which are generally deposited in a replication-coupled fashion and are expressed in the S-phase of the cell cycle. Replacement of canonical subunit with the histone variants contributes to the transcriptional regulation of genes [2]. Histone chaperones are responsible for the recruitment of canonical histones and these histone variants at different loci of the genome and further indulge in the regulation of gene expression.

Till date different subtypes of histone chaperones have been discovered. They are generally classified on the basis of replication-coupled or uncoupled mechanism of action. As mentioned

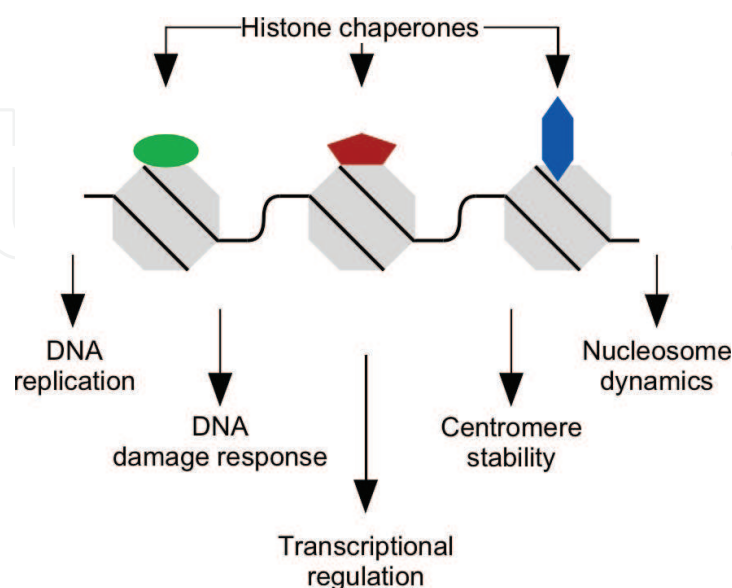


Figure 1. Overview of chromatin functions regulated by histone chaperones.

earlier replication coupled are the ones associated with deposition of newly synthesized canonical histones into the nucleosome, recycling them throughout the cell cycle and that include Chromatin Assembly Factor 1 (CAF1), Facilitates Chromatin Transcription (FACT), Suppressor Of Ty6 (Spt6), among others [2]. Whereas the other phenomenon of DNA repair, centromere deposition, transcriptional regulation and maintenance of heterochromatin dynamics are performed in a replication uncoupled fashion and that include Histone regulation A (HIRA), Anti Silencing Function 1A (ASF1A), Death domain-associated protein 6 (DAXX) among others [2]. Detailed information on the same could be referred from a recent review by Hammond et al. [2].

Replication-independent functioning of histone chaperones has a profound impact on the chromatin structure and as well as in different cellular processes. Such is the influence in different cellular context that many knockout phenotypes of histone chaperones demonstrated embryonic lethality. Among these cellular process, in this chapter, we will discuss in detail how histone chaperone influence different mammalian developmental stages and in the context of cellular reprogramming.

2. Histone chaperones in mammalian development

Mammalian development initiates upon the formation of single celled zygote from the sperm and ovum. The zygote undergoes division to form two-celled to four-celled and finally to a stage called morula. Till this stage the embryo is termed as totipotent, that can differentiate into any lineage. But, from the formation of blastocyst, the potency to differentiate becomes restricted from pluripotent to multipotent to finally unipotent (**Figure 2**, top panel). Intricate molecular mechanisms encompassing different transcriptional and epigenetic factors regulate the different stages of mammalian development from the pre-implantation embryo till the attainment of adulthood.

2.1. Pre-implantation and early embryonic development

From one-celled zygote to the formation of totipotent morula, functionally different histone chaperones influence the developmental stages. Even before fertilization, histone chaperone HIRA depletion lead to inhibition of male pronucleus formation due to a lack of nucleosome assembly in the sperm genome [3]. Hira mutant oocytes in *Xenopus*, lack the potential to divide parthenogenetically [4]. Basically, HIRA-dependent H3.3 incorporation in the paternal genome is a prerequisite for their reprogramming into the development to the mouse zygote [5]. Progression to the two-celled stage is absolutely dependent on the histone chaperone HIRA. Loss in maternal HIRA leads to complete inability to deposit the core histones on to the paternal genome thus resulting in a compromised maternal genome reactivation in mice [5]. Also, ASF1B has been found to be required for retaining the female reproductive capacity in mouse where in loss of ASF1B could introduce change in meiotic entry thereby resulting in a discrepancy in gonad development [6]. The development of morula to blastocyst occurs around E3.5 and E5.5 in mouse and human respectively (**Figure 2**). This is the first cellular differentiation wherein the blastocyst could be partitioned into the inner cell mass (ICM)

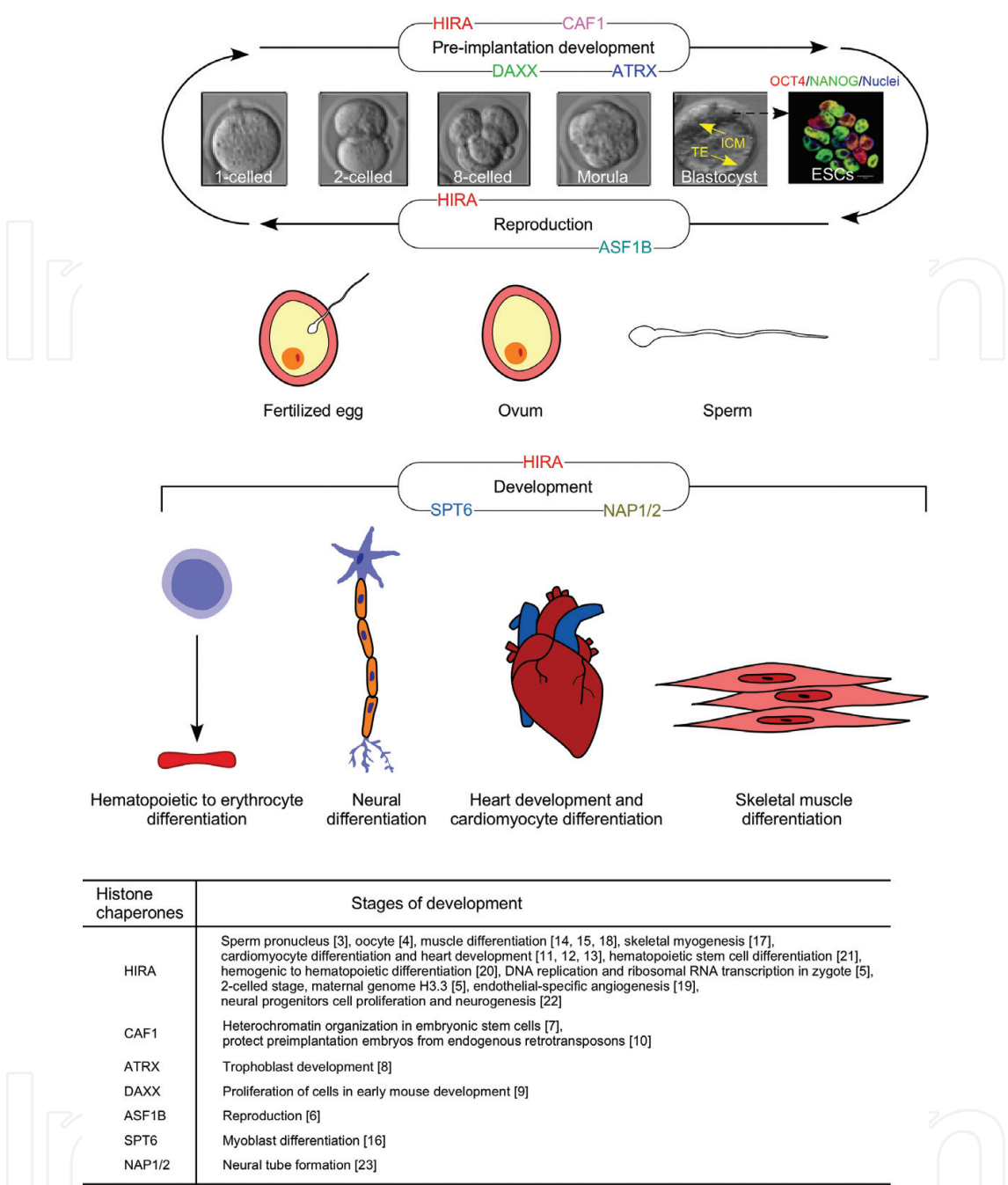


Figure 2. Histone chaperones implicated in mammalian developmental processes. (Top panel) Pre-implantation development—single-celled mouse embryo undergoes division to form blastocyst at embryonic day 3.5, E3.5. Inner cell mass (ICM) and trophectoderm (TE) constitute the blastocyst surrounding the blastocoel. ICM are source of embryonic stem cells (ESCs). Mouse ESCs when derived from ICM express core pluripotency factor OCT4 and NANOG, as visualized by immunofluorescence microscopy. (Middle panel) The structural organization of the chromatin of sperm and oocyte are also regulated by different set of histone chaperones. (Lower panel) Further development and differentiation to all three lineages, after post-implantation, have been attributed to the proper functioning of histone chaperones. Histone chaperones associated with the regulation of the developmental stages have been summarized in the table.

comprising of epiblast and hypoblast and the outer layer of trophectoderm (TE) covering the blastocoel (Figure 2). ICM gives rise to the embryo proper and the TE develops into the extraembryonic tissue forming the link between the embryo and the mother. Embryonic stem

cells (ESCs) are developed *in vitro* from the ICM and with time have evolved into the best tool to understand development as well as serve as the best alternative to be exploited for the generation of cells of different lineages due to the pluripotent nature of these cells. Pluripotent ESCs can differentiate into any lineage except to extraembryonic lineage. Loss in CAF1 leads to the mislocalization, loss of clustering, and decondensation of pericentric heterochromatin domains in ESCs and an altered histone methylation mark at the level of pericentric heterochromatin is formed [7]. Basically, CAF1 targeted mutation led to the developmental arrest at the 16-cell stage due to severe alteration in the nuclear organization of constitutive heterochromatin. The reason was the non-maturation of the heterochromatin and the retention of the 4-celled stage in the 16-cell stage leading to stalled development. So, CAF1p150 is needed for the development of preimplantation embryo [7]. Conditional mutagenesis of the histone chaperone ATRX demonstrated a failure in the development of TE in murine embryo [8]. A defect in methylation pattern and growth pattern lead to the embryonic lethality of mouse embryo. Thus, ATRX serve as one of the deterministic factor for the successful differentiation of embryo into TE and hence in the formation of extraembryonic tissues. During the early embryonic development, histone chaperone DAXX has been associated in the suppression of apoptosis in the embryo wherein its loss resulted in enhanced apoptosis thus resulting to embryonic lethality [9]. In the late pre-implantation stage, the embryo is significantly hypomethylated. Retrotransposons present in the mammalian genome stay in a silenced fashion to avoid their activation that could result in the loss of genomic integrity. In this stage of development, CAF1 mediated deposition of histone variant H3.1/3.2 and repressive histone marks, including H4K20me3 and H3K9me3, at retrotransposon regions repressed the activation of these elements thereby aiding in the proper development of embryo [10]. So, different histone chaperones specifically regulate stages of pre-implantation development or early mammalian development (**Figure 2**).

Development of the embryo to an adult further instils another set of histone chaperones in regulatory mechanism associated with differentiation into different lineages. A classic example is the histone chaperone HIRA. Targeted mutagenesis of HIRA demonstrated that HIRA is indispensable during murine embryogenesis. The defects of mutation were prominently visible during gastrulation, abnormal placentation, cardiac morphogenesis and finally leading to embryonic lethality [11]. There are different lineages, which are preferentially being targeted by few histone chaperones, and in the next section we will discuss the relation between subtypes of histone chaperones in development (**Figure 2**).

2.2. Cardiac differentiation and heart development

Mesodermal differentiation to cardiac progenitors demonstrated a substantial role of histone chaperone HIRA. Cardiomyocyte-specific *Hira* conditional-knockout mice did not disturbed the heart development, but instead resulted in cardiomyocyte hypertrophy and susceptibility to sarcolemmal damage [12]. Cardiomyocyte degeneration led to focal replacement fibrosis and hence resulted in the impaired cardiac function. Gene expression profile in *Hira* conditional-knockout hearts indicated impairment in pathways associated with responses to cellular stress, DNA repair and transcription and could hence implicate HIRA in maintenance of cardiomyocyte homeostasis [12]. HIRA could also individually regulate locus-specific

effects on cardiac-specific genes. Conditional ablation of Hira in the cardiogenic mesoderm of mice demonstrated dysregulation of *Tnni2* and *Tnni3*, Troponin genes, involved in the cardiac contractility [13]. HIRA bind to the enhancer elements of Troponin genes that are already bound by the cardiac-specific transcription factor NKX2.5. Hence, absence of HIRA during cardiac differentiation results in several defects including edema, which finally aggravate to embryonic lethality [13].

2.3. Muscular differentiation

Transcription factor MyoD is essential in myoblast differentiation. Histone chaperone HIRA and H3.3 play pivotal roles in MyoD regulation [14]. HIRA, phosphorylated by Akt kinase, thereby modulates a switch between its phosphorylated and non-phosphorylated state and thereby dictate the expression of myogenic genes during myogenesis [15]. Another histone chaperone SPT6, through cooperation with RNA Pol II and histone demethylase KDM6A, orchestrates removal of repressive H3K27me3 mark from MyoD, thereby facilitating the expression of MyoD and thus controlling gene expression associated with development and cell differentiation [16]. Simultaneously, HIRA interact with another transcription factor MEF2C and contributes to its activation during muscle differentiation [17]. ASF1A forms a complex with HIRA for MEF2C dependent transcription and is indispensable for myoblast differentiation. Basically, at the chromatin level, HIRA mediated enrichment of active histone modification marks within the myogenin promoter regulated myoblast differentiation [17]. Myofibers lacking HIRA suffer oxidative stress and generate a hypertrophic response in skeletal muscle thereby exposing the myofibers to stress-induced degeneration [18].

2.4. Endothelial/hematopoietic differentiation

Endothelial cells are differentiated from mesodermal lineage. They constitute crucial partners in angiogenesis, the development of new blood vessels. Angiogenic factors like basic fibroblast growth factor and epidermal growth factor could incite angiogenic response in endothelial cells with the significant increase in expression of Vascular endothelial growth factor receptor 1 (VEGFR1) [19]. Interestingly, mechanistic analysis showed that HIRA mediated incorporation of histone H3.3 variants upon acetylated at H3K56 induces the VEGFR1 level in mouse yolk sac endothelial cells. Loss in expression of HIRA eventually reduced *in vitro* angiogenesis and pathological angiogenesis in the choroidal neovascularization model [19].

RUNX1 (Runt-related transcription factor 1) has been attributed to be a pre-requisite for the hemogenic to hematopoietic transition. This cellular transition is transient in nature, but recently it has been shown to be the authentic source for the emergence of hematopoietic stem cells (HSCs). Since, HIRA could influence angiogenesis related endothelial-specific genes, we investigated the role of the histone chaperone HIRA in hemogenic to hematopoietic transition. In mouse hemogenic endothelial cells, HIRA physically interact with RUNX1 and thus could regulate the downstream targets of RUNX1 including *Pu.1*, *Gfi1*, and *Gfi1b* that are implicated in the functioning of HSCs [20]. The *Runx1* + 24 mouse conserved noncoding element, an intronic enhancer, is essential for the expression of *Runx1* during endothelial to hematopoietic transition. The locus is active upon incorporation of histone variant H3.3 in a HIRA-dependent

fashion. Thus, HIRA regulate RUNX1 in driving the hemogenic to hematopoietic transition. Earlier studies in leukemia cells, demonstrated that HIRA interacting with transcription factor EKLF could regulate the β -globin gene expression associated with adult definitive erythropoiesis [21]. It could also control the expression of EKLF and GATA1 by regulating the chromatin modification at the corresponding regulatory genes during differentiation.

2.5. Neuronal differentiation

Neural progenitor cells are enriched in histone chaperone HIRA and have been associated with neural progenitor cell proliferation, terminal mitosis and cell cycle exit. Loss of HIRA leads to premature differentiation in neural progenitors [22]. HIRA is involved in the increased β -catenin expression due to the enrichment of H3K4me3 mark within its promoter by inducing the recruitment of Setd1A methyltransferase at the promoter. Thus, HIRA could regulate neurogenesis. Interestingly, deletion of the neuronal Nap1/2 (nucleosome assembly protein 1-like 2) gene, another histone chaperone, in mice causes neural tube defects [23]. Nap1/2 actually enhance the histone acetylation at H3K9/14 within the *Cdkn1c* locus, responsible for neuronal differentiation.

3. Histone chaperones regulate reprogramming

In 2006, Takahashi and Yamanaka demonstrated how cell fates could be manipulated with the expression of four-transcription factor Oct4, Klf4, Sox2 and c-Myc or OSKM [24, 25]. These Yamanaka factors, when ectopically expressed in terminally differentiated fibroblasts led to the generation of induced pluripotent stem cells (iPSCs) [24, 25]. iPSCs are similar in character to ESCs. The major ethical concern associated with exploitation of embryo to derive ESCs could be successfully avoided by the use of iPSCs. Till now, they have been derived from different mammalian species, including human, utilizing varied protocols. Technically, human iPSCs has been proven to be a very efficient tool in understanding mechanistic basis of several diseases, for drug screening and finally to serve as the huge potential of cells needed for replacement therapy or in regenerative medicine. Although a vivid literature is available on the methods to generate iPSCs, nevertheless it is associated with lot of complexities including its low turnover and the distinct set of cellular machinery that result in reversing the clock. Even after a decade, the molecular mechanisms underlying the reprogramming process remains unclear and hence substantial amount of information still remain elusive.

Functionality of cell depends upon how the chromatin is arranged in each cell type. So, the parts of genome, which are essential for the function of a specialized cell, should be in accessible state to the transcription factors and other chromatin associated proteins. Actively differentiating cell changes its chromatin states progressively toward its mature state. The reversible nature of epigenome to embryonic state is proved with the seminal somatic cell nuclear experiments by the oocyte factors. Further the erasure of epigenetic modifications to pluripotency state is attained with the ectopic expression of transcription factors. iPSCs technology revealed the importance of transcription factors in cell fate change. Different transcription factor cocktail to induce pluripotency have been tried and still OSKM remains the

preferred cocktail mixture to study the cell fate change. These reprogramming factors are transcription factors, which are highly expressed, in embryonic stem cells and they maintain the circuits in the chromatin to change a state to pluripotent, which is likely to differentiate into different lineages including neuron, blood, etc. Thus, transcription factors and chromatin remodeling factors are key elements in cell fate change, which can drive the cell toward other state when maintained in a controlled environment. Thus it is essentially to identify the key factors to reprogram the cells directly into other cell type by bypassing the pluripotent state.

Along with transcriptional regulation, reprogramming entails global epigenetic remodeling. Reprogramming of somatic cells to pluripotent state is marked with change in the global epigenome, with the erasure of donor cell epigenetic modifications. Thus, inducing a somatic cell to reprogram into pluripotent one largely involves structural change at the chromatin in creating a signature expression pattern of genes associated with the generation of iPSCs. The histone code comprising of different histone modification patterns indicates the status of the genomic loci by the presence of active or repressive marks. After the OSKM transduction, initial days of reprogramming results in the loss of somatic cell characteristics, which are due to binding of the OSKM factors. With the OSKM binding the regions of the condensed chromatin leads to the genome wide chromatin changes. A plethora of histone modification marks encompasses different stages of reprogramming including active H3K4me2, H3K3me3 or repressive H3K27me3 [26]. The remodeling of pluripotent gene promoter initiates at an early stage to facilitate the chromatin accessibility for the binding of different remodeling factors.

Histone modifying enzymes responsible for the corresponding histone modifications interact with the core pluripotency factors like OCT4 and promote the activation marks to facilitate attainment of pluripotency.

Interestingly, the barrier for the reprogramming process lies in the histone marks with the repressive function, preventing their removal and directing a shift from change the chromatin structure from heterochromatin to euchromatin. This is an uphill task to be achieved only by the set of transcription factor. This criterion adds up to prolonged time span required to accomplish the process of reprogramming with the low production efficiency.

The non-canonical or replacement histone variants have been implicated in the regulation of cellular reprogramming. Deposition of histone variants by replacing their canonical histones along the genomic sequences changes the expression profiles thereby giving a cell a new identity. MacroH2A histone variants have been shown to resist reprogramming [27]. On the contrary, histone variants TH2A/TH2B, highly enriched in oocytes, typically enhance the reprogramming process [28]. These histone variants are known to be expressed in testes, oocytes and zygotes are associated with open chromatin [28].

But, upon looking into a close proximity, we could infer that basically, it is the tight packaging of nucleosomes that regulates the genetic information driving the morphological change of one phenotype of cell to the other one during reprogramming. And the players regulating the assembly or disassembly of nucleosomes are the histone chaperones. Although, late, but the first report on the role of histone chaperones in cellular reprogramming was established in 2014 [29].

After that, two other reports came out in the simultaneous years proving their role as activator or barrier to reprogramming thereby entrusting another regulatory behavior for the gene expression in mammalian cells. In the following paragraphs, we will understand what molecular mechanism drives the regulation of iPSCs formation as a function of histone chaperone.

3.1. ASF1A: essential for induction of pluripotency

Anti Silencing Function 1 (ASF1) is the most conserved histone H3 and histone H4. It has been implicated in almost all the functions of histone chaperone described in earlier sections. In mammals, ASF1 has two paralogs, ASF1A and ASF1B. The human oocyte in metaphase II is highly enriched in ASF1A and this state of oocyte has been attributed in having a greater reprogramming potential than any cells driven to pluripotency by the addition of exogenous factors [29]. Global transcriptomic profiling demonstrated ASF1A as the most vital oocyte-reprogramming factor across different mammalian species [29]. In mammals, it forms complex with other histone chaperones including HIRA and CAF1 and its role in cellular reprogramming of human dermal fibroblasts was analyzed for the first time. ASF1A is required for the generation of iPSCs from adult human dermal fibroblasts. The promoters of *NANOG*, *OCT4* and *SOX2* are significantly enriched with the active acetylated H3K56 mark during reprogramming. This enrichment was further enhanced upon overexpression of ASF1A while the level was significantly reduced upon its downregulation. So, functionally, ASF1A mediates the maintenance of H3K56ac level in reprogramming cells within the promoters of core pluripotency genes and those pluripotent factors as a consequence bind to their target genes to accomplish the process. OSKM combination is added for the induction of reprogramming, but ASF1A along with only OCT4 and GDF9 (Growth Differentiation Factor 9) could contribute to the generation of authenticated iPSC colonies. GDF9 is again an oocyte specific growth factor. This study proved that ASF1A is required for the reprogramming phenomenon in human dermal fibroblasts and how a histone chaperone could ultimately influence and regulate the transcriptional machinery in cellular reprogramming [29].

3.2. CAF1: restrict induction of pluripotency

Chromatin assembly factor 1 (CAF1) associate with the deposition of newly synthesized histone H3/H4 on DNA. Chaf1a and Chaf1b, two subunits of CAF1, have been recently associated with reprogramming [30]. Downregulation of CAF1 induced the generation of iPSC clones within 5 days of OSKM addition in human dermal fibroblasts. Even the efficiency in formation of iPSCs enhanced from 0.1% in control fibroblasts to 1–5% in *Caf1*-shRNA cells. But, this increase in number does not reflect an accelerated proliferative capacity of cells upon downregulation of CAF1. However, CAF1 presence was needed during the initial period of reprogramming and hence an optimal dosage of CAF1 determines the effect on the reprogramming process. SON-seq and ATAC-seq data revealed that CAF1 downregulation result in the enrichment of accessible ES-cell specific super enhancer elements. This implied that CAF1 regulate the local chromatin structure of the ES-cell specific enhancer elements. Thus, CAF1 regulate the gene expression thereby modulating the chromatin for accessibility of transcription factors. Pluripotency-associated transcription factor, SOX2 binding increased

across the lineage-specific super enhancer elements in CAF1-downregulated cells. Also, CAF1 downregulation resulted in an upregulation of OCT4 independent of OSKM-induced cell-fate changes. CAF1 deprivation resulted in a local depletion of the repressive histone modification mark, H3K9me3 at a subset of somatic heterochromatin areas termed 'reprogramming-resistant regions', linked to those sites that associated with low efficiency in somatic cell nuclear transfer. So, CAF-1 inhibition primed the change in chromatin to a more accessible form being further efficient in transcriptional activation.

3.3. APLF: a barrier of reprogramming

Aprataxin-PNK like Factor was first discovered as a DNA repair factor associated with the Non-homologous End Joining (NHEJ) repair process [31, 32]. Upon DNA damage, APLF aids in the recruitment of Ku, XRCC4 and Lig4 at the damaged site and accelerates the repairing process [31, 32]. On exposure to ionizing radiation, myeloid neoplasms were impeded in APLF-deprived mice, with a minute effect in DNA repair capacity [33]. So, APLF although a part of NHEJ complex, is dispensable and its dosage if modulated could be used in the advantage of diseased state of cancer. In 2011, APLF was demonstrated to possess histone chaperone activity. It could bind to histone H3/H4 and as well as the repressive MacroH2A variants [34]. We observed that the level of APLF was almost undetectable in mouse ESCs whereas, a significant expression was evident in the mouse embryonic fibroblasts (MEFs) [35]. On downregulation of APLF, the efficiency of reprogramming of MEFs to iPSCs was significantly enhanced to ~10 times. The average time for the generation of iPSC clones from APLF-depleted MEFs reduced to half the time required for the control cells to achieve the same. Mechanistically, it influenced mesenchymal-to-epithelial transition (MET) during the process of forming iPSCs from MEFs. Fibroblasts are typically mesenchymal in nature whereas the ESC-like iPSC clones demonstrate epithelial characteristics [36]. So, generation of iPSCs from MEFs involve this cellular transition and is one of the earliest event in the phases of reprogramming. E-Cadherin or CDH1 is the major player for the cells to demonstrate epithelial morphology whereas a group of other transcription factors SNAI1/SNAI2/ZEB along with N-cadherin or CDH2 drives the reverse phenomenon of epithelial-to-mesenchymal transition (EMT). Downregulation of APLF induced the expression of CDH1 while the same resulted in reduced expression of the other transcription factors associated with EMT. The enhanced expression of CDH1 resulted from the loss in recruitment of repressive MacroH2A.1 variant within the *Cdh1* promoter. Histone variant macroH2A.1 drives the compaction of chromatin thereby resulting in a repressed locus. APLF-downregulated cells demonstrated increased level of pluripotency genes, *Nanog* and *Klf4*, in comparison to the control cells. Histone modification H3K4me2 level was significantly enriched within these promoters of core pluripotency genes; one of the earliest histone marks in reprogramming that facilitates the cellular transition of fibroblasts to iPSCs [37]. So, histone chaperone APLF proved to be a barrier in reprogramming and its downregulation did not interfere with the DNA repair capacity whereas induced proliferation, kinetics and efficiency of reprogramming (Table 1).

Histone chaperone	Histone/histone variants	Reprogramming process	Transcription target/signaling target	Role	Chromatin status	Histone modification
ASF1A [29]	—	Human fibroblasts	GDF9/K56ac increased at Nanog/Sox2/Oct4 regulatory regions	Enhancing	Open	H3K56ac
CAF1A [30]	—	Mouse fibroblasts, direct conversion of B cells into macrophages and fibroblasts into neurons	Increased binding of Sox2 to pluripotency targets	Inhibitory	Heterochromatin inducer	H3K9me3
APLF [35]	MacroH2A.1	Mouse fibroblasts	MET targets, core pluripotency factors	Inhibitory	Heterochromatin inducer	H3K4me2

Table 1. Histone chaperones in reprogramming.

4. Conclusion

Basic fundamental molecule histone chaperones constitutes one of the epigenetic modulator responsible for different extent of epigenetic modifications [1, 2, 38]. They work in conjunction with histone modifying enzymes, histone modification and in few instances with transcription factors to induce the change occurring within the chromatin. Regulatory roles of histone chaperones are recently being highlighted and if exploited to their full potential, could serve as target molecules to be modulated in diseases as well. Again on that front also, a limited number of studies were performed. Being a component that take care of the entire histone metabolism and which in turn drives the major chunk of epigenetic status of a cell demands a better viewing and hence we initiated this approach for this chapter on gene expression in mammalian cells. We could only cover the development part as this field represents the most dynamicity in present day and the days to come. But, their role in replication, repair and heterochromatin is far from fully exploited. More studies are required to understand the importance of histone chaperones in mammalian system. Histone chaperones have the tremendous potential to modulate epigenetic changes and understanding their functions would give insights into how a cell converts its epigenome into another and can further unravel the secrets of developmental processes.

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Abbreviations

ESCs	embryonic stem cells
iPSCs	induced pluripotent stem cells
CENP-A	centromere protein A
CAF1	chromatin assembly factor
Spt6	Suppressor of Ty's
FACT	facilitates chromatin transcription
HIRA	histone cell cycle regulator A
DAXX	death associated protein 6
ASF1	antisilencing factor
ICM	inner cell mass
TE	trophectoderm
ATRX	alpha thalassemia/mental retardation syndrome X-linked
NKX2.5	NK2 transcription factor related, locus 5
KDM	lysine (K)-specific demethylase
MEF2C	myocyte-specific enhancer factor 2C
VEGFR1	vascular endothelial growth factor receptor
RUNX1	Runt-related transcription factor
Gfi1	growth factor independent protein 1
EKLF	erythroid Krüppel-like factor
GATA1	GATA-binding factor 1
NAP1/2	nucleosome assembly protein 1;2
CDKN1C	cyclin-dependent kinase inhibitor 1C
OSKM	Oct4-Sox2-Klf4-cMyc
OCT4	octamer 4
TH2A/TH2B	testis-specific counterparts for canonical H2A and H2B
SOX2	sex determining region Y-box 2
GDF9	growth differentiation factor
ATAC-seq	assay for transposase accessible chromatin with high-throughput sequencing

XRCC4	X-ray repair cross-complementing protein 4
LIG4	DNA ligase 4
NHEJ	non homologous end joining
MEF	mouse embryonic fibroblast
CDH1	Cadherin-1
CDH2	N-Cadherin
SNAI1/2	snail family transcriptional repressor 1/2
ZEB	zinc finger E-box-binding homeobox
EMT	epithelial-mesenchymal transition
APLF	aprataxin-PNK-like factor
PNK	polynucleotide kinase

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References

- [1] Gurard-Levin ZA, Quivy JP, Almouzni G. Histone chaperones: Assisting histone traffic and nucleosome dynamics. *Annual Review of Biochemistry*. 2014;**83**:487-517. DOI: 10.1146/annurev-biochem-060713-035536
- [2] Hammond CM, Strømme CB, Huang H, Patel DJ, Groth A. Histone chaperone networks shaping chromatin function. *Nature Reviews. Molecular Cell Biology*. 2017;**18**:141-158. DOI: 10.1038/nrm.2016.159
- [3] Loppin B, Bonnefoy E, Anselme C, Laurençon A, Karr TL, Couble P. The histone H3.3 chaperone HIRA is essential for chromatin assembly in the male pronucleus. *Nature*. 2005;**437**:1386-1390
- [4] Jullien J, Astrand C, Szenker E, Garrett N, Almouzni G, Gurdon JB. HIRA dependent H3.3 deposition is required for transcriptional reprogramming following nuclear transfer to *Xenopus* oocytes. *Epigenetics & Chromatin*. 2012;**5**:17. DOI: 10.1186/1756-8935-5-17

- [5] Lin CJ, Koh FM, Wong P, Conti M, Ramalho-Santos M. Hira-mediated H3.3 incorporation is required for DNA replication and ribosomal DNA transcription in the mouse zygote. *Developmental Cell*. 2014;**30**:268-279. DOI: 10.1016/j.devcel.2014.06.022
- [6] Messiaen S, Guiard J, Aigueperse C, Fliniaux I, Tourpin S, Barroca V, Allemand I, Fouchet P, Livera G, Vernet M. Loss of the histone chaperone ASF1B reduces female reproductive capacity in mice. *Reproduction*. 2016;**151**:477-489. DOI: 10.1530/REP-15-0327
- [7] Houliard M, Berlivet S, Probst AV, Quivy JP, Héry P, Almouzni G, Gérard M. CAF-1 is essential for heterochromatin organization in pluripotent embryonic cells. *PLoS Genetics*. 2006;**2**:e181
- [8] Garrick D, Sharpe JA, Arkell R, Dobbie L, Smith AJ, Wood WG, Higgs DR, Gibbons RJ. Loss of Atrx affects trophoblast development and the pattern of X-inactivation in extra-embryonic tissues. *PLoS Genetics*. 2006;**2**(4):e58
- [9] Michaelson JS, Bader D, Kuo F, Kozak C, Leder P. Loss of Daxx, a promiscuously interacting protein, results in extensive apoptosis in early mouse development. *Genes & Development*. 1999;**13**:1918-1923
- [10] Hatanaka Y, Inoue K, Oikawa M, Kamimura S, Ogonuki N, Kodama EN, Ohkawa Y, Tsukada Y, Ogura A. Histone chaperone CAF-1 mediates repressive histone modifications to protect preimplantation mouse embryos from endogenous retrotransposons. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;**112**:14641-14646. DOI: 10.1073/pnas.1512775112
- [11] Roberts C, Sutherland HF, Farmer H, Kimber W, Halford S, Carey A, Brickman JM, Wynshaw-Boris A, Scambler PJ. Targeted mutagenesis of the Hira gene results in gastrulation defects and patterning abnormalities of mesoendodermal derivatives prior to early embryonic lethality. *Molecular and Cellular Biology*. 2002;**22**:2318-2328
- [12] Valenzuela N, Fan Q, Fa'ak F, Soibam B, Nagandla H, Liu Y, Schwartz RJ, McConnell BK, Stewart MD. Cardiomyocyte-specific conditional knockout of the histone chaperone HIRA in mice results in hypertrophy, sarcolemmal damage and focal replacement fibrosis. *Disease Models & Mechanisms*. 2016;**9**:335-345. DOI: 10.1242/dmm.022889
- [13] Dilg D, Saleh RN, Phelps SE, Rose Y, Dupays L, Murphy C, Mohun T, Anderson RH, Scambler PJ, Chappier AL. HIRA is required for heart development and directly regulates Tnni2 and Tnnt3. *PLoS One*. 2016;**11**:e0161096. DOI: 10.1371/journal.pone.0161096
- [14] Yang JH, Song Y, Seol JH, Park JY, Yang YJ, Han JW, Youn HD, Cho EJ. Myogenic transcriptional activation of MyoD mediated by replication-independent histone deposition. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;**108**:85-90. DOI: 10.1073/pnas.1009830108
- [15] Yang JH, Song TY, Jo C, Park J, Lee HY, Song I, Hong S, Jung KY, Kim J, Han JW, Youn HD, Cho EJ. Differential regulation of the histone chaperone HIRA during muscle cell differentiation by a phosphorylation switch. *Experimental & Molecular Medicine*. 2016;**48**:e252. DOI: 10.1038/emm.2016.68

- [16] Wang AH, Zare H, Mousavi K, Wang C, Moravec CE, Sirotkin HI, Ge K, Gutierrez-Cruz G, Sartorelli V. The histone chaperone Spt6 coordinates histone H3K27 demethylation and myogenesis. *The EMBO Journal*. 2013;**32**:1075-1086. DOI: 10.1038/emboj.2013.54
- [17] Yang JH, Choi JH, Jang H, Park JY, Han JW, Youn HD, Cho EJ. Histone chaperones cooperate to mediate Mef2-targeted transcriptional regulation during skeletal myogenesis. *Biochemical and Biophysical Research Communications*. 2011;**407**:541-547. DOI: 10.1016/j.bbrc.2011.03.055
- [18] Valenzuela N, Soibam B, Li L, Wang J, Byers LA, Liu Y, Schwartz RJ, Stewart MD. HIRA deficiency in muscle fibers causes hypertrophy and susceptibility to oxidative stress. *Journal of Cell Science*. 2017;**130**:2551-2563. DOI: 10.1242/jcs.200642
- [19] Dutta D, Ray S, Home P, Saha B, Wang S, Sheibani N, Tawfik O, Cheng N, Paul S. Regulation of angiogenesis by histone chaperone HIRA-mediated incorporation of lysine 56-acetylated histone H3.3 at chromatin domains of endothelial genes. *The Journal of Biological Chemistry*. 2010;**285**:41567-41577. DOI: 10.1074/jbc.M110.190025
- [20] Majumder A, Syed KM, Joseph S, Scambler PJ, Dutta D. Histone chaperone HIRA in regulation of transcription factor RUNX1. *The Journal of Biological Chemistry*. 2015;**290**:13053-13063. DOI: 10.1074/jbc.M114.615492
- [21] Soni S, Pchelintsev N, Adams PD, Bieker JJ. Transcription factor EKLF (KLF1) recruitment of the histone chaperone HIRA is essential for β -globin gene expression. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;**111**:13337-13342. DOI: 10.1073/pnas.1405422111
- [22] Li Y, Jiao J. Histone chaperone HIRA regulates neural progenitor cell proliferation and neurogenesis via β -catenin. *The Journal of Cell Biology*. 2017;**216**:1975-1992. DOI: 10.1083/jcb.201610014
- [23] Attia M, Rachez C, De Pauw A, Avner P, Rogner UC. Nap1l2 promotes histone acetylation activity during neuronal differentiation. *Molecular and Cellular Biology*. 2007;**27**:6093-6102
- [24] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;**126**:663-676. DOI: 10.1016/j.cell.2006.07.024
- [25] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;**131**:861-872
- [26] Papp B, Plath K. Epigenetics of reprogramming to induced Pluripotency. *Cell*. 2016;**152**:1324-1343
- [27] Gaspar-Maia A, Qadeer ZA, Hasson D, Ratnakumar K, Leu NA, Leroy G, Liu S, Costanzi C, Valle-Garcia D, Schaniel C, Lemischka I, Garcia B, Pehrson JR, Bernstein E. MacroH2A histone variants act as a barrier upon reprogramming towards pluripotency. *Nature Communications*. 2013;**4**:1565. DOI: 10.1038/ncomms2582

- [28] Shinagawa T, Takagi T, Tsukamoto D, Tomaru C, Huynh LM, Sivaraman P, Kumarevel T, Inoue K, Nakato R, Katou Y, Sado T, Takahashi S, Ogura A, Shirahige K, Ishii S. Histone variants enriched in oocytes enhance reprogramming to induced pluripotent stem cells. *Cell Stem Cell*. 2014;**14**:217-227. DOI: 10.1016/j.stem.2013.12.015
- [29] Gonzalez-Muñoz E, Arboleda-Estudillo Y, Otu HH, Cibelli JB. Cell reprogramming. Histone chaperone ASF1A is required for maintenance of pluripotency and cellular reprogramming. *Science*. 2014;**345**:822-825. DOI: 10.1126/science.1254745
- [30] Cheloufi S, Elling U, Hopfgartner B, Jung YL, Murn J, Ninova M, Hubmann M, Badeaux AI, Euong Ang C, Tenen D, Wesche DJ, Abazova N, Hogue M, Tasdemir N, Brumbaugh J, Rathert P, Jude J, Ferrari F, Blanco A, Fellner M, Wenzel D, Zinner M, Vidal SE, Bell O, Stadtfeld M, Chang HY, Almouzni G, Lowe SW, Rinn J, Wernig M, Aravin A, Shi Y, Park PJ, Penninger JM, Zuber J, Hochedlinger K. The histone chaperone CAF-1 safeguards somatic cell identity. *Nature*. 2015;**528**:218-224. DOI: 10.1038/nature15749
- [31] Grundy GJ, Rulten SL, Zeng Z, Arribas-Bosacoma R, Iles N, Manley K, Oliver A, Caldecott KWAPLF. Promotes the assembly and activity of non-homologous end joining protein complexes. *The EMBO Journal*. 2013;**32**:112-125. DOI: 10.1038/emboj.2012.304
- [32] Macrae CJ, McCulloch RD, Ylanko J, Durocher D, Koch CA. APLF (C2orf13) facilitates nonhomologous end-joining and undergoes ATM-dependent hyperphosphorylation following ionizing radiation. *DNA Repair (Amst)*. 2008;**7**:292-302
- [33] Tong KI, Ota K, Komuro A, Ueda T, Ito A, Anne Koch C, Okada H. Attenuated DNA damage repair delays therapy-related myeloid neoplasms in a mouse model. *Cell Death & Disease*. 2016;**7**:e2401. DOI: 10.1038/cddis.2016.298
- [34] Mehrotra PV, Ahel D, Ryan DP, Weston R, Wiechens N, Kraehenbuehl R, Owen-Hughes T, Ahel I. DNA repair factor APLF is a histone chaperone. *Molecular Cell*. 2011;**41**:46-55. DOI: 10.1016/j.molcel.2010.12.008
- [35] Syed KM, Joseph S, Mukherjee A, Majumder A, Teixeira JM, Dutta D, Pillai MR. Histone chaperone APLF regulates induction of pluripotency in murine fibroblasts. *Journal of Cell Science*. 2016;**129**:4576-4591
- [36] Li R, Liang J, Ni S, Zhou T, Qing X, Li H, He W, Chen J, Li F, Zhuang Q, Qin B, Xu J, Li W, Yang J, Gan Y, Qin D, Feng S, Song H, Yang D, Zhang B, Zeng L, Lai L, Esteban MA, Pei D. A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. *Cell Stem Cell*. 2010;**7**:51-63. DOI: 10.1016/j.stem.2010.04.014
- [37] Barrero MJ, Sese B, Kuebler B, Bilic J, Boue S, Martí M, Izpisua Belmonte JC. Macrohistone variants preserve cell identity by preventing the gain of H3K4me2 during reprogramming to pluripotency. *Cell Reports*. 2013;**3**:1005-1011. DOI: 10.1016/j.celrep.2013.02.029
- [38] Avvakumov N, Nourani A, Côté J. Histone chaperones: Modulators of chromatin marks. *Molecular Cell*. 2011;**41**:502-514. DOI: 10.1016/j.molcel.2011.02.013