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Emerging roles of the histone chaperone CAF-1 in cellular plasticity

Sihem Cheloufi[1,2,3,4,5] and **Konrad Hochedlinger**[1,2,3,4,5]

[1]Department of Molecular Biology, Massachusetts General Hospital, 185 Cambridge Street, Boston, MA 02114, USA

[2]Center for Regenerative Medicine, Massachusetts General Hospital, 185 Cambridge Street, Boston, MA 02114, USA

[3]Cancer Center, Massachusetts General Hospital, 185 Cambridge Street, Boston, MA 02114, USA

[4]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA

[5]Harvard Stem Cell Institute, 1350 Massachusetts Avenue, Cambridge, MA 02138, USA

Abstract

During embryonic development, cells become progressively restricted in their differentiation potential. This is thought to be regulated by dynamic changes in chromatin structure and associated modifications, which act together to stabilize distinct specialized cell lineages. Remarkably, differentiated cells can be experimentally reprogrammed to a stem cell-like state or to alternative lineages. Thus, cellular reprogramming provides a valuable platform to study the mechanisms that normally safeguard cell identity and identify factors whose manipulation facilitates cell fate transitions. Recent work has uncovered the chromatin assembly factor complex CAF-1 as a potent barrier to cellular reprogramming. In addition, CAF-1 has been implicated in the reversion of pluripotent cells to a totipotent-like state and in various lineage conversion paradigms, suggesting that modulation of CAF-1 levels may endow cells with a developmentally more plastic state. Here, we review these exciting results, discuss potential mechanisms and speculate on the possibility of exploiting chromatin assembly pathways to manipulate cell identity.

Introduction

Development of multicellular organisms encompasses discrete stages of patterning and lineage specification, resulting in the production of all specialized cell types of the adult body. In mammals, fertilized zygotes and blastomeres of the cleavage stage embryo represent the developmentally most plastic state and are thus coined “totipotent,” which

Correspondence: khochedlinger@mgh.harvard.edu.

defines the ability of cells to produce all embryonic and extra-embryonic lineages of the developing organism [1] (Fig. 1). Fertilization represents a natural reprogramming process whereby the gametes' chromatin undergoes dramatic chromatin reorganization in order to prepare the genome for embryonic development [2–5]. Seminal work by John Gurdon, Ian Wilmut and others demonstrated that the transfer of differentiated nuclei into enucleated oocytes using somatic cell nuclear transfer (SCNT) recapitulates this process and endows the somatic nucleus with a zygote-like state capable of supporting the development of a cloned animal [6,7].

Following cleavage divisions and formation of the blastocyst embryo in mammals, cells residing within the inner cell mass (ICM) give rise to all embryonic lineages including the germ line. ICM cells are therefore called "pluripotent" [1]. Pluripotency can be captured *in vitro* by explanting blastocysts and deriving embryonic stem cells (ESCs)[8,9], which self-renew indefinitely in culture while retaining the potential to give rise to an entire animal upon reintroduction into host blastocysts [10,11]. Similar to zygotes, pluripotent cells are characterized by an open chromatin structure that reflects their potential to give rise to all embryonic cell types *in vitro* or *in vivo*. By contrast, differentiated cells exhibit a much more closed chromatin structure that correlates with their limited developmental potential and the establishment of specialized transcriptional programs [12](Fig. 1). Remarkably, Takahashi and Yamanaka demonstrated that pluripotency can be re-established in somatic cells by the ectopic expression of ESC-associated transcription factors such as Oct4, Klf4, Sox2 and c-Myc (OKSM), giving rise to induced pluripotent stem cells (iPSCs) [13]. The generation of iPSCs also reinvigorated earlier attempts of transcription factor-mediated cell fate change within somatic lineages [14,15] and led to more recent efforts to directly convert one mature cell type into another mature cell type using lineage-specific transcription factors or small molecules, a process termed direct lineage conversion or transdifferentiation [16].

The reprogramming of somatic cells to pluripotency or totipotency remains an ineffective process, suggesting that epigenetic barriers are established during development to safeguard somatic cell identity and resist cell fate change. Over the past decade, several chromatin pathways such as DNA methylation and histone H3K9 methylation, which are typically enriched within differentiated cells (Fig. 1), have been recognized as major roadblocks to nuclear reprogramming in the context of SCNT and iPSC generation [17,18]. However, the mechanisms by which chromatin modifications and overall chromatin accessibility act together to impede reprogramming and therefore protect cell identity remain incompletely understood. Here we review recent studies that have uncovered the chromatin assembly factor-1 (CAF-1) as a barrier to iPSCs generation and alternative cell fate transitions and thus implicate this essential complex in the regulation of cellular plasticity [19,20]. CAF-1 was identified almost three decades ago as a catalyzer of nucleosome assembly during DNA replication and repair [21,22]. Biochemical analyses further showed that CAF-1 is composed of three major subunits, p150, p60 and RbAp48 (also known as p48) that orchestrate complex interactions with histones and chromatin-modifying enzymes [23].

CAF-1 and cell fate control

The silencing of the somatic program occurs efficiently and rapidly in differentiated cells expressing the Yamanaka factors, OKSM [24]. By contrast, activation of the pluripotency-associated program is inefficient and slow. ChIP-seq analyses during early stages of human reprogramming revealed that transcription factors predominantly bind to distal elements that correspond to DNase I-resistant enhancers [25]. This observation is consistent with the idea of “pioneer factors”, which bind to closed chromatin and gradually recruit additional cofactors and remodeling enzymes to activate silenced target genes [26]. A subsequent study explored transcription factor occupancy during early stages of mouse reprogramming and confirmed binding of OKSM to enhancer elements, although no preferential binding to inaccessible enhancers was observed [27]. Specifically, this study found that OKSM facilitate the rapid silencing of somatic enhancers by physically associating with somatic transcription factors, leading to their redistribution to sites elsewhere engaged by OKSM. In addition, OKSM bind to pluripotency enhancers in a step-wise and collaborative manner, resulting in their gradual activation. The discrepancy between these studies with regards to the pioneering activity of OKSM could be due epigenetic differences between the somatic starting cells that were used, differences between the analyzed stages of reprogramming or species-specific differences. Despite these differences, a common conclusion is that the early engagement of transcription factors with chromatin fails to immediately activate a pluripotency-specific transcriptional program, suggesting that the pre-existing chromatin structure and associated modifications provide a profound impediment to effective transcriptional activation. In support of this notion, the manipulation of repressive and activating chromatin factors such as Setdb1, Dnmt1, PRC2 and SWI/SNF synergizes with OKSM to activate pluripotency-associated genes [28].

Additionally, several unbiased loss-of-function screens have been conducted during iPSCs generation to identify novel chromatin-associated factors that resist reprogramming [20,29–33]. In one such effort, our lab uncovered the two largest subunits of the chromatin assembly factor complex CAF-1, Chaf1a (p150) and Chaf1b (p60), as major roadblocks to iPSCs derivation (Fig. 2) [20]. Specifically, suppression of Chaf1a or Chaf1b enhanced the efficiency and speed of reprogramming by several orders of magnitude compared to other factors involved in heterochromatin maintenance or DNA methylation such as Dnmt1 and Setdb1. As CAF-1 is essential for cellular viability, optimal suppression was necessary to detect enhanced reprogramming and preserve a normal proliferative potential. This observation may also explain why previously identified chromatin barriers to reprogramming that are essential in somatic cells such as Mbd3 did not score prominently in our screen. Importantly, iPSCs generated with CAF-1 knockdown supported the development of high-grade germ-line chimeras, suggesting that transient CAF-1 suppression does not compromise the genomic or epigenetic integrity of cells. However, given that CAF-1 has previously been implicated in DNA damage [23], and genome stability is reportedly challenged during cellular reprogramming [34], additional experiments are warranted to rule out subtle changes to the genomic stability of iPSCs following CAF-1 depletion. Together these observations implicated CAF-1 for the first time in somatic cell

fate control and raised the question of whether this complex may also be involved in other cell fate transitions.

Indeed, Torres-Padilla and colleagues recently found that CAF-1 suppression endows ESCs with a more primitive developmental state that resembles totipotent 2-cell (2C) stage blastomeres of the cleavage stage embryo (Fig. 2)[19]. Interestingly, CAF-1 depleted ESCs were also more efficient at generating cloned blastocysts following SCNT, supporting the idea that hypomorphic CAF-1 expression endows ESCs with a chromatin state that is more amenable to reprogramming [19]. However, as blastocyst formation is not a very stringent readout for cloning efficiency [35], it will be informative to assess whether CAF-1 depleted nuclei also promote the postnatal survival of cloned mice. It will be equally interesting to assess the ability of CAF-1 depleted 2C-like cells to contribute to the extra-embryonic lineage upon injection into cleavage stage embryos, as has been shown for other cell populations with totipotent-like features [36,37]. Another question of interest is why only a small fraction of ESCs converted to the 2C-like state upon CAF-1 knockdown and whether this reflects different levels of CAF-1 depletion, a differential intrinsic susceptibility of some ESCs to revert or the acquisition of alternative cell states.

Interestingly, depletion of CAF-1 in ESCs not only endows them with a more primitive state but also appears to prevent their differentiation upon withdrawal from culture conditions that support self-renewal (Fig. 2). This conclusion is based on preliminary data from several recent RNAi screens aimed at identifying factors whose suppression delays or blocks ESCs differentiation [38–41]. Although no mechanism was provided on how CAF-1 loss impacts differentiation, it is tempting to speculate that CAF-1 depleted ESCs differentiate less due to the acquisition of a totipotent-like state, which may be less responsive to differentiation-inducing cues.

Notably, the loss of CAF-1 also facilitates other induced cell fate transitions that do not involve a pluripotent or totipotent state. For example, our lab recently showed that CAF-1 suppression enhances the conversion of B cells into macrophages upon overexpression of the myeloid transcription factor C/ebpa and of fibroblasts into neurons upon overexpression of the neuronal transcription factor Ascl1, representing two well-established transdifferentiation paradigms [20](Fig. 2). In addition, the smallest subunit of CAF-1, Rbbp4, scored as an epigenetic barrier in a chromatin-focused *in vivo* reprogramming screen in C. elegans (Fig. 2)[42]. Specifically, loss of the Rbbp4 ortholog Lin-53, together with overexpression of the neuronal-specific transcription factor CHE-1, led to the direct conversion of germ cells into neurons, indicating that Lin-53 normally safeguards germ cell fate. However, since Rbbp4 is not an exclusive component of the CAF-1 complex, it cannot be ruled out that the observed phenotypes were due to other chromatin complexes containing Rbbp4 such as the NURD and NURF nucleosome remodeling complexes and the SIN3A transcriptional repressor complex [23]. It should further be interesting to repeat this *in vivo* lineage conversion assay by knocking down the worm orthologs of the Chaf1a and Chaf1b subunits.

Altogether, these results are consistent with the notion that hypomorphic CAF-1 expression increases the epigenetic and developmental plasticity of cells in different cell lineages and

across multiple model organisms (Fig. 2). A fundamental question emerging from these observations is how manipulation of CAF-1 facilitates cellular plasticity, which will be discussed in the following two sections.

Roles of CAF-1 in nucleosome assembly, heterochromatin maintenance and epigenetic memory

CAF-1 was originally characterized as a heterotrimeric complex that promotes the assembly of nucleosomes on replicating Simian Virus 40 (SV40) plasmid DNA using a cell-free replication system (Fig. 3) [21]. Subsequent complementation assays with human and Xenopus cell extracts demonstrated that CAF-1 is also involved in restoring nucleosome assembly after DNA repair [22]. CAF-1 directly binds to newly synthesized histones H3.1 and H4, supporting its replication-dependent function during nucleosome assembly [43,44]. In addition, CAF-1 localizes to replication foci through p150's interaction with Proliferating Cell Nuclear Antigen (PCNA), which forms the replication clamp [45,46]. Notably, RNAi-mediated depletion of CAF-1 or overexpression of dominant negative mutants in immortalized cell lines decreases the assembly of newly replicated DNA into nucleosomes, stalling the replication process, activating DNA damage checkpoints and resulting in S phase arrest during cell division [47–49].

In addition to its conserved function in nucleosome assembly, the CAF-1 complex also contributes to heterochromatin maintenance by forming distinct chromatin silencing complexes (Fig. 3). The p150 subunit contains domains that interact with the heterochromatin reader proteins HP1α and HP1γ (MOD1) and the H3K9 methyltransferase Setdb1 [50,51]. Mutational analyses of these HP1 interaction domains in mouse cells confirm their role in the maintenance of heterochromatin and this function also appears to be essential for cell viability [52]. Importantly, defective HP1 recruitment in these CAF-1 mutants does not seem to alter global nucleosome assembly. Thus, CAF-1 participates in at least two crucial and separable functions, nucleosome assembly and heterochromatin maintenance. To understand CAF-1's molecular role in a given biological system, including reprogramming and transdifferentiation, it is therefore important to dissect the individual contribution of both processes. A case in point is the drosophila system where loss of CAF-1 causes defects in proliferation of mitotic and endocycling cells during larval development [53,54]. While these phenotypes were initially attributed to CAF-1's classical chromatin assembly function, defects in heterochromatin maintenance could not be ruled out initially. Indeed, a more recent study showed that expression of a newly identified and evolutionarily conserved HP1 interacting domain within the large CAF-1 subunit rescues the embryo-lethal null phenotype, unmasking an unprecedented role of CAF-1 during oogenesis [55].

Recent evidence suggests that maintenance of gene silencing at heterochromatin loci and Polycomb targets is maintained through self-propagation of the respective histone marks or sequence-specific recruitment of silencing factors to replicated chromatin [56–59]. Considering the dual effect of CAF-1 in nucleosome assembly and heterochromatin maintenance, it is tempting to speculate that the complex contributes to this process by propagating silenced gene expression patterns through cell division and thus perpetuating an

epigenetic memory. In support of this hypothesis, reduction of CAF-1 in Drosophila suppresses heterochromatin-dependent silencing and Polycomb-depedent gene repression [54]. It will certainly be interesting to test the role of CAF-1 in epigenetic memory in the context of cellular reprogramming [60].

Possible mechanisms by which CAF-1 depletion may induce cellular plasticity

Analysis of genome-wide chromatin accessibility and Sox2 binding during iPSC reprogramming revealed that CAF-1 loss acts locally by facilitating the opening of chromatin and binding of Sox2 to enhancer elements (Fig. 4). The examination of somatic heterochromatin domains indicated that CAF-1 depletion also leads to the dilution of H3K9 trimethylation across so-called Reprogramming-Resistant Regions (RRRs). These heterochromatic RRR domains are normally active at the 2C stage in fertilization-derived embryos, yet remain silenced in SCNT embryos and thus provide an impediment to the efficient generation of cloned mice [61]. However, CAF-1 suppression did not affect H3K9me3 deposition elsewhere in the genome, nor the expression of transposable elements during the generation of iPSCs. This observation is consistent with previous CAF-1 knockdown experiments in fibroblasts [62] and argues that alternative mechanisms are in place in somatic cells to keep retro-elements silent. By contrast, acute CAF-1 suppression in undifferentiated ESCs does perturb heterochromatin organization. Specifically, repressive H3K9me3 and H4K20me3 histone marks as well as HP1α are lost from pericentric heterochromatin in CAF-1 depleted ESCs, while overall nucleosome organization remains unperturbed [62]. These results suggest that at least some of the phenotypes observed in CAF-1 depleted ESCs (e.g., differentiation block) are due to perturbed heterochromatin maintenance rather than chromatin assembly.

Two recent studies established an intriguing link between CAF-1-dependent chromatin decondensation, repetitive element regulation and the ectopic expression of a 2C-like transcriptional program [19,63](Fig. 4). The activation of transposable elements in cleavage stage embryos has been proposed as a mechanism to rewire transcriptional networks and thus regulate stem cell identity [37,64,65]. Supporting this idea, Torres-Padilla and colleagues discovered that loss of CAF-1 in ESCs activates a subset of transposable elements that influence the expression of neighboring 2C stage-associated loci. The authors of that study further proposed that the reversion of ESCs to a 2C-like state depends on the nucleosome assembly function of CAF-1 during cell division. In further agreement with CAF-1's role in repressing transposable elements and thus preserving the ESC state, Loh and colleagues identified CAF-1 as a major player in a genome-wide RNAi screen for regulators of retroviral silencing in ESCs [63]. CAF-1 depletion led to the de-repression of newly integrated proviruses and the reactivation of several endogenous retroviruses (ERVs) in that study. Expression analysis of CAF-1 knockdown ESCs as well as functional and biochemical characterization of CAF-1-associated complexes suggest that both CAF-1's histone deposition function as well as its role in the recruitment of chromatin silencing complexes are involved in retro-element silencing. Strikingly, CAF-1 recruitment to different classes of ERVs seems to be regulated by co-binding to distinct cofactors such as Setdb1,

Kdm1a and HDACs. However, the effect of CAF-1 on retro-element silencing in ESCs does not appear to be global as other subtypes such as intra-cisternal A particles (IAP) are not affected [19,63]. Interestingly, a recent report implicated H3.3 replication-independent histone chaperones, including a-thalassaemia/mental retardation syndrome X-linked (ATRX) and death-domain-associated protein (DAXX), in the silencing of IAP elements [66].

In summary, given the functional diversity of the CAF-1 complex and the recent characterization of additional domains within its subunits [23,55], it will be important to dissect the interdependence between nucleosome assembly and heterochromatin regulation in the context of reprogramming, transdifferentiation and pluripotency maintenance in the future. For instance, it should be informative to assess whether mutations within the newly identified HP1-interacting domain of CAF-1, discussed above, or within regions affecting binding to Kdm1a and HDACs affect the reversion of ESCs to a totipotent state, which has thus far been ascribed to CAF-1's nucleosome assembly function [19].

Developmental roles of CAF-1

The composition and biochemical activities of the CAF-1 complex are evolutionarily conserved across human, mouse, amphibian, chicken, drosophila and yeast [23,67]. A pertinent question is therefore whether CAF-1 may function as a stabilizer of cell identity during normal development and tissue homeostasis in different multicellular organisms. However, addressing this question is challenging due to the early embryonic lethality of CAF-1 mutant animals. For example, CAF-1 knockout mice arrest between the 8- and 16-cell stage of pre-implantation development and no conditional allele has yet been reported [62]. Cytological analysis of CAF-1 mutant embryos using DAPI and HP1 staining implies a defect in constitutive heterochromatin domains, which are normally established after the second cleavage division. In support of this observation, two recent studies reported defects in heterochromatin organization and the repression of transposable elements upon CAF-1 knockdown during mouse pre-implantation development, possibly due to impaired distribution of histone H3 variants [68,69]. Interestingly these studies suggest that alternative deposition of the replication-independent histone variant H3.3 on chromatin is responsible for the perturbation of heterochromatin organization and activation of transposable elements upon CAF-1 knockdown. Strikingly, the embryonic lethality could be partially rescued by inhibition of reverse transcriptase activity, supporting the important role of CAF-1 in safeguarding the integrity of transcriptional networks during pre-implantation development by maintaining heterochromatin domains [69].

CAF-1 may also be required to maintain cell identity at later stages of development and in the adult. For example, CAF-1 is more abundantly expressed in stem cells compared to differentiated cells, suggesting that its downregulation may be important for cellular differentiation and tissue regeneration [63,70]. In support of this notion, CAF-1 scored as one of the top hits in a chromatin-focused RNAi screen for factors that prevent planarian regeneration by neoblasts, which serve as a model system for adult stem cells [70]. However, it remains to be determined whether the observed regeneration defect of CAF-1-depleted animals is due to a change of neoblast identity. Moreover, CAF-1 reportedly shuttles from the nucleus to the cytoplasm in developing mouse germ cells [71]. Since germ cells undergo

major chromatin changes during gametogenesis, it is plausible that exclusion of CAF-1 from the nucleus facilitates epigenetic reprogramming through the deposition of histone variants by alternative histone chaperones. Further functional analysis of CAF-1 subunits during development using conditional and tunable genetic perturbation systems that circumvent early lethality will be critical to understand how this and other chromatin assembly pathways contribute to cellular plasticity during mammalian development.

Analysis of CAF-1 mutants in zebrafish and worms also points to a role in cellular differentiation and lineage specification [72–74]. Despite the embryonic lethality of CAF-1 mutants in both species, embryos develop to a stage that allowed probing the function of CAF-1 in early cell fate decisions. In zebrafish, CAF-1 loss leads to cell cycle arrest and differentiation defects in several organs including the retina, pectoral fins and head skeleton [72]. It is unclear at this point whether these phenotypes are due to CAF-1's role in chromatin assembly or heterochromatin regulation. By contrast, the phenotypic similarity of mutants within histone H3 and CAF-1 in the worm points to an unprecedented role for CAF-1 histone deposition activity in generating bilateral asymmetry during embryonic development [73]. More recently, this lineage determination process has been ascribed to CAF-1's role in suppressing Notch signaling [74]. Notably, CAF-1 may also promote Notch signaling during fly development where it reportedly collaborates with the transcriptional activator Suppressor of Hairless Su(H) [75].

Taken together, the loss-of-function phenotypes of this conserved and essential molecule across different animal models indicates that the action of CAF-1 as a transcriptional activator or repressor is highly context-dependent and points to additional nuclear functions for CAF-1 beyond histone deposition and heterochromatin maintenance.

Role of other histone chaperones in cellular plasticity and outlook

A number of additional histone chaperones have evolved to control the deposition of a wide repertoire of histone variants, raising the question of whether they might also influence reprogramming, development and cellular plasticity in mammals [76–78]. Indeed, histone chaperones are important for different developmental processes such as gastrulation, myogenesis and neurogenesis [76,79]. Surprisingly, these alternative histone chaperones did not score prominently in unbiased screens of reprogramming, although the manipulation of individual members such as HIRA reportedly influences SCNT [80,81]. It is plausible that CAF-1 scores more frequently and strongly in cell transition assays because it acts as a general chromatin factor affecting both nucleosome assembly and heterochromatin organization. Moreover, CAF-1 is proposed to affect a wide range of activating and repressive histone marks including H3K56ace, H3K9ace, H3K27ace, H3K9me3 and H3K4me2/3 [62,63,82]. It also remains poorly understood how CAF-1 interacts with alternative histone deposition pathways. For example, analysis of histone deposition in CAF-1 depleted HeLa cells indicates that alternate deposition of the histone variant H3.3 by the histone chaperone HIRA provides a gap-filling mechanism to compensate for CAF-1 loss [83]. Interestingly, previous work suggested that replacement of H3.3 and the concomitant activation of pluripotency genes during amphibian SCNT are, in part, mediated by the histone chaperone HIRA [80]. More recently, H3.3 deposition within the donor

nucleus was also shown to facilitate mouse SCNT, indicating functional conservation of this mechanism across species [84]. It will be important to test whether CAF-1 depletion in somatic donor cells or in cells undergoing reprogramming into iPSCs leads to elevated H3.3 incorporation into chromatin and whether this underlies the increase in reprogramming efficiency.

In addition to CAF-1, the histone chaperone Asf1a has been implicated in cellular reprogramming and the maintenance of ESCs in human [81]. Asf1a acts upstream of CAF-1 by transferring newly synthesized and acetylated histones to CAF-1 [85]. In contrast to CAF-1, Asf1a overexpression rather than downregulation enhances human iPSCs formation. It is tempting to speculate that this phenotype is mediated by increased deposition of acetylated histones, thus inducing a more accessible chromatin state. It would further be interesting to test whether Asf1a overexpression overrides the repressive function of CAF-1 during iPSCs reprogramming.

Accumulating evidence suggests that common epigenetic mechanisms control cell fate change in the context of reprogramming, carcinogenesis and aging [28,86,87]. Given the profound effect of CAF-1 loss on the speed and efficiency of iPSCs formation, it will be interesting to test whether this complex also plays a role in cancer and aging. In support of this notion, recent studies found a connection between histone chaperone pathways and different types of cancers [77,79,88,89]. For example, the H3 chaperones ATRX and DAAX are mutated in pediatric glioblastoma, and CAF-1 levels are dysregulated in different solid tumors. As these findings are purely correlative, further studies are warranted to test whether alteration in histone chaperone pathways functionally contribute to tumor initiation, progression or maintenance. The observation that CAF-1 dosage is critical for reprogramming raises the intriguing possibility that CAF-1 may either promote or suppress tumor formation depending on cellular context. Indeed, while CAF-1 is typically overexpressed in solid tumors [90], a recent study found that its depletion endows epithelial cells with increased motility and invasive-like properties [91]. Finally, CAF-1 may play a role in ageing since both CAF-1 and histone levels decrease with cellular age. While a functional role for CAF-1 in ageing remains to be established, the recent observation that H3K9me3 levels also decrease with age suggests that CAF-1 may counter ageing by maintaining heterochromatin and thus safeguarding cell identity [92].

Altogether, these observations suggest that the effect CAF-1 perturbation on cell fate is context-dependent and influenced by several factors including the chromatin state of the target cell, environmental signals and chronological age. Although CAF-1 was discovered nearly three decades ago, its function in maintaining and safeguarding cell fate is only beginning to be recognized. Dissecting the mechanisms by which CAF-1 controls chromatin structure and function will be instrumental for a better understanding of the principles of cellular plasticity in health and disease.

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Highlights

- Recent RNAi screens have identified CAF-1 as a barrier to cell fate change in various cellular and developmental systems
- Effects of CAF-1 suppression are cell context-dependent, facilitating either differentiation, dedifferentiation or lineage conversion
- CAF-1 suppression influences cellular plasticity by altering local or global chromatin states

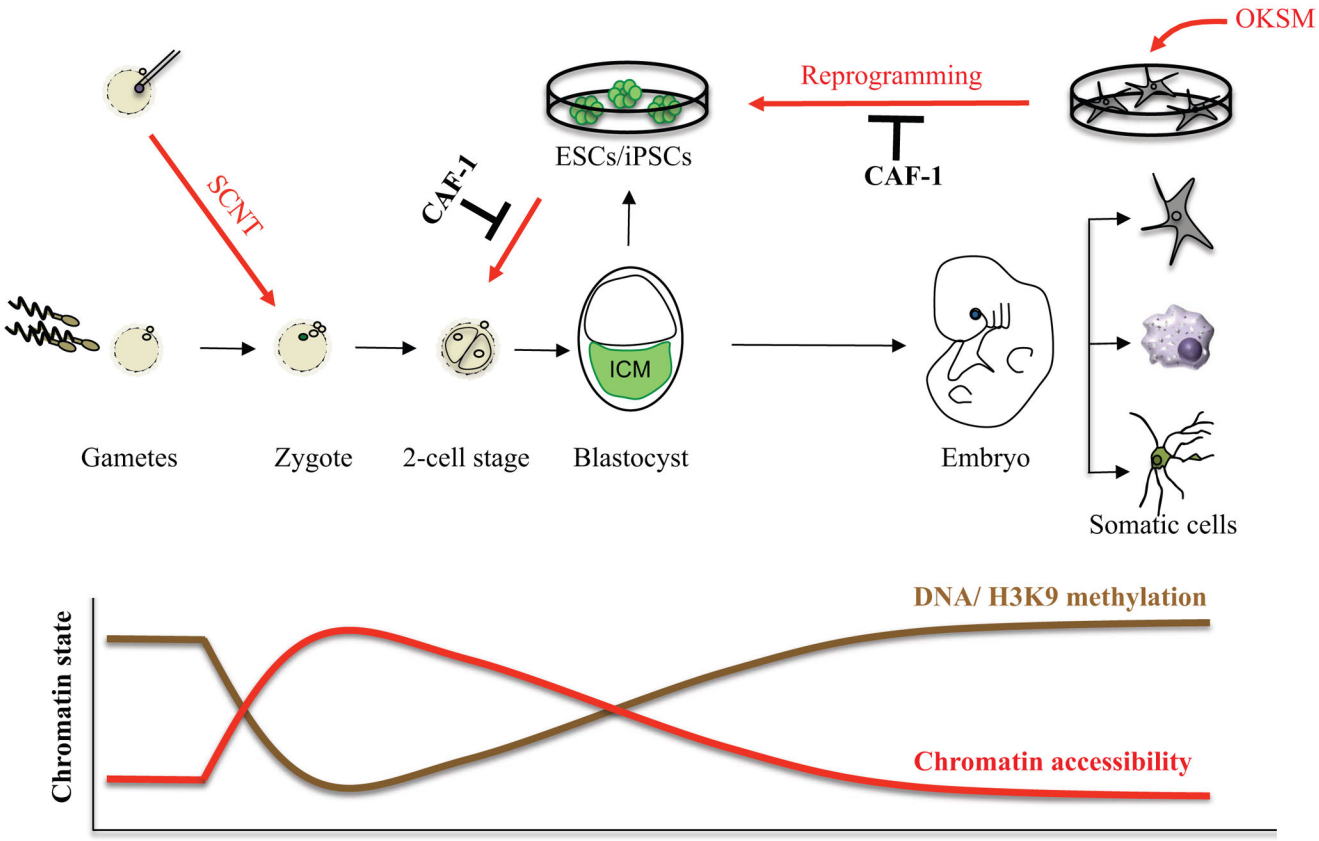

Figure 1. Chromatin accessibility and modifications during development and nuclear reprogramming

Development is accompanied by a gradual increase in chromatin compaction and the acquisition of repressive histone and DNA methylation patterns, which stabilize somatic cell fate and function as barriers to cellular reprogramming. Reprogramming to pluripotency and totipotency reverses these processes by chromatin decompaction (red arrows) and loss of silencing marks. The overexpression of transcription factors in somatic cells yields induced pluripotent stem cells (iPSCs) while the injection of somatic nuclei into oocytes by somatic cell nuclear transfer (SCNT) yields totipotent cells. CAF-1 suppression enhances the reprogramming of somatic cells to iPSCs and of ESCs/iPSCs to a totipotent-like state.

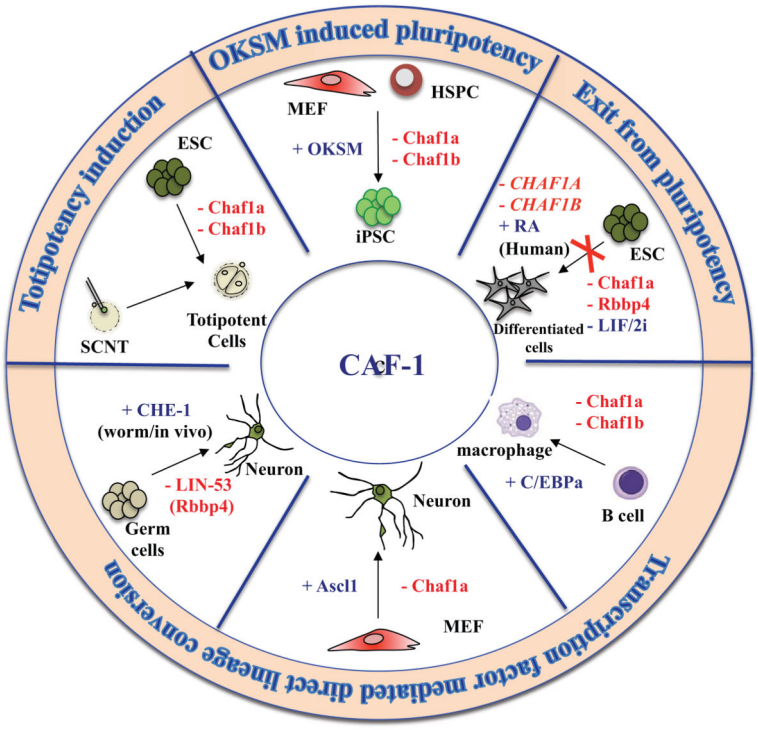

Figure 2. Roles of CAF-1 in controlling cellular plasticity

CAF-1 suppression facilitates cell fate change in different cellular systems with or without ectopic expression of transcription factors. Most cell fate switches were performed *ex vivo* using mouse cells unless noted. MEF (Mouse Embryonic Fibroblast), HSPC (Hematopoetic Stem and Progenitor Cell)

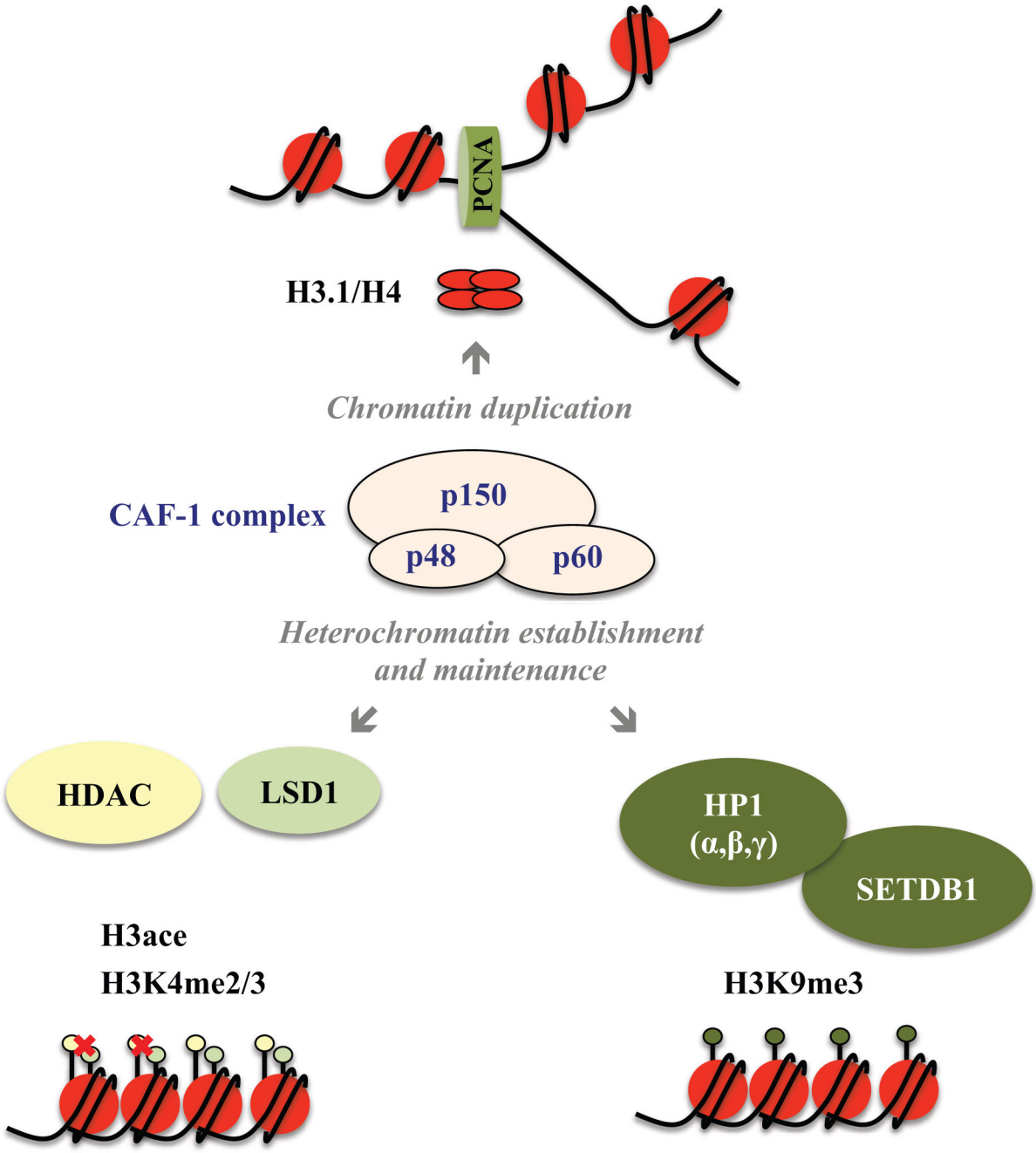

Figure 3. Functional diversity of the CAF-1 complex and its influence on chromatin structure and histone modifications

Depiction of CAF-1 complex composition, highlighting its function as (1) a replication-dependent histone chaperone via its interaction with PCNA and association with H3/H4 histone tetramers, (2) heterochromatin silencing factor via recruitment of silencing complexes such as HP1/Sedtb1, which influence H3K9me3 deposition and LSD1 and HDAC, which influence erasure of H3K4 di- and tri-methylation and H3 acetylation.

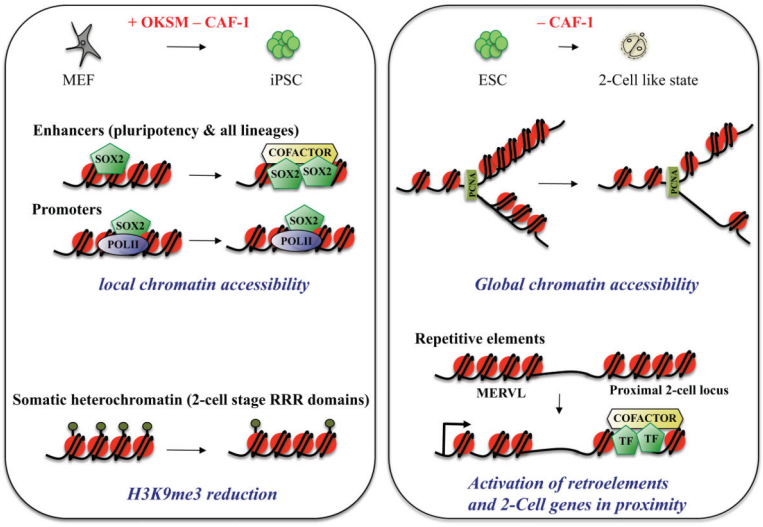

Figure 4. Mechanisms by which suppression of CAF-1 facilitates acquisition of a pluripotent or totipotent-like state

Shown are models of how CAF-1 modulation may influence chromatin accessibility and histone modifications over distinct chromatin domains. During the reprogramming of somatic cells to iPSCs, suppression of CAF-1 acts locally at enhancer elements, making them more accessible to transcription factor binding. CAF-1 suppression also results in a local reduction of the H3K9me3 silencing mark at 2-cell (2C) stage-associated "reprogramming resistant regions" (RRRs), which are normally repressed in somatic cells. During the conversion of ESCs to a 2C-like state upon CAF-1 suppression, chromatin becomes more accessible globally, resulting in activation of endogenous retro-elements, such as MERVL transcripts, and neighboring genes.