

With or Without them: Essential Roles of Cofactors in ES Cells

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Abstract

Embryonic stem cells (ESCs) are pluripotent cells which are capable of differentiating into many cell types. A complex transcriptional network consisting of Oct4, Sox2 and Nanog maintains ESCs in undifferentiated state while being poised to be directed into different committed cell types. Recently, tremendous efforts have been made to elucidate the functions of cofactors in ESC identity and differentiation. More and more evidence has shown that cofactors are crucial for both pluripotency maintenance and differentiation capacity in ESCs. Cofactors do not bind to DNA directly. Rather, they are usually recruited to target sites by transcription factors or epigenetic marks. Transcriptional cofactors including coactivators or corepressors serve as critical components of the regulatory circuitry that ensures unique gene expression program in ESCs. In this review, we will highlight recent findings on the functions of transcriptional cofactors and the underlying molecular mechanism that maintains ESC identity.

Keywords: Cofactors; ESCs; ES cell identity; Coactivator; Corepressor; Mediator

Introduction

Embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of the blastocyst-stage embryos [1,2]. They are characterized by pluripotency and self-renewal. Hence, ESCs provide a good *in vitro* system for the study of early embryonic development and molecular pathways involved in pluripotency and differentiation. Additionally, by understanding how ESCs can be manipulated to differentiate into specific cell types, ESCs also have great promise in regenerative medicine and gene therapy [3,4].

A complex transcriptional regulation network consisting of Oct4, Sox2, Nanog and epigenetic modifications have been known to be crucial in maintaining ESC identity [5,6]. Genome-wide studies have discovered that master regulators Oct4, Sox2 and Nanog act in cooperation by co-occupying two sets of genes: genes involved in pluripotency and genes associated with development [6-10]. They maintain ESC identity by activating transcription of pluripotency-associated genes via recruitment of co-activators such as p300, chromatin remodelling complexes and the transcriptional machinery [9,11,12], while repressing developmental regulators by engaging Polycomb (PcG) complexes as well as other co-repressor complexes, including NuRD, Sin3A and Pml complexes [11-15]. In addition, from protein-protein interactome studies, many transcriptional cofactors have been identified as binding partners of Oct4 and Nanog [11,12,16,17], highlighting the importance of transcriptional cofactors in the core transcriptional network in ESCs. Transcriptional cofactors do not have DNA sequence-specific binding abilities, and hence are usually recruited to target sites by transcription factors or epigenetic marks. Transcriptional cofactors can be co-activators or co-repressors, depending on their effect on transcription, and can act through a variety of mechanisms such as histone modification, nucleosome remodelling, or recruitment of transcriptional machinery. It has well been established that activators and repressors serve as critical components of the regulatory circuitry that ensures accurate transcription of a given gene according to the needs of a particular cell [18]. Since ESCs have a unique transcriptional network which maintains their undifferentiated state while keeping them poised to differentiate into all specific lineages, studying the roles of cofactors in ESC-specific transcription regulation is of great interest. Recently, tremendous efforts have been made to elucidate the functions of cofactors in ESC identity and differentiation.

In this review, we will highlight recent findings on the functions of transcriptional cofactors and the underlying molecular mechanism that maintain ESC identity.

Coactivators

Histone acetyltransferase complexes

Many coactivators have histone acetyltransferase (HAT) activity to create decondensed chromatin structure, thus facilitating the recruitment of transcriptional machinery and other regulatory proteins to activate transcription [19]. The p300/CBP complex and Tip60-p400 have been discovered as important factors in ES pluripotency and differentiation.

p300/CBP complex: The coactivators CREB-binding protein (CBP) and p300 are closely related functional homologs, which act together in histone acetylation and non-histone acetylation [20]. The p300/CBP coactivator complex has been shown to be crucial in cell growth, transformation and development [20]. Interestingly, *p300*-depleted ESCs show no defects in self-renewal [21], which may be credited to its functional redundancy with CBP. Silencing of both *p300* and *CBP* in ESCs would provide further insights into the functional roles of this complex. However, *p300* can modulate Nanog expression, possibly through histone modifications at the regulatory region of *Nanog* [21]. On the other hand, forced expression of p300 promotes neural differentiation of ESCs [22], suggesting the involvement of p300/CBP in differentiation. Indeed, multiple studies have revealed that p300/CBP mediates the activation of lineage genes during the differentiation

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Received May 24, 2012; Accepted June 27, 2012; Published June 29, 2012

Citation: Ma H, Ow JR, Chen X, Wu Q (2012) With or Without them: Essential Roles of Cofactors in ES Cells. J Stem Cell Res Ther S10:006. doi:10.4172/2157-7633.S10-006

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of ESCs into neurons, skeletal muscles and cardiac myocytes [22-27]. A recent study proposed a model in which phosphorylation of p300 drives the switch from Wnt/ β -catenin/CBP-mediated transcription to Wnt/ β -catenin/p300-mediated transcription, and initiates the differentiation of mouse ESCs [28]. Importantly, genome-wide mapping of p300 binding sites has shown that p300, usually associated with the H3K4me3 mark, is recruited to stem cell-specific enhancers by Oct4, Sox2 and Nanog. This suggests that p300 is an important component of the ESC transcription factor network [9]. Another study showed that p300 co-localizes and interacts with the chromatin remodeler CHD7, thus fine-tuning gene expression levels in ES cells [29].

Tip60-p400: Tip60-p400 is a multi-subunit remodeling complex possessing both histone acetyltransferase and chromatin-remodeling activities [30]. The complex consists of two core catalytic components, Tip60 and p400, and knocking out either of them causes embryonic death before implantation [31-33]. Tip60-p400 is found to interact with Oct4 [12], and knock-down of Tip60-p400 complex subunits affects ESC identity, with phenotypes including altered cell morphology, decreased proliferation rates, reduced alkaline phosphatase activity and defects in teratoma formation [34]. In addition, Tip60-p400 co-localizes with histone H3K4me3 and largely shares targets with Nanog [34]. These raise the possibility that Tip60-p400 can maintain ESCs in an undifferentiated state by cooperation with H3K4me3 or Nanog [34]. Although histone acetylation is usually a mark for active transcription, the observation that Tip60-p400 acetylates histone H4 not only at active promoters, but also at many PcG complex-occupied genes in undifferentiated ESCs indicates an additional repressive activity of Tip60-p400 in transcription regulation through some unknown mechanisms [35]. Taken together, Tip60-p400 likely possesses dual regulatory functions to maintain the balance between pluripotency and differentiation in ESCs.

Yes-associated protein (Yap)

First discovered as an interacting partner of Yes tyrosine kinase [36], YAP was found to be involved in establishing trophectodermal fate during the pre-implantation blastocyst stage [37-38]. The importance of YAP in maintaining ESC identity was recently revealed when YAP over-expression inhibited ESC differentiation [39]. YAP was suggested to act downstream of Yes, which in turn was activated by LIF [40], and together with TEAD2, activates the expression of pluripotency genes, including the master regulators *Oct4* and *Nanog* as well as their target genes [39,41]. While the exact mechanism by which YAP functions as a co-activator has yet to be elucidated, it appears that YAP-TEAD2 complex can be integrated into the functional regulatory network in ESCs.

Oct4-associated DNA repair proteins

MutS homolog (MSH) 2 and MSH6 form heterodimers that bind DNA mismatches [42-45] and are part of the DNA mismatch repair mechanism [46]. In recent protein-protein interactome studies in ESCs, hMSH2 and hMSH6 were recognized as Oct4-interacting partners [11,12,17]. Though it is not clear if this interaction is a result of a yet unknown role of Oct4 in DNA repair, the hMSH2-hMSH6 dimer may function as a "sliding clamp" that disassembles histone octamers, in a method aided by histone acetylation [45]. Given that the hMSH2-hMSH6 dimer binds DNA mismatches more efficiently than DNA without mismatches [42-45], it can be speculated that Oct4 may recruit the hMSH2-hMSH6 dimer to non-mismatched DNA for its nucleosome remodelling function and transcriptional regulation.

A stem cell coactivator complex (SCC) formed by nucleotide

excision repair (NER) proteins XPC, RAD23B and CETN2 were recently discovered as a coactivator which is directly recruited by Oct4 and Sox2 [47]. SCC is located to the *Nanog* and *Oct4* promoters as well as a majority of genomic regions that are occupied by Oct4 and Sox2. Depletion of SCC/XPC compromises both pluripotency in ES cells and somatic cell reprogramming [47]. This function does not require the DNA-binding ability normally required for the DNA repair activity [47]. In addition, multiple NER proteins, including XPC, XPA, and XPG, were found to be involved in mediating transcription, possibly by regulating histone modifications, such as histone H3K4 methylation and H3K9 and H3K14 acetylation that are marks of active transcription [48].

FACT complex

The FACT (facilitates chromatin transcription) complex, first found to be essential for *in vitro* transcription [49], is involved in many aspects of transcriptional regulation and histone disassembly [50-53]. FACT consists of two subunits: SPT16 and SSRP1 [54]. SSRP1 was found to be essential for the formation of ICM in early embryogenesis [55]. Both SPT16 and SSRP1 can interact with Oct4 in ESCs [11,17]. SSRP1 also binds c-Myc [56], which is another factor that is important for ESC identity [57,58]. While the precise function of the FACT complex in ESCs and its role in pluripotency has yet to be determined experimentally, it can be speculated that Oct4 and c-Myc may engage the FACT complex, which in turn mediates the transcriptional regulation activities of Oct4 and c-Myc.

Corepressors

Sin3a/HDAC complex

Sin3a, which binds to class I HDACs HDAC1 and HDAC2, is associated with multiple repressors through its paired amphipathic helix (PAH) domains, thus recruiting HDAC activity to target promoters in transcription repression [59-63]. The Sin3a/HDAC complex is involved in maintaining cell proliferation potential, DNA repair, and termination of cell differentiation [64-66]. Sin3a-mediated repression has critical roles in diverse developmental pathways (as reviewed in [67]), and *Sin3a* knock-out mouse embryos die between E3.5 and E6.5 due to impaired ICM and trophectoderm formation [64,65,68].

The Sin3a/HDAC complex is essential for the proliferation, viability, and genomic integrity of pluripotent ESCs [34,68]. However, the underlying mechanisms have not been studied in detail. In ESCs, Sin3a is found to be associated with cell cycle control and DNA damage response proteins, which might contribute to the cell cycle misregulation and apoptosis observed in *Sin3a*-null ESCs [68]. Besides, the Sin3a/HDAC complex can positively regulate *Nanog* expression, possibly through interactions with Sox2 or *Nanog* itself [15,69]. The fact that Sin3a stimulates *Nanog* expression might indicate a transcription activation role in ESC pluripotency regulation in the form of alternate co-repressor complexes [70].

NuRD complex

Nucleosome remodeling deacetylase (NuRD) complex couples ATP-dependent chromatin remodeling component Mi2 β and class I histone deacetylase HDAC1 and HDAC2 to induce repressive chromatin structure [71]. Mbd3, a core component of NuRD complex, is essential for mouse early embryogenesis, and is required for the derivation of mature epiblast from the ICM, as well as the acquisition of the pluripotent ESC state [72,73]. Mbd3 is indispensable for stable formation of the NuRD complex - ESCs lacking *Mbd3* are viable and

maintain expression of *Pou5f1* and *Nanog*, but are unable to exit self-renewal and fail to commit to developmental lineages during ESC differentiation [74]. Moreover, it is reported that Mbd3 represses pre-implantation genes, such as *Pramel6*, and also trophoctoderm-specific genes, such that loss of Mbd3 induces ESCs to differentiate towards trophoctoderm lineage [15,75]. Thus Mbd3 is essential for the full differentiation competency of ESCs. Furthermore, a recent study has reported that Mbd3 is essential for transcriptional heterogeneity and the dynamic range of a set of pluripotency genes in ESCs [76].

Moreover, Mbd3 also physically interacts with Brg1, a SWI/SNF component associated with active transcription, to antagonistically regulate a group of common target genes [77,78]. Mbd3-mediated deacetylation of histone H3K27 facilitates recruitment of PcG Complex 2 (PRC2), which subsequently stimulates H3K27me3 and enables the silencing of specific genes in ESCs [76]. Considering that there is some evidence to show that the NuRD and PcG complexes interact, these two complexes may act together to reinforce a gene silencing effect [79,80]. It is noteworthy that Mbd3 is also enriched at hydroxymethylated genes and seems to mediate some effects of hydroxymethylation on gene expression in ES cells [78]. Collectively, Mbd3 collaborates with diverse epigenetic factors in gene regulation in ESCs.

In ESCs, NuRD complex subunits *Mta1/2* and *Gata2a/b*, but not Mbd3, can form a repressive complex with *Nanog* and *Oct4* known as NODE (*Nanog* and *Oct4* associated deacetylase) [11,12,15]. NODE is recruited to *Nanog* and *Oct4* target genes independently of Mbd3. Knock-down of NODE component *Mta1* up-regulates endoderm lineage markers and induces differentiation, which is different from the observation upon *Mbd3* depletion, yet similar to that of *Nanog* depletion. This indicates that in ESCs, NODE functions distinctly from the canonical Mbd3-containing NuRD complex. Furthermore, NuRD subunits *Mta1/2*, *Mi2 β* and *RbAP46* were identified as *Sall4* interacting proteins, and thus the NuRD complex is implicated in *Sall4* transcription repression [81].

CCR4-Not complex

The CCR4-Not complex is a multi-subunit, multi-functional complex which can regulate gene expression mainly via ubiquitination and deadenylation [82]. The first indication of the role of the CCR4-Not complex in ESC identity was during a genome-wide screen for genes that perturbed ESC pluripotency upon RNAi-mediated knock-down. *Cnot3*, one of the subunits of the complex, was highlighted as being essential for ESC pluripotency [83]. This was confirmed upon further discovery that in addition to *Cnot3*, knock-down of *Cnot1* and *Cnot2* also induced differentiation [84]. Interestingly, while the collective presence of *Cnot1*, 2 and 3 are required to prevent differentiation, knock-down of the other subunits of the CCR4-Not complex did not induce any changes, consistent with the observation that only *Cnot1*, 2 and 3, and not any of the other subunits were down-regulated during differentiation of ESCs [84]. This may be explained by the fact that *Cnot1* serves as the scaffold of the complex [85], and that *Cnot2* may be required for the binding of *Cnot3* to the rest of the CCR4-Not complex [86], creating a functional interaction between these three components.

Interestingly, knock-down of *Cnot1*, *Cnot2* and *Cnot3* resulted in trophoctodermal differentiation of ESCs [84], resembling the phenotype for *Oct4* knock-down [87,88]. Nevertheless, immunoprecipitation experiments indicated there were no binding interactions between *Cnot1-3* and *Oct4* [84], in agreement with the finding that *Cnot3* is integrated into a transcriptional network that includes *c-Myc* and *Zfx*,

but not *Oct4*, *Nanog* or *Sox2* [83]. On a similar note, over-expression of *Oct4* did not rescue knock-down of *Cnot1*, *Cnot2* and *Cnot3* and knock-down of *Oct4* or *Sox2* did not cause any immediate change in expression levels of *Cnot1*, *Cnot2* and *Cnot3* [84]. This strengthened the idea that the CCR4-Not complex acts independent of the *Oct4-Sox2-Nanog* master regulators. Still, the phenotype observed upon knock-down of either of the *Cnot* subunits suggests that the complex may repress trophoctodermal genes, in accordance with its role in transcriptional repression [89,90]. However, the repression of trophoctodermal genes may be indirect, given ChIP-chip results showing that *Cnot3* does not bind to promoter regions of trophoctodermal genes [83]. Thus, it would be of interest to elucidate the mechanism and enzymatic activities by which the CCR4-Not complex regulates ESC identity.

Tri-partite motif-containing protein 28 (TRIM28)

TRIM28, also known as KAP1 or TIF1 β , has been identified as a cofactor for KRAB domain-containing zinc finger proteins [91]. Its role in pluripotency was first hinted at when it was detected as a *Nanog*-binding protein [16]. It was subsequently identified to be essential for pluripotency regulation through RNAi screens [34,83]. TRIM28 also interacts with *Oct4* when phosphorylated at serine 824 [92], which was the form previously ascertained to modulate the decondensation of chromatin conformation during DNA damage [93]. This is likely to be due to the recruitment of ATP-dependent chromatin remodelling complexes, in particular the ESC-specific SWI/SNF complex esBAF. This was in turn associated with the expression of pluripotency genes as well as the increased efficiency of pluripotency induction [92].

In contrast to its role as a co-activator above, TRIM28 is essential as a co-repressor for the function of ZFP57 [94], which is required to maintain both maternal and paternal imprints in ESCs [95] and in embryos [96]. This involves the binding of ZFP57 to specific hexanucleotide sequence found at imprinting control regions [97], where it recruits TRIM28 via the KRAB domain [98]. TRIM28 then acts to recruit DNA methyltransferases to maintain the DNA methylation patterns [98] and facilitates the establishment of higher order heterochromatin structure by engaging histone H3K9 methyltransferase SETDB1 and heterochromatin protein 1 γ (HP1 γ) to maintain transcriptional silence in an allele-specific manner [97,99]. It seems that association with the HP1 family of proteins is required for transcriptional repression facilitated by TRIM28 [100]. There also appears to be a role for histone deacetylation, from the observation that the NuRD complex plays a part in TRIM28 transcriptional repression [101].

TRIM28 is also a cofactor for ZFP809, another KRAB-containing zinc finger protein, and is similar to ZFP57 in its function in repressing the murine leukemia virus (MLV) in ESCs. ZFP809 binds to the primer binding site (PBS) that is required for the transcription of the retrovirus, and subsequently recruits TRIM28 through the KRAB domain [102,103]. Given the involvement of ESET in ZFP809-mediated silencing of retroviruses, the mechanism by which retroviruses are repressed may also involve the formation of heterochromatin [104]. Other than MLV, TRIM28 also represses other retroviruses, including those that harbour PBS sequences different from MLV, such as visna, spuma, and the Mason-Pfizer monkey virus [105], and endogenous retroviruses (ERVs) that are active in the mammalian genome [106]. However, the transcription factor that recruits TRIM28 in these other instances is not ZFP809 [105], but most likely some other unidentified KRAB domain-containing zinc finger proteins.

Mediators

Mediators are multi-subunit complexes which generally act as coactivators in RNAPII transcription. Mediators interact with both DNA-binding transcription factors and RNAPII machinery and form chromatin loops between the enhancers and promoters of active genes [107,108]. Recent studies have identified some roles for mediators in the regulation of ES pluripotency. A RNAi screen for regulators necessary for ESC state identified 12 subunits of the Mediator complex, including Med6, Med7, Med10, Med12, Med14, Med15, Med17, Med21, Med24, Med27, Med28, Med30, which are required for Oct4 maintenance in ESCs [109]. ChIP-seq results indicated that mediators contribute to gene regulation in the Oct4-Sox2-Nanog core circuitry, given the evidence that they can associate with promoters of *Pou5f1*, *Nanog* and other actively transcribed genes at sites bound by crucial transcription factors of the circuitry [6,109].

Among these subunits, Med12 is essential for early mouse embryogenesis, since its absence causes embryonic arrest at E7.5, with the loss of canonical Wnt/ β -catenin signaling [110]. Human *MED12* mutations cause Lujan-Fryns syndrome and Opitz-Kaveggia syndrome [111,112]. In ESCs, Med21 is found to be indispensable for ESC viability and homozygous disruption of *Med21* is lethal in ESCs [113]. *Med12* knock-down ESCs display phenotypes and gene expression profiles similar to those resulting from *Nanog* depletion [114]. In addition, Med12 is a Nanog-associated protein and co-occupies Nanog target promoters. Therefore Med12 may facilitate Nanog trans-activation functions in gene regulation [114].

Med15 is essential for the Smad2/3 transcriptional response [115]. As a nuclear partner of TAZ, Med15 retains TAZ in the nucleus and helps to maintain TAZ-mediated nuclear accumulation of Smad and expression of pluripotency markers *Pou5f1* and *Nanog* in hESCs [116].

CDK8, Cyclin C, Med12 and Med13 can form a sub-complex known as the CDK8 sub-module which negatively regulates mediators in the recruitment and activation of RNAPII [117]. The CDK8 sub-module can also recruit G9a methyltransferase associated with RIP140 repressive complex, jointly mediating gene inactivation [118]. Recently, an important role of CDK8 in ESCs was revealed, in which CDK8 serves as activator of several signaling pathways, such as the Wnt/ β -catenin pathway, Notch pathway, and the TGF β /BMP signaling pathway, which are key regulators in ESCs [119-122]. CDK8 also maintains ESC pluripotency through the regulation of *Myc* and *Myc* target genes [123].

Mediators can also regulate gene transcription through synergizing chromatin remodelers with chromosome conformation modification in ESCs. For instance, mediators are necessary for the recruitment of CHD1, a H3K4me3-associated chromatin remodeler, and therefore facilitates RNAPII transcription initiation of active genes in ESCs [124]. Furthermore, mediators and the Ada-Two-A-containing complex (ATAC) histone acetyltransferase can form a highly stable meta-coactivator complex (MECO), which regulates transcription of RNAPII-transcribed non-coding RNA (ncRNA) genes in ESCs [125].

In summary, there is compelling evidence showing that mediators are required for ESC pluripotency regulation. The underlying mechanisms include regulation of RNAPII, contributing to the ESC-specific transcription circuitries by mediating functions of core transcription factors like Nanog and *Myc*, facilitating signaling transductions and acting as chromatin remodeler partners.

Cohesin

The Cohesin complex is composed of four core subunits: Smc1a, Smc3, Stag2 and Rad21, and is primarily required for sister chromatid cohesion and chromosome compaction during meiosis and mitosis [126]. Studies in model organisms and evidence that cohesin is associated with developmental abnormalities have extended the role of cohesin to the regulation of gene expression [127,128].

How cohesin contributes to executive gene expression programs is not well understood. ChIP-seq results have shown that cohesin co-occupies the same binding sites as CCCTC-binding factor (CTCF) in mammalian cells and may contribute to CTCF insulator function by stabilizing DNA loop formation [129-131]. Similarly, cohesin is required for estrogen-dependent transcription regulation in breast cancer cells and mediator-activated transcription in ESCs [109,132].

Previous studies have identified the important roles of cohesin in ESC pluripotency regulation. As shown in two different ESC identity RNAi screens, the level of Oct4 is highly sensitive to reduced levels of cohesin subunits and the cohesin loading factor, Nipbl, which loads cohesin onto DNA [83, 109]. As such, cohesin may bind to promoters of key pluripotency-related genes, including *Pou5f1* and *Nanog* [109]. Furthermore, ChIP-seq results determined co-localization of cohesin with CTCF and mediators in their genomic regions in ESCs [109]. However, there are still nearly one-quarter of cohesin binding sites which are not occupied by either CTCF or mediators, implying that cohesin has other possible partners [109]. Based on what has been found, we may hypothesize that cohesin regulates ESC chromatin architecture through two possible mutually exclusive mechanisms: (i) cohesin can repress gene transcription via CTCF in the same way as in other mammalian cells, or (ii) cohesin can specially interact with mediators at the actively transcribed gene locus, stabilizing the enhancer-core promoter loops brought about by mediators.

Another study found that the loss of RAD21 affects pluripotency and induces differentiation of ESCs. RAD21 co-occupies binding sites bound by pluripotency-associated transcription factors independently of CTCF and is an integral component of the ESC transcriptional network, which is further demonstrated by the observation that the change of gene expression profile upon *RAD21* knock-down resembles that upon *Nanog* depletion [133].

Moreover, cohesin subunits are interacting partners of Oct4 and Nanog-Smc1a is identified as an Oct4 interaction protein [12] and Nanog is found to bind to cohesin subunit Stag2 and cohesin complex regulatory protein Wapl, which is necessary for cohesin removal from the chromatin [16,133].

Although cohesin has important roles in maintaining ESC-specific chromatin architecture (via either mediators or CTCF) and mediates ESC-specific gene transcription activation or repression, the molecular mechanism remains to be further elucidated. Besides, considering that cohesin co-localizes with many key transcription factors in ESCs, together with the observed binding interactions of Oct4 with Smc1a and Nanog with Stag1 and Wapl, pluripotency transcription factors may mediate the placement of cohesin at CTCF-independent binding sites. For instance, Nanog may recruit cohesin to its binding sites through the interaction with Stag1 and/or Wapl [133]. Conversely, cohesin may also assist these core transcription factors in their functions.

Condensin

Similar to cohesin, condensin is also a structural maintenance of

Cofactor	Gene Symbol	Functions in ESCs	Reference			
Coactivators						
FACT complex	SPT16	Oct4-associated protein Recruitment of TBP and TFIIIB of transcription pre-initiation complex	[11,17] [50]			
	SSRP1	Oct4-associated protein, and also c-Myc binding partner	[11,17,56]			
Yes-YAP-Tead pathway	YAP	Maintains ES identity, as OE inhibited ESC differentiation	[39]			
		Activates pluripotency genes	[41]			
DNA repair proteins	MSH2/MSH6	Oct4-associated proteins	[11,12,17]			
		Acts as a "sliding clamp" that disassembles histone	[45]			
	XPC-RAD23B-CETN2	Located at <i>Oct4</i> and <i>Nanog</i> promoters	[47]			
		Required for ESC identity and somatic cell reprogramming	[47]			
Mediators	Med6 Med7 Med10 Med14 Med17 Med24 Med27 Med28 Med30	Required for Oct4 maintenance	[109]			
				Med12	Required for Oct4 maintenance	[109]
					Nanog-associated protein, regulates Nanog and Nanog target genes	[114]
				Med15	Required for Oct4 and Nanog expression in hESCs	[109,116]
				Med21	Required for Oct4 maintenance	[109]
					Required for ESC survival	[113]
				CDK8	Regulates Myc and Myc target genes	[123]
				MECO	Regulates transcription of ncRNA genes	[125]
	Cohesin and associated proteins	Smc1a	Oct4-associated protein, Required to maintain ESC identity and viability	[12] [133,137]		
			Smc3	Required to maintain ESC identity	[133]	
		Stag2	Nanog-associated protein	[133]		
		Rad21	Required to maintain ESC identity Regulates Nanog and Nanog target genes	[133] [16]		
		Nibpl	Required for Oct4 maintenance	[109]		
		Wapl	Nanog-associated protein	[16,133]		
	Condensin	Smc2	Required for ESC survival	[34]		
Smc4		Involved in interphase high-order chromatin organization	[137]			
Histone acetyltransferases (HATs)	P300/CBP complex	Component of ESC transcription factor network and modulates Nanog expression	[9,21]			
		Cooperates with CHD7 Involved in ES cell differentiation	[29] [22,28]			
	Tip60-p400	Oct4-associated protein Cooperates with Nanog or H3K4me3	[12] [34]			
Corepressors						
CCR4-NOT complex	Cnot1	Prevents ESC differentiation, repression of trophectodermal genes	[84]			
	Cnot2					
	Cnot3		Essential for ESC pluripotency for inducing c-Myc and Zfx, independently of Oct4/Nanog/Sox2 Repression of trophectodermal genes	[83,84] [84]		
KRAB-ZFP-Trim28	Trim28	Interacts with Nanog and Oct4, essential for ES pluripotency	[16,34,83,92]			
		Chromatin de-condensation by recruitment of chromatin remodelling complexes	[92]			
		Co-repressor to ZFP57 for maintenance of genomic imprinting, possibly by recruiting DNA methyltransferases and HP1 family	[97-99]			
		Co-repressor to ZFP809, and other un-identified transcription factors, for repressing retrovirus expression	[102-106]			
Histone deacetylases (HDACs)	Sin3a	Essential for proliferation, viability and genomic integrity of ESCs	[34,68]			
		Interact with Sox2 and Nanog, and positively regulates Nanog	[15,69]			
	NuRD complex	Essential for derivation of ESCs	[73]			
		Represses lineage genes to maintain the full pluripotency of ES cells	[15,73,75]			
		Forms a repressive ESC-specific NODE complex together with Oct4 and Nanog	[15]			
		Interacts with other chromatin modifiers to mediate gene regulation	[76-78]			
	Implicated in Sall4-mediated transcription repression	[81]				

Table 1: Known functions of cofactors in ES cells.

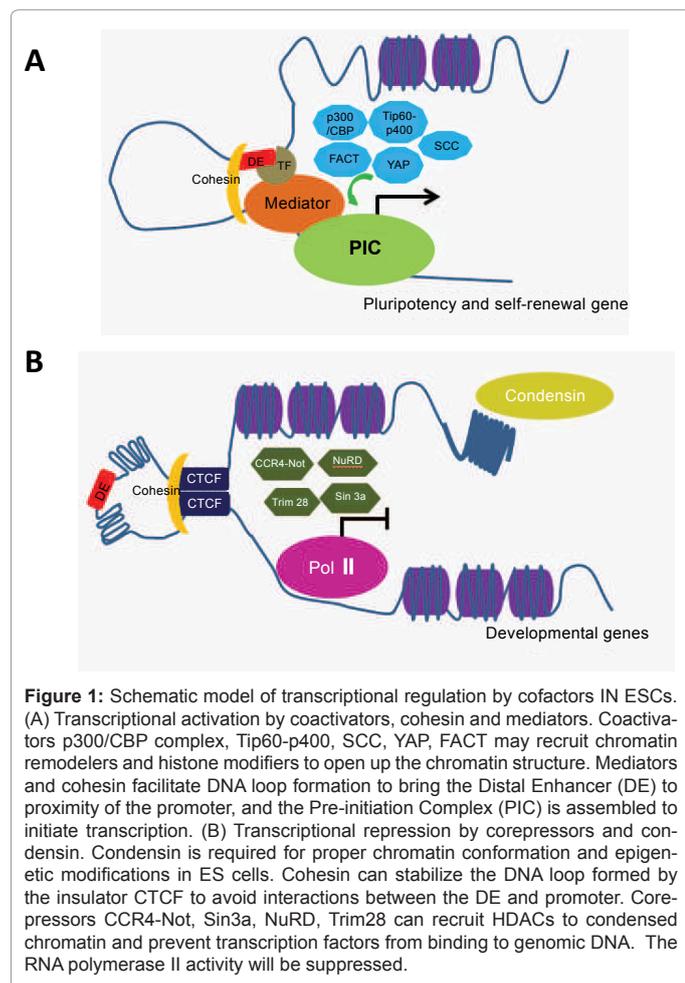


Figure 1: Schematic model of transcriptional regulation by cofactors in ESCs. (A) Transcriptional activation by coactivators, cohesin and mediators. Coactivators p300/CBP complex, Tip60-p400, SCC, YAP, FACT may recruit chromatin remodelers and histone modifiers to open up the chromatin structure. Mediators and cohesin facilitate DNA loop formation to bring the Distal Enhancer (DE) to proximity of the promoter, and the Pre-initiation Complex (PIC) is assembled to initiate transcription. (B) Transcriptional repression by corepressors and condensin. Condensin is required for proper chromatin conformation and epigenetic modifications in ES cells. Cohesin can stabilize the DNA loop formed by the insulator CTCF to avoid interactions between the DE and promoter. Corepressors CCR4-Not, Sin3a, NuRD, Trim28 can recruit HDACs to condensed chromatin and prevent transcription factors from binding to genomic DNA. The RNA polymerase II activity will be suppressed.

chromosomes (SMC) protein complex. It is required for chromosome compaction and meiotic condensation, chromosome segregation and removal of cohesin during cell division [134]. Studies in model organisms have suggested a role of condensin in gene regulation, possibly through repression of RNAPII transcription [135,136].

Condensin has a role in maintaining the specific interphase chromatin structure in ESCs, suggesting that condensin plays a role in transcription regulation. Indeed, knock-down of *Smc2* and *Smc4*, which are core catalytic subunits of mammalian condensin complexes, causes ESC-specific apoptosis [34,137]. Enlarged nuclei, altered higher-order chromatin conformation and epigenetic modification changes were also observed in the knocked-down ESCs, but not in somatic cells [137]. This suggests that ESCs require condensin for their unique chromosome folding, which is different from somatic cells. Thus, there is still much to discover about how condensin functions in transcription and chromosome regulation in ESCs.

Summary and Perspectives

In summary, cofactors including coactivators, corepressors, mediators, cohesin and condensin that function through histone modification, chromatin remodeling or the recruitment of transcription machinery, are vital for the regulatory activity of transcription factors (Table 1, Figure 1). Transcription factors are in turn responsible for recruiting the cofactors to their target genes (Figure 1). Many of these cofactors are also present in differentiated cells and serve to play

different roles when compared to their function in ESCs. This is likely due to the presence of cell type-specific factors that trigger the cell type-specific roles of these cofactors in different context. Therefore, it would be worthwhile to further determine how the interactome of these proteins leads to their specific functions and how it integrates into their regulation of the transcriptional network in ESCs. In particular, more structural studies on cofactor complexes and combination of genome-wide location analysis with computational biology will provide novel insights into cellular functions of cofactors in ES cells.

Acknowledgement

We thank Eunice Lin and Yun Hwa Lee for critical reading of the manuscript. Research in Q. Wu's lab is supported by National University of Singapore (NUS) and Singapore Ministry of Education.

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This article was originally published in a special issue, **Embryonic and Induced Pluripotent Stem Cells** handled by Editor(s). Dr. Jianlong Wang, Mount Sinai School of Medicine, United States