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Review Article

The Oct4 protein: more than a magic stemness marker

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Abstract: The Oct4 protein, encoded by the *Pou5f1* gene was the very first master gene, discovered 25 years ago, to be absolutely required for the stemness properties of murine and primate embryonic stem cells. This transcription factor, which has also been shown to be essential for somatic cell reprogrammation, displays various functions depending upon its level of expression and has been quoted as a "rheostat" gene. Oct4 protein is in complexes with many different partners and its activity depends upon fine post-translational modifications. This review aims at revisiting some properties of this protein, which has not yet delivered all its potentialities.

Keywords: *Oct4/ Pou5f1*, embryonic stem cells, pluripotency, cell lineages, cancer stem cells

Oct-4: a member of the POU family

Oct-4, a transcription factor also known as Oct-3, Oct-3/4, Otf3 or NF-A3, is encoded by the *Pou5f1* gene (located on chromosome 6 in human and 17 in mouse) and belongs to the POU (Pit, Oct, Unc) family of DNA binding-proteins. These proteins regulate the expression of target genes by binding to the octamer motif ATGCAAAT within their promoter or enhancer regions [1, 2]. Oct4, whose expression is associated with pluripotent properties of stem cells, is an essential factor controlling early stages of mammalian embryogenesis [3-5].

Oct4 expression in early embryos

Maternal murine *Oct-4* mRNA and protein (352 AA), deposited in the oocyte, are inherited by the zygote and are necessary for development until the 4-cell stage. Proteins are present at low levels at these early stages of murine embryogenesis. Transcription of zygotic *Pou5f1* gene is activated at the 4- to 8-cell stage. Consequently, high level of nuclear Oct-4 protein is detected in all blastomeres until morula stage. Upon blastocyst formation, Oct4 expression remains high in the inner cell mass (ICM) and is not expressed in the trophectoderm (TE). After implantation of the mouse embryo, transient upregulation of *Oct4* in a group of cells of the ICM triggers their differentiation into primitive endoderm (hypoblast) cells. Subsequently, *Oct4* expression is down- regulated in these cells [6-8]. During gastrulation, *Oct4* is down- regulated and, after day 8 of gestation, it is confined to primordial germ cells [7, 9, 10]. *In vitro*, *Oct4* is highly expressed in undifferentiated embryonic stem (ES) cells, embryonic carcinoma (EC) cells and embryonic germ cells. Upon differentiation of these cells induced by Leukemia Inhibitory Factor (LIF) withdrawal or in the presence of retinoic acid, *Oct4* expression is down-regulated with different kinetic [11-13].

In humans, *Oct-3/4*, unlike in mice, encodes 2 isoforms that are generated by alternative splicing of *Pou5f1* mRNA [14, 15]. These isoforms, Oct4-IA and Oct4-IB (360 and 265 amino acids, respectively), of which the 225 amino acids at the C-termini are identical, differ in sequence at their N termini [15]. Critical amounts of human Oct4-IA are required to sustain stem cell self-renewal and it has been shown that Oct4-IB is not related to stemness. In humans, *Oct4* mRNA is present throughout all stages from the unfertilized oocyte to the uncompacted morula [16-18]. These stages display a variable expression pattern of *Oct4* mRNA, between individual blastomeres of the same cell stage with only cytoplasmic localization of Oct4 proteins. No

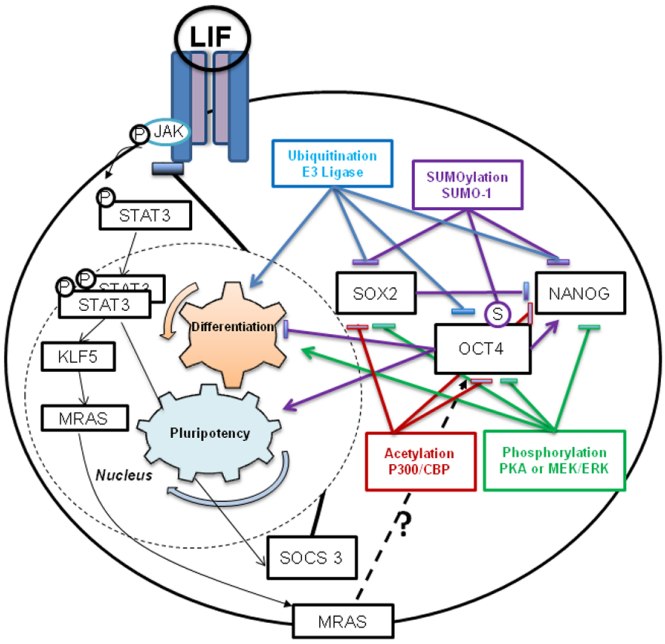

Figure 1. LIF signaling and post-translational modifications of some stemness transcription factors. The maintenance of self-renewal and pluripotency in mESC is controlled by extrinsic signaling pathway (LIF) and intrinsic self-renewal factors (eg: Oct4, Sox2 and Nanog). LIF binds to its heterodimeric receptor which leads to the activation of receptor associated Janus kinases (JAKs) which phosphorylate receptor docking sites and Stat3 on tyrosine 705 (Tyr705). Then, dimers of Phospho-Stat3 translocate to the nucleus and activate the transcription of target genes that are essential for ES cell self-renewal (eg: Klf4, Klf5 which induces expression of Mras GTPase). The activated Stat3 leads also to expression of its own repressor like Socs3, which serves as a negative feedback signal. Phosphorylation, Ubiquitination, SUMOylation and Acetylation of Oct4, Sox2 and Nanog control protein activity, expression, and stability, which result in modulation of ESC self-renewal activity. S stands for "stabilization" of the protein. Arrow indicates "activation" while the line indicates "repression".

Oct4 proteins are found in the nucleus during these stages [11]. During compaction, the expression of Oct4 protein becomes ubiquitous and abundant in the nuclei of all blastomeres of the morula. In blastocysts, *Oct4* transcripts and proteins are present in the ICM [18]. As in murine model, Oct4 is present in human embryonic stem (hES) cells [19], human embryonic carcinoma cells [20] as well as in human embryonic germ cells [21].

Oct4 is a gatekeeper for ES cell pluripotency

Pluripotent embryonic stem cell identity is governed by a core of transcription factors involving Oct4 that acts as a key regulator of pluripotent cells across mammalian species. *Oct-4* -/- embryos developed to blastocysts only composed of trophoblast cells and they are dead at the time of implantation [4, 22]. In mouse ES cells, *Oct4* knockdown results in an up-regulation of genes associated with endoderm differentiation such as *Gata6* and *a-fetoprotein* and those associated with trophoblast differentiation such as *Cdx2* [23, 24].

Recent investigation by elegant disruption of maternal or zygotic *Oct4* show also the crucial role of this gene for lineage priming *in vivo* [25]. In addition, *Oct-4* is required to maintain the pluripotency in hES cells where it acts as a repressor of the gene encoding for hCG (human chorionic gonadotropin), a placental marker in hES cells. Down- regulation of *Oct4* in hES cells, was coincident with a significant increase in transcription of genes associated with trophoblast and endoderm lineages [26, 27]. Furthermore, Oct4, Sox2 and Nanog cooperate to maintain the self-renewal and pluripotency of mouse and human ES cells. These three transcription factors, together bound to the promoters of their own genes [28, 29], allow their activation. In addition, they activate the transcription of genes involved in maintaining ES cell self-renewal. *Dppa4, Tdgf1, Oct4, Nanog, and Lefty2* are positively regulated by Oct4, Sox2 and Nanog and preferentially expressed in ES cells [28]. On the other hand Oct4, Sox2 and Nanog co-occupy the promoters of genes that have key roles in differentiation and development and promote their inactivation [30].

The intersection between the transcriptional core (Oct4, Sox2 and Nanog) and LIF signaling

In addition to Oct4, Sox2 and Nanog, the LIF/ STAT3 signaling pathway is also known to be critical for the maintenance of pluripotency in murine ES (mES) cells while it seems dispensable in hES cells, despite the expression and the functional activation of the LIF/ STAT3 in these cells [31]. Since these first observations, it has been demonstrated that hES cells are closer to murine Epiblast stem cells (primed cells) than to naïve mES cells [32, 33]. In addi-

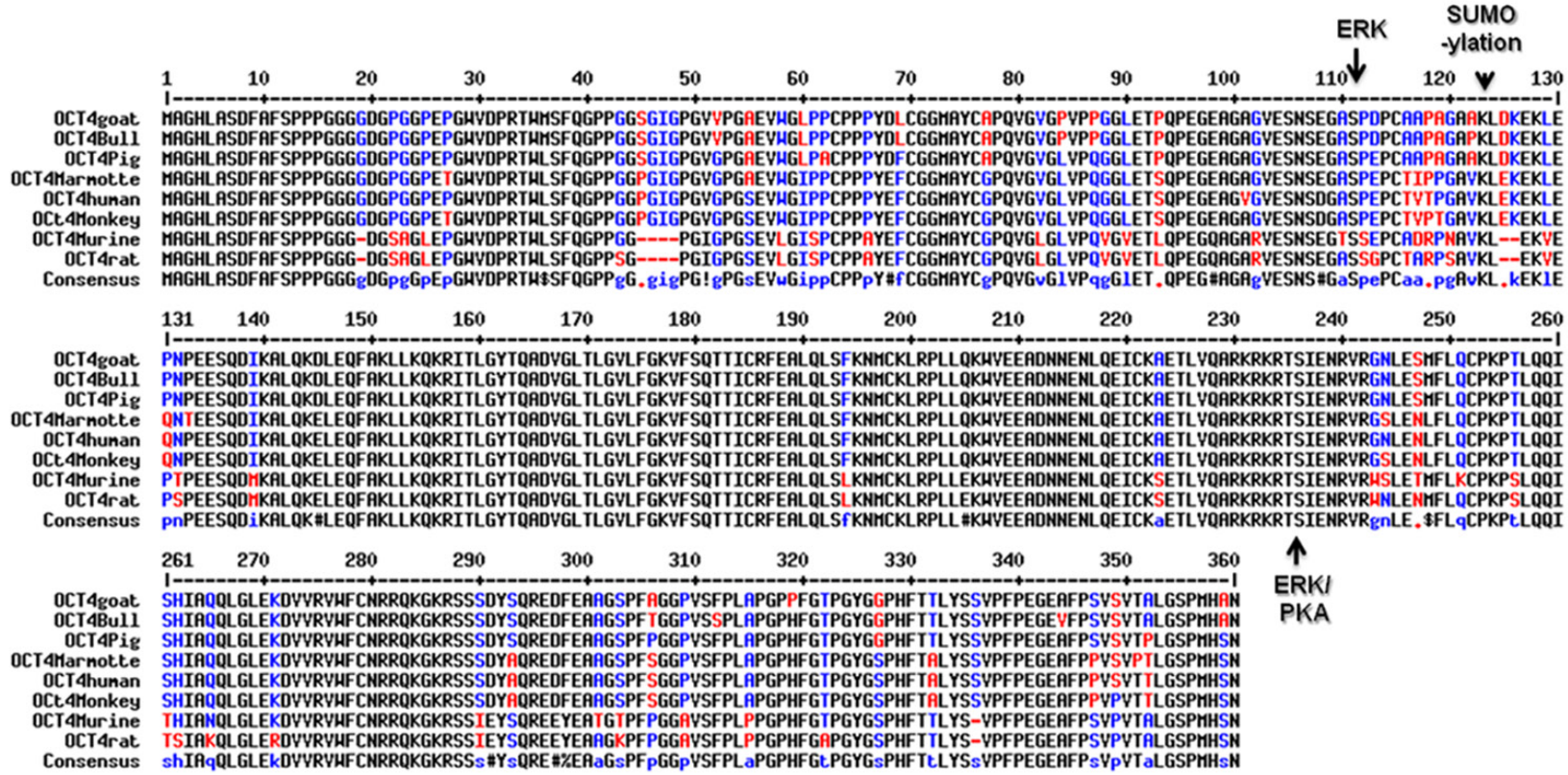

Figure 2. Protein sequence alignment of mammalian OCT4 proteins. Protein sequences of the different mammalian proteins, retrieved from GenBank, have been aligned with the MultiAlign software [81]. The conserved ERK, PKA and SUMOylation sites are indicated.

tion, recent studies have demonstrated a key role of LIF to induce the reversion of primed to naïve states in the human model [12, 34, 35]. An important challenge of this research field is to characterize the molecular mechanisms of ES cell plasticity.

In mES cells, LIF (Leukemia inhibitory factor), via activation of JAK1/ STAT3 pathway, is an essential cytokine that prevents differentiation. Stat3 activates the expression of a significant number of pluripotency-related genes in ES cells [12, 36-39]. Furthermore, some of the Stat3 target genes are also regulated by Oct4. For example, Oct4 and Stat3 directly bind to the promoter region of *Eed* gene (Embryonic ectoderm development) which encodes for a core component of Polycomb repressive complex 2 [40, 41]. In addition, *Klf4* (Krüppel-like transcription factor 4), a direct downstream target of Oct4 and Stat3, is required for ES cell self-renewal and maintenance of pluripotency [42-44]. These data revealed that the cooperation between the intrinsic Oct4 network and extrinsic LIF pathway is crucial to regulate ES cell self-renewal and pluripotency. However Oct4 and LIF's targets are regulated in different ways upon cell differentiation triggered by LIF withdrawal: indeed, *Oct4* mRNA and protein remains at high level for few days upon LIF withdrawal. This is in contrast with many of the LIF 's targets (named *Pluri* genes like *Mras, Ceacam1* and *2, Irak3, Esrrb*) whose mRNA expression decreased on the first day of LIF withdrawal concomitantly with cell differentiation [12, 13, 39]. It has also recently been shown that disregulated *Mras* expression, a small GTPase of ras family, lead to an alteration in *Oct4* expression, indicating a potential link between this small GTPase and Oct4 regulation [45], see **Figure 1**. More generally, how the *Pluri* gene cluster, which encodes various types of protein regulators, modulates Master gene activity remains to be resolved.

Oct4 and its dosage effect on the cell fate

Oct4 dosage is important in the determination of the mES cell fate. Depending upon Oct4 expression level, mES cells maintain their pluripotency or differentiate towards trophoblast (low or no *Oct4* expression) or primitive endodermal and mesodermal (high *Oct4*) lineages [23, 46, 47]. This rheostat behavior of gene, which has also been shown for *Sox2* [48], revealed that these genes exerts a dose-dependent action. In addition, it has been well documented that neuronal differentiation of ES cells, under the serum-free culture condition, is enhanced because of the sustained overexpression of *Oct4* in ES cells [49]. Moreover, the involvement of *Oct4* in the mesendoderm differentiation and cardiac commitment of ES cells was also proved. Indeed, transient increase in *Oct4* expression upon TGFβ induction, in undifferentiated ES cells and in the epiblast of mouse embryos leads to establishment of cardiogenic lineages [46]. Also, by different approaches it was shown that, depending upon its expression level, Oct4 could form different protein complexes with members of the SOX family: the Sox2/ Oct4 complex, which binds to a canonical binding site, induces expression of genes involved in the maintenance of pluripotency. In contrast, an increase in *Oct4* or *Sox17* expression level leads to a switch of partners, Sox2 being replaced by Sox17. This complex then binds to a different compressed DNA motif at promoter of genes involved in primitive endoderm and mesendoderm differentiation [46, 47, 50, 51]. Whether the Oct4 protein, along with Sox2 or Sox17, displays various functions as a complex, depending upon their post-translational modification, remains unknown.

Regulation of Oct4 stability at the post-translational level

The mechanisms through which Oct4 protein activity is regulated are largely unclear. Potential phosphorylated or sumoylated conserved residues, present among the mammalian Oct4 proteins, are shown in **Figure 2**. Oct4 can be phosphorylated by protein kinase A and/or ERK MAPK at a highly conserved residue, Ser 229 (murine) or Ser 236 (human) within the POU DNA-binding homeodomain, (see **Figure 2**) [52]. Phosphorylation at this Ser residue sterically hinders both DNA binding and homodimer assembly [53, 54]. Furthermore, ubiquitination of Oct4 (shown for both murine and human proteins) is also a post-translational modification that dramatically reduces its transcriptional activity. During ubiquitination process, the E3 ligase interacts directly with target proteins and promotes the Ubiquitin transfer. WWP2, an E3 ubiquitin ligase that specifically interacts with Oct4 through its Tryptophan-based WW domains, has been identified in murine and human embryonic

stem cells. WWP2 promotes the ubiquitination of Oct4 and its degradation both *in vivo* and *in vitro* [55-59]. Additionally, MEK/ERK signaling, activated upon ES cell differentiation, is involved in the negative regulation of human Oct4-IA protein, through its phosphorylation at Ser111 which alters stability and its subcellular localization [60]. Oct4 is also a target for small ubiquitin-related modifier (SUMO) -1 that occurs at a highly conserved K among the mammalian proteins (K118 in mice and K123 in primates), see **Figure 2**. This conserved lysine is located at the end of the amino- terminal transactivation domain and next to the DNA -binding domain. Sumoylation of Oct4, which does not alter its subnuclear localization, enhances its stability, DNA binding and transactivation functions [61, 62]. Whether sumoylation of Oct4 is involved in its increased level, coupled with its function in primitive endoderm differentiation, remains to be established. Interestingly, it has also been shown that SUMOylation of Oct4 and Sox2 regulated Nanog in an opposing manner: SUMOylation of Oct4 enhanced Nanog expression, while SUMOylated Sox2 inhibited its expression. Moreover, SUMOylation of Oct4 by Pias2 or Sox2 by Pias3 impaired the interaction between Oct4 and Sox2 [63]. All these findings reveal that the post-translational modifications of Oct4, act as a regulatory signal to control its activity and stability in a wide variety of cellular processes. This also applies to other stem cells factors, such as Sox-2 and Nanog (**Figure 1**).

Oct-4 and tumorigenicity

It is well documented that overexpression of Oct4, Sox2 and Nanog, together or separately, led to tumorigenicity, tumor metastasis, and even distant recurrence after chemoradiotherapy in different types of cancer [64-66]. High expression of Oct4 was detected in Prostate [67] and Breast cancer stem cells [68] and in the tumor initiating cells in a p53-/- tumor mice model [69]. Oct4 has a critical role in the survival of these tumor cells. Generally, these transcription factors are more frequently overexpressed in poorly differentiated tumors (compared to well differentiated tumors) and expression level of these stemness-involved factors decreases with the differentiation of cells [70-73]. There are probably conserved molecular mechanisms which could explain dedifferentiation of somatic cells, as observed in cancers, and somatic cell reprogrammation [74-76]. So far, reprogrammation has not yet been demonstrated to occur, normally, in particular situation in the adult body. However, we could hypothesize that this potential normal processus, if it exists, should be constantly under strict control in adult body [for exemple, co-expression of Oct4, Nanog, Sox2 and myc, one of the *in vitro* reprogrammation cocktails, [75, 77-79] should never occur in normal differentiated adult cells]. We could then hypothesize that cancer formation is the result of uncontrolled reprogrammation, both involving Oct4 and many other stemness genes [80].

Conclusion

Oct4, first discovered and characterized 25 years ago [1, 5], is not only a key stemness marker but it is also involved in lineages specification and it is a cell ressetor involved in somatic cell reprogrammation *in vitro* [33, 74]. Oct4 is also reexpressed in different types of cancer stem cells, which are tumor cell clusters at the origin of chemotherapy tumor resistance and recidive of cancers. Though, the precise understanding of the molecular mechanisms of Oct4 regulation and particularly of its switch ON and OFF in tissues, depending upon microenvironment, is a challenge in fundamental and applied research fields, for regenerative medicine and cancer therapy.

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Disclosure of conflict of interest

None.

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