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Perspective

Post-translational modulation of pluripotency

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The maintenance of pluripotency relies on an intricate transcriptional network hinged on a key set of transcription factors. Pluripotent stem cells have been shown to be sensitive to modulations of the cellular abundance and transcriptional activity of these key pluripotency factors. Recent evidence highlights the important role of post-translational modifications, including ubiquitination, sumoylation, phosphorylation, methylation, and acetylation, in regulating the levels and activity of pluripotency factors to achieve a balance between pluripotency and differentiation.

Pluripotency of stem cells, including induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), is defined as the ability of the cells to differentiate into all types of cells of an organism. PSCs rely on a coordinated network of transcription factors to simultaneously maintain pluripotency and suppress lineage-specific differentiation. Central to these processes are the proteins Oct4, Sox2, and Nanog. Studies have also discovered that the four transcription factors, Oct4, Sox2, c-Myc, and Klf4, can reprogram differentiated somatic cells to iPSCs, which further highlights the significance of a coordinated transcriptional network in the control of pluripotency. These pluripotency-associated transcription factors are highly expressed in pluripotent cells and become down-regulated during differentiation. There are a variety of regulators that ensure the intracellular activity of these transcription factors, including those functioning at the transcriptional, translational, and post-translational levels. In this perspective, we will summarize the recent studies on post-translational modifications of transcription factors (Oct4, Sox2, Klf4, etc.) in maintaining pluripotency, including ubiquitination, sumoylation, phosphorylation, methylation, and acetylation.

Ubiquitination is the main pathway responsible for eliminating short-lived regulatory proteins. In mouse ESCs, Oct4 can be ubiquitinated by an HECT-type E3 ubiquitin ligase, Wwp2, via the Lys63 linkage (Xu et al., 2004; Liao and Jin, 2010). Wwp2 regulates Oct4 level by mediating its ubiquitination and degradation during ESC differentiation. Both the Wwp2 and Oct4 levels decrease when ESCs are induced to differentiate (Xu et al., 2004). Wwp2 catalyzes Oct4 ubiquitination in a dosage-dependent manner and also regulates its own ligase activity by auto-ubiquitination (Liao and Jin, 2010). Subsequent study shows that the human counterpart of Wwp2, WWP2, ubiquitinates OCT4, and promotes its degradation through the 26S proteasome in human ESCs (Xu et al., 2009). c-Myc has also been reported to be regulated by ubiquitin–proteasome system (UPS). Its N-terminal sequences, which include the first 158 residues, are necessary for ubiquitination and proteasomal degradation, whereas a centrally located PEST sequence motif enriched in proline (P), glutamic acid (E), serine (S), and threonine (T) is necessary for the rapid degradation but not ubiquitination of c-Myc (Gregory and Hann, 2000). A recent study shows that knockdown of TRIM6, a RING finger family ubiquitin ligase E3 that is selectively expressed in ESCs, enhances the transcriptional activity of c-Myc, resulting in the promotion of ESC differentiation (Sato et al., 2012). Nanog is also among the transcription factors that are crucial for pluripotency of ESCs. A recent study reports that in human ESCs, Nanog is regulated by UPS through ubiquitination of a PEST motif that lies in the N-terminal region of Nanog from amino acid 47 to 72, although the specific ubiquitin ligase E3 and the exact conjugate residues are still unknown (Ramakrishna et al., 2011).

The small ubiquitin-related modifier (SUMO), which is structurally related to but functionally divergent from ubiquitin, can modify many nuclear proteins to affect their subcellular localization, thus altering their interaction with cooperative molecules. Studies show that Oct4 can be sumoylated at a single lysine, lysine 118, which is located at the end of the N-terminal transactivation domain and next to the POU DNA-binding domain (Zhang et al., 2007). Sumoylation of Oct4 significantly increases its stability, DNA binding, and thus the transcriptional activity without altering its subnuclear localization (Wei et al., 2007). Sox2 can be sumoylated at a single lysine, lysine 247 (Tsuruzoe et al., 2006; Van Hoof et al., 2009). Unlike its effect on Oct4, sumoylation of Sox2 inhibits DNA binding, thus negatively regulates its transcriptional activity (Tsuruzoe et al., 2006). Further study shows that this lysine 247 residue is adjacent to a serine triplet (Ser249, 250, and 251) and phosphorylation of this serine triplet directly triggers the sumoylation of Sox2 (Van Hoof et al.,

2009), indicating a crosstalk between two different types of post-translational modification of Sox2. Klf4 can be modified by SUMO in both the non-covalent and covalent manners. The physical interaction of Klf4 with SUMO-1 through its SIM is responsible for the transactivation of Klf4 (Du et al., 2010). Mutations within the SIM or knockdown of SUMO-1 can both dramatically lower the transcriptional activity of Klf4 in yeast or mammalian systems (Du et al., 2010). Klf4 can also be sumoylated at lysine 275 by the SUMO E3 ligase PIAS1. However, this sumoylation does not seem to affect the nuclear localization, transcriptional activity, or the stability of Klf4 (Kawai-Kowase et al., 2009). The transcription factor Nr5a2 (orphan nuclear receptor, also known as liver receptor homolog-1, Lrh-1) can replace Oct4 in the generation of iPSCs from mouse somatic cells and enhance reprogramming efficiency. A study in rat granulosa cells has shown that Nr5a2 can be sumoylated at five lysine residues, Lys173, 213, 289, 329, and 389 (Yang et al., 2009). Nr5a2 is transported to transcriptionally inactive nuclear bodies after sumoylation. A lysine-to-arginine mutation at the SUMO conjugating site increases the transcriptional activity of Nr5a2. Sumoylation-deficient Nr5a2 has been shown to greatly increase reprogramming efficiency. While replacing Oct4 with wild-type Nr5a2 shows a 4-fold enhancement of reprogramming efficiency, K173R/K289R and K173R/K213R/K289R/K329R/K389R Nr5a2 mutants increase reprogramming efficiency to around 7- and 11-folds, respectively (Heng et al., 2010). Together these results suggest that sumoylation negatively regulates the transcriptional activity of Nr5a2.

Phosphorylation is one of the most classic post-translational modifications known to link to most if not all cellular signaling and transcription events. Oct4 can be phosphorylated by protein kinase A at Ser229. This serine residue lies within the POU DNA-binding homeodomain of Oct4, which spans amino acids 230–289. The phosphorylation of Ser229 sterically hinders both the DNA binding and homodimer assembly of Oct4 (Saxe et al., 2009). Oct4 can also be phosphorylated by the Abl kinase at Tyr327. Mutation of this tyrosine to alanine or phenylalanine results in hyperactive transactivation of all tested Oct4 target gene promoters, indicating that this phosphorylation may act to fine-tune Oct4 transcriptional activity (Saxe et al., 2009). A phosphoproteome analysis shows that Oct4 can be phosphorylated at Ser236 in human ESCs (Swaney et al., 2009). Similar to the phosphorylation at Ser229, this site is contained within the POU homeodomain of Oct4 and thus phosphorylation at Ser236 might influence the DNA binding and transcriptional activity of Oct4. The same phosphoproteome analysis also shows that Sox2 is phosphorylated at Ser246, 249, 250, and 251, which is consistent with the result of another similar phosphoproteome analysis using quantitative mass spectrometry (Van Hoof et al., 2009). Interestingly, this phosphorylation consequently triggers the sumoylation of Sox2 at Lys247 (Van Hoof et al., 2009), which in turn results in the inhibition of Sox2 DNA-binding activity (Tsuruzoe et al., 2006). A recent study shows that Sox2 can also be phosphorylated at Thr118 by Akt. This phosphorylation not only promotes Sox2 stability by blocking ubiquitination, thus enhancing the self-renewal capacity of mouse ESCs, but also enables Sox2 to reprogram mouse embryonic fibroblasts more efficiently (Jeong et al., 2010). A recent study discovered that Nanog can be phosphorylated at four Ser/Thr-Pro motifs and these phosphorylation events promote the interaction between Nanog and the prolyl isomerase Pin1, which in turn suppresses the ubiquitination of Nanog and thus stabilizes Nanog. Inhibiting Pin1 activity or disrupting of Pin1–Nanog interaction in ESCs suppresses the capability of Nanog in maintaining self-renewal (Moretto-Zita et al., 2010). Three of the four Ser/Thr-Pro phosphorylation sites are located within the PEST motif of the human Nanog, which is exactly the target motif for the ubiquitination of Nanog (Ramakrishna et al., 2011), further confirming a competitive relationship between phosphorylation and ubiquitination. Unlike phosphorylation of Nanog which antagonizes ubiquitination, phosphorylation of Klf4 triggers the ubiquitination and degradation of Klf4 (Kim et al., 2012). Klf4 phosphorylation by ERK1 or ERK2 at Ser123 leads to the recruitment of βTrCP1 or βTrCP2, an F-box protein with E3 ubiquitin ligase activity, to its N-terminal domain, thus resulting in the ubiquitination and degradation of Klf4 (Kim et al., 2012). These studies also provide examples of crosstalk between two different types of post-translational modifications, which jointly control the stability and activity of transcription factors essential for pluripotency maintenance. The transcriptional activity of Nr5a2 can also be regulated by phosphorylation. Human Nr5a2 can be phosphorylated at the hinge domain serine 238 and 242 after being treated with mitogenic stimuli such as phorbol myristate (PMA). Preventing phosphorylation by S238A/S243A double mutations decreases PMA-induced Nr5a2 transactivation, whereas mimicking phosphorylation by the S238D/S243D mutation increases basal transactivation of Nr5a2 (Lee et al., 2006). These results suggest that phosphorylation of Nr5a2 may be involved in controlling the pluripotency of ESCs and its phosphorylation mimicking mutant could be used to improve the reprogramming efficiency towards pluripotency.

The methylation of arginine residues is catalyzed by a family of protein arginine methyltransferases (PRMTs), among which coactivator-associated arginine methyltransferase 1 (CARM1, also referred to as PRMT4) catalyzes the asymmetric dimethylation of arginine residues in a variety of proteins, including histones and certain key transcription factors. ESCs with depleted CARM1 gradually lose their pluripotency (Wu et al., 2009). A recent study shows that CARM1 is in association with Sox2 and directly methylates Sox2 at Arg113 (Zhao et al., 2011). This methylation, although does not affect Sox2 subcellular localization or stability, enhances Sox2 self-association through its HMG-box domain. The retention of Sox2 on chromatin restricts the methylation level of Sox2 (Zhao et al., 2011).

In addition to protein ubiquitination, sumoylation, phosphorylation, and methylation, acetylation of lysine residues acts as a regulatory signal to control pluripotency of ESCs. p300/cAMP-response-element-binding protein (CBP) is a well-known acetyltransferase of both histones and non-histone proteins. Sox2 is reported to be acetylated by p300/CBP at Lys75 in ESCs (Baltus et al., 2009). Blocking acetylation by K75A mutation results in the

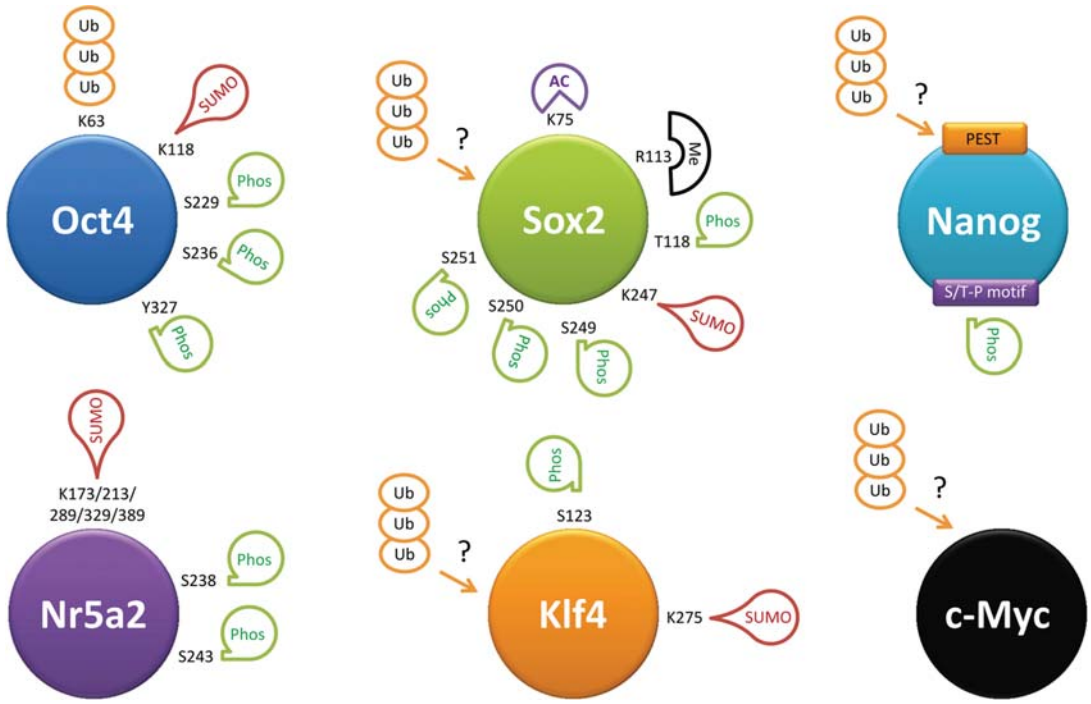

Figure 1 Post-translational modifications of pluripotency-associated transcription factors. Ub, ubiquitination; SUMO, sumoylation; Me, methylation; AC, acetylation; Phos, phosphorylation.

retention of Sox2 in the nucleus and thus sustains induction of its target genes, while mimicking acetylation by the K75Q mutation enhances the interaction of Sox2 with the nuclear export machinery. Interestingly, an increase of cellular acetylated Sox2 by treatment with deacetylase inhibitors promotes Sox2 ubiquitination and subsequent proteasomal degradation. This provides another example of how different types of post-translational modifications jointly control the stability and activity of transcription factors that are essential for maintaining the pluripotency of ESCs.

Ever since the Yamanaka group discovered that four transcription factors, Oct4, Sox2, c-Myc, and Klf4, can reprogram differentiated somatic cells to iPSCs, a lot of efforts have been put into studying the underlying molecular mechanisms through which these transcription factors are controlled to maintain pluripotency or initiate differentiation. Post-translational modifications play an important role in these processes by regulating the activity, stability, and cellular distribution of transcription factors that control pluripotency. PSCs appear to prefer employing various post-translational modifications to regulate specific functions of a single transcription factor, which seems to enable a more precise regulation. Having taken Sox2 as an example, it is a key component of the pluripotency-associated transcription factor network that undergoes a variety of post-transcriptional modifications. These modifications indeed coordinate the dynamic regulation of both the subcellular distribution and protein stability of Sox2 (Figure 1, Supplementary Table S1). Oct4 lies at the center of the mechanisms that maintain the self-renewal and pluripotency of ESCs. Similarly, the activity of Oct4 is cooperatively controlled by ubiquination, sumoylation, and phosphorylation (Figure 1, Supplementary Table S2). It would be interesting to know the spatial and temporal regulation of pluripotency-associated transcriptional networks via various post-translational modifications, and their implications in stemness maintenance and differentiation initiation. Further detailed mapping of the crosstalk between various post-translational modifications in the regulation of ESC functions leads us to an exciting topic for future studies. The hyperactive mutants of pluripotency factors based on these studies could be used to optimize the reprogramming procedure and generate iPSCs in a more efficient and safe way. The unraveling of post-translational modifications of lineage-specific transcription factors may also contribute to transdifferentiation studies. It will be also interesting to see whether small molecule drugs that target specific post-translational modifications could enhance our ability to generate clinical relevant cell types.

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