Cell Research (2009) 19:527-528.

www.nature.com/cr

RESEARCH HIGHLIGHT

OCT4: Less is more

Ryan T Wagner[1], Austin J Cooney[1]

[1]*Department of Molecular and Cellular Biology, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, USA*
Cell Research (2009) **19**:527-528. doi: 10.1038/cr.2009.48; published online 4 May 2009

Embryonic Stem Cells (ES cells) exhibit vast potential that is just beginning to be realized with regard to both clinical therapies, and use in the laboratory as a model system for the study of development and cancer progression. Reaching this point has required thorough investigations into identifying the distinct cellular properties and molecular mechanisms that convey their unique ability to differentiate into every somatic cell lineage as well as the germ cell lineage; a property defined as pluripotency. In the decade since the isolation of the first human ES cell line in 1998, investigations into pluripotency have largely focused on transcriptional networks within the ES cell. While this narrow scope has proven successful, ultimately giving rise to the new field of somatic cell reprogramming, other cellular processes of the pluripotent ES cell, such as post-translational signaling, remain largely unknown.

The transcriptional regulation of pluripotency falls primarily to the transcription factors OCT4, NANOG, and SOX2. Together, these three factors form a variety of hetero-dimeric complexes at target gene promoters where they mediate transcriptional activation of targets that promote pluripotency, or repression of targets that mediate differentiation. Additionally, they comprise a rather unique positive feed back loop in the regulation of their own transcription. Together these factors bind their own promoters to reinforce their own expression thereby forming a robust autoregulatory loop [1]. Viral transduction of OCT4 and SOX2 alongside KLF4 and c-MYC is sufficient to reprogram somatic cells into induced pluripotent stem cells (iPS cells) in both mouse and human fibroblasts [2, 3]. Interestingly, reprogramming can occur independent of SOX2 and NANOG, however, the requirement of OCT4 is consistently reiterated for both pluripotency and reprogramming.

The importance of transcriptional networks in the ES cell has been well documented, however recently laboratories have begun to investigate the proteomic interactions and signaling events that mediate these networks [4, 5]. In this issue of *Cell Res,* a report by Xu *et al*. [6] becomes the first to characterize a post-translational regulation event occurring on OCT4 in a hES cell system. Largely translating their previous work in mouse to human ES cells, the group identified a conserved mechanism in the regulation of OCT4 turnover. WWP2, a HECT domain E3 ubiquitin ligase, was found to directly interact with OCT4 *in vitro* by GST-pull down, as well as complex with OCT4 *in vivo* in co-immunoprecipitation experiments. WWP2 is so named due to its four tandem tryptophan rich domains known as WW domains. Previously identified in NEDD4 and NEDD4-like proteins, these WW domains are reported to mediate protein-protein interactions with proline rich motifs. Employing transient transfections assays, WWP2 was found to ubiquitinate OCT4 thereby promoting its subsequent degradation by the 26S proteasome. Experiments performed in undifferentiated hES cells showed that OCT4 is indeed ubiquitinated *in vivo*, and that RNAi knockdown of WWP2 causes an increase of OCT4 at the protein level [6].

Although WWP2 is expressed at high levels in undifferentiated hES cells, its expression is not specific to them, and it was found to be highly expressed in many somatic tissues. Intriguingly, the authors observed upon differentiation of hES cells, by embryoid body formation, that WWP2 expression is rapidly repressed. In fact, the kinetics of repression occur faster than that of OCT4 [6]. This suggests that the primary role for WWP2 is in the regulation of pluripotency, with little or no role in regulating differentiation. Taken together with the RNAi experiments, WWP2 is likely playing an important role in mediating pluripotency by fine-tuning OCT4 levels.

While it may seem somewhat counter-intuitive that WWP2 could promote pluripotency by driving OCT4 degradation, previous findings support this sort of regulatory feedback. Niwa *et al*. [7] previously observed that Oct4 levels must exist within a specific range to maintain self-renewal. They observed divergent differentiation in a mES cell line genetically recombineered to con-

Correspondence: Austin J Cooney
E-mail: acooney@bcm.edu

ditionally over-express, or repress Oct4 expression in the presence or absence of tetracycline. Specifically it was found that a less than two-fold increase in Oct4 levels caused differentiation into primitive endoderm and mesoderm, while suppressing Oct4 expression caused the ES cells to adopt a trophectodermal fate.

When considering this finding alongside the nature of the OCT4, NANOG, and SOX2 signaling axis, it seems essential that mechanisms exist to counter OCT4 transcription. The autoregulatory loop described for OCT4 ensures that transcription will occur largely unabated in self-renewing ES cells. Without negative feedback, it seems that OCT4 levels would simply accumulate to a threshold point after which precocious differentiation into primitive endoderm would ensue. WWP2 may play a significant role in blocking primitive endoderm differentiation by keeping OCT4 levels balanced in the pluripotent ES cell.

Other post-translational modifications of Oct4 have been reported in the mES cell system, namely: sumoylation and phosphorylation. Sumoylation of Oct4 reportedly has the opposite effect of ubiquitination; it is thought to promote Oct4 stability, DNA binding, and transcriptional activity [8]. Phosphorylation of Oct4 has been reported to mediate various heterodimer and homodimer configurations thereby altering its transcriptional activity and target specificity [9]. Previous work in mES cells by the group highlighted here found that in addition to promoting Oct4 turnover, ubiquitination also significantly represses the transcriptional activity of Oct4 *in vitro* [10]. Other post-transcriptional mechanisms reported in ES cells include microRNA targeting of Nanog, Oct4, and Sox2, as well as targeted cleavage of Nanog by Caspase-3 and -9 [11, 12]. However, these mechanisms differ fundamentally from the current study in that they function to suppress pluripotency and are active during early differentiation.

It remains to be determined to what extent these mechanisms will translate to the hES cell system, or what impact they will have on the field of regenerative medicine. Ultimately, the post-translational modifications of key factors such as OCT4 may prove beneficial in improving the efficiency of reprogramming, or alternatively in differentiating cells towards specific lineages.

References

1 Boyer LA, Lee TI, Cole MF, *et al*. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* 2005; **122**:947-956.

2 Takahashi K, Yamanaka S. Induction of mouse pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**:663-676.

3 Takahashi K, Tanabe K, Ohnuki M, *et al*. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**:861-872.

4 Wang J, Rao S, Chu J, *et al*. A protein interaction network for pluripotency of embryonic stem cells. *Nature* 2006; **444**:364-368.

5 Liang J, Wan M, Zhang Y, *et al*. Nanog and Oct4 associate with unique transcription repression complexes in embryonic stem cells. *Nat Cell Biol* 2008; **10**:731-739.

6 Xu H, Wang W, Li C, *et al*. WWP2 promotes degradation of transcription factor OCT4 in human embryonic stem cells. *Cell Res* 2009; **19**:-.

7 Niwa H, Miyazaki J, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat Genet* 2000; **24**:372-376.

8 Wei F, Schöler HR, Atchison ML. Sumoylation of Oct4 enhances its stability, DNA binding, and transactivation. *J Biol Chem* 2007; **282**:21551-21560.

9 Saxe JP, Tomilin A, Schöler HR, Plath K, Huang J. Post-translational regulation of Oct4 transcriptional activity. *PloS ONE* 2009; **4**:e4467.

10 Xu HM, Liao B, Zhang QJ, *et al*. WWP2, an E3 ubiquitin ligase that targets transcription factor Oct-4 for ubiquitination. *J Biol Chem* 2004; **279**:23495-23503.

11 Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I. MicroRNAs to Nanog, Oct4, and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature* 2008; **455**:1124-1128.

12 Fujita J, Crane AM, Souza MK, *et al*. Caspase activity mediates the differentiation of embryonic stem cells. *Cell Stem Cell* 2008; **2**:595-601.