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Export and Expression: mRNAs Deliver New Messages for Controlling Pluripotency

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Fine-tuning of the pluripotency program is executed by a multitude of cellular processes. Two recent studies published in *Cell Stem Cell* (Wang et al., 2013; Tahmasebi et al., 2014) provide novel insights into the post-transcriptional and translational regulatory mechanisms controlling stem cell pluripotency and somatic cell reprogramming.

In the past several years, a plethora of work has been directed toward dissecting the molecular mechanisms governing the establishment and maintenance of the pluripotent state. It is well known that protein-protein and protein-DNA interactions, as well as specific miRNAs and posttranslational modifications of core transcription factors, can exert potent control over the pluripotency program by promoting protein degradation or by blocking translation (Fabian et al., 2010). However, significantly less is known about potential regulatory mechanisms that may control core pluripotency factor gene expression at the posttranscriptional and translational levels. Two recent papers in *Cell Stem Cell*, Wang et al. (2013) and Tahmasebi et al. (2014), provide insights into posttranscriptional and translational regulation of pluripotency genes, as well as key cell cycle regulators, demonstrating that these processes are essential for promoting pluripotency and blocking differentiation.

Wang, Hu, and colleagues (Wang et al., 2013) identified a posttranscriptional mechanism that regulates pluripotency and differentiation as well as somatic cell reprogramming, in which nuclear export of pluripotency gene mRNAs is positively coupled to their expression. This is coordinated by the THO/TREX (*tr*anscription/*ex*port) complex, which functionally links mRNA biogenesis to the nuclear export of mature transcripts (Katahira, 2012). A previous report from Hu and colleagues identified Thoc5 in a genome-wide siRNA screen for embryonic stem cell (ESC) self-renewal factors (Hu et al., 2009), whereas Thoc2 was discovered in a similar fashion through a genome-wide RNAi screen for regulators of ESC identity (Ding et al., 2009). Thoc2 and Thoc5 are members of the THO/TREX complex, and Wang and colleagues found that the expression patterns of *Thoc2* and *Thoc5* paralleled that of *Oct4* before and after ESC differentiation. Knockdown of *Thoc2* or *Thoc5* caused ESC differentiation, confirming the requirement of both factors for ESC maintenance (Wang et al., 2013). Remarkably, although loss of *Thoc2* or *Thoc5* caused a significant reduction in protein expression of the pluripotency factors Nanog, Sox2, Klf4, and Esrrb, their mRNA levels remained relatively unchanged. Such discordant expression between mRNA and protein levels has been previously described at a systems level during cell fate change of mouse ESCs upon Nanog depletion (Lu et al., 2009) and demonstrates the multilayered nature of gene expression regulation in pluripotent cells.

The authors then observed nuclear retention of *Nanog*, *Sox2*, *Klf4*, and *Esrrb* transcripts in *Thoc2* and *Thoc5* knockdown ESCs, indicating a failure of the THO complex to export these transcripts out of the nucleus for translation. Interestingly, they further found that depletion of *Thoc5* led to a drastic reduction in the amounts of *Nanog*, *Sox2*, *Klf4*, and *Esrrb* transcripts bound to Thoc2 (Wang et al., 2013). Collectively, these data demonstrate the critical role of the THO complex in properly directing key pluripotency transcripts for export from the nuclear transcriptional apparatus toward the cytoplasmic translation machinery in a Thoc5-dependent manner (Figure 1).

Tahmasebi, Sonenberg, and colleagues investigated the role of mRNA translational control during the process of reprogramming mouse embryonic fibroblasts (MEFs) to induced pluripotent stem cells (iPSCs) with "Yamanaka factors" (Tahmasebi et al., 2014). It was previously shown that human fibroblasts can be efficiently reprogrammed via transfection of synthetic reprogramming factor mRNAs containing a 5′ cap analog to enhance translation efficiency, suggesting a significant role of translational control in reprogramming (Warren et al., 2010). The eukaryotic initiation factor 4F (eIF4F) complex broadly controls protein translation, including a specific subset of mRNAs encoding genes involved in proliferation. This process is tightly regulated in self-renewing ESCs, and during differentiation, ESCs undergo a widespread increase in transcript abundance and translation initiation (Sampath et al., 2008). These findings prompted Tahmasebi and colleagues to determine whether an inversely correlated regulation of translation could be observed during somatic cell reprogramming.

eIF4E binding proteins (4E-BPs) are key regulators of translation, and activated, dephosphorylated 4E-BPs bind and inhibit the mRNA 5′ cap binding factor eIF4E to repress translation. These authors found that phosphorylation levels of 4E-BPs were much lower in pluripotent cells compared to MEFs, consistent with tight regulation of protein translation in ESCs. Knockdown of 4E-BPs 1 and 2 significantly lowered reprogramming efficiency (Tahmasebi et al., 2014). The importance of 4E-BPs in reprogramming

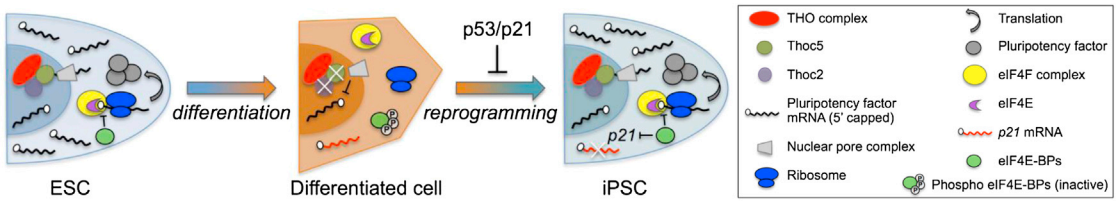

Figure 1. Posttranscriptional and Translational Regulation of Pluripotency Gene Expression
ESCs express high levels of pluripotency factors whose transcripts are exported by the THO complex in a Thoc5-dependent manner. Thoc2 and Thoc5 are downregulated during ESC differentiation, thereby compromising the export of pluripotency factor mRNAs, and eventually leading to the loss of the pluripotency gene expression program. The eIF4F complex recognizes and binds the 5′ cap of mRNAs via eIF4E to initiate translation. The p53/p21 pathway serves as a major roadblock for efficient somatic cell reprogramming, and eIF4E-BPs are essential for this process by blocking the eIF4E-mediated translation of *p21* transcripts.

was then confirmed by reprogramming *4E-BP1/2* double knockout (DKO) MEFs, which displayed a similar inefficiency. Because the p53/p21 pathway acts as a barrier for efficient reprogramming (Hong et al., 2009), Tahmasebi and colleagues checked the expression levels of p53 and p21 in wild-type and DKO MEFs. They found that both p53 (a direct activator of *p21*) and p21 protein levels, but not mRNA levels, were increased, which could explain the reprogramming defect observed upon removal of 4E-BPs. These results suggested that 4E-BPs were directly suppressing the translation of *p21* transcripts and that removing 4E-BPs during reprogramming effectively derepressed the expression of *p21*.

To test this hypothesis, the authors removed this barrier from their system by knocking out p53 in their DKO MEFs, generating triple knockout (TKO) MEFs (DKO + *p53* KO). Infection of TKO MEFs with Yamanaka factors not only rescued the loss of reprogramming efficiency observed in DKO MEFs, but also significantly enhanced reprogramming efficiency above that of wild-type and *p53* KO MEFs. These results demonstrated that 4E-BPs were critical for efficient reprogramming by blocking the translation of *p21* mRNA transcripts (Figure 1). Next, the authors showed that Oct4 expression alone was sufficient to reprogram TKO MEFs due to increased translation of *Sox2* and *c-Myc* transcripts; this effect was not observed in *p53* KO MEFs (Tahmasebi et al., 2014). They further characterized Oct4-induced TKO iPSCs and found that they were fully reprogrammed, as demonstrated by their multilineage differentiation in teratoma assays, their ability to contribute to chimeric animals, and their competency to support germline transmission. This work sheds light on translational control during the establishment of pluripotency and also connects this critical layer of regulation to the p53/p21 signaling pathway, a well-known roadblock during reprogramming.

Wang et al. (2013) and Tahmasebi et al. (2014) provide valuable insights into the regulatory processes that occur downstream of transcriptional activation of pluripotency genes, yet additional questions remain. For instance, posttranscriptional mechanisms controlling *Oct4* expression remain unclear. *Oct4* nuclear export was unaffected by loss of either Thoc2 or Thoc5 (Wang et al., 2013), and expression of Oct4 alone was sufficient to reprogram *4E-BP1/2* and *p53* TKO MEFs (Tahmasebi et al., 2014). Delineating the specific mechanisms that control Oct4 processing at the posttranscriptional and translational levels may reveal novel mechanisms of gene regulation. It will also be beneficial to identify other eIF4E-sensitive genes, as these may point to unique modes of pluripotency control. Additional work is needed to elucidate downstream mechanisms within the hierarchy of gene activation, which will aid in designing more efficient reprogramming strategies. Finally, a thorough examination of genome-wide cross-talk between transcription and translation will certainly lead to many more new and important insights, creating a better understanding of the molecular mechanisms underlying stem cell pluripotency and somatic cell reprogramming.

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