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The pluripotency transcription factor network at work in reprogramming

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Pluripotency-associated transcription factors possess a pivotal role to maintain pluripotency in pluripotent stem cells as well as to induce pluripotency in somatic cells. They direct specific pattern of gene expression from the genome by co-operating with the genetic and epigenetic mechanisms. Recent findings revealed that these mechanisms possess unique features in pluripotent stem cells, which is different from that in somatic cells either qualitatively and quantitatively. To reprogram somatic cells, pluripotency-associated transcription factors should modulate the co-operating machineries to establish the optimal environment for their function to maintain pluripotency-associated transcription factor network.

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Introduction

Pluripotency is defined as an ability of a cell to differentiate into all somatic cell types. It is observed in limited numbers of cells in pre- and early-post implantation embryos in developmental context. Once cultured *in vitro*, these cells derive pluripotent stem cells with continuous self-renewal. Somatic cells including stem cells of post-natal mice never give rise to pluripotent stem cells without reprogramming. Reprogramming is an event that resets the genetic and epigenetic information in a genome-wide manner. Reprogramming of somatic cell nuclei to totipotent state is achieved by the nuclear transfer technique [1], whereas reprogramming of somatic cells into pluripotent stem cells is induced by the over-expression of pluripotency-associated transcription factors [2]. It has been reported that several molecules are involved in the reprogramming event, but it is still unclear how the pluripotency-associated transcription factor network is finally established to maintain the pluripotent state induced by reprogramming. Here the recent findings were reviewed to understand the reprogramming event from the point of view of the transcription factor network transition.

Transcription factor network in pluripotent stem cells

Mouse embryonic stem (ES) cells continue self-renewal in the presence of leukemia inhibitory factor (LIF). LIF signal integrates into ES cells and activates three different intracellular signal transduction pathways, which target the expressions and activities of multiple pluripotency-associated transcription factors such as *Klf4*, *Tbx3* and *Tfcp2l1* [3,4]. Wnt signal cooperates with LIF signal to promote the maintenance of pluripotency via transcriptional activation of the transcription factor *Esrrb* [5]. These transcription factors form a network in which they regulate each other and process the signal integration to direct self-renewal (or to block differentiation which is a default) by maintaining the expressions of the core transcription factors that consist of *Oct3/4* and *Sox2* (Figure 1). The core transcription factors compose auto-regulatory loops to maintain their own expressions and regulate the components of the pluripotency-associated transcription factor network in a signal integration-dependent manner [6]. Moreover, transcription factor network possesses a positive feedback to amplify LIF signal integration via transcriptional repression of *Socs3*, a negative regulator of Stat3 activation, by Nanog [7]. The essence of the function of the transcription factor network is the maintenance of pluripotency.

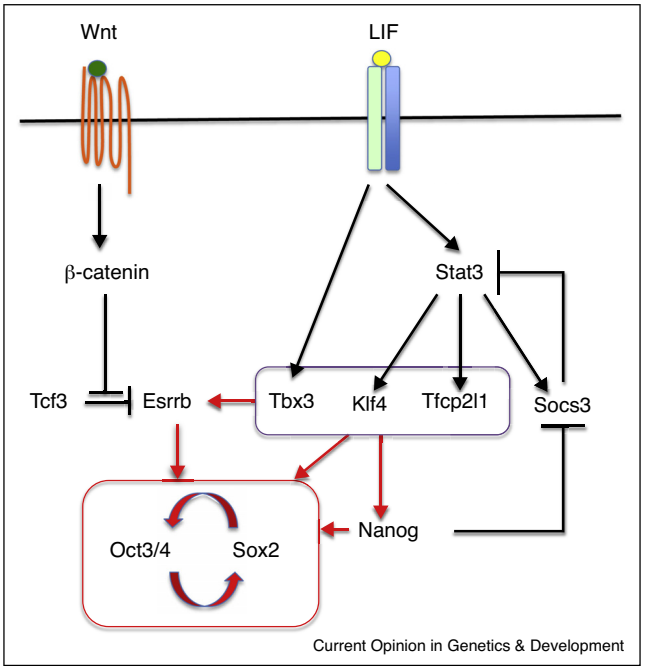
Transcriptional activation by transcription factors

Mediating the functions of pluripotency-associated transcription factors involves many parameters that define their abilities to activate or repress the target gene expression. This includes the interactions among the transcription factors, transcriptional co-factors and general transcription factors.

Interaction of the transcription factors

It has been reported that Oct3/4 and Sox2 form a heterodimer to activate the transcription of the target genes including their own. The direct interactions of transcription factors define their specific functions [6]. For example, Sox2 is expressed in a variety of cell types during development including trophoblast stem (TS) cells [8]. In TS cells, Sox2 functions to mediate the mitogen-activated protein kinase (MAPK) signal to maintain self-renewal

Figure 1



Models of pluripotency-associated transcription factor network. LIF and Wnt signals are integrated into mouse ES cells to direct the transcriptional activation of pluripotency-associated transcription factors Tbx3, Klf4, Tfcp2l1 and Esrrb. These transcription factors then enhance the auto-regulation of the core transcription factors Oct3/4 and Sox2 either directly or indirectly via Nanog. Nanog also works as a positive feedback loop to amplify LIF signal.

[9] although it is known that the MAPK signal negatively regulates pluripotency in ES cells [10]. Moreover, Sox2 regulates a set of target genes that are different from ES cells, including TS cell-specific transcription factors, and the differential mode of function is partly due to the interaction of Sox2 with Tfap2c in TS cells [9]. Therefore, a proper combination of the transcription factors is required to direct their specific function, and the combination of Sox2 with Oct3/4 confers the function to regulate the pluripotency-associated genes. In ES cells, Klf4 is known to act as a partner of Oct3/4 and Sox2 to activate the transcription of particular target genes such as *Lefty1* [11], which might partly explain the requirement of Klf4 as a reprogramming factor. However, it is still unclear by how many transcription factors the complete function is determined, since the addition of transcription factors to the Yamanaka factors (Oct3/4, Sox2, Klf4 and Myc) still showed weak synergistic effect to promote reprogramming efficiency [12].

Recently, the concept of 'super-enhancer' has been established [13,14••]. On the tissue-specific enhancers, multiple (~8) transcription factors form a large complex to direct transcriptional activation. This concept supports the idea that the 4 transcription factors are NOT sufficient to direct full activation of the target genes.

Mediator complex

To direct transcriptional activation, multiple transcription factors bind to the enhancer located at the distal site of the promoter. The binding of these transcription factors is transduced by the mediator complex to recruit general transcription factors to the promoter, which promote the recruitment of RNA polymerase II complex (polII) [15] (Figure 2). Mediator complex consists of ~30 components and is regarded as a general transcriptional machinery shared by all types of cells. However, it was reported that the knockout of the components show cell-type-specific defect and that the tissue-specific transcription factors interact with different components of the mediator complex, and the expression levels of the components could vary in different cell types [15]. Therefore, it is possible that proper combinations of the transcription factors and the mediator components are required for proper function of the transcription factors to establish and maintain the transcription factor network, and the functions of the ectopically-expressed transcription factors could be blocked by the endogenous transcription factors by competing for the common interaction partner of the mediator complex. Moreover, it has been reported that the mediator complex interacts with cohesin and CTCF to shape 3D organization of the genome [16••,17], suggesting the importance of the interaction to the mediator complex beyond the direct regulation of transcription.

General transcription factors

The general transcription factors bind to the promoter to recruit polII and are regarded to have ubiquitous functions in all cell types [18] (Figure 2). TFIID is a basal transcription factor complex containing TATA-binding protein (TBP) and 13 TBP-associated factors (TAFs) [18]. Pijnappel *et al.* demonstrated that TBP and many TAFs are expressed at higher levels in ES cells than in somatic cells and their rather high expression levels are required for the maintenance of pluripotency as well as the reprogramming of somatic cells [19••]. Interestingly, some of the TFIID subunits like *TAF4* are directly regulated by Oct3/4, indicating that their high expression levels are partly conferred by the pluripotency-associated transcription factor network thus forming a positive feedback loop for the maintenance of pluripotency [19••]. Since there are cell-type-specific paralogs of TFIID subunits [18], the composition of TFIID could also affect the transcriptional activity of the ectopically-expressed pluripotency-associated transcription factors in somatic cells.

Remodeling complex

Nuclear remodeling is an essential part of proper transcriptional activation since its function alters the accessibility to the genomic DNA for both tissue-specific and general transcription factors as well as polII (Figure 2). It