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Oct-4: The Almighty POUripotent Regulator?

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Oct-4 plays an essential role as a central regulator of the undifferentiated state. Grinnell *et al.* demonstrate for the first time that Oct-4 by itself has the ability to reprogram committed somatic cells, inducing their dedifferentiation by reverting them to a more developmentally potent state. This study provides evidence that Oct-4 might be the master regulator of the pluripotent state in mammalian cells.

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Embryonic stem (ES) cells possess a vast developmental potential that allows them to self-renew and give rise to any cell type within an organism (that is, they are pluripotent). Somatic stem cells are undifferentiated cells found among differentiated cells of a specific tissue and are considered to be multipotent. If we understood more about the biology of stem cells, it would be possible to isolate, grow, and manipulate them in order to generate specific tissues, such as muscle, heart, lung, or kidney, which could be used to repair damaged and diseased organs. It is widely accepted that ES cells have a greater developmental potential than somatic stem cells, making them an attractive research target in regenerative medicine. However, the use of ES cells remains under heated ethical debate, and the use of somatic stem cells is hampered by the inability to isolate a large pure population of these cells in many tissues.

In a recent landmark study, Takahashi and Yamanaka (2006) showed that induced expression of certain ES cell-specific transcription factors in somatic cells is capable of reprogramming them into a more ES cell-like state. By using a clever and rational approach, they were able to identify four genes, *Oct-4*, *Sox-2*, *c-Myc*, and *Klf4*, that can orchestrate reprogramming to a pluripotent state when simultaneously introduced into mouse embryonic and adult fibroblasts. However, the study by Grinnell *et al.* (2007, this issue) provides the first example of the reprogramming of committed somatic cells to a more ES cell-like state with the use of a single factor. In particular, the authors demonstrate that transient transfection of Oct-4 into mouse interfollicular epidermal basal keratinocytes has the ability to endow the cells with properties characteristic of more developmentally potent cells. For example, the Oct-4-transfected keratinocytes express a group of genes important for self-renewal and maintenance of the undifferentiated state in stem cells. Furthermore, the Oct-4-transfected keratinocytes are capable of differentiating into a different cell type (that is, a neuronal cell type not found in the skin) when exposed to the appropriate culture conditions. The findings from these two studies are of paramount importance for therapeutic cell and/or tissue replacement applications, because they suggest that we could generate pluripotent cells from committed somatic cells derived from readily accessible adult tissues such as the skin.

Oct-4 is a mammalian POU family transcription factor that is normally expressed in ES cells, maintaining them in an immature state (Pesce and Scholer, 2001). Oct-4 has also been shown to be expressed in germ cells and to be required for their pluripotency (Nichols *et al.*, 1998; Niwa *et al.*, 2000). Loss of Oct-4 expression allows ES cells to differentiate into different cell fates. For example, during the development of keratinocytes from cultured human ES cells (Green *et al.*, 2003), loss of Oct-4 expression coincides with the appearance of p63, a transcription factor required for the establishment of the keratinocyte cell fate (Koster *et al.*, 2004). In addition, previous reports have detected Oct-4 expression in germ-cell tumors (Gidekel *et al.*, 2003; Looijenga *et al.*, 2003). More surprisingly, Oct-4 expression has recently been detected in somatic-cell tumors (reviewed in Hochedlinger *et al.*, 2005), which suggests that reactivation of Oct-4 expression may also be associated with the formation and maintenance of tumor stem cells.

It is well established that Oct-4 downregulation is associated with ES cell differentiation. On the other hand, it has been previously demonstrated that concomitant Oct-4 reactivation and reprogramming occur in somatic cells after cell fusion with ES cells (Tada *et al.*, 2001; Kimura *et al.*, 2004), or after nuclear transfer into oocytes (Boiani *et al.*, 2002; Bortvin *et al.*, 2003). In addition, Shimazaki *et al.* (1993) showed that re-expression of Oct-4 is associated with dedifferentiation in hybrid-cell experiments. However, none of these studies showed whether reprogramming and dedifferentiation occur as a result of Oct-4 activity.

> The first example of the reprogramming of committed somatic cells to a more embryonic stem cell-like state with the use of a single factor

Grinnell *et al.* (2007) report that transient transfection of mouse epidermal interfollicular basal keratinocytes with mouse Oct-4 leads to nuclear localization of the transcription factor and a temporally regulated expression pattern mimicking the Oct-4 expression pattern seen during embryonic

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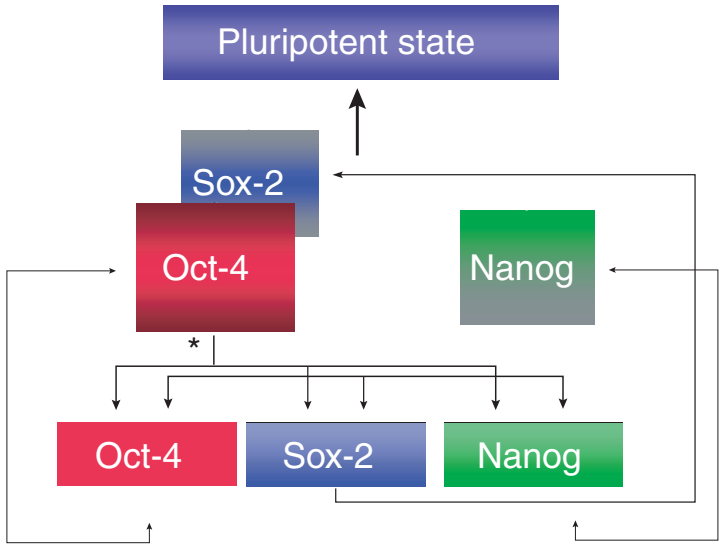

Figure 1. Model for establishment and regulation of the pluripotent state by Oct-4 and Nanog. Multiple studies suggest that Oct-4 and Nanog are required for the maintenance of pluripotency. Grinnell *et al.* (2007) suggest that Oct-4 alone may be sufficient to establish the pluripotent state in keratinocytes. Proteins are represented by ovals; genes are represented by rectangles. Solid arrows represent protein binding to gene regulatory regions; dotted arrows represent gene expression.

development. The expression of Oct-4 peaks at 48 hours, and it is almost totally downregulated between 120 and 168 hours after transfection. Oct-4 is a dose-dependent determinant of pluripotency in ES cells. Therefore, the authors monitored gene expression and protein-level changes in the transfected keratinocytes that were associated with different levels of Oct-4 expression.

Oct-4 orchestrates a relatively defined expression profile characteristic of the pluripotent state. Therefore, Grinnell *et al.* (2007) hypothesized that if Oct-4 induced dedifferentiation of mature keratinocytes, reverting them to a more ES cell-like state, the expression profile would change as this process occurred. They showed that Oct-4 induced expression of ES cell genes in mouse epidermal interfollicular basal keratinocytes. Specifically, they showed that Oct-4 was capable of reactivating the expression of *Sox-2*, *Utf1*, *Rex-1*, and *Nanog*, which are early developmental genes under direct Oct-4 regulation in pluripotent ES cells. Interestingly, the expression of *Utf1* and *Rex-1* was still maintained even after Oct-4 expression was downregulated. The expression of *Sox-2*, which is a cofactor of Oct-4, was temporally similar to that of *Oct-4* and *Nanog*. The regulation and maintenance of the pluripotent state are areas of intense research, and it is clear that Oct-4 and Nanog are key regulators in these processes. The observations put forth by Grinnell *et al.* (2007) are of significant importance because they show that in keratinocytes, and probably in other differentiated somatic cells, Oct-4 has the ability to induce expression of ES cell genes by itself, suggesting that Oct-4 is at the cusp of the pluripotent signaling cascade. However, in a recent study, Loh *et al.* (2006) presented extensive and convincing binding and genetic evidence showing that Nanog regulates the expression of *Oct-4* and *Sox-2* in mouse ES cells. It is possible that there exist cell-specific differences in the pluripotent state, or that there are built-in redundant mechanisms that control this state, as indicated by the inability of Oct-4 by itself to induce the pluripotent state in mouse embryonic or adult fibroblasts (Takahashi and Yamanaka, 2006). Further studies are warranted to clarify these issues in different cell types and species. Nevertheless, a clearer picture is emerging in which Oct-4, Nanog, and Sox-2 are key regulators of the pluripotent state (Figure 1).

Not only is Oct-4 capable of inducing an ES cell-like expression profile, it is also capable of increasing the developmental potency of mouse keratinocytes. Indeed, Grinnell *et al.* (2007) showed that Oct-4 expression allows mouse keratinocytes to achieve a neuronal phenotype and acquire a different gene expression profile in response to neuroectodermal growth conditions. The Oct-4-transfected keratinocytes expressed the neuronal markers nestin, neuN, and Sox-1 after culture in neuroectodermal medium.

This study has broad implications for the field of cell biology and regenerative medicine. It shows for the first time that a single factor is capable of inducing dedifferentiation of mature somatic cells. Also, it suggests that Oct-4 is the master regulator of the pluripotent state, as its transient expression is capable of increasing the developmental potential of lineage-committed somatic cells (that is, inducing the dedifferentiation of mouse keratinocytes). The idea of increasing the potency of differentiated somatic cells opens the possibility of developing a vast array of cell and tissue replacement therapy applications. Needless to say, these possibilities must be explored on a tissue- and species-specific basis, because it is possible that human cells and tissues will respond differently.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Human Keratinocyte Toll-like Receptors Promote Distinct Immune Responses

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It has been well established that Toll-like receptors (TLRs) are expressed by keratinocytes and respond to their respective ligands to initiate immune responses. However, it appears that keratinocytes, via differential activation of TLRs, may play a key role in determining the type of subsequent cutaneous immune response generated against a particular pathogen.

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Human Toll-like receptors (TLRs, numbered 1–10) are found on a variety of different cell types and can recognize various components of microorganisms, subsequently initiating signaling pathways important in the generation of cytokines, chemokines, antimicrobial peptides, and upregulation of adhesion and costimulatory molecules involved in innate and acquired immune responses (Kaisho and Akira, 2006). Previous studies have demonstrated that human keratinocytes express TLRs 1–6 and 9 (Kawai *et al.*, 2002; Mempel *et al.*, 2003; Song *et al.*, 2002; Pivarcsi *et al.*, 2003; Baker *et al.*, 2003; Miller *et al.*, 2005; Kollisch *et al.*, 2005). In addition, some of these studies have demonstrated that TLRs on keratinocytes are functional and respond to their respective ligands to produce cytokines, and chemokines, and to activate NF-κB. For example, several studies have reported that TLR2 and TLR4 are expressed by human keratinocytes and can be activated by their ligands, bacterial lipopeptides and lipopolysaccharide, respectively (Kawai *et al.*, 2002; Mempel *et al.*, 2003; Song *et al.*, 2002; Pivarcsi *et al.*, 2003; Kollisch *et al.*, 2005). Furthermore, additional studies have demonstrated that TLR3 and TLR5 are also expressed by human keratinocytes and can be activated by their ligands, double-stranded RNA (poly-I:C) and bacterial flagellin, respectively (Dai *et al.*, 2006; Baker *et al.*, 2003; Miller *et al.*, 2005; Kollisch *et al.*, 2005). Lastly, previous studies have also identified that human keratinocytes express TLR9 and can respond to CpG motifs of bacterial DNA (Mempel *et al.*, 2003; Miller *et al.*, 2005).

Lebre *et al.* (2007, this issue) confirm previous reports demonstrating that human keratinocytes express certain TLRs, including TLRs 1–6, 9, and 10. A particular strength of this study is that the authors evaluated TLR expression and activation on cutaneous keratinocytes derived from plastic surgery skin specimens and not mucosa-derived keratinocytes from human foreskin specimens. Taken together, these data provide additional evidence that keratinocytes not only act as a barrier to infectious microorganisms but also detect components of these organisms and initiate immune responses via activation of TLRs. However, the authors also demonstrate that certain immune responses generated by activation of TLRs 3, 4, 5, and 9 on keratinocytes are indeed distinct (summarized in Table 1). Although activation of TLRs 3, 4, 5, and 9 all induced expression of the proinflammatory cytokine tumor necrosis factor-α, the neutrophil chemotactic factor IL-8 (CXCL8), the monocyte and basophil chemokine CCL2, and macrophage inflammatory protein-3 (CCL20), of particular interest was the differential production of chemokines by these TLRs. Activation of TLR3 and TLR5 selectively induced CCL27, a chemokine that promotes memory T-

> TLRs on keratinocytes may participate in immune responses and host defense against viruses and bacteria.

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