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Develop-WNTs in Somatic Cell Reprogramming

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Two manuscripts published recently in *Cell Stem Cell* (Lluis et al., 2008 [this issue]; Marson et al., 2008) show that Wnt-β-catenin signaling stimulates nuclear reprogramming. These two studies, using distinct reprogramming methods, offer insights into the mechanisms underlying acquisition and maintenance of pluripotency.

With the demonstration of induced pluripotent stem cell (iPSC) techniques (Takahashi and Yamanaka, 2006), Yamanaka and colleagues started a race to generate pluripotent cells that could be used to develop new cell-based therapies for human disease. The use of oncogenes and DNA-integrating viruses to generate iPSCs offer obvious hurdles because they introduce a proven risk of oncogenic transformation of differentiated cells derived from iPSCs. The race has progressed primarily along two related tracks; first, by elucidating the molecular events important for reprogramming, and second, by applying new techniques and knowledge to prevent oncogenic events. Despite breathtaking progress in just two years, we still know relatively little about the underlying mechanisms by which somatic cell nuclei are reprogrammed to pluripotency. The combined findings reported by the Cosma (Lluis et al., 2008 [this issue of *Cell Stem Cell*]) and Jaenisch (Marson et al., 2008) groups constitute progress along both tracks of inquiry by identifying molecular components of Wnt signaling that stimulate nuclear reprogramming.

The canonical Wnt signaling pathway centers on the stability of β-catenin (Figure 1A). In the absence of a Wnt signal, the GSK-3β kinase phosphorylates β-catenin and targets it for ubiquitin-mediated destruction. Activation of the pathway by a Wnt ligand inhibits GSK-3β activity and results in the accumulation of β-catenin. Stable β-catenin can interact with DNA-binding Tcf factors in the nucleus, where the complex activates transcription of target genes. Treating ES cells with Wnt or inhibiting GSK-3 activity can stimulate ESC self-renewal and support pluripotency (Sato et al., 2004).

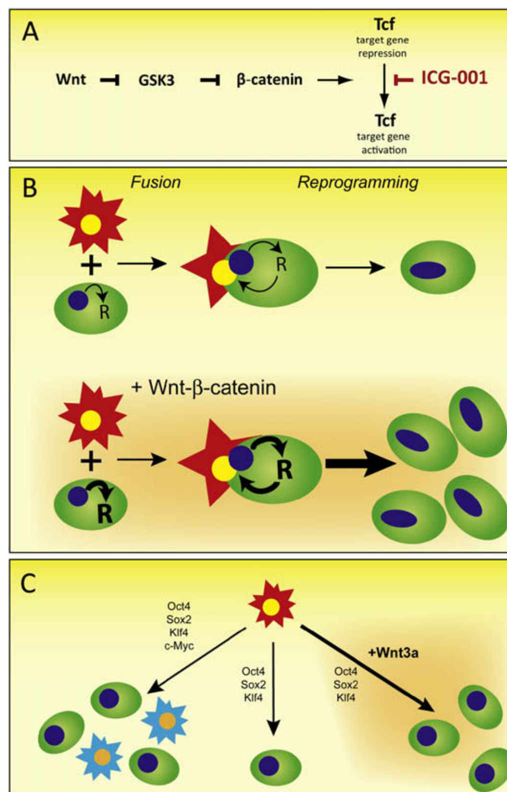

Figure 1. Wnt Signaling Stimulation of Reprogramming
(A) Diagram depicting the relationship between some members of the Wnt-β-catenin signaling pathway.
(B) A reprogrammer activity (R) found in ESC (green with blue nucleus) converts the nucleus of the differentiated cell (red with yellow nucleus) to a pluripotent state after cell fusion. Wnt3a, inhibition of GSK-3, or β-catenin expression in ESC or fused cells stimulated reprogrammer activity (thick arrows).
(C) Differentiated cells can be reprogrammed to ESC-like state by viral transduction of Oct4, Sox2, Klf4, and c-*myc*. Some cells (light blue with orange nucleus) continue to proliferate after viral transduction but fail to become ESC-like. Addition of Wnt3a media allowed more efficient reprogramming in the absence of c-*myc*-expressing virus.

In this issue, the Cosma group tested whether Wnt signaling could also stimulate nuclear reprogramming (Lluis et al., 2008). Instead of taking the popular iPSC approach, they induced cell fusion between ESCs and other nonpluripotent cell types (neural stem cells [NSCs]), thymocytes, or fibroblasts). Treating cells with Wnt3a or an inhibitor of GSK-3 activity elevated reprogramming frequencies up to 80-fold relative to nontreated controls (Figure 1B). Increased reprogramming occurred either when the ESCs were treated prior to fusion, or when the fused cultures were treated soon after fusion. In contrast, treating the differentiated cells with Wnt prior to fusion failed to improve the efficiency of reprogramming. The authors concluded that Wnt signaling turned on a "reprogrammer" factor from pluripotent nuclei that allowed more effective conversion of differentiated cell nuclei to a pluripotent program.

A similar effect of Wnt signals was reported by the Jaenisch group using the iPSC method of nuclear reprogramming (Marson et al., 2008). Without drug selection, the iPSC induction process (using Oct4, Sox2, Klf4, and c-*myc* viruses; OSKC) generates heterogeneous colony types; some are ESC-like, and others continue to proliferate, but are neither pluripotent nor resemble ESCs. Reprogramming without c-*myc* virus (OSK) increases the percentage of ESC-like clones within the total number of colonies; however, a large decrease in total colony number effectively reduces the rate of pluripotent clones relative to combined OSKC virus treatment (Nakagawa et al., 2008). Adding

Wnt3a media to OSK-infected cells enhanced reprogramming efficiency about 20-fold while maintaining a high specificity for ESC-like colonies (Figure 1C; Marson et al., 2008). The gains were abolished by adding an inhibitor (ICG-001) that targets β-catenin-CBP complex transcriptional activity, suggesting that target genes of β-catenin-dependent transcription provide a ''reprogrammer'' function.

While the identity of the ''reprogrammer'' downstream of β-catenin activity remains unknown, the most likely candidates have already been examined. Since c-*myc* is a Tcf-β-catenin target gene in cancer cells, and Wnt3a effectively replaced c-*myc*-expressing viruses in iPSC experiments, it might have been that the observed Wnt-β-catenin effects were due to an elevation of c-*myc* expression in treated cells. However, there was no detectable increase in c-*myc* expression in either iPSC or fusion experiments (Lluis et al., 2008; Marson et al., 2008). Nanog was another good candidate for the ''reprogrammer'' because it can elevate fusion-mediated reprogramming (Silva et al., 2006) and is also a downstream target of Tcf3-mediated repression (Pereira et al., 2006). And yet, Nanog expression was also not altered in response to the levels of Wnt-β-catenin that improved reprogramming during cell fusion. Thus, while we do not yet understand the molecular consequences of β-catenin-mediated reprogrammer activity, fusion experiments tell us that β-catenin cannot act alone, as it requires some other factor(s) present in ESCs, but absent from NSC, thymocytes, or fibroblasts.

One important clue that may help elucidate how β-catenin stimulates reprogramming comes from the analysis of different levels of Wnt-β-catenin activity present during fusion experiments. Reprogramming failed when fusion was performed with ESCs harboring complete genetic knockout of GSK-3 activity, or with ESCs that expressed high levels of β-catenin. Treatments that caused relatively modest increases in β-catenin levels and reporter gene activity provided the greatest benefit for reprogramming efficiency. These results were consistent with reports that very high levels of Wnt activity found in GSK-3 double-knockout cells coincided with differentiation of ESCs (Ying et al., 2008). In addition, the requirement for a specific range of β-catenin activity could provide an explanation for why β-catenin's effect was not discovered in the original Yamanaka screen (Takahashi and Yamanaka, 2006), and why inhibiting GSK-3 activity failed to stimulate iPSC reprogramming (Marson et al., 2008). Perhaps, in the case of β-catenin signals, it is possible to have too much of a good thing?

Collectively, these findings leave us with the interesting question of what β-catenin does differently when present at high levels versus low levels. The answer to this question could apply to other, non-pluripotent stem cells, as nuanced responses to varying Wnt-β-catenin levels have been described in physiologically relevant contexts with hair follicle stem cells and hematopoietic stem cells (Lowry et al., 2005; Fleming et al., 2008). I believe it is safe to say that the common depictions of Wnt signaling in models (i.e., Figure 1) are guilty of simplifying the effects of β-catenin to a singular effect on Tcf proteins. To accommodate the dose-dependent effects of β-catenin, the field must consider that different concentrations of activated β-catenin either could mediate distinct Tcf protein effects, or even impact non-Tcf partners. Improved understanding of the specific molecular consequences of varying levels of β-catenin activity in stem cells will require direct investigation and may clarify the range of cellular responses that are observed in each case.

REFERENCES

Fleming, H.E., Janzen, V., Lo Celso, C., Guo, J., Leahy, K.M., Kronenberg, H.M., and Scadden, D.T. (2008). Cell Stem Cell *2*, 274–283.

Lluis, F., Pedone, E., Pepe, S., and Cosma, M.P. (2008). Cell Stem Cell *3*, this issue, 493–507.

Lowry, W.E., Blanpain, C., Nowak, J.A., Guasch, G., Lewis, L., and Fuchs, E. (2005). Genes Dev. *19*, 1596–1611.

Marson, A., Foreman, R., Chevalier, B., Bilodeau, S., Kahn, M., Young, R.A., and Jaenisch, R. (2008). Cell Stem Cell *3*, 132–135.

Nakagawa, M., Koyanagi, M., Tanabe, K., Takahashi, K., Ichisaka, T., Aoi, T., Okita, K., Mochiduki, Y., Takizawa, N., and Yamanaka, S. (2008). Nat. Biotechnol. *26*, 101–106.

Pereira, L., Yi, F., and Merrill, B.J. (2006). Mol. Cell. Biol. *26*, 7479–7491.

Sato, N., Meijer, L., Skaltsounis, L., Greengard, P., and Brivanlou, A.H. (2004). Nat. Med. *10*, 55–63.

Silva, J., Chambers, I., Pollard, S., and Smith, A. (2006). Nature *441*, 997–1001.

Takahashi, K., and Yamanaka, S. (2006). Cell *126*, 663–676.

Ying, Q.L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P., and Smith, A. (2008). Nature *453*, 519–523.