regulatory network defined by the currently known pluripotency factors (reviewed in Young, 2011). Because pluripotency factors are known to be involved in cellular reprogramming, it is also worthwhile to test the role of FoxO proteins in induced pluripotent stem cell derivation. Finally, recent work has revealed that pluripotency factors play additional roles in driving differentiation into specific lineages (Thomson et al., 2011; discussed in Loh and Lim, 2011); it would therefore be of interest to analyze the dynamics of transcriptional regulatory networks when ESCs exit the self-renewal state and enter lineage-specific differentiation.

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REFERENCES

Chia, N.Y., Chan, Y.S., Feng, B., Lu, X., Orlov, Y.L., Moreau, D., Kumar, P., Yang, L., Jiang, J., Lau, M.S., et al. (2010). Nature *468*, 316–320.

Gonzales, K.A., and Ng, H.H. (2011). Biochim. Biophys. Acta *1809*, 337–349.

Loh, K.M., and Lim, B. (2011). Cell Stem Cell *8*, 363–369.

Thomson, M., Liu, S.J., Zou, L.N., Smith, Z., Meissner, A., and Ramanathan, S. (2011). Cell *145*, 875–889.

van den Berg, M.C., and Burgering, B.M. (2011). Antioxid. Redox Signal. *14*, 607–621.

Yamaji, M., Seki, Y., Kurimoto, K., Yabuta, Y., Yuasa, M., Shigeta, M., Yamanaka, K., Ohinata, Y., and Saitou, M. (2008). Nat. Genet. *40*, 1016–1022.

Young, R.A. (2011). Cell *144*, 940–954.

Zhang, X., Yalcin, S., Lee, D.F., Yeh, T.Y., Lee, S.M., Su, J., Mungamuri, S.K., Rimmele, P., Kennedy, M., Sellers, R., et al. (2011). Nat. Cell Biol. 10.1038/ncb2293.

Committing to a Hairy Fate: Epigenetic Regulation of Hair Follicle Stem Cells

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Chromatin modifications are important for embryonic stem cell (ESC) pluripotency, but their functions in adult stem cells are less clear. In this issue of *Cell Stem Cell*, Lien et al. (2011) delineate histone methylation patterns in hair follicle stem cells and show that these marks differ from those of ESCs.

Histones are subject to numerous modifications that affect chromatin structure and alter transcription factor accessibility. Among these, trimethylation of histone H3 on lysine 4 (H3K4me3) in promoter regions is associated with transcriptional initiation, whereas broadening of H3K4me3 marks and dimethylation of histone H3 at lysine 79 (H3K79me2) characterize active transcription. Conversely, trimethylation of histone H3 lysine 27 (H3K27me3) marks certain genes that possess low or absent transcriptional activity (Suganuma and Workman, 2011).

Histone methylation and demethylation are carried out by multiprotein complexes whose components ensure target specificity, efficiency of modification, and interactions with other chromatin modifiers and transcription factors. Polycomb group (PcG) repressive complexes (PRC) have attracted interest because they associate with cell-fate regulators. H3K27 is specifically trimethylated by the PRC2 enzymes EZH1 and EZH2, which ultimately promotes histone H2A ubiquitination, chromatin condensation, and gene silencing (Suganuma and Workman, 2011).

PRC2 proteins are required for establishing and maintaining the undifferentiated state and pluripotency of embryonic stem cells (ESCs) (Fisher and Fisher, 2011). Unexpectedly, genome-wide mapping of histone methylation sites revealed that, in addition to genes marked by H3K4me3 or H3K27me3, several thousand "bivalent" genes display both H3K4me3 and H3K27me3 marks in human and mouse ESC populations. This gene set is enriched for developmental regulators that are repressed by pluripotency-associated factors, leading to the idea that bivalency marks genes as "poised" for subsequent transcription or repression (Fisher and Fisher, 2011). More recent experiments suggest that bivalency in ESC populations may reflect their underlying heterogeneity and plasticity, rather than the existence of both activating and repressive marks at a given locus within an individual cell or DNA strand (Hong et al., 2011). Understanding the precise nature of bivalency and its underlying mechanism will likely require analysis at the single-cell level (De Gobbi et al., 2011).

Bivalent genes are also detected in other progenitor populations, including hematopoietic progenitors, mesenchymal stem cells, neural progenitor cells (NPCs), and murine embryonic fibroblasts (MEFs), where their identities reflect developmental potential; for instance, genes related to adipogenesis are often bivalent in MEFs but not in NPCs (Fisher and

Fisher, 2011). However, little is understood regarding the patterns and functional relevance of histone methylation in most adult stem cell populations in vivo.

To address this question, Lien et al., (2011) examine the role of chromatin modifications in adult stem cells of the well-characterized hair follicle system. Hair follicles undergo cycles of growth, regression, rest, and regrowth that depend on epithelial stem cells residing in a niche known as the bulge. Bulge cells are generally quiescent but proliferate transiently at the onset of each growth period (anagen). Activated bulge cell progeny surround the hair follicle dermal papilla (DP) and form a matrix cell population that divides rapidly before terminally differentiating to produce a hair shaft and surrounding inner root sheath (IRS). The bulge produces only follicle cells under homeostasis, but following skin wounding contributes transiently to interfollicular epidermis (Ito et al., 2005).

Loss of PRC2 components in mouse or human skin epithelia results in defects in epidermal proliferation and maintenance, indicating the importance of PcG modifications (Ezhkova et al., 2011; Sen et al., 2008). To explore this further, Lien et al. (2011) carried out chromatin immunoprecipitation (ChIP)-Seq for H3K27me3 and H3K4me3 using cells isolated by fluorescence-activated cell sorting (FACS) from the resting hair follicle bulge (quiescent bulge population), early anagen bulge (''activated'' bulge population), and anagen matrix. These experiments revealed how the global distribution of H3K27me3 and H3K4me3 changes as stem cells progress from quiescence to activation and formation of a transient amplifying population in vivo.

Lien et al. (2011) found that in quiescent bulge stem cells, PcG repressive marks are absent from transcription factor genes that possess important bulge cell functions, whereas hair differentiation lineage regulators and nonskin regulators are PcG repressed. Interestingly, and in contrast to ESCs and MEFs, quiescent bulge stem cells display only a small number of bivalently marked genes (89 among 15,284 analyzed).

The transition from quiescent to activated bulge cells is characterized by relatively few changes in PcG marks. Instead, activated bulge cells gain H3K79me2 marks at nonPcG regulated cell-cycle regulatory genes. The relative paucity of changes in PcG marks between these states could explain in part how activated stem cells can return to quiescence after midanagen. However, it is worth noting that only a minority of bulge cells actively cycle during anagen; thus the ''activated bulge'' population isolated from anagen hair follicles was likely contaminated with quiescent cells, and changes in PcG marks associated with the switch from quiescence to activation may therefore be underrepresented.

As cells transit from activated bulge to matrix, H3K27me3 PcG repressive marks are acquired by hair follicle stem cell genes such as *Cd34* and *Fgf18* and lost from regulators of hair shaft and IRS formation, suggesting that PcG activity plays important roles in the transition to differentiation. In parallel, many cell-cycle regulators were unmarked by H3K27me3 in bulge cells and acquired H3K4me3 in the matrix, indicating that their regulation is PcG independent.

The authors found that in *Ezh1/2* mutant hair follicles, bulge stem cell markers were derepressed in the matrix and matrix regulators were derepressed in bulge cells. However, these genes were not expressed at the levels seen in normal bulge and matrix, respectively. Thus removal of PcG activity is insufficient for complete dysregulation, likely due at least in part to the absence of cell-type-specific transcription factors. Future experiments to determine whether or not other repressive mechanisms including histone deacetylation and DNA methylation are still operational at these loci in the absence of PcG activity would be informative in beginning to unravel how diverse chromatin modulatory functions are coordinated in adult stem cells.

This elegant study has several important implications for our understanding of the functions and potential of skin stem cells. In particular, although the nature and roles of bivalency remain controversial (Hong et al., 2011), the finding that bulge cells possess few bivalently marked genes could suggest that the developmental potential of this population is restricted, consistent with its limited contributions within the skin, and perhaps with its inability to contribute permanently to interfollicular epidermis. In an interesting contrast, cells of the hair follicle DP are multipotent and readily differentiate in vitro into adipogenic, neuronal, and osteogenic lineages (Biernaskie et al., 2009). It would be fascinating to determine whether DP cells possess a histone methylation profile more similar to that of ESCs or MEFs than to that of bulge cells. This said, further investigation of the 89 loci that are bivalent in bulge stem cells may shed further light on the mechanisms underlying the choice that activated bulge daughter cells make between self-renewal and progression to a transient amplifying state. It will also be interesting to discover whether novel regulators that associate with the PcG machinery, such as long noncoding RNAs (lincRNAs) (Khalil et al., 2009), are involved in hair stem-cell-fate decisions. Finally, the authors were able to use the results of their chromatin mapping studies to identify roles for the signaling molecules Gremlin1, GDF10, Activin B, and follistatin. Although genetic approaches will be needed to test the in vivo functional relevance of these factors in adult hair stem cells, these experiments support the utility of chromatin state mapping as a general tool for identifying novel regulatory factors.

REFERENCES

Biernaskie, J., Paris, M., Morozova, O., Fagan, B.M., Marra, M., Pevny, L., and Miller, F.D. (2009). Cell Stem Cell *5*, 610–623.

De Gobbi, M., Garrick, D., Lynch, M., Vernimmen, D., Hughes, J.R., Goardon, N., Luc, S., Lower, K.M., Sloane-Stanley, J.A., Pina, C., et al. (2011). Epigenetics Chromatin *4*, 9.

Ezhkova, E., Lien, W.H., Stokes, N., Pasolli, H.A., Silva, J.M., and Fuchs, E. (2011). Genes Dev. *25*, 485–498.

Fisher, C.L., and Fisher, A.G. (2011). Curr. Opin. Genet. Dev. *21*, 140–146.

Hong, S.H., Rampalli, S., Lee, J.B., McNicol, J., Collins, T., Draper, J.S., and Bhatia, M. (2011). Cell Stem Cell *9*, 24–36.

Ito, M., Liu, Y., Yang, Z., Nguyen, J., Liang, F., Morris, R.J., and Cotsarelis, G. (2005). Nat. Med. *11*, 1351–1354.

Khalil, A.M., Guttman, M., Huarte, M., Garber, M., Raj, A., Rivea Morales, D., Thomas, K., Presser, A., Bernstein, B.E., van Oudenaarden, A., et al. (2009). Proc. Natl. Acad. Sci. USA *106*, 11667–11672.

Lien, W.H., Guo, X., Polak, L., Lawton, L.N., Young, R.A., Zheng, D., and Fuchs, E. (2011). Cell Stem Cell *9*, this issue, 219–232.

Sen, G.L., Webster, D.E., Barragan, D.I., Chang, H.Y., and Khavari, P.A. (2008). Genes Dev. *22*, 1865–1870.

Suganuma, T., and Workman, J.L. (2011). Annu. Rev. Biochem. *80*, 473–499.