Unraveling the score of the enhancer symphony

Thomas P. Zwaka[1]
Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX 77030

One of the most fascinating and daunting challenges of contemporary developmental biology is to fully classify diverse cell types on the basis of a quantifiable genetic profile. Although compiling an inventory of such identifying features seems feasible for some specific cell types (e.g., those of the hematopoietic system), a robust and universal platform has yet to emerge. Indeed, in most cases, a series of confirmatory physiological assays are still needed to verify cell identity, yet such assays are available for only a small subset of classifiable cells and can be quite difficult to conduct and interpret. A study by Creyghton et al. (1) provides a possible solution to this conundrum. It gives us a first and unique glimpse of the regulatory complexity of enhancers at the epigenetic level and suggests that histone marks allow discrimination between at least two kinds of enhancers: those that are truly active and whose activities correlate closely and specifically with cell identity and those that are poised to engage in future enhancer activity, thus allowing predictions about the lineage decisions that the cell is facing.

Over the last 5–10 y, it has been become apparent that specific combinations of epigenetic marks may provide a much-needed bar code specific to a given cell type. Epigenetic features such as covalent histone tail modifications seem especially useful in this context, because they (*i*) are typically transmitted very faithfully from one cell generation to the next, (*ii*) are imposed and removed in a highly dynamic fashion, and (*iii*) allow storage of information at relatively high densities. Although early studies emphasized the histone marks found within promoter regions, it has become clear that histone modifications closely linked to distal enhancers can provide stunningly accurate predictions of lineage specification (2–6). This is not surprising at all, because enhancer elements have long been considered key to highly modular patterns of specific gene expression. Indeed, the cross-talk between promoters and enhancer elements is the subject of much recent intensive research (7). Although systematic cataloging of enhancers based on DNA sequence information has proven difficult, in part because such elements do not always display the necessary level of DNA conservation needed for such analyses (2), specific histone marks (monomethylation of lysine 4 of histone H3 or H3K4me1 and acetylation of lysine 27 on the same histone or H3K27ac) have become extraordinarily useful surrogate markers for identifying enhancers. The success of this method is well illustrated by the ability of an H3K4me1-based system to enable mapping of all enhancers in any given cell, irrespective of the activity of the enhancer (over 10^5–10^6 enhancers have been mapped to date) (8). However, such knowledge, although impressive, does little to clarify the current and future developmental potential of the cell. To draw an analogy from the world of symphony music, one might know all of the individual notes in a sonata but would be hard pressed to identify the dominant theme without a proper score.

Creyghton et al. could open the window to a more profound understanding of the molecular mechanisms essential in lineage decisions.

Cells Select Specific Enhancers to Aid Lineage Decisions

While attempting to refine chromatin modifications associated with enhancers, Creyghton et al. (1) revisit the histone mark landscape of H3K4me1 and H3K27ac and the activity of nearby genes in ES cells—the tabula rasa prototype of cell fate decisions (9). Although no significant association could be attributed to H3K4me1 and acetyltransferase p300 (another factor previously linked to enhancer elements), the study identified an unexpected coalition between H3K4me1 and H3K27ac at enhancers near productive genes. These investigators then extend their observations to other cell lineages, including lymphocytes, neural precursor cells, and adult liver, and found that, although H3K4me1 alone is sufficient to identify enhancers in general, the H3K4me1/H3K27ac comark discriminates active from inactive enhancer elements containing H3K4me1 alone. Motif search analysis and additional DNA binding studies at these enhancer sites confirmed the tissue-specific distribution of the newly identified enhancer subsets and hence their relevance to cell lineage specification.

Gene ontology (GO) studies revealed that genes near enhancers accentuated by H3K4me1 alone participate in functions quite distinct from those associated with both marks. Neural progenitor genes with H3K4me1 enhancers, for example, were broadly associated with more mature adult neuronal states and GO categories, such as synaptic transmission and neuronal receptor activity, than were active genes associated with H3K4me1/H3K27ac enhancers, which were generally enriched for categories such as multipotent stem cells, anatomic structure development, and nervous system development. This observation reiterates to some extent the previously discovered bivalent domain, a histone modification associated with gene promoters that are thought to be poised for transcription (10). Similarly, the authors contend that some H3K4me1-only enhancers are associated with genes destined to become activated and to acquire H3K4me1/H3K27ac positivity. If this scenario is correct, then one would predict an epigenetic resetting of the enhancer-associated histone modifications during cellular reprogramming with Oct4, Sox2, Klf4, and c-Myc, a transcription factor mixture that reverses the developmental program of somatic cells to an ES-like state. This was exactly the case, because such reprogrammed, induced pluripotent stem (iPS) cells (11) possessed the enhancer chromatin architecture of embryonic cells. Finally, the authors present evidence that the binding of certain transcription factors, such as Rfx1, to enhancers in neuronal precursors correlates with enhancer gene activity, with others correlating with reduced activity.

Generation of an Enhancer Roadmap

The picture emerging from this research is that relatively few enhancers may be active at any given time, but many seemingly inactive elements are, in fact, poised to respond to environmental cues signaling the initiation of new developmental programs. The authors' data support the notion that H3K27ac distinguishes between these two enhancer states. However, the precise mechanisms by which the regulatory events at the enhancers contribute to

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[1]E-mail: tpzwaka@bcm.tmc.edu.

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specific patterns of gene expression remain to be defined.

The study by Creyghton et al. (1) is well in line with other observations supporting the idea that enhances play an essential role in cell fate determination (5). The concept of poised vs. active enhancer elements is likely to be refined in the near future into additional categories that may ultimately yield a cellular enhancer code, including not only new histone modifications found near these enhancer sites but also specific transcription factors and other proteins that are bound to these elements. The resultant road map would be extraordinarily useful in augmenting or perhaps replacing contemporary means of predicting cell identity. Using a global, specific quantitative map of enhancer qualities, together with transcriptional profiling, might allow one to precisely fix the position of a given cell in a lineage hierarchy. It might also permit more detailed determination of the aberrations that characterize partially or erroneously reprogrammed iPS cells (12, 13) or cancer cell genomes. Ultimately, the discovery reported by Creyghton et al. (1) could open the window to a more profound understanding of the molecular mechanisms essential in lineage decisions and might catalyze the construction of a far more comprehensive outline of lineage development than is currently available.

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