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Transcription factors and CD4 T cells seeking identity: masters, minions, setters and spikers

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Summary

Naive T cells differentiate and become distinct subsets in response to changes in the cytokine milieu. Such specialization arises through a complex and dynamic utilization of *cis*-regulatory enhancer elements. In this brief essay, we review recent findings on the relative contributions of sensors of the cytokine milieu, especially the signal transducer and activator of transcription family transcription factors, 'master regulators', and other transcription factors in the enhancer architecture of T cells. These findings provide new insights into how signal transduction impinges upon the genome.

Keywords: enhancers; epigenomics; master regulators; STATs; T helper cells.

Introduction

Understanding the basis of cellular differentiation is a fundamental issue in developmental biology, but it is also a relevant topic for host defence. In fact, the palette of developmental decisions for naive CD4 T cells is a critical aspect of immunoregulation and elimination of microbial pathogens. However, for those not directly studying the subtleties of options available to CD4 T cells, the range of options may seem bewildering. From T helper type 1 (Th1), Th2 and Th17 cells to regulatory T (Treg) cells and follicular helper T cells, the extent to which each of these states represents lineage commitment, metastable states, sub-specialization or cells just making cytokines may appear to be an impenetrable morass. However, advances in our understanding of the epigenetic aspects of helper cell specification have begun to provide some insights into mechanisms by which different classes of transcription factors contribute to helper cell identity. In this brief essay, we will focus on one aspect of the organization of the genome: the creation of enhancer elements and how this relates to specialization of helper T cells. Recent advances in understanding the factors that drive distinctive enhancer landscapes will be emphasized. A major surprise is how master regulators of helper cells work – rather than driving enhancer architecture they exploit the action of other factors. Sensors of the cytokine milieu and other factors do the hard work and master regulators serve to fine-tune the landscape.

Genomic enhancer landscapes

Although cell identity is often equated with gene expression, genes themselves account for only a small portion of genome; most of the genome consists of instructions or regulatory elements that turn genes on and off. So,

when discussing issues related to cell identity, it seems logical to consider the contribution of extra-genic portions of the genome.

Enhancers are extra-genic DNA sequences that serve as physical platforms for the combinatorial recruitment of transcription factors to 'enhance' transcription of cognate target genes, regardless of their location or orientation. Elucidating the contributions of enhancer elements as determinants of cell identity has been limited by the ability to identify them. Strategies for enhancer census have only been devised in the past decade, when many fully sequenced genomes have become available for comparison. However, the operational definition of enhancers simply based on the presence of known transcription-factor-binding sites is ambiguous because transcription factor consensus motifs are considerably degenerate.[1] The approach also relies on the tacit assumption that sequence conservation across evolution implies regulatory function.[2] An additional serious limitation of this method is that DNA sequence does not predict function of the putative regulatory elements.

Transcription factors bind to open chromatin regions, so mapping the accessible chromatin landscape can be used to measure the tissue-specific activity of enhancers. The accessible parts of the genome are hypersensitive to digestion by DNaseI. Previously, scans of gene loci for hypersensitive sites had been slow and laborious, but recent advances have allowed the mapping of nuclease hypersensitivity genome-wide using microarrays or high-throughput sequencing.[2] For example, the full population of accessible regions in Treg cells was profiled using DNaseI hypersensitive site sequencing (DNase-seq).[3]

While enhancers are the accessible part of the genome in a given cell type, not all accessible elements are enhancers. Profiling of hypersensitive sites alone cannot distinguish enhancers from other regulatory elements such as insulators, locus control regions, or promoters. Recent studies revealed that histone modification signatures that are not associated with other functional elements can be useful in distinguishing enhancer elements. A pioneering study reported H3K4me1-high, H3K4me3-low as a chromatin signature of enhancers in human cells.[4] Notably, active promoters are associated with H3K4me1-low, H3K4me3-high. The H3K4me1 signature has been highly correlated with enhancer activity in gain-of-function assays when this histone signature is combined with another indicator of enhancer activity, the acetyltransferase p300.[5] The predictive ability of p300-based enhancer signature has been tested using a large series of transgenic mice in which enhancer activity correlated with the tissue-specific p300 binding.[5]

Functionally distinct classes of enhancers are now being defined based on the chromatin status of these elements (Fig. 1). Two consecutive studies in embryonic stem cells defined two classes of putative enhancers: poised versus active.[5,6] Both classes of regulatory elements were defined to be H3K4me1-high and p300-high. The difference between the two classes was that the active enhancers were enriched for H3K27Ac whereas poised enhancers were flanked with H3K27me3-high regions. Poised enhancers were also shown in the haematopoietic progenitors: substantial fractions of lymphoid and myeloid enhancers are premarked by H3K4me1 in multipotent progenitors.[7] Therefore, in haematopoietic progenitors, multi-lineage priming of enhancer elements precedes commitment to the lymphoid or myeloid cell lineages. Apart from active and poised elements, 'latent enhancers' are proposed as a new category of enhancers.[8] Latent enhancers are defined as regions of the genome that in terminally differentiated cells are unbound by transcription factors and lack the chromatin signature of enhancers but acquire these features, including H3K4me1 and H3K27Ac, in response to stimulation such as changes in the cytokine milieu.

Transcription factors shaping enhancer landscapes

The importance of enhancers is well-appreciated and the technology to recognize such elements has improved dramatically, but what is less well-understood are factors that influence the activity of elements in the genome. If the non-coding part of the genome constitutes the instructions for genes to be turned on and off, what makes the cell decide the combination of regulatory elements to be used?

With the advent of chromatin immunprecipitation-sequencing technology and the recognition that master regulator transcription factors bind at thousands of sites throughout the genome,[9] it has been argued that the pervasive action of such factors supervises the creation of the enhancer landscape.[10] A priori, it would seem to be a reasonable expectation, given the nature of master regulators and their ability to specify cellular phenotype in a cell autonomous manner. Like other differentiated cells, distinct differentiated CD4 T cells express cognate master regulators, which have been argued to be the major drivers of cell identity.[11] In this context, it would be logical to expect that these master regulator transcription factors determine what portion of the genome should be used in the relevant T-cell population. Strikingly though, three independent studies uncovered that this is not the case. Deletion of T-bet, retinoic acid receptor-related orphan receptor γt (RoRγt) and Forkhead box p3 (Foxp3) mostly led to no change in the enhancer landscape of Th1, Th17 and Treg cells.[3,12,13]

Foxp3 goes where it is told in regulatory T cells

Many lines of evidence point to the role of Foxp3 as being necessary and sufficient for Treg-cell development,[14] though the notion of a single master regulator controlling

the Treg-cell phenotype has been called into question.[15] Arguing against the ability of Foxp3 to drive enhancer landscapes is the recent work of Samstein *et al.*[3] Using genome-wide DNaseI hypersensitive profiling, it was found that T cells expressing Foxp3 have a DNaseI accessibility profile similar to those that do not express it. Moreover, this work argues that, rather than creating the enhancer landscape, Foxp3 exploits an already established open chromatin landscape in naive T cells. Hence, at later stages of differentiation, Foxp3 specifies 'Treg-ness' by exploiting pre-formed elements, instead of generating new ones. Through the use of genomic footprinting, Foxp3-bound regions were found to be enriched for specific DNA-binding motifs of other transcription factors, including ETS (E-twenty-six) and RUNX (runt-related transcription factor) family proteins. A related transcription factor, Foxo1, seems to act as a place-holder at Foxp3 binding sites and is displaced once Foxp3 is expressed.

Master regulators and effector cell enhancer landscapes: 'you're not my boss'

Th1, Th2 and Th17 cells are the major effector cell fates attained by naive T cells. Their respective master regulators are T-bet, GATA3 and RoRγt. Using p300 to define active enhancer landscapes, recent work shows that in Th1 and Th17 cells, T-bet and RoRγt have a minor role on genomic enhancers.[12,13] A few enhancers including those in the *Ifng* locus were missing in the absence of T-bet. For the most part though, the role of T-bet largely involves limiting p300 recruitment on enhancers of opposite lineages rather than establishing the enhancer elements of Th1 cells.[13] RoRγt also plays minor roles in generating the enhancer elements of Th17 cells. In fact, deletion of RoRγt in T cells resulted in no major change in the recruitment of p300 in Th17 cells. Instead, only a handful of loci including *Il17a*, *Il17f*, and *Il23r* are highly dependent on RoRγt.[12] For both RoRγt and T-bet the lesson seems to be that they have relatively narrow actions, serving as modulators. The perception that they are master regulator transcription factors relates to their important actions on signature cytokines. However, their global action with respect to cell identity is surprisingly restricted.

Environmental sensors and pioneering factors create enhancer landscapes

Taken together, these recent studies argue strongly, despite using different technologies, that the master regulators involved in helper cell specification are not the

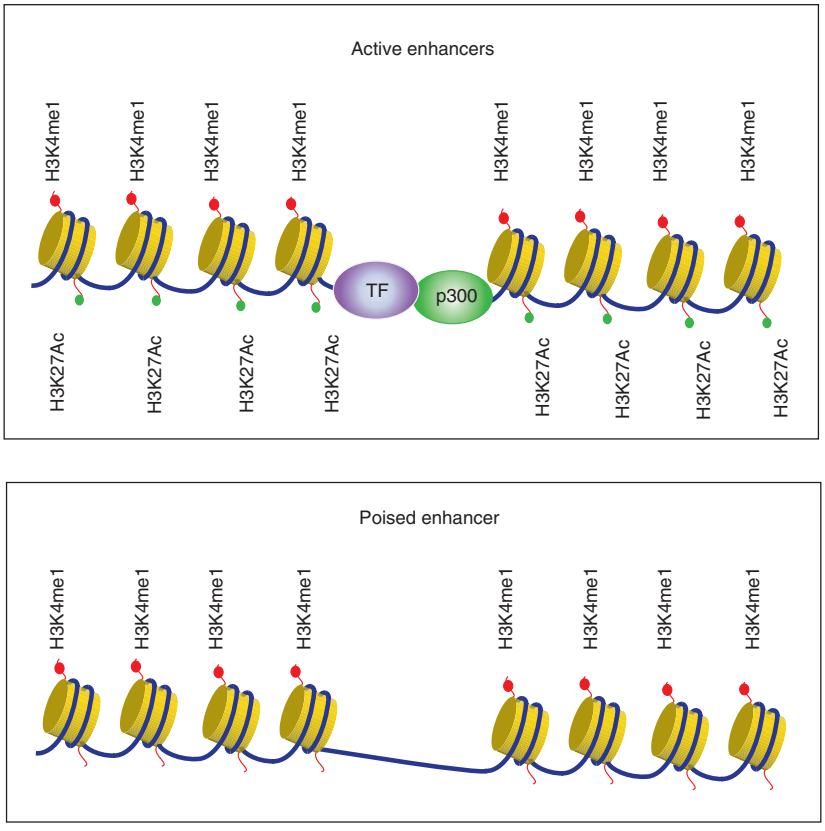

Figure 1. Chromatin signature of active and poised enhancers. Poised enhancers are marked by deposition of H3K4me1 while active elements acquire the acetylation through binding of acetyltransferase p300.

factors that shape the enhancer landscape of $CD4^+$ T-cell subsets. This begs the question then, which are the driving factors?

Before answering this question, it is useful to consider the steps that are involved in T helper cell differentiation. CD4 T cells arise in the thymus after appropriate selection. In the periphery, they are activated by dendritic cells and other antigen-presenting cells and so receive multiple signals in their differentiation. Signals generated by engagement of their antigen receptors are the first step in cellular activation. In fact, in T-cell receptor (TCR) - activated $CD4^+$ T cells, basic leucine zipper transcription factor, an activating protein 1 (AP-1) family protein along with another factor, interferon regulatory factor 4, appear to act as pioneering factors in pre-patterning the enhancer landscape of Th17 cells.[12] This is of interest in that pre-patterning of chromatin by AP-1 family proteins has been reported in other cell types.[16] Hence, TCR-dependent signals appear to be key in generating the initial phase of creation of enhancer repertoires; in this way, TCR-activated transcription factors function as pioneering factors. An important point is that activation-dependent transcription factors have a major role in this process.

A key aspect in the acquisition of distinct T helper cell phenotypes is the cytokine milieu. Upon encountering diverse microbial pathogens, dendritic cells and other cells of the innate and adaptive immune system produce cytokines, which serve to instruct distinct T-cell fates. The major specifying cytokines exert their effect through signal transducer and activator of transcription (STAT) family transcription factors. Strikingly, the majority of differentially active enhancers in Th1 and Th2 cells were STAT4- or STAT6-dependent and a proportion were direct targets of STATs.[17] This was also the case in Th17 cells, in which STAT3 had a major role in p300 recruitment.[12] Importantly, reconstitution of STAT4- and STAT6-deficient cells with the master regulators T-bet and GATA3 failed to recover the active enhancer landscapes, again arguing for a primary role of environmental sensors in dictating global landscapes. In addition, these results also revealed a direct role of STATs in limiting p300 binding. A similar study in macrophages also revealed that STAT1 and STAT6 play key roles in depositing H3K27Ac and H3K4me1 in response to interferon-γ and interleukin-4.[8] In Th1 and Th2 cells, deletion of STATs had a variable role in the creation of H3K4me1-positive poised enhancer landscape. The presence of poised enhancer elements suggests that STATs work in conjunction with pioneering factors to establish poised elements.

As we come to understand the multistep processes involved in specialized cells such as differentiated T helper cells and activated macrophages on a more sophisticated level, it seems likely that enhancer landscapes will represent the concerted action of a multiplicity of factors. The stepwise process of firing enhancers can be visualized as a volleyball game: a team of factors sequentially 'set' the play. The actions result in the formation of a permissive enhancer landscape. This allows environment-sensing factors to 'spike' the ball, creating the active enhancer elements. In this scheme, master regulators serve as specialists; they are called out under defined circumstances. This interpretation does not 'diss' master regulators; rather, it clarifies their functions as having discrete, focused roles rather than pervasive roles in cell identity. At the same time, master regulators should not be haughty about their status and 'diss' the minions, when it is the latter who are doing the heavy lifting. Cell identity presumably represents the integration of signals from a network of transcription factors, with those that respond to environmental signals having a major impact. In this way, signal transduction can be linked to chromatin biology and epigenetic regulation.

Concluding remarks

With recent advances in genomics, comprehensive maps of enhancers are being generated in different cell types. Despite such progress, we still know relatively little about the factors responsible for activating a specific subset of regulatory elements in each cell type; however, these gaps are quickly being filled. Nonetheless, even though enhancers were discovered more than 30 years ago, they are still puzzling parts of the genome with respect to their ability to activate transcription from promoters over large genomic distances. Unravelling the progressive creation of the three-dimensional chromatin architecture and the factors responsible will certainly be a challenge. Nonetheless, it certainly appears that the tools are available to begin to understand this on a more sophisticated level.

Disclosure

The authors declare that they have no financial disclosures or competing interests.

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