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# Epigenetic Flexibility Underlying Lineage Choices in the Adaptive Immune System

Dimitris Kioussis<sup>1\*</sup> and Katia Georgopoulos<sup>2</sup>

Although fundamental models have emerged in recent years describing how chromatin and transcription regulation interface with one another in the developing immune system, the order of events and their biological impact are still being resolved. Recent advances have provided a flexible, rather than static, view of chromatin regulation to reveal how both positive and negative forces work concomitantly to establish specific chromatin structures and regulate gene expression. The challenge will now be to explore new epigenetic models and validate them during lymphocyte development, with the ultimate goal of unraveling the long-sought mechanisms that support the emerging complexity of the adaptive immune response.

Lymphoid development is ultimately determined by a succession of gene expression programs and by stage-specific networks of classical transcriptional factors, which act as drivers in the progression to specific immune cell types (1, 2). The activity of such cell fate-determining transcription factors is intimately linked to dedicated chromatin modifiers that alter accessibility of lineage-specific gene loci and provide ultimate control over this process (3). A consideration of the regulated development of the adaptive immune system from a nuclear perspective must take into account the extended potential to choose between alternative fates that characterizes lymphoid cells, from their earliest stages of development to their later specialization as immune cell types (Fig. 1). Such cell fate choices, acting as they do at numerous branching points, result in a diverse yet balanced immune system and depend on the coordinated acquisition of a gene expression program that favors one cell fate over another. These programs are the result of a combination of gene-activating and gene-silencing events and provide the molecular “signature” for a particular cell fate. At cell stages preceding fate choices, a subset of genes within a program may be transcriptionally primed (expressed) or poised (not expressed but readily activatable) for transcription, providing cells with the potential for differentiation into alternative lineages (Fig. 1). This state of affairs has been increasingly recognized as lineage priming that is regulated at both the transcriptional (4–6) and epigenetic (7, 8) level. At these pre-decision stages, apparently opposing chromatin structures may coexist on a gene locus but resolve in subsequent stages into exclusively activating or silencing structures (Fig. 1). This type of

resolution may be decided at the point of cell division, when asymmetric distribution of regulatory proteins in daughter cells can lead to differential gene expression patterns (9). The retention of opposing chromatin structures on lineage-specific gene loci may provide a potential at a later point for further differentiation by allowing epigenetic flexibility on key differentiation factors. Activation or repression of genes may be stably maintained through the rest of the differentiation process, or they may be “flexible,” reverting to earlier states at later steps in the pathway.

During lymphocyte development, a certain set of “fixed” transcriptional decisions appears to coexist with flexible changes in gene expression. For example, T cell receptor expression is activated at the double negative (DN) stage and is maintained at subsequent stages of T cell differentiation, whereas expression of the CD8 and CD4 co-receptors fluctuates during the development of cytotoxic and helper T cell lineages.

Epigenetic flexibility may endow developing immune cells with their extended potential for alternative effector fate choices during terminal differentiation and may allow early progenitors and their late progeny to share key molecular properties. For example, both primitive hematopoietic stem cells (HSCs) and memory immune cells survive for extended periods, possibly by using similar genetic programs that contribute to their capacity to self-renew (10, 11).

## Modes of Epigenetic Regulation

The outcome of genetic programs set up during lymphocyte development is influenced in part by the developmentally regulated gain or loss of expression of nuclear factors that modulate basal transcription. It is also controlled by specific changes in chromatin structure in the vicinity of lineage-specific genes. Chromatin structures have been classified as closed or open-permissive, depending on whether the genes included are silenced or expressed (12). And a number of histone mod-

ifications (known as histone code) have been associated with such states (13).

Silenced chromatin, largely heterochromatic, contains a number of restrictive histone modifications, such as meH3K9, meH3K27, meH4K20, and histone deacetylation, which allow for a higher-order packing and inaccessibility to transcription factors. In addition, silenced chromatin frequently contains hypermethylated DNA. Conversely, open-permissive chromatin with histone modifications, such as meH3K4, meH3K36, acH3, and acH4, contains genes that are actively transcribed and is perceived to be accessible to regulators of transcription. Recent studies in embryonic stem (ES) cells have provided evidence for a third type of chromatin, referred to as “bivalent,” as a way of generating developmental plasticity through epigenetic flexibility (Fig. 1). This type combines the characteristics of both closed (meH3K27) as well as permissive (meH3K4) chromatin structures and marks lineage-specific genes poised for later lineage-specific activation (7, 8). In a model deduced from these studies, the repressive chromatin modifications keep lineage-specific genes in a transcriptionally inactive state, while the permissive chromatin modifications keep them poised for activation once the former influence is removed. Conversely, removal of the permissive chromatin modifications may also allow the repressive chromatin modifications to prevail, thus establishing gene silencing. Increased expression of relevant transcription regulators during development may also aid the resolution of bivalent epigenetic structures into their respective activating or repressing states in a permanent fashion.

## Epigenetic Flexibility in Differentiating Immune Cells?

The existence of bivalent chromatin structures and their role in providing cell fate flexibility during somatic cell differentiation and during development of the immune system need further exploration. Lineage-specific genes with flexible expression are observed throughout the lymphoid pathway, from the earliest pre-commitment steps to the later pre-effector cell stages, and these genes represent excellent candidates for testing the presence and role of bivalent epigenetic states during lymphocyte development as well as maturation to effector states (Fig. 1). For example, components of the antibody-producing machinery (e.g., immunoglobulin J and sterile transcripts from the immunoglobulin heavy chain constant regions implicated in immunoglobulin class switching) required in terminally differentiated plasma cells are expressed in early hematopoietic multipotent progenitors and are temporarily repressed during early B cell differentiation (to be reactivated in the periphery at later stages of B cell development) (14). Similarly, in early thymocyte precursors that lack both CD4 and CD8 co-receptors (DN), the genes for each co-receptor may acquire a bivalent chromatin state poised for

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expression. This state would then resolve into activating structures at the double positive (DP) stage of T cell differentiation. After thymocytes have reached the DP ( $CD4^+ CD8^+$ ) stage of development, these genes may again become temporarily poised, before their fate is determined by permanently resolving the bivalent state in a complementary fashion in mature cytotoxic (CD8) and helper (CD4) T cells (15). It is possible that

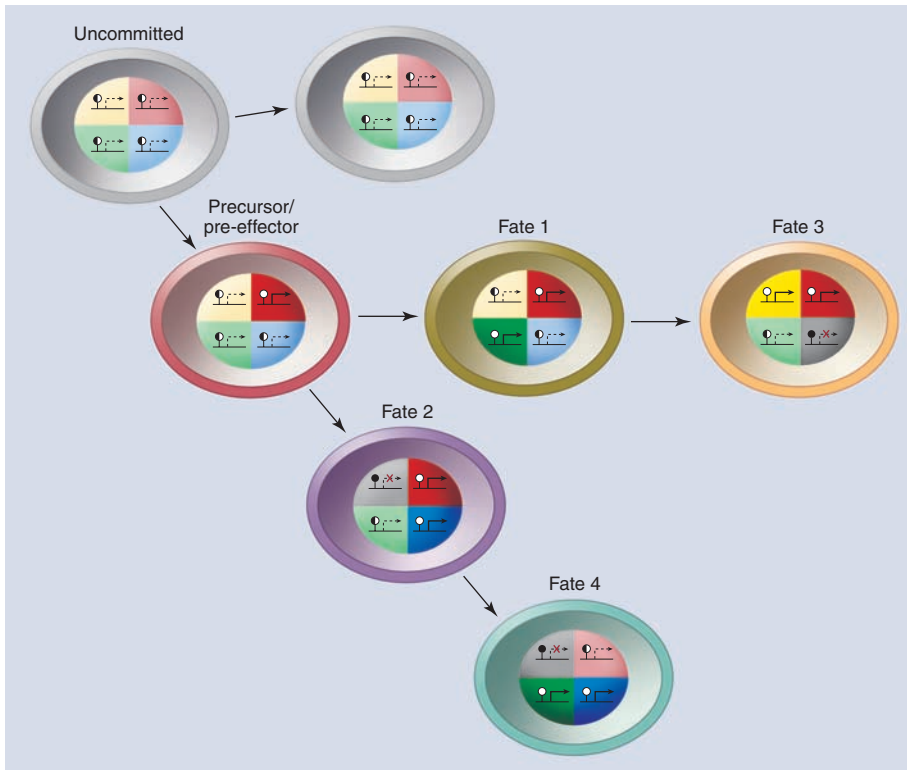
may offer yet further examples of genetic programs poised for activation and help us define their mode of establishment and resolution. For example, it will be important to determine whether the resolution to a permissive or silenced state is permanent, or whether these can revert back to a bivalent state, which may indicate restoration of previous potential. This type of epigenetic regulation may also explain the relative ease with which lym-

phocytes can be reprogrammed through much of their ontogeny to their closer relatives within the hematopoietic system: the myeloid lineage. For example, the inappropriate introduction or removal of antagonistic lineage-specific transcriptional regulators (e.g., loss of PAX-5 in precursor B cells and ectopic expression of C/EBP $\alpha$  in mature B cells) may allow activation of a myeloid genetic program that may exist in a bivalent state in these cells (18–20).

It is possible that epigenetic flexibility may be brought about by the concomitant action of functionally opposed chromatin regulators occupying the same chromosomal site. Precedence for such a mode of regulation is again provided in ES cells, where positive chromatin regulators and members of the trithorax complex coexist with negative regulators of the Polycomb group on lineage differentiation genes, several of which have been shown to exist in a bivalent chromatin configuration (21). The *CD4* and *CD8* genes lend themselves as potential paradigms for identifying such dueling epigenetic regulators during T cell development. The coexistence of opposing chromatin regulators on the *CD4* locus and its regulatory elements provides a ground for identifying such a “bimodal” regulatory network composed of competing activities and responsible for setting bivalent chromatin states and flexible transcriptional outcomes during development (22–24).

Another example for generating poised chromatin is suggested by the structure of the nucleosome remodeling and deacetylase (NuRD) complex, in which the adenosine triphosphate-dependent chromatin remodeler Mi-2 $\beta$  that provides chromatin fluidity coexists with histone deacetylases that are usually associated with repressive chromatin structures (25, 26). The coexistence of antagonistic enzymatic activities within a protein complex may ensure both proper chromatin regulation and epigenetic flexibility. The chromatin remodeler Mi-2 $\beta$  in the NuRD complex is one such potential direct bimodal regulator of *CD4* gene expression during T cell development (24, 27). A direct partner of Mi-2 $\beta$  in this NuRD-based chromatin-remodeling complex is Ikaros: a sequence-specific DNA binding factor implicated in early lymphocyte development (26, 28, 29). Ikaros, through its association and gene-specific targeting of such a bivalent complex in the HSC and its immediate progeny, may confer lineage plasticity and the potential for differentiation to these cells. It would be important to determine whether such an Ikaros bimodal complex effects the priming or poising of lineage-specific gene expression programs in the HSC and its early progeny.

One further challenge will be to determine whether and how opposing activities within a protein complex on a given genetic locus are regulated. For example, DNA bound chromatin-modifying complexes and their components may be amenable to modifications, such as those to histones, that could influence their overall activity and, therefore, chromatin dynamics. Another challenge is to obtain a more global view on the recruitment of such chromatin regulators to gene loci associated with lineage-specific expression signatures and determine the DNA binding activities or chromatin (histone code) platform that determines such targeting.



**Fig. 1.** Developmental progression from an uncommitted state and a precursor–pre-effector state to distinct cell fates from a gene expression–chromatin perspective. Four programs in gene expression (shown in compartments within the nucleus), whose combinatorial acquisition allows for distinct cell fate choices (depicted by the differently colored cell perimeters) to be made, are shown from the early to the later steps of the pathway. The poised (not expressed but activatable) or primed (low expression) state of the genes in these programs is indicated by a half-black, half-white circle representing bivalent chromatin and a dashed bent arrow for low or no transcription. Activated genes are indicated by a solid white circle and a solid bent arrow for transcription. Genes repressed in a permanent fashion are indicated by a solid black circle and a block (indicated by “x”) on the transcriptional arrow. Different scenarios in the resolution of poised lineage-specific genetic programs are entertained, leading to distinct cell fate choices. Fate choices with poised genetic programs may be reversible, whereas others without such programs may be permanent.

expression of regulatory genes such as *GATA-3*, *Tbet*, *Fox-P3*, and *RORgamma*, which influence decisions both in early T cell development and at later steps of T helper lineage maturation (1), may also be modulated through such flexible epigenetic mechanisms.

This handful of known examples lends evidence for a broader flexibility in the genetic programs that bestow lymphocytes with an extended differentiation potential. Genome-wide investigations on developmentally relevant cells [for example, with the use of chromatin immunoprecipitation-on-chip technology (16, 17)] in the concurrence of restrictive and permissive chromatin modifications at various branch points of the lymphoid pathways

phocytes can be reprogrammed through much of their ontogeny to their closer relatives within the hematopoietic system: the myeloid lineage. For example, the inappropriate introduction or removal of antagonistic lineage-specific transcriptional regulators (e.g., loss of PAX-5 in precursor B cells and ectopic expression of C/EBP $\alpha$  in mature B cells) may allow activation of a myeloid genetic program that may exist in a bivalent state in these cells (18–20).

#### Regulators of Epigenetic Flexibility

To date, the molecular players and their interactions that generate and regulate bivalent flexible chromatin structures and their resolution are not well de-

#### Nuclear Compartmentalization of Genetic Programs

In addition to the mechanisms described above, classical competition between opposing regulators



for the same DNA binding sequence at a chromosomal site or for an established chromatin (histone code) domain may also determine the transcriptional activity of a regulated gene. In these cases, fluctuating concentrations of nuclear factors with disparate activities during development can allow for a transcriptional flexibility that would obey mass action rules by establishing working equilibria between activating and silencing components (12). Such equilibria may be modified not only by varying the production of any particular factor but also by regulating the rate of their synthesis, stability, and degradation, as well as by sequestering them in different nuclear compartments. In the latter case, gene activity could be determined by moving genes into nuclear compartments where different types of regulators predominate. Indeed, the positioning of gene loci within nucleus subdomains has emerged as a potentially important determinant of gene activity (27, 30, 31). Genes associated with heterochromatic regions of the nucleus (perinuclear, centromeric clusters) seem to be silent. So far, the association is correlative, and it is unclear whether the silencing precedes or is the result of this localization. Better characterization (composition and dynamics) of such active or silencing regions will require the identification of molecules responsible (i) for setting up the environment in these domains and (ii) for the movement of genes from one region

of the nucleus to another. Improvements in resolution and specificity of the tools needed for the identification and visualization of these components will be one of the most formidable technological challenges in the forthcoming years.

## Concluding Remarks

The hematopoietic system, in which cell lineage choices are well characterized and a substantial number of transcription regulators of cell fate and their targets have been identified, provides an excellent paradigm to study the mechanisms that underlie lineage progression and plasticity. Initial steps in such studies are already identifying epigenetic states by which lineage priming and plasticity are achieved and are suggesting that the three discrete states of chromatin may be achieved by different mechanisms at different stages in the hematopoietic lineage. The ability to use alternative mechanisms at multiple steps during differentiation makes the hematopoietic system an important contributor to future research on epigenetic models of gene regulation in normal development and disease.

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## PERSPECTIVE

# Division of Labor with a Workforce of One: Challenges in Specifying Effector and Memory T Cell Fate

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In the course of the immune response against microbes, naïve T cells proliferate and generate varied classes of effector cells, as well as memory cells with distinct properties and functions. Owing to recent technological advances, some of the most imposing questions regarding effector and memory T cell differentiation are now becoming experimentally soluble: How many classes of antigen-specific T cells exist, and how malleable are they in their fate and in their functional state? How might a spectrum of cell fates be imparted to the clonal descendants of a single lymphocyte? Where, when, and how does pathogen-associated information refine the instruction, selection, and direction of newly activated T cells as they perform their tasks in different locations and times? Some surprising new glimpses ahead on these subjects and other yet-unanswered questions are discussed.

Specific immunity adapts to the threat of pathogen attack with vigorous clonal expansion of a selected lymphocyte whose antigen receptor binds microbial peptide in the

context of self major histocompatibility molecules. The culmination of specific immunity is the generation of effector cells that are responsible for acute elimination of the pathogen and memory cells that patrol their various tissue domains in search of evidence of re-attack.

Heterogeneity is a hallmark of antigen-specific T cells. CD4<sup>+</sup> T cells make effector choices to become T helper cell 1 (T<sub>H</sub>1), T<sub>H</sub>2, or T<sub>H</sub>17 cells and might likewise choose to become antigen-specific regulatory cells (1–3). In addition to

choice of cytokine repertoire, effector CD4<sup>+</sup> T cells exhibit diversity in homing, such as migration to peripheral nonlymphoid tissue versus transit to lymph node follicles to promote B cell help (4). Heterogeneity of CD8<sup>+</sup> T cell effector gene expression has been described (5), although it is not clear whether this represents physiologically distinct cell fates or simply fluctuation in activation state. Memory T cells are heterogeneous, with central memory cells that patrol secondary lymphoid tissues, recapitulating the surveillance of their naïve progenitor, and effector memory cells that act as sentinels standing guard at frontline barriers (6).

Although the role and function of effector and memory subsets in protection or pathology and the nature of polarizing signals required for their differentiation are becoming increasingly clear, there are still outstanding questions that need to be addressed that relate to the mechanism of T cell fate specification. Many of these questions deal with fundamental uncertainties that are common to many areas of blood differentiation, such as the extent of fate diversity, the ontogeny and lineage relationship between opposing and kindred fates, and the degree of natural and therapeutic plasticity at different stages of differentiation.

## “One Cell, One Fate” Versus “One Cell, Multiple Fates”

Signaling and transcription during T cell activation have traditionally been viewed as a uniform process. Any given naïve precursor cell could be

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