ligation of an as-yet-undescribed ligand, as well as the persistence of specific antigen, help to maintain the memory T_{FH} cell population in the draining node[7,8].

The results of these studies raise many issues. Although CXCR3 ligands promote the homing of $CD8^+$ T_E and T_{EM} cells to HEVs in reactive nodes[2], it is still not apparent what molecular interactions are involved in the sticking and rolling of these cells on the HEV lumen and their migration into T cell area of the node (**Fig. 1b**). In addition, it is not known how long the $CD8^+$ effector T cells persist in reactive lymph nodes. Also, the homing of effector $CD4^+$ T cells, which apparently traffic by different mechanisms, is not addressed in these studies.

One chief unresolved issue is the relative importance of the phenomenon of DC killing in the node by cognate $CD8^+$ effector T cells. Is this an important means of negative feedback? In addition, what is the main site of DC elimination by effector $CD8^+$ T cells; is this reactive lymphoid tissue or the periphery? A published study has reported that DC elimination by this mechanism occurs in the periphery but not in lymph nodes[6].

Another important issue is how to reconcile the observed killing of cognate DCs in reactive nodes with the established effectiveness of many booster vaccination regimens[9]. What makes some DCs stimulatory, whereas some DCs apparently become targets? One possible explanation is that DCs can be rendered resistant to killing by cytolytic T lymphocytes depending on the maturation stimulus. A published study has shown that DCs activated by lipopolysaccharide, CD40L or T helper type 1 $CD4^+$ T cells (but not those activated by T helper type 2 $CD4^+$ T cells) are rendered resistant to killing by cytolytic T lymphocytes[10]. This effect is mediated by upregulation in the DC of a specific serum protease inhibitor (Spi-6) that inhibits the function of granzyme B.

There are also many unresolved issues about the development and function of memory T_{FH} cells. $CD4^+CXCR5^+$ T cells are also found in the circulation[4,11]. So what leads to the retention of some T_{FH} cells in lymphoid tissue and the release of other T_{FH} cells to the blood? In particular, the precise MHC class II–positive antigen-presenting cell associated with and perhaps maintaining T_{FH} cell memory is not known. It is not a follicular DC (which do not process antigen or express MHC class II molecules); perhaps myeloid DCs or a subset of B cells are responsible (**Fig. 1b**). The function of CD69 in the retention of memory T_{FH} cells and the location of these memory T_{FH} cells in the node also must be elucidated.

Many issues raised by both reports are relevant to vaccination studies. Can adjuvants be selected for that will enhance beneficial effects such as the development of T_{FH} cells but avoid unwanted effects such as DC killing? How should booster vaccinations be administered without inadvertent promotion of negative feedback and downregulation of specific immunity? In addition, in vaccination studies, peripheral blood is typically monitored for the presence of high-affinity T cells after vaccination. But if a large proportion of these cells remain localized or are recruited to lymph nodes draining inoculation sites, how will this affect the assessment of vaccine efficacy? These issues must be considered in the design of future vaccine studies in animal models and in human trials.

COMPETING INTERESTS STATEMENT
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1. Sallusto, F., Geginat, J. & Lanzavecchia, A. *Annu. Rev. Immunol.* **22**, 745–763 (2004).
2. Guarda, G. *et al. Nat. Immunol.* **8**, 743–752 (2007).
3. Fazilleau, N. *et al. Nat. Immunol.* **8**, 753–761 (2007).
4. Vinuesa, C.G., Tangye, S.G., Moser, B. & Mackay, C.R. *Nat. Rev. Immunol.* **5**, 853–865 (2005).
5. Hosmalin, A. *et al. Blood* **97**, 2695–2701 (2001).
6. Yang, J., Huck, S.P., McHugh, R.S., Hermans, I.F. & Ronchese, F. *Proc. Natl. Acad. Sci. USA* **103**, 147–152 (2006).
7. Shiow, L.R. *et al. Nature* **440**, 540–544 (2006).
8. Zammit, D.J., Turner, D.L., Klonowski, K.D., Lefrancois, L. & Cauley, L.S. *Immunity* **24**, 439–449 (2006).
9. Badovinac, V.P., Messingham, K.A., Jabbari, A., Haring, J.S. & Harty, J.T. *Nat. Med.* **11**, 748–756 (2005).
10. Medema, J.P. *et al. J. Exp. Med.* **194**, 657–667 (2001).
11. Breitfeld, D. *et al. J. Exp. Med.* **192**, 1545–1552 (2000).
12. Lindquist, R.L. *et al. Nat. Immunol.* **5**, 1243–1250 (2004).

Epigenetic regulation of *Ifng* expression

Charalampos G Spilianakis & Richard A Flavell

Intensive characterization of the locus encoding interferon-γ provides new insight into how proper gene expression is achieved in polarizing T cells.

Epigenetic regulation of gene expression is an important mechanism that controls transcriptional activation or repression of the *Ifng* locus (encoding interferon-γ (IFN-γ)). In this issue of *Nature Immunology*, two groups report specific epigenetic changes that regulate the activation of *Ifng* during the process of the differentiation of $CD4^+$ T helper cells into T helper type 1 (T_H1) cells[1] and also the silencing of *Ifng* in differentiating T_H2 cells[2]. In the comprehensive analysis presented by Schoenborn *et al.*, specific histone-methylation patterns of chromatin surrounding *Ifng* in an extended region of over 100 kilobases are used to explain the permissive chromatin environment for *Ifng* expression in differentiated T_H1 cells relative to the nonpermissive chromatin environment for *Ifng* expression in T_H2 cells[1]. Additionally, Chang and Aune report potential epigenetic mechanisms that drive silencing of *Ifng* in differentiating T_H2 cells[2]. The dynamic nature of the epigenetic changes that drive the silencing of *Ifng* is dependent on two key transcriptional activators of T_H2 cell differentiation: GATA-3 and STAT6.

T lymphocytes regulate the mammalian adaptive immune response. Effector T cells provide protection against intracellular and extracellular pathogens as well as tumor cells and enable many other effector mechanisms, including antibody production. The pathological consequences of overaggressive T cell response have been linked to allergy, autoimmunity and transplant rejection. Much of the activity of T cells is controlled transcriptionally. Two main populations of T lymphocytes mediate adaptive immunity and have been studied extensively to elucidate gene regulation in terms of *trans*-acting factors and to target the chromatin of the T_H1- and T_H2-specific genes to identify the transcriptional regulatory

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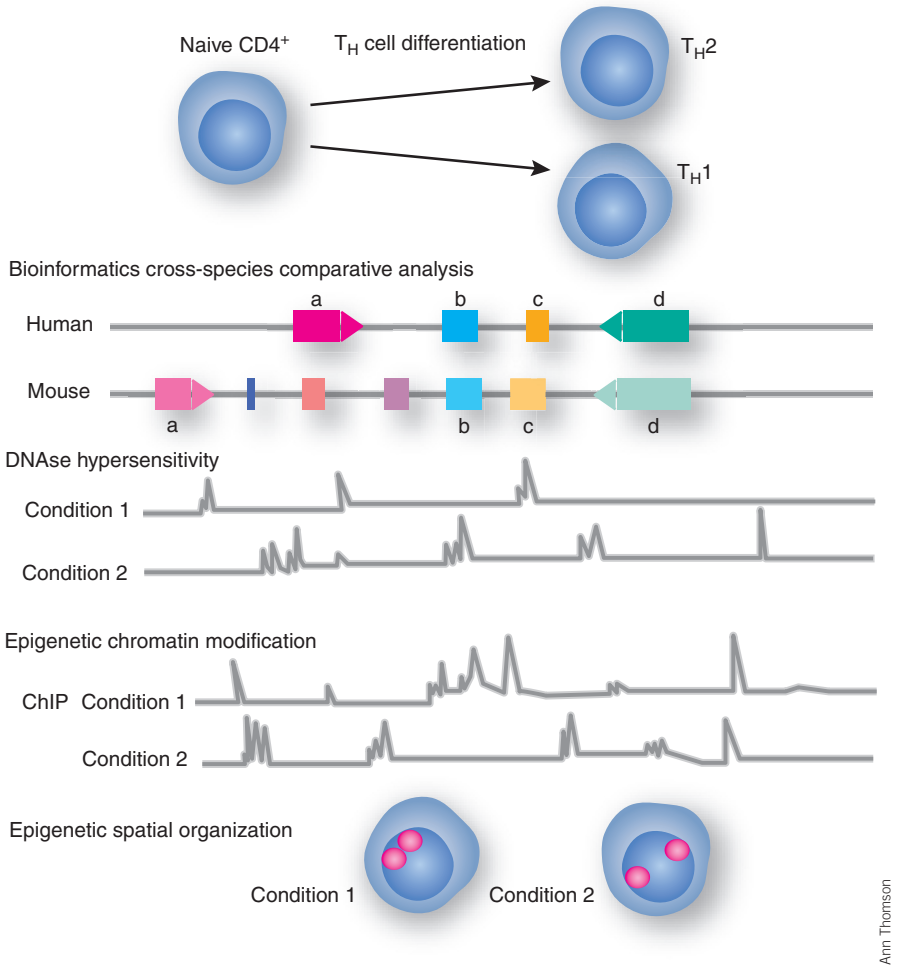

Figure 1 T helper cell differentiation: a model system for the study of epigenetics. A combination of approaches can be used to identify and functionally characterize regulatory elements important for gene regulation. Cross-species comparative sequence analysis in combination with assays of DNAse I hypersensitivity and DNA methylation narrows down the chromatin regions in which histone modifications can be studied. This first level of epigenetic gene regulation is accompanied by the spatial organization of the genome; the second level of epigenetics can influence gene expression.

mechanisms that lead to the transcriptional activation of specific genes[3]. These T cell populations, which develop in the thymus from a common lymphoid progenitor, are the major histocompatibility complex (MHC) class II–restricted $CD4^+$ helper T cells and MHC class I–restricted $CD8^+$ cytotoxic T cells.

The differentiation of naive $CD4^+$ T cells gives rise to T_H1, T_H2 and 'T_H-17' cells, characterized by secretion of the 'signature' cytokines IFN-γ, interleukin 4 (IL-4) and IL-17, respectively. T_H1 cells also produce tumor necrosis factor and lymphotoxin-α and are responsible for cell-mediated immunity, whereas T_H2 cells also produce IL-5, IL-6, IL-10 and IL-13 and are important in antiparasitic and antibody-dependent immune responses[4]. The naive $CD4^+$ T cell is believed to be pluripotent and can follow a T_H1, T_H2 or T_H-17 differentiation pathway, depending on the cytokine environment it experiences or the stimuli it encounters. At the onset of initial $CD4^+$ T cell activation, if a naive $CD4^+$ T cell is activated in the presence of IL-12, which is produced by antigen-presenting cells such as macrophages and dendritic cells through Toll-like receptors in response to microbial stimulation, the transcription factor STAT4 will be phosphorylated and translocated to the cell nucleus, where transcription of *Ifng* is triggered. The production of IFN-γ leads to the transcriptional activation of target genes, including *Il12rb2* (encoding the IL-12 receptor-β2) on T cells and the induction of key T_H1-specific transcription factors such as T-bet. T-bet binds to regulatory elements at several loci, including *Ifng*, and transactivates *Ifng*, creating a positive feedback loop. In contrast, IL-4, which is secreted mainly by $CD4^+$ T cells and mast cells, promotes the differentiation of T_H2 cells through the activation of STAT6 and the subsequent mRNA upregulation of GATA-3, which promotes T_H2 differentiation.

Studies of *cis*-regulatory elements such as the promoter and short regions upstream of *Ifng* suggest that such elements cannot confer proper T cell subset–specific expression, in contrast to bacterial artificial chromosomes containing a human *Ifng* transgene. These studies suggest that the elements responsible for proper expression of the *Ifng* locus are dispersed in an extended region of over 100 kilobases covering the gene. Several approaches have been used to identify and functionally characterize regulatory elements for a specific locus (**Fig. 1**). Schoenborn *et al.* report here a complete and comprehensive analysis using such a combination of approaches to identify regulatory elements for *Ifng* expression[1]. As described before[5], an extensive computational analysis identifying cross-species sequence conservation was the first step in identifying potential chromatin regions with regulatory function. High-throughput quantitative analysis of DNAse I–hypersensitive sites surrounding *Ifng* indicated additional regulatory regions and conserved regions with potential functional relevance, as DNAse I hypersensitivity usually characterizes regions with an open chromatin conformation where transcription factors can bind and transcriptionally regulate a gene. The DNA-methylation profile of specific regions in the extended *Ifng* locus was correlated to the activated or silenced status of *Ifng*, as DNA demethylation is usually linked to the transcriptionally active status of a gene. And to make things clearer, or more complicated, certain epigenetic changes can be checked, such as the acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation profile of the amino-terminal tails of histones in specific chromatin regions. Histones H3 and H4 can be methylated in six known positions of lysine residues at which specific methylation patterns or combination of methylation patterns characterize transcriptionally active genes or euchromatin, whereas other patterns characterize transcriptionally silent genes and heterochromatin. Schoenborn *et al.* have used a combination of the approaches mentioned above with many functional assays to characterize the regulatory elements responsible for the transcriptional regulation of *Ifng* in T_H1 and differentiating T_H2 cells[1]. They have used chromatin immunoprecipitation experiments to detect histone H3 dimethylated at Lys4, a stable 'mark' associated with 'poised' or actively transcribed chromatin, and H3 trimethylated at Lys27,

a repressive histone modification associated with the establishment of Polycomb-mediated silencing. They find that whereas dimethylation of H3 at Lys4 is mainly a T_H1-specific modification associated with the conserved regions characterized in the *Ifng* locus, trimethylation of H3 at Lys27 is broadly distributed throughout the locus in T_H2 cells.

Chang and Aune show that the epigenetic changes on the *Ifng* locus, represented by the histone-methylation patterns of the locus, are very dynamic and correlate with cellular differentiation[2]. Specifically, initial methylation of H3 at Lys9 is activation dependent and is maintained throughout T_H1 cell differentiation but is diminished in T_H2 cells in a GATA-3-dependent way. In the differentiating T_H2 cells, loss of methylation of H3 at Lys9 of the *Ifng* locus is accompanied by recruitment of STAT6 and GATA-3 as well as recruitment of the methyltransferase enzyme Polycomb EZH2, which initiates methylation of H3 at Lys27. The $CD4^+$ T cell differentiation system is a good model system for studying the onset of epigenetic changes over time in which a precursor, undifferentiated, naive cell is poised for transcription, and this is reflected in certain epigenetic traits of the cytokine loci or their regulatory elements. The dynamics of epigenetic regulation can be studied during the differentiation process of the naive cell to a T_H1 or T_H2 cell in opposite conditions.

How do the histone- or chromatin-modifying enzymes 'know' where they must be recruited so that they confer their locus- and tissue-specific action? And how can the same enzymes or transcription complexes coordinately regulate different families of gene loci? Possibly the spatial organization of the genome and the multiple interactions between chromosome regions or interactions between specific gene loci and foci of transcription factors in the cell nucleus represent an additional level of epigenetic regulation of gene expression. In human lymphocytes and fibroblasts, 'gene-poor' chromosomes tend to localize toward the nuclear periphery, whereas it has been reported that after their activation, genes tend to relocalize from the periphery to the inner part of the cell nucleus (**Fig. 1**). Silent genes in developing B cells and T cells are repositioned in the nucleus at pericentromeric heterochromatin[6,7], showing a direct link between the subnuclear localization of a gene locus and a given cellular process such as differentiation. Several regulatory elements such as locus-control regions, enhancers or insulators also act by repositioning specific genetic loci to regions with active or silent transcription, in addition to their function in regulating gene activity *in cis*. It has been shown that the β-globin locus-control region is responsible for the proper relocalization of the locus before activation[8]. Furthermore, sequence-specific DNA-binding proteins may accomplish their functions by directly repositioning such loci to the relevant chromatin compartments. Future studies will need to determine the mode of action of transcription or remodeling complexes recruited on the regulatory elements of the *Ifng* locus and how they confer their tissue- and differentiation-specific action in creating large chromatin domains and regulating gene expression.

COMPETING INTERESTS STATEMENT
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1. Schoenborn, J.R. *et al. Nat. Immunol.* **8**, 732–742 (2007).
2. Chang, S. & Aune, T.M. *Nat. Immunol.* **8**, 723–731 (2007).
3. Lee, G.R., Kim, S.T., Spilianakis, C.G., Fields, P.E. & Flavell, R.A. *Immunity* **24**, 369–379 (2006).
4. Glimcher, L.H. & Murphy, K.M. *Genes Dev.* **14**, 1693–1711 (2000).
5. Nardone, J., Lee, D.U., Ansel, M.K. & Rao, A. *Nat. Immunol.* **5**, 768–774 (2004).
6. Brown, K.E. *et al. Cell* **91**, 845–854 (1997).
7. Brown, K.E., Baxter, J., Graf, D., Merkenschlager, M. & Fisher, A.G. *Mol. Cell* **3**, 207–217 (1999).
8. Ragoczy, T., Telling, A., Sawado, T., Groudine, M. & Koxak, S.T. *Chromosome Res.* **11**, 513–525 (2003).