progeny seems to be limited. Based on these points, it seems more appropriate to consider whether ETPs and CLPs represent two stages of development within a common developmental pathway.

In such a model, ETPs and bone marrow CLPs would derive from a common precursor termed the "pre-CLP." Pre-CLPs would be able to generate B and T cells with a comparable, high degree of efficiency. They might also be able to generate myeloid progeny, although this potential should be markedly attenuated compared with the PHSC. The fate of the pre-CLP and/or its progeny would depend on whether they are exposed to the bone marrow or thymic microenvironment. In the former case, exposure to signals from bone marrow stromal cells would result in assumption of a B lineage fate with attenuated T lymphoid potential. These CLPs, which would be equivalent to those previously described[2], are termed herein the CLP-B. On the other hand, exposure of pre-CLP or their progeny to thymic stroma would result in specification to the T cell lineage with diminished B lymphoid potential. Such cells would be the ETPs described by Allman *et al.*, which we now refer to as CLP-T (**Fig. 1b**).

Although this model redefines the nature of the CLP as originally described[9], it is in accord with features of another recently formulated plan of early lymphoid development that proposes the existence of a primitive lymphoid progenitor upstream from the CLP (**Fig. 1b**)[10], and allows the findings of Allman *et al.* to be assimilated. For example, their study demonstrates that the ETP or CLP-T is more efficient at generating T cells than B cells. Interestingly, ETP-derived B lineage cells have TCR D_β-J_β gene rearrangements, suggesting that they are being programmed away from a B lineage fate. Allman *et al.*[3] further demonstrate that the bone marrow CLP as originally defined[2], and now termed CLP-B, is more efficient than the ETP at generating B cells.

Is there any evidence to support the existence of a pre-CLP? The recently described $Lin^{neg}c\text{-}kit^{hi}Sca\text{-}1^{+}flt3^{+}IL\text{-}7R\alpha^{neg}$ progenitor population is of interest in this regard[11] (**Fig. 1b**). These cells have a considerable potential to generate B and T cells and could be the pre-CLP candidate. If one assumes they are in a lymphoid lineage pathway, CMPs in this case would derive independently from the PHSC. However, the $Lin^{neg}c\text{-}kit^{hi}Sca\text{-}1^{+}flt3^{+}IL\text{-}7R\alpha^{neg}$ cells retain transient myeloid repopulating potential, so it may be more appropriate to consider them as part of the PHSC pool. In this case, the pre-CLP, along with CMP, could be generated from these $Lin^{neg}c\text{-}kit^{hi}Sca\text{-}1^{+}flt3^{+}IL\text{-}7R\alpha^{neg}$ precursors. The proposed scheme of hematopoiesis makes no prediction regarding the pre-CLP phenotype (**Fig. 1b**). However, as the CLP-Ts are postulated to derive from them, the pre-CLP would be expected to be $Lin^{neg}c\text{-}kit^{hi}IL\text{-}7R\alpha^{neg/lo}$. In addition, the pre-CLP would have some myeloid potential to account for the observation that ETPs can generate myeloid cells. However, unlike CLP-Ts, whose B cell developmental potential is attenuated, pre-CLPs would be able to generate B and T cells to a comparable degree. A final prediction is that the generation of pre-CLPs would be an Ikaros-independent event, in view of data from Allman *et al.* that ETPs develop normally in Ikaros-deficient animals[3].

This model assumes that as lymphoid lineage commitment progresses, the expression of myeloid-associated genes is gradually downregulated. Thus, residual myeloid developmental potential could still be demonstrated in seemingly lymphoid committed cells, such as the pre-CLP, whose ultimate destiny is to generate B and T cells. At this point, the proposed model (**Fig. 1b**) remains hypothetical, but a test of it will be whether a candidate pre-CLP can be isolated and shown to generate CLP-Ts (that is, ETPs) and CLP-Bs (CLPs) with the properties predicted herein. This would be a reasonable task to undertake, because continuing advances in flow cytometry and cell culture have provided an appropriate arsenal of experimental tools, in human[12] as well as in mouse. As a result, it seems likely that further refinements of current schemes of early lymphopoiesis will be forthcoming.

1. Akashi, K. *et al. Nature* **404**, 193–197 (2000).
2. Kondo, M., Weissman, I.L. & Akashi, K. *Cell* **91**, 661–672 (1997).
3. Allman, D. *et al. Nat. Immunol.* **4**, 168–174 (2003).
4. Wu, L. *et al. J. Exp. Med.* **174**, 1617–1627 (1991).
5. Godfrey, D.I. *et al. J. Immunol.* **150**, 4244–4252 (1993).
6. Matsuzaki, Y. *et al. J. Exp. Med.* **178**, 1283–1292 (1993).
7. Donskoy, E. & Goldschneider, I. *J. Immunol.* **148**, 1604–1612 (1992).
8. Abramson, S., Miller, R.G. & Phillips, R.A. *J. Exp. Med.* **145**, 1567–1579 (1977).
9. Prohaska, S.S. *et al. Sem. Immunol.* **14**, 377–384 (2002).
10. Kincade, P.W. *et al. Sem. Immunol.* **14**, 385–394 (2002).
11. Adolfsson, J. *et al. Immunity* **15**, 659–669 (2001).
12. Payne, K.J. and Crooks, G.M. *Immunological Rev.* **187**, 48–64 (2002).

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Everything is E(Z): linking histone methylation to B cell development

Dale Ramsden and Yi Zhang

The histone methyltransferases E(Z) and Ezh2 may control developmental fate by regulating homeobox gene expression. Emerging evidence suggests Ezh2 also plays a role in B cell development by controlling V(D)J recombination.

In *Drosophila* the spatially and temporally restricted expression of homeobox (HOX) genes is required for embryonic patterning and cell lineage determination[1]. HOX expression patterns are established early in embryonic development by transiently expressed segmentation genes and are maintained for the rest of development by Polycomb group (PcG) and trithorax group (trxG) proteins[1]. Generally, PcG proteins are transcriptional repressors that maintain the "off state," and trxG proteins are transcriptional activators that maintain the "on state." The transcriptional state maintained by PcG and trxG proteins is linked to their ability to mediate the methylation of specific lysine residues located at the N-terminal tails of histone H3 (**Fig. 1a**). The PcG proteins E(Z) and Ezh2 methylate H3 on lysine27[2–5], and a mammalian trx homolog, MLL (mixed lineage leukemia), methylates H3 on lysine4[6,7]. In this issue of *Nature Immunology*, Su *et al.* now provide evidence that murine Ezh2 also has an important role in B cell development, that of controlling V(D)J recombination[8].

B cells develop in bone marrow and require the highly regulated assembly of immunoglobulin genes at both heavy and light chain loci to complete their maturation[9]. Assembly of a mature immunoglobulin gene is mediated by rearrangement of variable (V), diversity (D) and joining (J) coding segments, a process termed V(D)J recombination. A productive heavy chain rearrangement requires first that a D_H segment

recombine to a J_H segment, producing a DJ_H junction. Next, one of several hundred different V_H segments is rearranged with the already recombined DJ_H junction. V_H genes can be grouped into 15 families based on sequence homology, and these families are clustered along the chromosome (**Fig. 1b**). The V_H family 7183 is located most proximal to D_H segments, whereas members of the much larger V_H family J558 are located furthest away from D_H segments. Previous studies have indicated that these two V_H families are treated differently by the V(D)J recombination machinery[10], but the underlying mechanism for this difference is still unclear.

Work by Su and colleagues sheds new light on this issue. They show that deletion of the catalytic domain of the mouse histone methyltransferase Ezh2 results in a mild impairment in B cell development, then present evidence that this impairment is a consequence of problems during the stage at which V_H-DJ_H recombination occurs. Interestingly, Ezh2 deficiency results in a block in V_HJ558 recombination, but has a negligible impact on V_H7183 recombination. Other recombinations, including D_H to J_H and V_H7183 to DJ_H, are not affected by Ezh2 deficiency, arguing against a defect in the general recombination machinery. Therefore, it is likely that Ezh2 has a specific role in targeting of recombination factors to the V_HJ558 family.

A phenotype similar to Ezh2 deficiency was previously reported in mice deficient for the receptor for interleukin 7 (IL-7)[11]. Moreover, transformed pro-B cells frozen at the stage at which V_H-DJ recombination occurs respond to IL-7 by hyperacetylating histones in chromatin where V_HJ558 genes are located[12]. Given the potential link between IL-7–dependent activation of V_HJ558 recombination and covalent modification of histones, and given that EZH2 is a histone methyltransferase[2,4], Su *et al.* assessed whether histone methylation also responds to IL-7. They showed that global levels of histone methylation are increased in response to IL-7 treatment, though only slightly, and that this increase is dependent on Ezh2. They further suggest that V_HJ558 segments are hyper-methylated relative to V_H7183 segments and that methylation of V_HJ558-containing chromatin is Ezh2-dependent. Similar to histone acetylation, methylation also seems to be an additional means by which IL-7 directs the activation of V_HJ558 recombination.

How might methylation of core histones by Ezh2 activate V_HJ558 recombination? A simple model might suggest that Ezh2 is selectively targeted to V_HJ558, leading to locally increased H3-K27 methylation. Methylated H3-K27 could function as a marker for the direct recruitment of the recombination machinery, much like E(Z)-dependent H3-K27 methylation in *Drosophila*, which acts as a marker for recruitment of Polycomb protein to Polycomb response elements[2]. A striking precedent for this model is found in programmed DNA elimination in *Tetrahymena*, a process with similarities to V(D)J recombination. In programmed DNA elimination, methylation at H3-K9 targets recombination by tethering factors required for this process[13].

Given that Ezh2 deficiency does not generally block V(D)J recombination, a more likely possibility is that Ezh2 controls the targeting of V(D)J recombination to V_HJ558 by altering chromatin accessibility. V(D)J recombination is initiated by the RAG-1 and RAG-2 proteins, which act together to introduce double-stranded breaks at specific recombination signal sequences found in both T cell receptor and immunoglobulin loci[12]. Whereas the RAG proteins and the presence of this signal sequence are sufficient to introduce double-strand breaks on naked DNA, targeting of RAG proteins in cells is highly dependent on the chromatin state. For example, in nuclei prepared from pre-B cells, the recombination sequences in immunoglobulin loci, but not T cell receptor loci, are accessible to the RAG proteins[14]. Thus, Ezh2-dependent H3-K27 methylation of chromatin in the V_HJ558 region might make this region more accessible to the RAG proteins. However, this runs counter to how E(Z) and Ezh2 proteins function in controlling HOX gene expression, where they repress transcription by reducing chromatin accessibility[1]. Moreover, Su *et al.* present evidence against Ezh2 deficiency causing alterations in the V_HJ558 region with respect to other markers of chromatin accessibility. In Ezh2-deficient cells, there is no change in histone acetylation in the V_HJ558 chromatin, and the transcription of V_HJ558 segments that precedes recombination (germline transcription) is actually greater in Ezh2-deficient cells than in cells with normal amounts of Ezh2.

Importantly, although Ezh2 deficiency resulted in a genome-wide decrease in H3-K27 methylation, Su *et al.* were unable to directly address the status of H3-K27 methylation at V_HJ558 in their experiments. It thus remains to be seen if altered methylation patterns in V_HJ558 observed with a pan-methyl-lysine antibody reflect changes in H3-K27 methylation. We therefore suggest an alternative model, in which Ezh2 indirectly affects targeting of V(D)J recombination. Ezh2 might methylate chromatin outside V_HJ558, which could help define a boundary that more effectively targets the activity of other

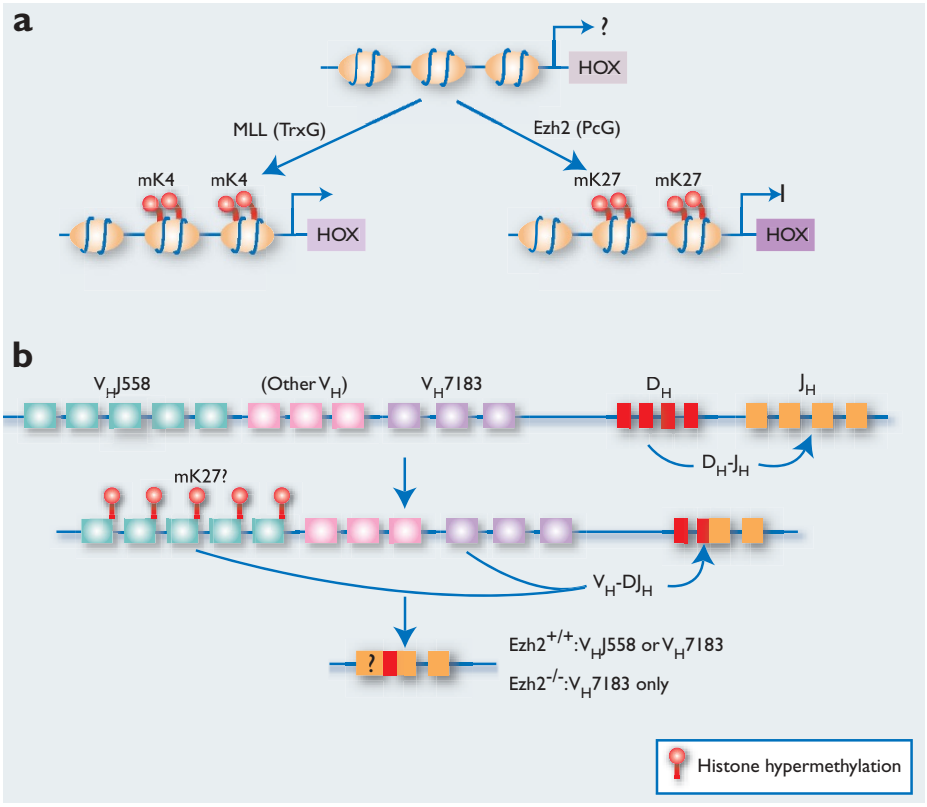

Figure 1. Control of developmental processes by histone methylation. (**a**) Differential regulation of HOX genes by PcG and trxG proteins by histone methylation. (**b**) Altered V_H-DJ_H recombination in Ezh2-deficient cells.

chromatin-modifying enzymes to V_HJ558. Intriguingly, the H3-K4 methyltransferase MLL is critical for earlier steps in hematopoiesis, and is further implicated in leukemogenesis in lymphoid cells (including B cells)[15]. Methylation of H3-K4 at HOX gene promoters by MLL activates HOX gene expression and, thus, opposes the effects of Ezh2-dependent H3-K27 methylation (**Fig. 1a**). MLL activity might be similarly required for increased accessibility of V_HJ558 genes, but in the absence of its antagonist, Ezh2, MLL activity is not appropriately targeted.

In summary, the work by Su *et al.* provides strong evidence in support of a link between histone methylation and targeting of V(D)J recombination. However, several important questions remain to be addressed. The possibility that the cleavage activity of RAG proteins at V_HJ558 recombination signal sequences is altered in nuclei from Ezh2-deficient cells should be tested directly. More significantly, what is the pattern of methylation at H3-K27 over the IgH locus? Definitive assessment of the targeting of Ezh2 activity is critical in distinguishing between direct and indirect mechanisms. Is the pattern of K4 methylation or the activity of MLL important in causing the phenotype of Ezh2 deficiency? It is worth noting that although Ezh2 deficiency does not affect histone acetylation at V_HJ558, Ezh2 deficiency does stimulate acetylation of V_H7183[8]. How else might patterns of methylation and acetylation at the IgH locus influence each other? If recent progress in the study of histone methylation and gene expression is any indication, we should expect the answers soon.

1. Francis, N.J. & Kingston, R.E. *Nat. Rev. Mol. Cell Biol.* **2**, 409–421 (2001).
2. Cao, R. *et al. Science* **298**, 1039–1043 (2002).
3. Czermin, B. *et al. Cell* **111**, 185–196 (2002).
4. Kuzmichev, A., Nishioka, K., Erdjument-Bromage, H., Tempst, P. & Reinberg, D. *Genes Dev.* **16**, 2893–2905 (2002).
5. Muller, J. *et al. Cell* **111**, 197–208 (2002).
6. Milne, T.A. *et al. Mol. Cell* **10**, 1107–1117 (2002).
7. Nakamura, T. *et al. Mol. Cell* **10**, 1119–1128 (2002).
8. Su, I. *et al. Nat. Immunol.* **4**, 124–131 (2003).
9. Bassing, C.H., Swat, W. & Alt, F.W. *Cell* **109** (Suppl.), S45–55 (2002).
10. Marshall, A.J., Wu, G.E. & Paige, G.J. *J. Immunol.* **156**, 2077–2084 (1996).
11. Corcoran, A.E., Riddell, A., Krooshoop, D. & Venkitaraman, A.R. *Nature* **391**, 904–907 (1998).
12. Chowdhury, D. & Sen, R. *EMBO J.* **20**, 6394–6403 (2001).
13. Taverna, S.D., Coyne, R.S. & Allis, C.D. *Cell* **110**, 701–711 (2002).
14. Stanhope-Baker, P., Hudson, K.M., Shaffer, A.L., Constantinescu, A. & Schlissel, M.S. *Cell* **85**, 887–897 (1996).
15. Ayton, P.M. & Cleary, M.L. *Oncogene* **20**, 5695–5707 (2001).

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Location, location, location

James Ferrara

Graft-*versus*-host disease results when donor T cells attack immunocompromised hosts. New data show the distinct environment of Peyer's patches triggers the activation of these aggressive anti-host responses.

One of the most complex puzzles to intrigue immunologists is that of graft-*versus*-host disease (GVHD). When injected into a suitably immunocompromised host, mature T cells (the graft) respond to foreign (allogeneic) antigens in the host and attack multiple target organs including the skin, liver, intestines and lung (but not the heart or kidney). The T cells initiate a complex cascade that eventually includes multiple cellular and cytokine effectors of this acute, often lethal syndrome. Acute GVHD represents a major morbidity of the clinical transplantation of allogeneic marrow or peripheral blood stem cells because T cells are mixed in with the donor graft[1]. After entering the blood stream, donor T cells can migrate anywhere in the host; thus, the unusual distribution of target organs remains perplexing. The study by Matsushima and colleagues in this issue of *Nature Immunology* provides an important piece to this puzzle and advances our understanding of the earliest events in acute GVHD. They demonstrate that the Peyer's patches (PPs) of the small intestine are key sites of antigen presentation to $CD8^+$ donor T cells[2]. If PPs are absent, or if donor T cell migration to PPs is blocked, lethal GVHD does not occur (**Fig. 1**).

Previous studies have shown that antigen-presenting cells (APCs) of the host must activate donor T cells to cause GVHD[3]. These host APCs are both necessary and sufficient to cause most GVHD reactions, even when the alloantigen is absent from epithelial target structures[4]. The natural assumption is that interactions between donor T cells and host APCs occur in the secondary lymphoid organs such as lymph nodes or the spleen. Indeed, the spleen has long been considered a window for observing the responses of donor T cells to host alloantigens[5]. Matsushima and colleagues show that two days after injection, donor T cells localized not only in the spleen, but also in the subepithelial dome of PPs, a region close to the mucosal epithelium. Peyer's patches are an integral component of gut-associated lymphoid tissue, which in aggregate constitute the largest secondary lymphoid organ in the body and which serve as the principal site of immune surveillance of foreign material in the gastrointestinal tract[6]. The authors use several approaches to show that PPs, rather than other secondary lymphoid organs, are the key site for donor T cell activation and the initiation of GVHD. Blockade of mucosal addressin cellular adhesion molecule 1 (MAdCAM-1), the receptor on venules that controls the migration of T cells to the intestinal mucosa, prevents the disease. Similarly, donor T cells lacking C-C chemokine receptor 5 (CCR5) cannot traffic to PPs and also fail to induce disease. Finally, the authors generated mice that lack PPs but whose other secondary lymphoid organs are normal. Remarkably, when these mice that lack PPs were used as hosts in two different GVHD models, donor T cells did not expand and lethal GVHD failed to develop.

Thus, in the development of GVHD, as in so many of life's opportunities, location is everything. Without the microenvironment of the PPs, systemic acute GVHD does not occur. This requirement is rather startling and suggests that the unusual target organ distribution of GVHD depends on the local microenvironment of the antigen presenting cells rather than tissue-specific histocompatibility antigens. Others have noted the importance of the gastrointestinal tract in acute GVHD, but have focused on its role in the amplification of inflammatory effectors[7,8]. Microorganisms and their components (such as endotoxin) are present in normal bowel flora and can serve as potent triggers of inflammatory cytokines and GVHD[9]. Indeed, the appearance of endotoxin in the bloodstream causes massive secretion of tumor necrosis factor-α (TNFα) and signals the final stages of systemic GVHD, including shock and death[7]. Protection of the intestine either by molecules tropic for mucosal epithelium (for example, keratinocyte growth factor) or by neutralization of inflammatory cytokines (for example, soluble TNF receptor) prevents lethal GVHD[4,10]. These strategies that