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From hematopoietic progenitors to B cells: mechanisms of lineage restriction and commitment

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Summary

The generation of B lymphocytes from hematopoietic progenitors requires lineage-specific transcription factors that progressively direct cell fate choices. Differentiation of hematopoietic stem cells to lymphoid progenitors requires Ikaros-dependent lineage priming and graded levels of PU.1, which are controlled by Ikaros and Gfi1. E2A drives expression of EBF1, which initiates B lineage specification. EBF1, in addition to Pax5, is involved in commitment to the B cell lineage. As a model of gene activation in early B lymphopoiesis, *mb-1* genes are activated sequentially by factors (e.g. EBF1) that initiate chromatin modifications prior to transcription. This review highlights the requisite interplay between transcription factors and epigenetic mechanisms in the context of B cell development.

Introduction

Pluripotent hematopoietic stem cells (HSCs) undergo successive rounds of lineage fate restrictions resulting in the specialized cells of the blood (Figure 1). HSCs yield multipotent progenitors (MPPs) that give rise to lymphoid-primed multipotent progenitors (LMPPs) and common lymphoid progenitors (CLPs). The transcription factors E box binding protein 2A (E2A) and Early B cell Factor-1 (EBF1) direct CLPs into the B cell developmental pathway. Together with Paired box protein 5 (Pax5) and Ikaros, these factors initiate the progressive steps of V(D)J recombination and expression of accessory proteins required for the display of pre- and mature B cell receptors (BCRs) [reviewed in 1]. Signaling via functional BCR complexes promotes the egress of immature B cells from the bone marrow. In the periphery, differentiation culminates with the production of antigen-experienced plasma and memory cells.

In coining the term `epigenetics,' Conrad Waddington asserted the importance of regulatory mechanisms above and beyond the genes themselves [2]. Expression of the B cell program requires lineage-specific transcription factors which recruit proteins that mediate epigenetic changes in chromatin structure, including post-translational modifications of histones and DNA methylation. This review highlights recent advances concerning factors that govern early B cell development by modulating gene expression.

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Regulation in progenitors prior to B lineage specification: Ikaros, PU.1 and E2A

HSC multipotency derives from low-level expression of lineage-affiliated genes associated with multiple hematopoietic backgrounds [3,4]. These genes possess `bivalent' chromatin in which both activating and repressive histone and DNA modifications are present simultaneously [5, *6, 7]. Primed regions of chromatin have been described at early developmental stages in many different systems, and are important in numerous developmental contexts for the rapid activation of poised genes after receiving appropriate cues [8]. Primed chromatin is characterized by greater accessibility, reduced nucleosome occupancy, low levels of associated RNAPII, and the presence of H3K4 mono-, di-, or trimethylation. In response to environmental stimuli, the expression of primed genes is modulated by a hierarchical network of transcription factors and epigenetic regulators. Consequently, dominant transcriptional programs emerge and direct differentiation of individual HSCs into discrete hematopoietic lineages. The transcription factors Ikaros, Purine box factor 1 (PU.1) and E2A are essential for priming lineage-associated genes and restricting fates to the B lineage [9–12].

Ikaros (encoded by *Ikzf1*) is a transcription factor containing variable numbers of Krüppel-like zinc fingers in two domains that mediate DNA binding and formation of dimers and multimeric complexes [13,14]. In Ikaros-deficient mice lymphoid cell development is arrested at the LMPP stage and myeloid development is perturbed [15,16]. Ikaros functions either as a transcriptional activator or repressor by recruiting various chromatin remodeling complexes (CRCs) including SWI/SNF (related to the yeast SWItch/Sucrose Non-Fermenter) or Mi-2/Nucleosome Remodeling and Deacetylase (Mi-2/NuRD) to DNA regulatory elements [17–19]. These associations are likely critical for Ikaros' roles in priming the expression of lineage-specific genes and regulating self-renewal in HSCs [**20,**21]. Subsequently, in LMPPs, Ikaros limits transcripts to the lymphoid and myeloid programs [**20,**22]. The lack of Ikaros in CLPs resulted in preferential development of NK cells at the expense of B and T cells [**21]. Other data suggest that Ikaros promotes B cell identity, because Ikaros-deficient pro-B cells expressing EBF1 and Pax5 were uncommitted [**23]. Following the expression of E2A and EBF1, Ikaros mediates chromatin accessibility necessary for V(D)J recombination. Ikaros also modulates the expression of early B cell-specific genes, including *Igll1* (λ5), by competing with EBF1 for DNA binding [**24]. Thus, Ikaros restricts the self-renewal program in early hematopoiesis, while it advances the B lineage program at later stages of development.

PU.1, an Ets family transcription factor encoded by *Sfpi1*, regulates the bifurcation between myeloid and B lymphoid development [25]. Low concentrations of PU.1 favor the B cell fate, while higher concentrations promote myeloid differentiation. Recent work illuminated pathways that modulate the expression of *Sfpi1*. Mice lacking the Growth factor independent 1 transcriptional repressor Gfi1 exhibited significant reductions of B lineage cells in the bone marrow and elevated levels of PU.1 in MPPs [26,**27]. The B cell potential of MPPs in *Gfi1*$^{-/-}$ mice is reduced substantially, but can be reversed by enforced expression of *Sfpi1*-specific shRNAs. Gfi1 thus represses *Sfpi1* expression by displacing PU.1 from its upstream autoregulatory element. Interestingly, the expression of Gfi1 is up-regulated by Ikaros in a subset of MPPs. Thus, Ikaros drives the production of B cells at the expense of granulocytes and macrophages.

In contrast, enforced expression of the C/EBPα transcription factor converted a progenitor B cell line into macrophages in vitro [**28]. Activation of a C/EBPα-estrogen receptor fusion protein rapidly stimulated dramatic alterations in transcription, including up-regulation of myeloid-associated genes and down-regulation of B cell-specific genes such as E2A, EBF1, and Pax5. Several chromatin modifiers were down-regulated, including *Hdac11*, Polycomb group protein *Ezh2* and the DNA methyltransferase DNMT3b. This finding underscores the

importance of chromatin modifications in maintaining lineage commitment. Similar to PU.1, C/EBPα must be restrained to allow B cell development.

E2A (encoded by *Tcfe2a*) is a basic helix-loop-helix transcription factor required for B cell development beyond the pre-pro stage [29›30]. It occurs in two splice variants, E12 and E47 [reviewed in 31]. E2A contributes to the maintenance of the HSC pool [32¯34]. Additionally, it is necessary for lymphoid-lineage priming in MPPs, as evidenced by severely reduced numbers of LMPPs and CLPs in the absence of both E2A proteins and reduced expression of lymphoid-specific genes in *Tcfe2a*$^{-/-}$ LMPPs [**33›**34]. E2A is not necessary for the expression of Ikaros, PU.1 and Gfi1; however, it activates genes in LMPPs synergistically with these factors. E2A is required for initiating and maintaining expression of EBF, Pax5 and the B cell-specific program in pro-B cells, as well as directing B cell maturation in germinal centers [*35]. Although both E12 and E47 are present in LMPPs and CLPs, E47 alone is required for B cell lineage specification [*36]. Ikaros, PU.1 and E2A are therefore essential for transcriptional priming that lays the foundation for commitment to the B cell lineage.

The role of EBF in B cell commitment

EBF1 (encoded by *Ebf1*) comprises novel DNA-binding and helix-loop-helix domains and is essential for the development of functional B cells [37¯39]. EBF1 is expressed first in CLPs and regulates many genes involved in B cell development including *mb-1* (*Cd79a*) and *Pax5* [40›41]. *Ebf1* transcription is mediated by two promoters that are differentially regulated in B cells [42›*43]. The distal (α) promoter is controlled by interleukin-7 signaling (STAT5), E47 and EBF1 autoregulation. The proximal (β) promoter appears to be the stronger of the two modules and is up-regulated by Pax5, Ets1 and PU.1. Consequently, activation of the *Pax5* gene by EBF1 up-regulates *Ebf1* transcription. Although EBF1 is not critical for the development of CLPs, EBF1-deficient CLPs displayed reduced expression of B lineage genes in both conventional and single cell expression analyses [*44]. In CLPs, expression of *Ebf1* initiates a pre-commitment process in which B cell-specific genes are primed for later expression.

Pax5 is essential for maintaining commitment to the B lineage program [45›**46]. Recently, a requisite role of EBF1 in this process was elucidated. MPPs cultured under B-lymphoid-promoting conditions retained T lymphoid and myeloid potential in the absence of EBF1 [**47]. Accordingly, enforced expression of EBF1 in wild-type MPPs generated B cells while suppressing myeloid cell development by attenuating expression of both PU.1 and C/EBPα. Significantly, EBF1 expression rescued the generation of B cells from *Pax5*$^{-/-}$ progenitors, while blocking promiscuous differentiation. Thus, the developmental plasticity previously demonstrated in *Pax5*$^{-/-}$ cells may result, in part, from reduced levels of EBF1.

EBF1 plays an additional role in maintaining B lineage development by preventing Id2- and Id3-mediated inhibition of E47 [**47›*48]. Exogenous expression of E47 in pre-pro B cells induced Id2 and Id3 expression. EBF1 was found to bind the *Id2* and *Id3* promoters and dramatically down-regulated Id2 and Id3 mRNAs. These results indicate that E47 mediates a negative feedback loop by activating Id2 and Id3 expression, while EBF1 maintains E47 activity through down-regulation of Id2 and Id3. Overexpression of either Id2 or Id3 in wild-type murine bone marrow cells resulted in developmental arrest at the pre-pro B cell stage, confirming that down-regulation of these factors is essential for appropriate B lymphopoiesis. Together, these findings demonstrate the importance of EBF1 in maintaining B lineage commitment.

Epigenetic regulation of the *Pax5* gene and maintenance of B cell identity

Pax5 has been extensively characterized as a commitment factor that maintains B cell identity through activation of B cell-specific genes and repression of genes associated with other lineages [45,49–**50]. Strikingly, conditional inactivation of Pax5 in mature B cells resulted in de-differentiation to lymphoid progenitors, which gave rise to functional T cells [46]. These effects were largely due to the loss of Pax5 repressive activities, which include recruiting histone deacetylases (HDACs) and Groucho family corepressors to modulate chromatin structure [51,52].

The potent activities of Pax5 suggest that its expression must be tightly governed throughout lymphopoiesis. However, little was known about the control of Pax5 expression until recently. Decker and colleagues defined a tissue-specific enhancer in intron 5 of the *Pax5* gene that reconstitutes Pax5 expression in concert with the *Pax5* promoter [**53]. Both the *Pax5* promoter and intron 5 enhancer are contained in regions of active chromatin in pro-B cells, as indicated by the presence of active histone marks (acetylated H3K9, di- and trimethylated H3K4) and absence of the repressive histone mark trimethylated H3K27. This active motif also was detected within hypersensitive regions of *Pax5* genes in E2A- or EBF1-deficient progenitor cells. These data suggest that an active chromatin configuration is present at *Pax5* enhancers in the absence of E2A and EBF1, though mechanisms that establish this epigenetic state have not yet been determined. CpGs in the *Pax5* enhancer are demethylated in MPPs during early hematopoiesis. In contrast, the *Pax5* promoter region possessed marks of inactive chromatin in E2A- or EBF1-deficient progenitor cells, and DNA methylation appeared to be less important for epigenetic regulation of the promoter. E2A binding was not detected at the *Pax5* promoter or enhancer in pro-B cells, suggesting that E2A regulates *Pax5* expression indirectly by inducing EBF1 expression. Thus, EBF1 is critical for initiating modifications that result in an active chromatin configuration at the *Pax5* promoter, but not at the enhancer.

Maintenance of Pax5 expression at later stages of development requires multiple factors. ChIP analyses demonstrated binding of PU.1 and the interferon regulatory factors (IRF) 4 and IRF8 to the *Pax5* enhancer in pro-B and mature B cell lines. IRF4 and 8 are each directly regulated by Pax5. Therefore, their activities at the *Pax5* enhancer constitute an indirect positive feedback loop. Together these data suggest that activation of the *Pax5* enhancer and promoter regions occurs in a stepwise fashion: PU.1 likely contributes to early remodeling of the *Pax5* enhancer [**53], where low levels of IRF4/8 may aid its activity. At the onset of B cell specification, the expression of EBF1 overcomes repression of the *Pax5* promoter by Polycomb group proteins, resulting in *Pax5* transcription.

Epigenetic regulation of *mb-1* and *Cd19* transcription by EBF and Pax5

The *mb-1* (*Cd79a*) gene encodes Igα, a signaling component of the pre- and mature B cell receptors that directs their assembly at the plasma membrane. Multiple transcription factors including E2A, EBF, Runx1/CBFβ, Pax5, and Ets family proteins are required for transcriptional activation of *mb-1* [40,54]. The *mb-1* promoter is hypermethylated at CpGs in HSCs, and becomes progressively demethylated and accessible to transcription factors over the course of B cell development [54]. EBF1 initiates chromatin remodeling necessary for the binding of Pax5 and its Ets protein partners to the *mb*-1 promoter. Additionally, transcriptional activation of the *mb-1* promoter requires the recruitment of SWI/SNF chromatin remodeling complexes (CRCs) by EBF and Pax5 [**55]. Brahma (Brm)-related gene-1 (Brg1), a catalytic (ATPase) component of SWI/SNF, was detected at *mb-1* promoters following their activation by EBF1. Furthermore, knock-down of Brm and Brg1 prevented increased chromatin accessibility mediated by EBF alone or in conjunction with Pax5. This suggests that SWI/SNF complexes directly remodel the *mb-1* promoter during activation. Enforced expression of EBF1

in *Ebf1*-deficient fetal liver cells activated endogenous *Pax5* expression and promoted demethylation, increased accessibility and transcriptional activation of *mb-1* genes. However, reconstitution of $Ebf1^{-/-}Pax5^{-/-}$ progenitors with EBF1 did not enable demethylation of the *mb-1* promoter or achieve transcriptional activation of the gene. These results identified mechanisms necessary for the observed synergy between EBF1 and Pax5.

Notably, demethylation of *mb-1* promoter DNA and efficient transcriptional activation (>1700-fold) were achieved only following knock-down of Mi-2β, a catalytic (ATPase) component of the Mi-2/NuRD CRCs. The dramatic increase in *mb-1* transcription corresponded with histone displacement/eviction and increased chromatin accessibility. These data suggest that Mi-2/NuRD CRCs function as a barrier to the activation of *mb-1* transcription. EBF, Pax5 and SWI/SNF were proposed to assist with the exclusion of Mi-2/NuRD. Thus, at *mb-1* promoters, SWI/SNF and Mi-2 NuRD function in opposition by shifting the balance between activating and repressive chromatin architecture (Figure 2).

Regulation of the *Cd19* locus was examined as a model for epigenetic priming of lymphoid genes in early progenitors [**56]. Chromatin remodeling was associated with E2A binding in MPPs at a newly-identified upstream enhancer of *Cd19*. The enhancer exhibited low levels of monomethylated histone H3K9 in the absence of Pax5, indicating chromatin that is poised for transcription before Pax5 expression. Interestingly, RNA polymerase II (RNAP II) was enriched at the enhancer even in the absence of EBF1 and Pax5. However, binding of Pax5 to the *Cd19* promoter was required for efficient transcription. The transient presence of RNAP II prior to transcriptional activation may enable rapid activation of the gene. Notably, demethylation of CpG dinucleotides at the *Cd19* enhancer in very early progenitors preceded demethylation of CpGs in the promoter. E2A remodels the enhancer prior to binding of the promoter by EBF and Pax5. These observations suggest that CpG demethylation initiates at discrete sites and spreads to proximal areas as differentiation proceeds. Thus, similar to the *Pax5* gene, activation of *Cd19* genes is characterized by “primed” chromatin at the enhancer prior to gene expression.

Conclusions and Perspectives

Although many of the details regarding the hierarchical regulation of B cell development by transcription factors have been elucidated, the epigenetic topography that regulates this process remains largely unknown. In the near future, mechanisms of epigenetic change will be revealed by global analysis of modifications using techniques involving deep sequencing technologies, such as ChIP-seq and genomic bisulfite sequencing. Resulting data will address regulation of gene targets by transcription factors described in this review, epigenetic consequences of their binding and changes in binding patterns throughout B cell development. Other areas of analysis include identification of proteins recruited to mediate epigenetic changes and the order of events necessary for propagation of DNA demethylation, histone modifications, and nucleosome eviction/displacement in various contexts. Ultimately, it will be important to address how expression of transcription factors and ensuing changes in the epigenetic landscape contribute to aberrant lymphocyte development, resulting in diseases such as cancer. As the answers to these and many other questions are revealed, a more detailed framework for understanding the control of lymphocyte development and the significance of regulatory steps will emerge.

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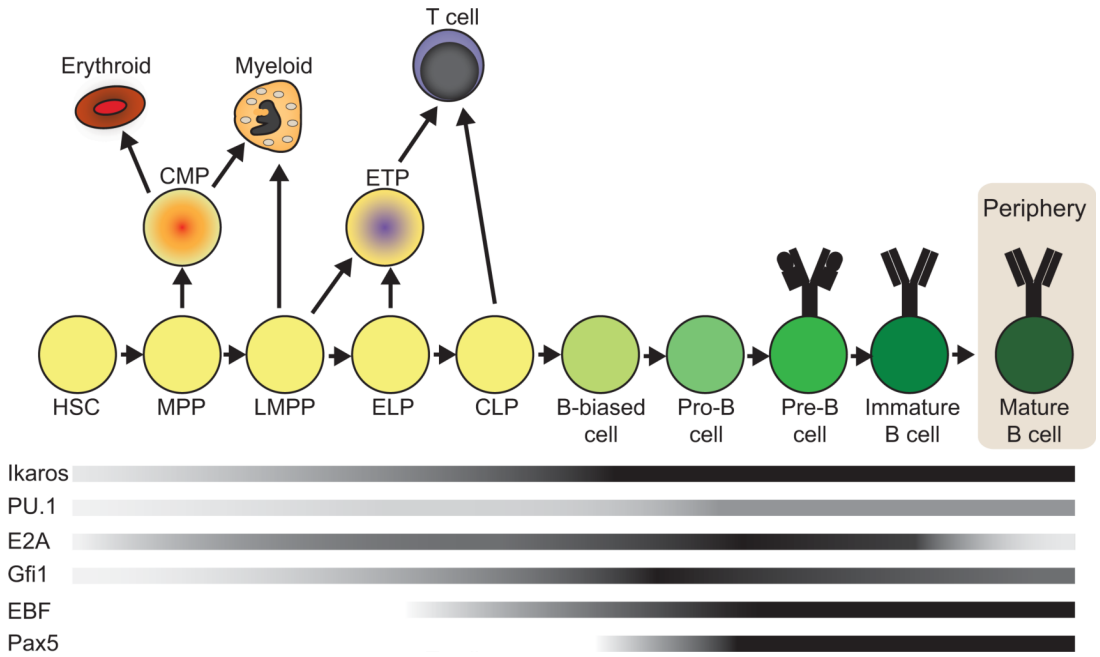

Figure 1.

Transcription factor expression during B lymphopoiesis. The progression of cells from hematopoietic stem cells through stages of B lymphopoiesis is shown. Shaded bars represent the levels of gene expression of transcription factors that are important in B cell development during the course of differentiation. HSCs (hematopoietic stem cells), MPPs (multipotent progenitors), LMPPs (lymphoid-primed MPPs), ELPs (early lymphoid progenitors), CLPs (common lymphoid progenitors), ETPs (early T lineage progenitors). Darker shading indicates increased gene expression. Important branch points during B lymphopoiesis are shown with arrows indicating alternative developmental pathways. Expression profiles were compiled using data from RefDIC (Reference Database of Immune Cells) http://refdic.rcai.riken.jp/profile.cgi and citations in the text.

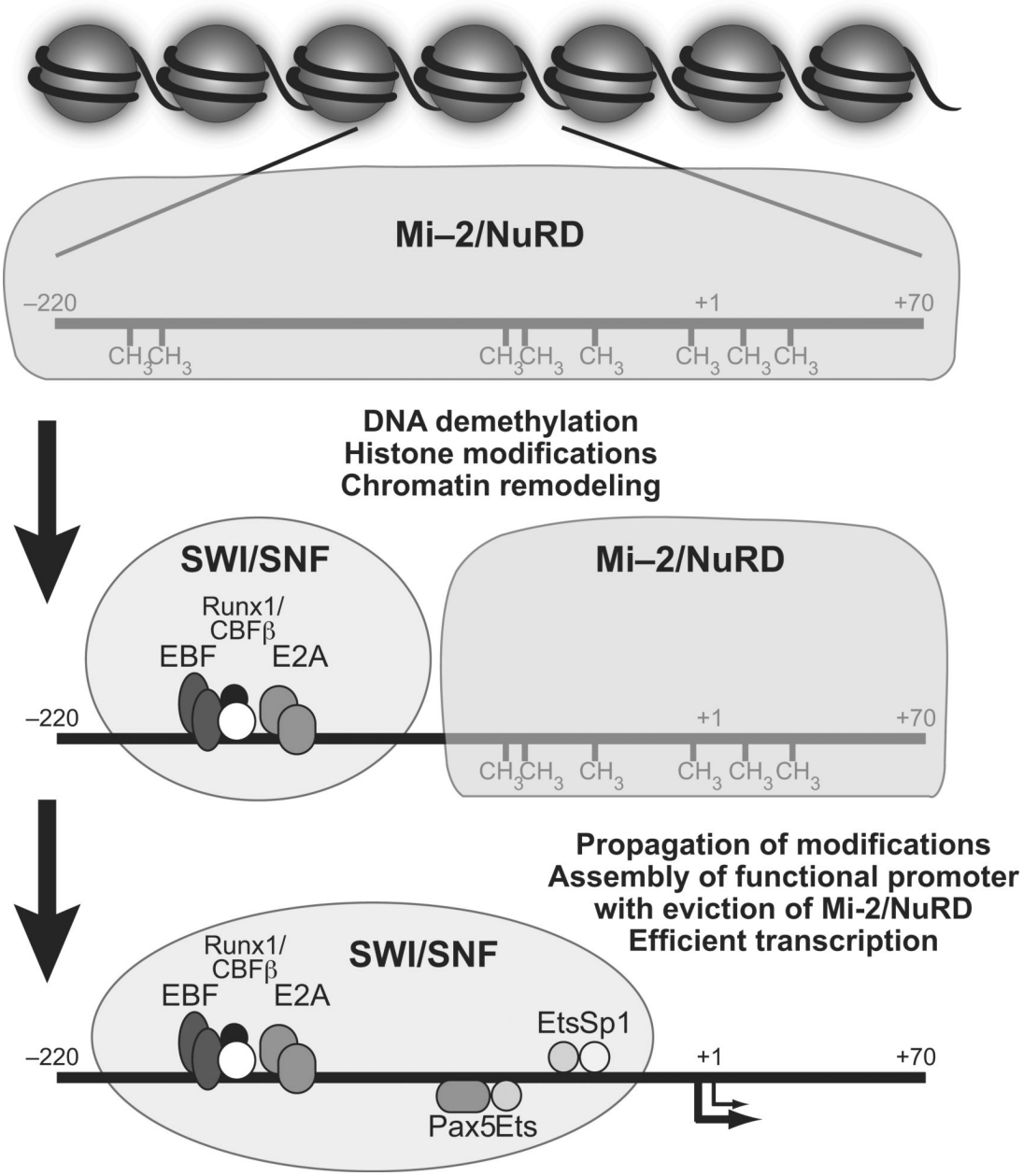

Figure 2.

Model of epigenetic regulation of the *mb-1* promoter. Sequential steps involved in *mb-1* transcriptional activation are shown. Association of chromatin remodeling complexes with promoter elements is indicated by shaded regions representing hypothesized Mi-2/NuRD and SWI/SNF occupancy. We propose that Mi-2/NuRD complexes are required to maintain hypermethylated, transcriptionally silent *mb-1* promoters (top). The *mb-1* promoter is primed for expression prior to transcriptional activation (middle). Priming following DNA binding of EBF, E2A and Runx1/CBFβ involves demethylation of CpGs at distal sites and chromatin remodeling following recruitment (by EBF1) of SWI/SNF. Efficient transcriptional activation of the *mb-1* gene (bottom) requires 5' to 3' propagation of DNA demethylation, which allows appropriate binding of Pax5, Ets and Sp1, displacement of Mi-2/NuRD, and further modifications of chromatin by SWI/SNF.