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Epigenetic dynamics in normal and malignant B cells: die a hero or live to become a villain

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Normal B cell development, activation, and terminal differentiation depend on the intricate dynamics of cooperating epigenetic and non-coding components to control the level and timing of expression of thousands of genes. Recent genome-wide studies have integratively mapped changes in the chromatin landscape, DNA methylome, 3-dimensional interactome, and coding and non-coding transcriptomes of normal and malignant B cells. Genetic ablation in human cells and mouse models has begun to elucidate the coordinated roles of essential epigenetic modifiers, key transcription factors, and long non-coding RNAs in B cell biology. Perturbation of these stewards of the epigenome drive B cell oncogenesis, but may be exploited to develop new avenues of therapy.

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Introduction

Long regarded as 'junk DNA', the non-protein-coding portion of the genome has been liberated from this denigrating moniker by high-throughput sequencing-enabled discoveries demonstrating its essential role in the regulation of genome integrity, structure, replication, and gene expression. Sometimes referred to as the 'regulome', these epigenetic mechanisms are exquisitely controlled and essential for development and homeostasis, and when disturbed, contribute to a range of diseases, from auto-immunity to cancer.

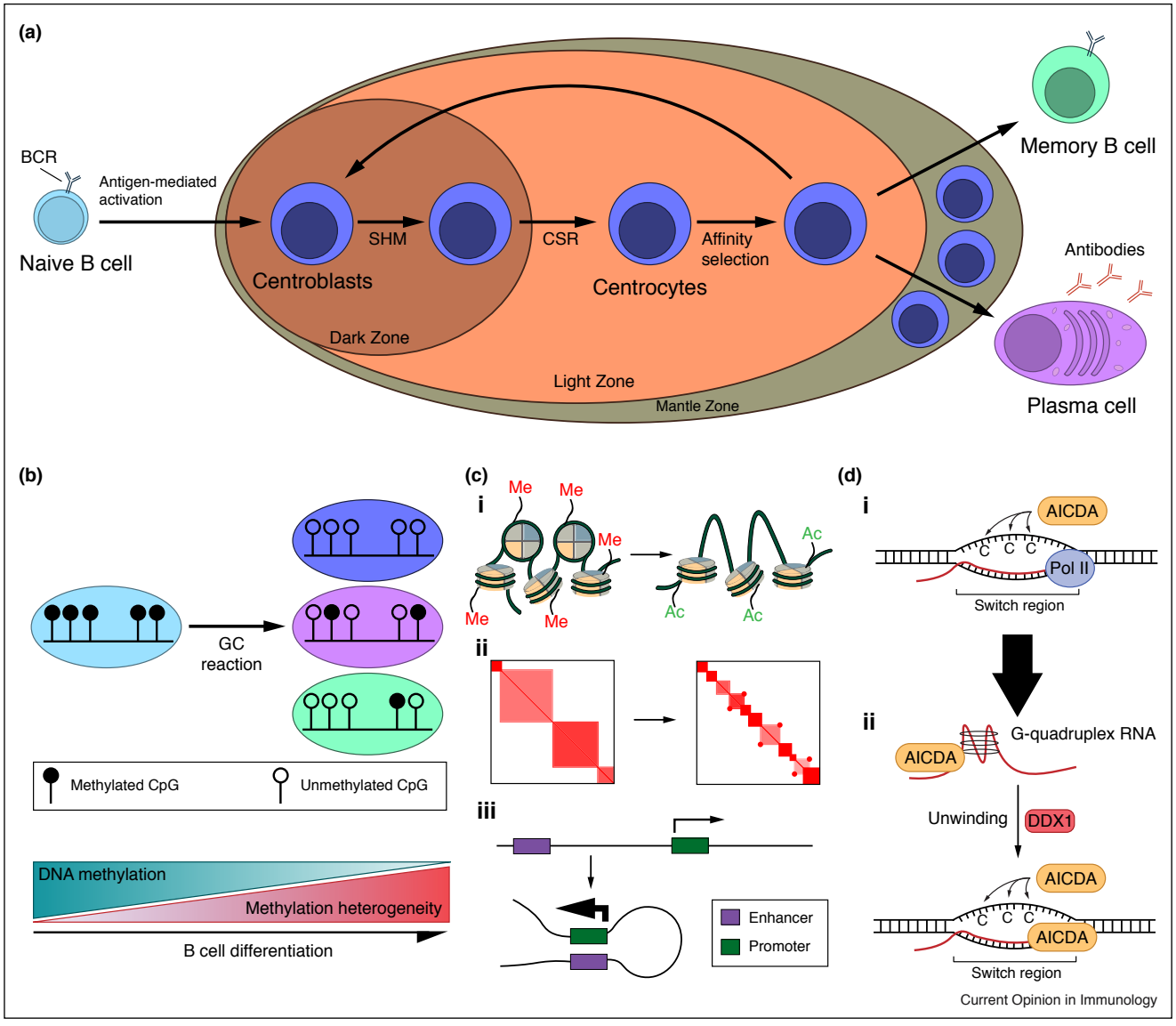
The following review discusses the most recent discoveries regarding the role of epigenetic regulation in B lymphocytes, focusing on latter stages of maturation and activation, as well as how mature B cell cancers subvert these mechanisms for their own survival and progression. Figure 1 provides a basic overview of each epigenetic mechanism and its role in B cell activation and Figures 2 & 3 illustrate how lymphomas hijack these mechanisms. We summarize and highlight three epigenetic modes of regulation and discuss each separately: first DNA methylation; second, histone modifications and chromatin architecture; and last, long non-coding RNAs.

Dynamic flux of the B cell epigenome throughout development and in B cell cancers

Genome-wide studies revealed the extensive plasticity of the DNA methylome during B cell development (Figure 1a), with dynamic methylation at nearly 5 million CpGs (30% of sites surveyed) [1,2]. These changes are temporally enriched in specific regulatory regions, with enhancers and heterochromatin preferentially demethylated in precursors and germinal center (GC) cells, respectively, and methylation gains in polycomb-repressed regions in the most differentiated B cells [3]. Dynamic methylome changes exhibited no direct correlation with gene expression, though demethylation of key B cell transcription factor (TF) binding sites correlated with expression of those TFs and their transcriptional programs (Figure 1b) [1,2,4,5]. The importance of enhancer demethylation was directly demonstrated by knockouts of TET family demethylase genes in early stage B cells, which prevented lineage-specific programmed demethylation of enhancers of B-cell lineage genes, causing decreased expression of these key genes, defects in development at the pro-B cell to pre-B cell stages, and impaired IgK rearrangement [6,7]. The importance of *de novo* methylation was shown by double knock-out of DNMT3A and DNMT3B in pre-B cells, which did not affect B cell development and maturation, but, upon immunization, increased cell activation and expansion of GC B cell and plasma cell (PC) populations by 2–3-fold [8•].

Given the essential nature of epigenetic control in B cell differentiation, it is not surprising that aberrant DNA methylomes are hallmarks of B cell cancers and the degree of methylation heterogeneity correlates with more aggressive disease [9,10•]. While earlier studies reported 'global hypomethylation' in tumors, more recent comparisons to normal B cell differentiation stages revealed that these widespread changes parallel those of each tumor type's 'cell of origin' (Figure 2a) [11,12]. Focusing instead on differences between B cell tumors and matched-stage normal cells demonstrated

Figure 1

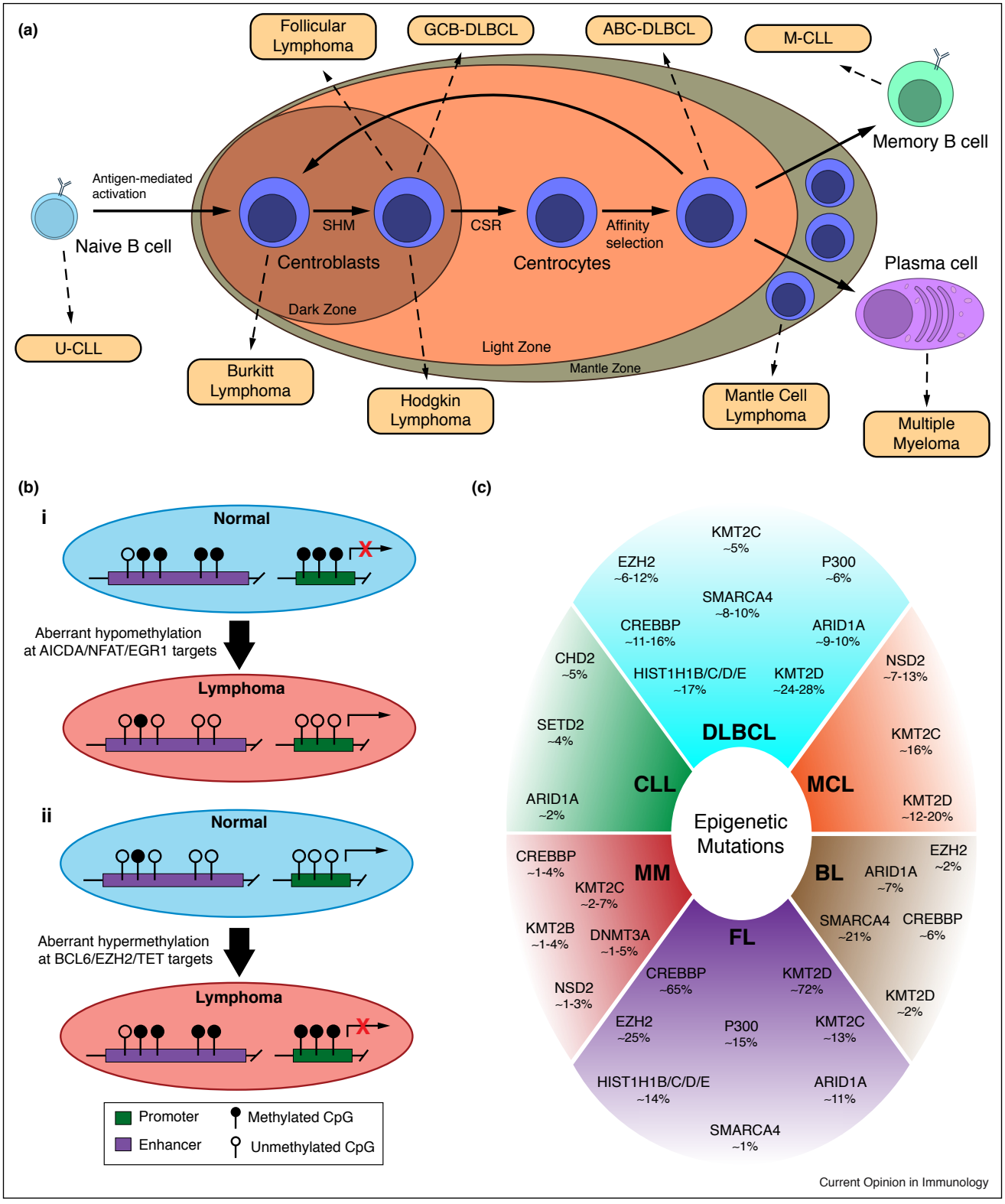


Epigenetic mechanisms regulating B cell activation and the GC reaction.
(a) Simplified diagram shows the stages of peripheral B cell activation and maturation in the GC reaction. **(b)** DNA hypomethylation leads to methylation heterogeneity during B cell maturation. **(c)** As naïve B cells are activated and form GCs, i) increased histone acetylation and loss of repressive methylation drastically alter the chromatin landscape, which ii) decompacts chromatin and generates chromatin loops, iii) facilitating increased promoter-enhancer contacts and a massive increase in gene expression. **(d)** lncRNA transcription at switch regions contributes to SHM and CSR through two mechanisms; i) R-loops — DNA-RNA hybrid and ssDNA — are formed during transcription and the ssDNA can be targeted by AID or ii) switch transcripts form G-quadruplexes that are bound by AICDA and unwound by DDX1 to facilitate R-loop formation at switch regions. SHM — somatic hypermutation; CSR — class-switch recombination; BCR — B cell receptor.

that altered DNA methylation is concentrated at enhancers, and particularly enriched in the binding sites of stage-specific and tumor-specific TFs that are crucial for B cell maturation and activation (Figure 2b) [2,12,13]. Importantly, methylation changes in these regulatory regions correspond to alterations in the transcriptional programs of the associated TFs [11,14].

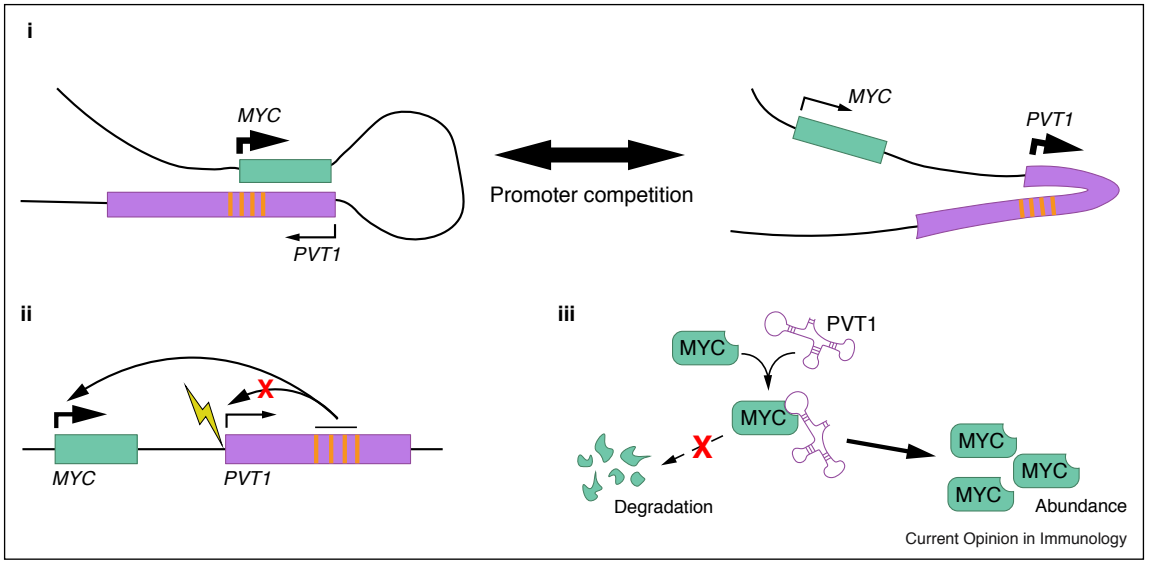
The underlying cause(s) of DNA methylation changes in B cell cancers is less clear. In GC B cells and GC-derived lymphomas, activation-induced cytosine deaminase (AID), which drives somatic hypermutation, an essential step in affinity maturation, also mediates DNA hypomethylation and increased methylome heterogeneity in intergenic and distal regions associated with essential B

Figure 2



Subversion of epigenetic mechanisms in B cell cancers.
(a) Overview of the cell of origin for many B cell cancers. **(b)** B cell lymphomas exhibit increased intra-sample DNA and inter-sample DNA methylation heterogeneity due to i) aberrant hypomethylation at major transcription factor and AICDA binding sites, and ii) aberrant histone and DNA hypermethylation at gene promoters regulatory elements that may result in transcriptional repression. **(c)** Frequencies of select mutations in

Figure 3



The complex, synergistic interplay between genetic and epigenetic aberrations of the PVT1/MYC locus.
(a) The *MYC* and *PVT1* promoters exhibit competitive binding to a group of enhancers (orange bars) located within the *PVT1* gene body. **(b)** Mutations to the *PVT1* promoter eliminate its interaction with this regulatory cluster, allowing the *MYC* promoter to freely interact with the enhancers and increase *MYC* expression. **(c)** Independently, IgH promoter/enhancer translocations or amplifications of the locus can result in PVT1 overexpression, which binds to the MYC protein and prevents its degradation.

lineage genes (Figure 1b) [3,15,16]. Over-expression of AID accelerates a mouse model of BCL2-driven lymphoma without increasing off-target mutations [17,18•]. However, most B cell cancers do not overexpress AID; mutations in DNA methylation and demethylation factors are not common — though chromatin modifiers are frequently mutated; and there is little association between genetic mutation and DNA methylation profiles (reviewed in Ref. [11]). Instead, the coincidence of DNA methylome changes with B cell TF binding sites and B lineage enhancers in all B cell cancers suggest a common mechanism, and one that is well-established in cancer: perturbations in the activities of key TFs and chromatin modifiers involved in B cell activation and maturation are major contributors to aberrant methylation patterns.

The 3-dimensional (3D) interactome and chromatin modifiers mediate transcriptional programs integral to B cell activation

Globally increased transcription and histone acetylation were linked to B cell activation more than 50 years ago [19]. More recently, integrative analysis of activating and repressive histone marks, chromatin accessibility, and gene expression have defined regulatory landscape transitions in normal development from human or murine naïve B through plasma cells, and have revealed key regulatory elements that are reprogrammed in human B cell cancers and may be prognostic [20••,21•,22,23]. The mechanisms driving these epigenetic changes have been elucidated through B cell specific knockout of histone modifier genes, many of which are recurrently mutated in B cell cancers (Figure 2c) [24–27]. Conditional deletion of the histone acetyltransferase CREBBP perturbed B cell development and accelerated the development of lymphoma in BCL2- and MYC-driven mouse models [28,29,30•,31]. These effects are likely related to CREBBP's role in programs crucial to the GC reaction, including counteracting the repressive effects of BCL6 on transcription via direct acetylation of the BCL6 protein itself, and by H3K27 acetylation at promoters and enhancers of BCL6 target genes [25,28,29]. Several histone methyltransferases are also commonly mutated in NHL and crucial for B cell development and GC formation [24,25]. EZH2, a component of the polycomb repressive

(Figure 2 Legend Continued) epigenetic modifiers, chromatin remodelers, and histone proteins in several B cell cancers. ABC-DLBCL — Activated B-cell-like subtype of diffuse large B cell lymphoma; GCB-DLBCL — Germinal center B-cell-like subtype of diffuse large B cell lymphoma; U-CLL — unmutated chronic lymphocytic leukemia; M-CLL — mutated chronic lymphocytic leukemia; FL — Follicular lymphoma; MM — Multiple myeloma; BL — Burkitt lymphoma; MCL — Mantle cell lymphoma.

complex 2 (PRC2) that mediates trimethylation of H3K27 (H3K27me3), interacts with a distinct set of TFs to direct stage-specific gene repression programs, such as BCL6 in GC B cells and Blimp-1/PRDM1 in plasma cells (PC) [32,33]. Conditional ablation of EZH2 in B cells led to inappropriate activation of immune response pathways, B cell transcription factors, and regulators of cell division and metabolism normally repressed during terminal differentiation of PC, resulting in decreased proliferation and poor antibody production [22,34]. Thus, several chromatin modifiers coordinate with key B cell TFs to temporally regulate developmental transcriptional programs, and, when mutated, are lymphomagenic.

In concert with dynamic epigenetic landscapes, higher order genome organization and nuclear sub-compartmentalization orchestrate transcriptional activity [35]. Chromosome conformation capture and super-resolution microscopy methods have enabled mapping of the dramatic reorganization of 3D genome architecture during normal B cell development and activation [36]. These changes include chromatin decondensation via amplification of histone acetylation and nucleosome spreading, as well as chromatin decompaction mediated by new short-range CTCF loop interactions (Figure 1c) [20••]. The latter changes facilitate increased promoter-enhancer contacts between, and augmented expression of, B cell activation genes and require the proto-oncogene Myc. Additional studies corroborated these findings and implicated lineage-specific transcription and non-coding regulatory regions as contributors to these changes in chromatin architecture [36,37••]. In GC B cells, a large (>100 kb) enhancer cluster upstream of BCL6 comprises a locus control region (LCR) that forms the crux of intra-chromosomal interactions with promoters and enhancers of hundreds of upregulated GC B cell-specific genes [37••]. Strikingly, this BCL6 LCR was necessary for GC formation, but was dispensable for BCL6 expression in non-B cells [37••]. Taken together, these epigenomic findings underscore the complex choreography of chromatin landscape and 3D-genome organization with TF identity, level and timing that is crucial for normal B cell differentiation.

The roles of long non-coding RNAs in B cell activation and lymphomagenesis

LncRNAs are a heterogeneous group of ncRNAs that exhibit remarkable cell type specificity and disease association. They are differentiated from other ncRNAs (miRNA, tRNA, etc.) based on their length (>200 nucleotides), and are sub-classified by genomic location (e.g. intergenic, antisense, and enhancer) and structure (e.g. un-/spliced, uni-/bi-directionally transcribed, circRNAs) [38•]. LncRNA's functional versatility includes epigenetic modification, nuclear domain organization, transcriptional control, regulation of RNA splicing and translation, and modulating protein activity (reviewed in Ref. [39]).

Developmentally-controlled and tissue-specific production of lncRNA 'germline' transcripts from unrearranged VH and V_K gene segments in B lineage cells was identified nearly four decades ago [40] and was subsequently linked to enhanced chromatin accessibility and recombination of VDJ loci (reviewed in Ref. [41]). More recently, complementary roles in class-switch recombination (CSR) and somatic hypermutation (SHM) have been shown for intronic and anti-sense lncRNAs derived from switch regions and divergent transcription at promoters and enhancers. These lncRNAs create triplex RNA/DNA hybrid R-loops and G-quadruplexes, recruiting AID, the RNA exosome complex, and the RNA helicase DDX1, thereby facilitating CSR (Figure 1d) [42•,43,44]. Some super-enhancer loci, such as *MYC* and *BCL6*, also exhibit high levels of divergent and convergent lncRNA transcription, which may underlie off-target AID-mediated hypermutation and translocations that are oncogenic [16,45,46].

Several profiling studies have catalogued the patterns of lncRNA expression across B cell development and activation in human and mouse. One surprising finding is the loss of the repressive lncRNA Xist from the inactive X (Xi) chromosome at the pro-B cell stage, leading to loss of heterochromatin and re-expression of immune genes from the Xi. Activation of mature B cells restored Xist RNA and heterochromatin to the Xi. These findings suggest that chromatin changes on the Xi during B cell development and the dynamic nature of Xi maintenance in mature B cells predisposes X-linked immunity genes to reactivation [47,48]. Across B cell differentiation, hundreds of lncRNAs are expressed in a cell-type/stage-specific manner, which is consistent with studies in other tissues and suggests a *cis*-regulatory function for many of them [38•,49•,50–55]. Compared to coding genes, lncRNAs exhibit significantly less sequence conservation, but alternative comparative methods, such as structure, profile, or positional conservation can detect homologues without primary sequence conservation [56]. In support of this approach, only 185 of 4516 lncRNAs expressed during murine B cell development had a human syntenic ortholog based on pairwise genomic alignments, but positional comparison of murine B cell eRNAs near orthologous coding genes revealed an additional 228 potentially orthologous lncRNAs [47]. One such syntenic lncRNA, RP11-132N15.3, lies in an intergenic enhancer region ~200 kb upstream of BCL6 within a cluster of lncRNAs that is highly and specifically expressed in both mouse and human GC B cells and strongly correlates with expression of the GC transcription factor BCL6 [23,37••,47,52]. The robust expression and conservation of this lncRNA suggest that it is involved in the *cis*-regulation of BCL6 expression. To

answer this question, future experiments are needed that compare suppression and truncation of the lncRNA transcript to epigenetic repression of the enhancer locus, to determine the impact on BCL6 expression.

Profiling and clinical retrospective studies in many lymphoma and leukemia subtypes identified altered expression of lncRNAs, suggesting a potential oncogenic role. MINCR (MYC-induced long noncoding RNA) is upregulated in MYC-rearranged or amplified primary lymphomas and cell lines. Knock-down of MINCR reduced MYC binding to the promoters of cell cycle genes and decreased their expression, impairing cell cycle progression [57]. Another lncRNA gene, PVT1, plays essential roles in the regulation of MYC-mediated growth and oncogenesis via epigenetic mechanisms (promoter competition) and direct stabilization of MYC protein levels (Figure 3) [58••,59]. Other lncRNAs, such as MALAT1, lncRNA-p21 (PANDAR), and NEAT1, have significant associations with clinicopathologic metrics of increased disease severity and decreased survival in lymphomas and MM and may be promising therapeutic targets [50,60,61]. NEAT1 is induced by p53 in response to DNA damage in CLL and lymphoma; primary CLL cells with low NEAT1 expression exhibited reduced cell death in response to DNA damage, even if TP53 was unmutated [60]. Depletion of NEAT1 in non-lymphoid cancer cells induced synthetic lethality with genotoxic chemotherapies as well as with activation of p53, suggesting that NEAT1 may be a promising target in B cell cancers as well [62]. The novel mechanisms of action exhibited by the few lncRNAs studied to date strongly suggest that these molecules hold vast potential for greater understanding of B cell biology, and new avenues for therapeutic development.

Conclusion

Significant progress has been made in charting the dynamic, non-coding portions of the epigenome and transcriptome in normal B lymphocytes and B cell cancers. However, outstanding questions remain. Though performed on sorted cells, the profiling studies to date are bulk analyses, which can only detect the average epigenomes and transcriptomes of component cells. Given the heterogeneity of differentiating B cells, single cell transcriptional and epigenetic profiling at many time points is necessary to definitively determine the causative links between temporally regulated epigenetic modifications, binding of regulatory factors, and gene expression levels. In addition, to more accurately elucidate their roles in lymphomagenesis, it is necessary to perturb multiple of epigenetic modifiers and transcription factors in a single model in the sequence that they are known to occur in human lymphomas. Finally, arguably the largest outstanding question is defining the functions of thousands of long non-coding RNAs with dynamic transcriptional patterns during B cell differentiation and/or in B cell cancers.

Conflict of interest statement

Nothing declared.

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Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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