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Review

Pre-BCR signals and the control of Ig gene rearrangements

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Abstract

Progenitor B lymphocytes that successfully assemble a heavy chain gene encoding an immunoglobulin capable of pairing with surrogate light chain proteins trigger their own further differentiation by signaling via the pre-BCR complex. The pre-BCR signals several rounds of proliferation and, in this expanded population, directs a complex, B cell-specific set of epigenetic changes resulting in allelic exclusion of the heavy chain locus and activation of the light chain loci for V(D)J recombination.

Keywords: B cell development; Pre-B cell receptor (pre-BCR); Allelic exclusion; V(D)J recombination; Transcriptional regulation

1. Introduction

B cell differentiation is a highly dynamic process that requires the coordinated action of various receptors, signaling molecules, transcription factors and the V(D)J recombinase. Completion of immunoglobulin heavy chain (μHC) gene rearrangement leads to surface expression of the pre-BCR complex which triggers a cascade of signaling events that alters the developing cell's response to growth factors and effects transcription and DNA rearrangement. μHC expression results in pre-B cell expansion and inhibits further rearrangement at the μHC locus, a process known as allelic exclusion. Pre-B cells subsequently exit the cell cycle and initiate of a second set of gene rearrangements at the immunoglobulin light chain (IgLC) loci. The timing of DNA rearrangements, which involve the generation and repair of DNA double stranded breaks interspersed with bursts of proliferative expansion, must be tightly regulated to ensure proper development and to prevent genomic instability and leukemic transformation. This review will focus on the function of the pre-BCR as the critical regulator of the pre-antigenic phase of B cell development.

2. Pre-BCR signalling—an overview

Pre-BCR signaling is initiated upon expression of a clonotypic immunoglobulin μ heavy chain (μHC) that can assemble a membrane complex with the surrogate light chain components (SLC) VpreB and λ5, and the transmembrane proteins Ig-α and -β. Signaling through this receptor is associated with its internalization and degradation [1–3]. The cytoplasmic domains of Ig-α and -β contain immunoreceptor tyrosine-based activation motifs (ITAMs) which, when clustered in the membrane, provide a docking site for the Syk kinase, Src family kinases (Fyn, Lyn, and Blk), the Tec family kinase, Btk, and the adaptor proteins Grb2 and BLNK. BLNK (also known as BASH and SLP-65) recruits Btk, which is then phosphorylated by Lyn, Syk, or both [4,5].

Phosphorylated Btk then phosphorylates PLCγ2, which hydrolyzes PIP_2 to IP_3 and diacylglycerol (DAG) causing calcium mobilization and resulting in activation of calcium dependent enzymes (reviewed in [6]). Proliferation is induced via activation of MAP kinase pathways, and allelic exclusion is imposed by a presently undefined mechanism [7,8]. Upon pre-BCR signaling, cells initially undergo a proliferative burst accompanied by down regulation of SLC components and RAGs, followed by exit from the cell cycle and transition from pro-B/large pre-B I (Hardy fraction C) to small pre-B II (Hardy fraction C′) [9]. Signaling also induces transcription factors such as NF-κB, Spi-B and IRF-4 as well as re-expression of RAGs, contributing to activation of the κ enhancers and κ germline transcription and rearrangement [10,11].

3. Pre-BCR signaling does not require exogenous ligand

Once the pre-BCR assembles on the cell surface, tyrosine kinase-dependent signaling begins. Ig-α/β heterodimer surface expression facilitated by the μHC and SLC seems to be sufficient

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to initiate signaling. Truncation mutants that lack extracellular domains can function to aggregate Ig-α/β and signal B cell differentiation [12,13]. In fact, pro- to pre-B cell development can be driven by the Ig-α and -β ITAMS (along with other conserved cytoplasmic motifs) targeted to the inner leaflet of the plasma membrane by fusion with a Lck myristoylation/palmitoylation sequence [14]. These observations, along with the fact that a single pre-B cell in the absence of any other stromal cells can proliferate in response to pre-BCR expression [15], calls into question the relevance of various extracellular ligands proposed to interact with the pre-BCR. However, soluble recombinant pre-BCR can bind stromal cells [16]. The Schiff group identified a human pre-BCR ligand, galectin-1, that may be involved in receptor aggregation and activation of tyrosine kinase activity localized at the pre-BCR [3]. Another study by the Jäck lab implicated stromal heparin sulfate as a ligand for murine pre-BCR [17] (see also contribution by Espeli et al. in this issue). Recent data has revealed another potential mechanism for pre-BCR signaling involving the SLCs. The conserved non-immunoglobulin portions of λ5 and VpreB may mediate constitutive receptor aggregation, signaling and internalization via homotypic ionic interactions [18]. Various truncated or unusually structured μHCs may inherently allow aggregation in an SLC-independent fashion providing an explanation for the truncated μHC transgene results mentioned above.

The absence of both Ig-α and -β causes a developmental arrest at the pro-B cell stage despite intact V(D)J recombination and μHC expression revealing the requirement for these molecules in pre-BCR signaling [19,20]. One role of the ITAMs is recruitment of BLNK to Ig-α. Fusing BLNK to a mutant Ig-α lacking these tyrosines reconstitutes downstream signaling events. Thus, simply the formation of the pre-BCR leading to the membrane localization of Ig-α and -β is sufficient to trigger pre-BCR signaling, allow downregulation of RAGs and SLC proteins, and upregulation of transcription factors that result in IgLC gene rearrangements.

The pre-BCR may serve yet another function, that of a molecular clock which eliminates B cell precursors that fail to generate a functional IgLC gene within a certain window of time or that express a μHC that is incapable of associating with any IgLC ([21,22]; see also contribution by C. Vettermann and H.-M. Jäck in this issue). Pre-BCR signaling is associated with internalization and degradation of the pre-BCR [1–3]. Since the genes encoding the SLC components λ5 and VpreB are inactivated upon pre-BCR signaling [23], progressively less pre-BCR complex can form on the surface as cells divide, culminating in reduced proliferative signals. If a pre-B cell fails to make an IgLC that associates with its μHC, then μHC expression on the surface will eventually be lost, presumably leading to the cell's arrested development and death [24]. The existence of this clock may help protect the genome from RAG-associated genomic instability.

4. Pre-BCR signals leading to proliferation

While there is debate as to whether the complete pre-BCR is required for IgHC allelic exclusion and pre-B cell differentiation [25], it is widely agreed that pre-BCR signaling is required for the proliferative expansion of pre-B cells expressing a successfully rearranged IgHC gene which can assemble with SLCs. This proliferation signal induces two to five rounds of cell division and eliminates the dependence of developing cells on IL-7 [15]. Pre-B cells are known to be cycling in vivo based on their rapid incorporation of the nucleotide analog BrdU [1,26,27]. Transgenic IgHC expression induces proliferation in pro-B cells cultured on stromal cells [28]. Targeted deletion of the Igμ membrane exon or disruption of the SLC genes results in the failure of pre-B cell proliferation [25].

The MAP kinase pathway is involved in pre-B cell expansion and perhaps various aspects of differentiation as well. In mature B cells, Ras signaling is induced upon BCR crosslinking [29,30]. Mice expressing a constitutively activated Ras mutant transgene on a RAG-null background bypass the pre-BCR checkpoint and contain almost normal numbers of B cells in their lymph nodes and spleen [31]. These RAS-transgenic B cells are abnormal in that they continue to express λ5 and RAG2, which are normally down-regulated upon pre-BCR signaling. Their κ germline transcript levels are similar to those observed in wild-type pre-B cells, but the transgenic cells also express some surface markers associated with more mature stages of B cell development. When the activated Ras transgene is bred onto a RAG sufficient but ΔJ_H background, κ rearrangement is induced despite a lack of HC expression [32]. The survival phenotype of Ras transgenic B cell precursors is similar to that of RAG-null B cells expressing transgenic μHC and Bcl-2. Indeed, levels of Bcl-2 are high in the activated Ras transgenic B cell precursors, a trait characteristic of mature B cells [31] perhaps allowing survival in the setting of both proliferation and ongoing Igκ locus rearrangement.

Mice expressing a transgenic human dominant negative Ras, H-rasN17, show a developmental arrest at a stage corresponding to Hardy fraction "A". This was not attributable to increased apoptosis. Over-expression of a membrane targeted, and thus constitutively active, partner of Ras, Raf-1(Raf-CAAX), on the H-rasN17 background overcame the block confirming the involvement of this MAP kinase pathway in B cell development [33]. Mimicking the pre-BCR signal with these transgenes does not, however, induce allelic exclusion since V-to-DJ rearrangement proceeds despite the premature proliferation signal.

Thus, the MAPK pathway is necessary for proper pre-BCR signaling, and when activated can substitute for the pre-BCR causing proliferative expansion, survival and κ activation, but not IgHC allelic exclusion, or λ5 and RAG down-regulation. These experiments demonstrate the existence of two separable pre-BCR signaling pathways; one causing proliferation and the induction of some transcription factors involved in differentiation, and the other mediating allelic exclusion.

5. Allelic exclusion

Allelic exclusion remains an enigmatic aspect of pre-BCR signaling. Preventing further μHC gene rearrangement in a cell that already expresses a μHC is necessary to ensure that each B cell has an unique antigen specificity. It is imposed at the

V_H-to-DJ_H step in recombination while D-to-J_H rearrangement is unaffected [34]. The ability of RAG proteins to selectively act at different loci depending on the cell type or stage in development has been attributed to regulated accessibility of each rearranging locus within chromatin structure [35]. RAG proteins cannot cleave a nucleosomal substrate in vitro, implicating the necessity of some sort of chromatin remodeling to render the DNA accessible to the recombinase [36–38]. The loci within purified nuclei that are accessible to cleavage by RAGs in vitro depend on the source of nuclei [39]. Thus, the accessibility of rearranging loci is a cell-type and stage-specific property of chromatin structure. The accessibility of the IgHC locus changes across the pro-B to pre-B cell transition, and pre-BCR signaling induces a permanent loss of IgH locus accessibility. The mechanism or signaling pathways employed for such chromatin remodeling have not been delineated.

5.1. Requirements and signals

Surface expression of a μHC induces allelic exclusion and mice carrying a mutation in the transmembrane region of the μHC gene are unable to turn off IgHC gene segment rearrangements [40]. Expression of a μHC transgene in mice prevents endogenous V_H-to-DJ_H rearrangements [41]. It is controversial, however, whether SLC expression is required for IgHC allelic exclusion. One group reported that targeted disruption of the gene encoding λ5 resulted in a failure of early pre-B cell proliferation and the loss of allelic exclusion at the level of μHC gene rearrangements. Surprisingly, however, these λ5-deficient mice still displayed allelic exclusion at the level of surface μHC expression [42]. Similar results were observed in mice lacking VpreB1, VpreB2 and λ5 [43,44]. This second set of researchers detected no difference in the frequency of pre-B cells with two μHC V(D)J rearrangements in the presence or absence of SLCs suggesting allelic exclusion at the level of gene rearrangement does not require full pre-BCR assembly. One potential explanation for this observation was provided in a report showing that precocious expression of IgLC could replace the need for SLCs to promote B cell development [45]. However, more compelling data shows that μHC can be detected on the surface of IgLC-negative SLC-deficient cells demonstrating that some μHC are capable of reaching the cell surface without SLC or LC [46–49]. The few cells that are able to express μHC on the surface despite the absence of SLC would then be capable of signaling progression as well as allelic exclusion. Although it is not yet clear how a μHC can reach the surface in the absence of SLC, it is possible that μHC can pair with other chaperone-type proteins in pre-B cells, transiting to the surface and signaling allelic exclusion and other changes in gene regulation, but not proliferation [46–49].

Differences in the ability of μHC gene products to be expressed on the cell surface may be responsible for the surprising observation that 4–8% of pre-B cells do express two endogenous rearranged μHCs, only one of which is expressed on the cell surface [22]. This may be due to inefficient pairing and thus weak surface expression of the first HC, resulting in a failure of allelic exclusion followed by productive rearrangement of a second IgHC allele. In this vein, differences in surface expression amongst individual μHC genes could account for the curious observation that some μHC transgenes allelically exclude very stringently while others allow continuation of D-proximal V_H gene segment rearrangement [50]. Transgene expression levels may also play a role.

Dμ, an endogenously generated truncated μHC protein formed by translation of a partially rearranged (D_H-to-J_H) IgHC allele [51] is also capable of signaling allelic exclusion. Dμ surface expression blocks further development of B cell precursors, a process known as Dμ selection [42,52–54]. This selection is thought to occur when Dμ expression causes μHC gene allelic exclusion, thus preventing the formation of a complete IgHC gene [55,56]. Proliferation is impaired in the Dμ transgenic mice, again pointing to different requirements for allelic exclusion and proliferation. Dμ can cause increased germline κ and diminished λ5 transcription, yet it cannot promote full differentiation to the pre-B cell stage [56]. Considering that Ig-β, λ5 and Syk are necessary for Dμ to signal, Dμ apparently forms a pre-BCR-like complex, but due to the lack of V_H structure, this pseudo-pre-BCR fails to mimic essential aspects of the pre-BCR signal and such cells eventually die from an inability to differentiate perhaps due to ineffective pairing with IgLC [56,57]. Pairing of Dμ to the SLC could be qualitatively different, and different requirements for the maturation and surface expression of the two types of pre-BCRs have been demonstrated [58]. But precisely how lack of the V regions in this circumstance has such a dramatic effect on B cell development when truncation mutants suffice to replace full-length proteins remains a mystery [12,13].

As mentioned above, proliferative signals transmitted through the MAPK pathway are not sufficient to induce allelic exclusion. Nor is BLNK required for allelic exclusion [59]. But Syk, the tyrosine kinase recruited and activated upon pre-BCR signaling upstream of BLNK, is necessary for normal B cell development and allelic exclusion. Irradiated mice reconstituted with $Syk^{-/-}$ μHC transgenic cells displayed incomplete μHC gene allelic exclusion, however ZAP-70, another Syk family kinase expressed in B cells, appears to play a partially redundant role with Syk. $Syk^{-/-}$ $ZAP\text{-}70^{-/-}$ double mutants completely arrest at the pro-B cell stage according to their cell surface marker phenotype and cannot allelically exclude in the presence of a μHC transgene [60].

BLNK is the adaptor protein downstream of Syk that is involved in cessation of proliferation and the activation of germline κ transcription and rearrangement (see contribution by R.W. Hendriks and R. Kersseboom in this issue). However, while Dμ selection is impaired in the BLNK knock out, IgHC allelic exclusion in that mutant remains intact [61]. This may be due to another partially redundant adapter, LAT, which can substitute to some extent for BLNK in pre-B cell development [62]. LAT is able to link Syk to downstream PLCγ signaling, just as BLNK does [63]. Coincidentally, mice heterozygous for PLCγ1 and missing PLCγ2 on an IgHC transgenic background fail to induce allelic exclusion as measured using a PCR assay for endogenous V_H gene-segment rearrangements [64]. The mechanism for PLCγs' involvement in allelic exclusion is not yet understood, but perhaps calcium flux induces activation of relevant

transcription factors. It would be interesting to know whether over-expression of PLCγ signaling cascade components could relieve the absolute requirement for HC surface expression in allelic exclusion.

5.2. Chromosomal choreography

Recently, large chromosomal movements have been correlated with allelic exclusion at the μHC locus. Fluorescent in situ hybridization (FISH) experiments revealed that the μHC locus associates with the nuclear periphery in most T cells, but is more centrally located in LPS-stimulated B cells [65]. This phenomenon was shown to be RAG independent but IL-7Rα-dependent as well as cell type specific, suggesting a role for such chromosomal relocation in the regulation of recombination. Remarkably, two-color FISH using probes from either end of the μHC locus revealed a higher level of compaction within the locus in B cells undergoing V-to-DJ rearrangement [65,66]. This compaction was lost in B cell precursors lacking the B cell-restricted transcription factor Pax5 correlating with a loss of rearrangement to distal V genes [67]. In transgenic T cells expressing Pax5, central nuclear relocation of IgHC genes occurred, but the further level of compaction did not. This Pax5 induced nuclear relocation stimulated D-to-J_H rearrangement in the T cells and allowed proximal V_H gene rearrangement that normally is not found in T cells [68,69]. Thus, nuclear relocalization and chromatin compaction may contribute to regulation of the recombination machinery. Allelic exclusion requires that this accessibility be reversed and indeed, FISH studies have now demonstrated that co-localization to gamma satellite sequences of one μHC allele coincides with allelic exclusion and is then relaxed but reestablished during LC rearrangement [68].

IL-7 signaling is associated with histone acetylation and germline transcription of the distal V gene segments, and is necessary for the large chromosomal movements of the IgH locus [65]. Loss of IL-7 responsiveness upon pre-BCR signaling is accompanied by deacetylation of the distal V gene segments, although subsequent exposure to IL-7 causes the locus to become acetylated once again [70]. Treatment of activated splenic B cells with IL-7 greatly diminished gamma satellite association of the IgH locus further implicating the shut-off of IL-7 receptor signaling in allelic exclusion [68]. Thus, adoption of new proliferation signals and loss of IL-7 responsiveness upon pre-BCR signaling may be necessary to establish allelic exclusion.

Correlating with the initial activation of the IgHC locus are antisense transcripts originating in intergenic regions between V_H gene segments [71]. These transcripts are turned off by the time B cell precursors reach the late pro-B stage (Hardy's fraction C, $CD19^+CD43^+BP\text{-}1^+$). Whether these antisense transcripts play a role in opening up the locus or in inducing chromatin compaction via an RNAi-dependent pathway has yet to be determined.

The gene expression profiles driving these developmentally regulated changes in μHC locus structure remain to be discovered. A microarray study designed to elucidate changes in transcription associated with B cell differentiation identified four genes encoding proteins each of which is either associated with SWI-SNF, involved in heterochromatin formation, or active in acetylation-dependent transcriptional regulation [72].

6. Turning on kappa

μHC expression causes several rounds of cell division followed by exit from the cell cycle, silencing of the μHC locus, and up-regulation of the RAGs (among other molecules involved in differentiation), as discussed above. The final step in differentiation induced by μHC is Igκ locus activation and V-to-Jκ recombination [34,73]. Signaling cascades emanating from the pre-BCR induce transcription factors and perhaps chromatin remodeling activities that bind *cis*-regulatory elements within the Igκ locus resulting in germline transcription and rearrangement. The mechanism of these events is currently the focus of intense research efforts.

It is generally agreed that Igκ rearrangement does not absolutely require μHC or pre-BCR expression. For example, one can detect low levels of Igκ rearrangement in JH-deleted pro-B cells [74] or in IL-7-dependent μHC^- pro-B cell clones upon acute withdrawal of IL-7 [75]. This has led to the suggestion that Igκ locus activation may be part of a developmental program that is independent of pre-BCR expression. It has been proposed that increases in Igκ rearrangement that are associated with pre-BCR expression may be a consequence of the proliferative expansion of these cells rather than a pre-BCR signal transmitted to the Igκ locus [25]. Strong data exists which contradicts this hypothesis, however. Pre-B cells positive for cytoplasmic μHC contain far higher levels of Jκ locus dsDNA breaks associated with active V-to-Jκ rearrangement than do pro-B cells on a per nanogram of DNA basis [73] and expression of a μHC transgene in RAG-deficient mice results in increases in germline transcription and recombinase accessibility in sorted populations of pre-as compared to pro-B cells [34,39]. Also, there is data linking the pre-BCR-mediated activation of the Ras-MAP kinase pathway to κ locus activation [76]. Additional experimentation will be required to determine whether the pathway from pre-BCR expression to κ locus activation is direct or indirect, however.

The two IgLC loci, κ and λ, are expressed at a ratio of approximately 20:1 in mice [77]. The more frequently rearranged, and therefore more frequently studied κ locus consists of approximately 140 Vκ gene segments, 5 Jκ gene segments (four of which are functional), two germline transcript promoters, and two enhancers (see Fig. 1). These two enhancers play partially redundant roles in activating the κLC locus as deletion of either one results in a decrease in the ratio of κ-to-λ expression (more so in the Eκ3′ deletion), but only when both enhancers are deleted is the phenotype dramatic. Deletion of both completely abolishes κ recombination, leaving only λ positive B cells [78]. κ germline transcripts are activated in pre-B cells and correlate with κ rearrangements [79].

6.1. Transcription factors and κ locus activation

Many studies of Igκ locus regulation have utilized Abelson murine leukemia virus (AMuLV)-transformed pro-B cell

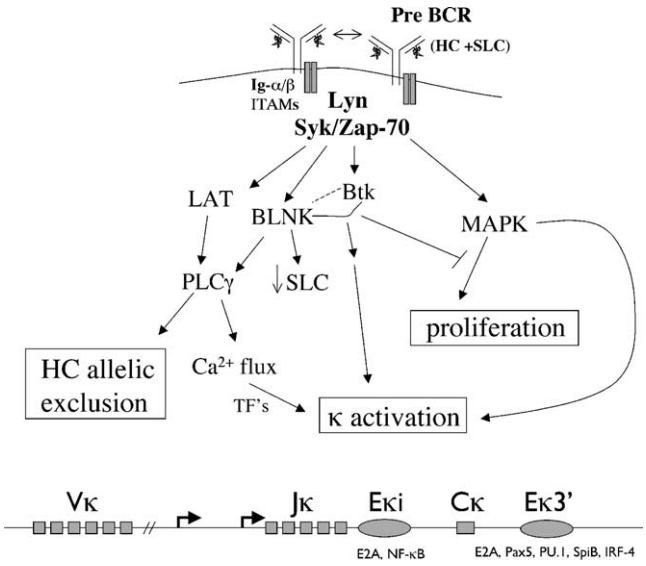

Fig. 1. Pre-BCR signaling activates pre-B cell proliferation, enforces allelic exclusion and activates Igκ locus rearrangement. The upper part diagrams the signaling cascades initiated by homotypic surrogate light chain (SLC)-dependent interactions. Although stroma cell-associated pre-BCR ligands have been identified, it is unclear whether this kind of interaction is critical for cells passing through the pre-BCR checkpoint. The lower part is a schematic of the germline κ locus including the Vκ and Jκ gene segments and the constant region exon (Cκ) in light grey and the intronic (Eκi) and 3′ (Eκ3′) enhancers in dark grey. Transcription factor binding sites are shown beneath their respective enhancers. Dark arrows represent germline κ transcript promoters.

lines. While recapitulating certain aspects of pre-B cell biology, these transformed cells are an imperfect model regarding gene regulation during the pro-to-pre-B cell transition. Treatment of cells with LPS or growth of temperature-sensitive AMuLV-transformed pro-B cells at the non-permissive temperature stimulates κ germline transcription and rearrangements [80,81]. This activation is dependent on the activation of NF-κB, a transcription factor composed of the five members of the Rel-family that can form various homo- and heterodimers. Indeed, in vivo footprinting of AMuLV-transformed cells revealed an LPS-dependent NF-κB footprint in the κ intronic enhancer [10]. A direct comparison of NF-κB site occupancy in primary pro- and pre-B cells showed that the NF-κB site is bound with protein in both pro- and pre-B cells. Gel shift analysis of pro- and pre-B cell nuclei revealed different levels of the distinct NF-κB complexes, but footprinting cannot distinguish the nature of the interacting protein.

Occupancy of binding sites within Eκ3′ changes across the pro-B to pre-B cell transition [10]. A Pax5 site is occupied in pro-B cells but not in pre-B cells. Likewise, pre-B cells showed hypersensitivity at binding sites that were not present in pro-B cells, namely those for Ets family factors, PU.1 or Spi-B, and CRE, a cyclic AMP response element. The binding sites for PU.1/IRF-4 [82], CRE, and Pax5 are in very close proximity to each other and the authors depict a model where Pax5 binding interferes with binding of other factors until pre-BCR signaling somehow reduces Pax5 DNA binding allowing CRE and PU.1/IRF-4 to bind the 3′ enhancer and activate the κLC locus [10].

AMuLV transformation arrests developing B cells in what appears to be an early pre-B cell-like stage by an unknown mechanism. The viral oncogene v-Abl immortalizes these cells rendering them IL-7 independent and apoptosis resistant, but blocks their further differentiation. Upon treatment of AMuLV-transformed cells with the abl kinase inhibitor STI-571 (commonly called Gleevec) these pre-B cells activate RAG and germline κ transcription and rearrange their κ and λ loci at very high rates [11]. By analyzing DNA microarray data derived from such cells prior to and after treatment with STI-571, IRF-4 and Spi-B were identified as key factors in activating the κLC locus [11]. Co-transfection of cDNA expression vectors encoding the two proteins was sufficient to induce κ germline transcripts in an AMuLV-transformed pre-B cell line [11]. PU.1$^{-/-}$Spi-B$^{-/-}$ primary pro-B cell cultures have lower levels of κ transcription than wild-type B cell lines, however IL-7 withdrawal still induced κ but not λ activation, suggesting that κ rearrangement might be unaffected by the absence of these factors [83].

PU.1 and IRF4 interact with the Eκ3′ enhancer in chromatin immunoprecipitation experiments [84,85]. Increased binding of these proteins was associated with the enhancer's increased accessibility to restriction endonucleases as well as increased association with acetylated histones in B cell lines mimicking various stages of B cell development, leading to the suggestion that accessibility has a greater affect on enhancer occupancy than protein levels [84]. IRF4 binds Eκ3′ but in the absence of IRF4, PU.1 binding is disrupted, suggesting coordinated binding of the two molecules [82,85]. There are no binding sites in the κ intronic enhancer for IRF4/PU.1, thus the resulting complete absence of κ rearrangements is intriguing. Interestingly, IRF4,8$^{-/-}$ B cell precursors fail to exit the cell cycle, cannot down-regulate SLC components, and express low levels of RAG proteins, all of which could contribute to the lack of κ activation [85]. Because of the similarity between this phenotype and that of the BLNK null mouse and the fact that BLNK levels are unaffected by IRF4,8 inactivation, the authors suggest that IRF4,8 might be downstream of BLNK in a signaling pathway. BLNK might promote exit from the cell cycle and differentiation as well as Eκ3′ factor binding causing κ activation. It would be interesting to know whether these IRF4,8$^{-/-}$ B cells are able to allelically exclude IgHC rearrangement.

Another well-studied transcription factor, E2A, can activate Igκ germline transcription and recombinase accessibility in non-lymphoid cell lines [86]. There are E2A binding sites (known as E-boxes) in both Igκ enhancers, and a recent study showed that two such sites in Eκi were critical for V-to-Jκ rearrangement [87]. While a direct role for E2A has not yet been demonstrated, involvement of this factor has attracted attention because of the abundance of E2A binding sites within Ig loci and their ability to recruit chromatin modifying factors [88]. Specific chromatin modifications associated with V(D)J recombination have yet to be identified, although regions of the IgHC locus poised for recombination associate with hyper-acetylated histones H3 and H4, and hypo-methylated histone H3 Lysine 9 [89–91].

6.2. κ locus activation and IgLC allelic exclusion

Recent attention has focused on the mechanism of IgLC allelic exclusion. While productive IgLC gene rearrangement leads to the formation of a BCR and inactivation of the recombinase, mechanisms must exist to prevent the near simultaneous rearrangement and expression of both κ alleles in a pre-B cell. Liang et al. [92] recently reported that high-level activation of the κ locus in pre-B cells occurs in only a small fraction of pre-B cells at any given time. Using a promoterless GFP cDNA reporter knocked into the coding region of Jκ1, these investigators measured the percentage of cells turning on a κ allele at each stage in development using flow cytometry. They found that pro-B cells from heterozygous mice did not express the reporter, while only 5% of small pre-B cells did. Further experiments showed that κ activation was mono-allelic and thus may contribute to allelic exclusion of IgLC rearrangement. The authors suggest that some threshold level of a combination of particular enhancer-binding transcription factors might allow for the rare and probabilistic activation and rearrangement of a single κ allele at a time.

In contrast, Bergman and co-workers found biallelic germline transcription in single-cell RT-PCR assays performed on pre-B cells [93]. They corroborated this finding with RNA-FISH performed on AMuLV-transformed pre-B cells stimulated with LPS, which revealed that 91% of cells expressed κLC from both alleles. These results conflict with those from Liang et al. discussed above who suggest monoallelic germline transcription precedes rearrangement. Differences between these two sets of results may have to do with the amounts of germline transcripts detected in two different assays (GFP expression versus high cycle-number PCR).

DNA cytosine methylation can inhibit recombinase activity [94] and monoallelic DNA de-methylation of the Igκ locus correlates with its activation [95,96]. DNA methylation status is not affected by loss of Eκ3′ but surprisingly both alleles are demethylated in Eκi$^{-/-}$ B cells. In the absence of both enhancers, however, alleles remain fully methylated [78]. Results from the Schlissel group corroborate the correlation of demethylation with activation, as the modified κ-GFP allele was unmethylated in GFP^{+} pre-B cells, but was highly methylated in GFP^{-} cells. Surprisingly, unrearranged κ alleles in mature B cells remain CpG methylated but are nonetheless highly transcribed casting doubt on causality in the relationship between DNA methylation and Igκ locus activation in this later stage.

The timing of replication during S phase is associated with gene expression [97]. At the Ig loci, where alleles appear to be activated one at a time, replication is asynchronous [98]. This observation has led to the hypothesis that early replication could be associated with the allelic choice for recombination. Asynchronous replication begins in early embryogenesis, thus pre-BCR signaling does not influence this phenomenon. Each of these epigenetic events is correlated with κLC rearrangement but the order of events needs to be established.

7. Conclusion

The transition of B cell precursors through the pre-BCR checkpoint (i.e. from the pro-B to pre-B cell stage) requires tightly coordinated signals emanating from surface pre-BCR that directs complex changes in the pattern of gene expression and even movement of entire chromosomes within the nucleus. Alterations in nuclear transcription then redirect the V(D)J recombinase to different rearranging Ig loci creating molecules that then replace the originating receptor with a new molecule, the BCR. While the earlier steps in signaling cascades are quickly being teased apart despite the complexity of redundant pathways, and enhancers responsible for IgHC and κ locus activation are known, pathways linking signaling to retargeting of the V(D)J recombinase remain vague at best. While over-expression of particular signaling molecules or transcription factors can mimic certain aspects of development, the nature of signaling pathways makes over-expression and knock-out studies difficult to interpret. This is particularly true in light of a stochastic model of κ activation, which requires the cumulative action of multiple factors. A better understanding of chromatin structure's precise role in accessibility and the coordinated activity of chromatin remodeling enzymes and transcription factors will help identify key components involved in transmitting signals from the pre-BCR to the μHC and κLC loci, and may also determine causality of these correlated events.

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