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J Immunol published online 23 July 2014
http://www.jimmunol.org/content/early/2014/07/23/jimmunol.1401275

Supplementary Material
http://www.jimmunol.org/content/suppl/2014/07/23/jimmunol.1401275.5.DCSupplemental

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Zfp318 Regulates IgD Expression by Abrogating Transcription Termination within the Ighm/Ighd Locus

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The protein Zfp318 is expressed during the transition of naive B cells from an immature to mature state. To evaluate its role in mature B cell functions, a conditional gene deficiency in Zfp318 was created and deleted in bone marrow lineages via Vav-Cre. B cell development was minimally altered in the absence of the protein, although transitional 2 (T2) B cell populations were depressed in the absence of Zfp318. Intriguingly, the analysis of IgM and IgD expression by maturing and mature naive B cells demonstrated an elevated level of IgM gene products and a virtual loss of IgD products. Transcriptome analysis of Zfp318-deficient B cells revealed that only two gene products showed altered expression in the absence of Zfp318 (Ighd and Sva), demonstrating a remarkable specificity of Zfp318 action. In the absence of Zfp318, Ighm/Ighd transcripts, which would normally encode IgM and IgD from heterogeneous nuclear RNA transcripts via alternative splicing, lack intron and exon sequences from the IgD (Ighd)–encoding region. This finding indicates that Zfp318, in a novel manner, functions by repressing recognition of the transcriptional termination site at the 3′ end of the terminal IgM-encoding exon, allowing for synthesis of the complete Ighm/Ighd heterogeneous nuclear RNA.

The Journal of Immunology, 2014, 193: 000–000.

The differentiation and maturation of immature transitional B cells of the marrow into mature but naive B cells requires the coordinated expression of a number of gene products (1). Pax5 and the E2 transcriptional regulators are widely regarded as central for inducing the expression of many of the early B cell–specific products (such as CD19 and IgM) in maturing B cells (2, 3). Later, as B cells enter the periphery, additional transcription factors, such as MeC2e, NF-κB family members, NFAT proteins, Ciita, and Notch signaling family members, continue the differentiation of immature B cells into mature follicular (FO) or marginal zone (MZ) cells (4, 5). A number of studies have sought to define the key transcriptional regulators and kinetics that control this pathway. In one such analysis, we examined the expression of transcriptional regulators during B cell differentiation, comparing B220+ B cells obtained from the marrow of 2-wk-old animals (thus lacking mature recirculating B cells), from spleens of 2-wk-old animals [enriched for transitional 1 (T1) and transitional 2 (T2) B cells], and from mature but naive spleens (enriched for FO and MZ B cells) (6). That gene expression screen identified a number of candidate transcriptional regulatory proteins whose expression increased with B cell maturation. Zfp318 was one such factor.

The Zfp318 gene encodes a primary protein of 2237 aa that includes domains encoding two C2H2 zinc fingers and a myosin II-homology sequence as well as regions rich in serine and basic amino acids (7, 8). A smaller truncated form of Zfp318 has also been described in the mouse, but only the full-length form has been found as a homolog in human tissues. Zfp318 has been most extensively studied in the testes (an alternative name for the protein is testicular zinc-finger, or TZF) where it is expressed during spermatogenesis. In transfection analyses, Zfp318 was shown to augment ligand-dependent androgen receptor (AR) control in a dose-dependent fashion (9). The possibility that Zfp318 could also play a role in B cell development via the AR was suggested by a number of studies showing B cell functions dependent upon AR (10, 11). In one such study, using a B cell–specific deletion of AR, B cell development from the marrow was inhibited, accompanied by enhanced autoimmune responses, using a collagen-induced arthritis model (12).

To determine if Zfp318 does have a role in the development and function of B cells, we created a mouse with a conditional (Flox’d) deletion of the gene. Using Vav-Cre–dependent deletion of the gene, we have found that B cells deficient in Zfp318 developed from marrow precursors virtually identical to that of wild type (WT). Zfp318-deficient, naive splenic B cells did, however, demonstrate a dramatic loss of both IgD-specific transcripts and protein. IgM and IgD are synthesized from alternatively spliced transcripts to produce the IgM product owing to RNA transcript termination prior to the IgD-encoding exons. The role of Zfp318 in regulating gene products is highly specific for IgD, in that genome-wide transcriptome analysis of B cells obtained from the Zfp318-deficient animal identified only a single additional gene with significantly altered gene expression.

As our data were being prepared for submission, a report by Enders et al. (15) detailed the results of an ethylnitrosourea mutagenesis screen followed by whole-exome sequencing that identified...
a single point mutation (a nonsynonymous T > C transition altering the protein sequence I1347T) within Zfp318 that inhibited the production of IgD. This mutation mapped to the long form of the protein, confirming its necessity versus that of the alternatively spliced, truncated form of the protein. The group also created a germline Zfp318 null animal by gene targeting that reproduced their point mutation analyses. Those data, along with the findings presented in this article, confirm the necessity of Zfp318 for the production of IgD.

Materials and Methods

Animal strains and care

Animals were housed in the Animal Resource Center (University of Utah Health Science Center, Salt Lake City, UT) according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animal protocols were reviewed and approved by the University of Utah Institutional Animal Use and Care Committee. For transcriptional profiling experiments, C57BL/6 mice were bred and maintained in house. B6.Cg-Tg (Vav1-cre)A2Kio/J mice (Stock #008449) were obtained from The Jackson Laboratory and maintained as heterozygotes. Zfp318+/- mice were bred to the Vav-Cre deleter strain to obtain progeny with a hematopoietic-specific deletion of Zfp318 [conditional knockout (cKO)]. Littermates approximating 4 wk of age were used for all experiments. Numbers of animals used per experiment are noted in the figure legends.

Generation of the Zfp318+/- KO animal

A targeting construct for Zfp318 was created using a 14-kb homologous fragment containing the first two exons of the gene obtained from a 129/Sv phage library. A neo targeting cassette (flanked by FRT sites) and a single LoxP site was inserted at the Hpa1 site located in the intron between exons 2 and 3. A single LoxP site was inserted at the BspE1 site 5’ of the transcription start site. 129/Sv ES clones possessing the targeted Zfp318 gene were confirmed by Southern blot using 5’ and 3’ probes. The neo cassette was removed by a single mating to a FRT deleter strain, leaving behind the Zfp318 KO allele. Animals were genotyped by PCR specific for the products and DNA isolated from either the tail or the bone marrow of heterozygote (Zfp318+/+) or homozygous (Zfp318/-) animals (Fig. 1B). Deletion of the sequences between the two LoxP sites would thus result in a conditional Zfp318-deficient animal, and analysis of B cell development in the absence of Zfp318.

FACS analysis and sorting of hematopoietic cell populations

Upon dissection, the plunger of a 5-ml syringe was used to dissociate thymus and spleen tissues. Cells were strained through a 100-μm filter and collected in 10 ml FACS buffer (1× PBS + 0.1% BSA). Bone marrow was collected from both tibias and tibiae by propping up the ends of each bone with a razor blade exposed bone cavities. Marrow was flushed from cavities using a 5/8-inch 25-gauge syringe and FACS buffer. Contents were collected in 8 ml FACS buffer. After centrifugation, erythrocytes were lysed on ice for 10 min using ACK buffer. Following lysis, cells were resuspended in FACS buffer and counted using a hemocytometer. Cells were stained on ice for 30 min using the appropriate Ab mixture. Stained cells were washed with FACS buffer, centrifuged, and resuspended in FACS buffer. To discriminate between live and dead cells, DAPI was added at a final concentration of 3 μM. The Abs used with their indicated dilutions are available in Supplemental Table I. Population analysis was performed on the FACSCanto II (BD Biosciences), and results for a given cell type are graphically represented as mean values ± SEM of total live cells or ratios of live cells analyzed per tissue. Cell sorting of select populations was performed on the Aria Cell Sorter (BD Biosciences) at the University of Utah Flow Cytometry Core.

Analysis of whole blood cell populations

Blood was collected in heparinized capillaries via the retro-orbital route. Complete blood counts were obtained using a Hemavet 950 FS (Drew Scientific). Results for a given parameter are listed in Supplemental Table II and are represented as mean values ± SEM.

RNA-sequencing analysis

A total of 5 × 10⁶ splenic CD19+ B cells were FACS sorted from three WT and three Zfp318 cKO mice. Total RNA was isolated as described above. A total of 50 ng total RNA was used for RNA-sequencing (RNA-Seq) library preparation, using the Illumina TruSeq RNA Sample Preparation kit with the polyA enrichment step omitted. Libraries were subjected to HiSeq2000 50 Cycle Single Read Sequencing. Greater than 20 × 10⁶ reads per sample were obtained and aligned to the mm10 (Ensembl build 75) transcriptome index, using Novoalign. Aligned reads were further processed for splicing and expression variance with the UsEquation 8.7.4 software package. Data tracks were visualized with the University of California–Santa Cruz genome browser.

Statistical analysis

For FACS, RT-PCR, and complete blood count data, two-tailed unpaired Student t tests were applied for all relevant statistical comparisons, using GraphPad Prism software. For RNA-Seq analysis, two-tailed Student t tests using two-sample equal variance were performed with Microsoft Excel. Statistical cutoffs are noted in the figure legends.

Results

Analysis of Zfp318 expression, the generation of a Zfp318-deficient animal, and analysis of B cell development in the absence of Zfp318.

Our previous screen of maturing and mature B cells for the expression of Zfp318 used gene grid array analysis of pooled age- and tissue-specific B cells (6). To expand and specify the time frame of Zfp318 expression during B cell development, we FACS sorted and isolated pro, pre, and newly formed (NF) B cells from the bone marrow, and T1, T2, MZ, and FO B cells from the spleen (Supplemental Fig. 1). RNA was extracted and analyzed for Zfp318-specific transcripts. As shown in Fig. 1A, the presence of the Zfp318 transcript parallels the maturity of the cell, similar to our previous observation. Of interest, although the FO cells showed the highest level of expression, the MZ cells had dramatically less Zfp318 transcript, similar to levels seen in the NF marrow population.

To create a conditional Zfp318-deficient animal, we generated a targeting construct that possessed a LoxP site upstream of the transcriptional start site and a second LoxP site ∼7000 bp 3’ (Fig. 1B). Deletion of the sequences between the two LoxP sites would thus remove the promoter sequences, the initiating ATG, and the first two coding exons of the gene. This construct was used to create a germ-line-targeted animal, which was then bred to the Vav-Cre deleter line (maintained as a heterozygote). Genotyping of this animal (Fig. 1C) for the WT and flox’d/knockin alleles was done using PCR primers specific for the products and DNA isolated from either the tail or the bone marrow of heterozygote (WT/flo) or homozygous Zfp318-targeted animals (flo/flo) with or without the Vav-Cre deleter transgene. With use of the P1 and P2 PCR primers, the 175-bp fragment of WT DNA is evident, as is the same sequence containing the inserted LoxP site in the tail of the heterozygote and KO. However, that sequence is entirely lost in the marrow of the KO animal possessing the Vav-Cre construct, indicating a very efficient deletion of those sequences in the tissue expressing the Vav-Cre transgene. The loss of Zfp318 expression was confirmed and quantified in the spleen of the cKO compared with that of WT (Fig. 1D), using the primers described in Supplemental Table I (4913/4914).
The development of B cells in the cKO animal was analyzed. The percentage of CD19+ cells and quantification of total cells of the marrow (Fig. 2A) were assessed using markers to define the pro, pre, NF, and mature, recirculating B cells (CD22+AA4.1+ in 4-wk-old animals. No significant difference was noted between the WT and cKO marrow samples. A similar analysis was carried out using the T1, T2, and FO populations of the spleen (Fig. 2B). Again, the cKO animals closely tracked with WT animals, except for a slight but significant depression in T2 B cells in the cKO animals. Hemavet analysis of peripheral blood also showed no

FIGURE 1. Zfp318 is highly expressed in mature follicular B cells and is conditionally deleted via Vav-Cre. (A) RT-PCR of cDNA derived from the indicated B cell subsets. Four biological replicates were assayed for each subset. *p < 0.05, ***p < 0.001. (B) Schematic diagram of the Zfp318flox conditional allele. Cre-mediated recombination of LoxP sites results in the deletion of the Zfp318 proximal promoter as well as exons 1 and 2 (black boxes). (C) Representative genotyping of animals possessing targeted alleles (fl/fl) or heterozygous alleles (WT/fl) in the presence or absence of the Vav-Cre deleter construct. Analysis of genomic DNA isolated from the tail or bone marrow shown using primer set P1/P2 (Supplemental Table I). The Zfp318fl/fl (Zfp318 KN) allele is deleted only in cells isolated from the bone marrow, demonstrating the hematopoietic specificity of gene deletion. (D) Loss of Zfp318 transcripts was validated using cDNA derived from WT and cKO total spleen tissues. Three biological replicates were performed per genotype. For (A) and (D), Zfp318 transcripts were normalized to Actb, and data are represented as the mean ± SEM. ND, not detected.

FIGURE 2. FACS analysis of B cell development in the Zfp318 cKO animal. (A) Bone marrow and (B) spleen tissues from WT and cDKO animals were assessed by FACS for the disruption of B cell development as a result of Zfp318 deletion. Three to four animals per group were analyzed. In (A) and (B), data represent mean percentage (left) or absolute cell count (right) ± SEM for an indicated cell population. *p < 0.05, **p < 0.01. Recirc, mature recirculating.
significant difference between WT and the Zfp318 cKO animal (Supplemental Table II).

The absence of Zfp318 results in the loss of IgD

The previous analyses indicated that the development of mature B cells in the cKO animal was very similar to that of WT, but did not determine if the lack of Zfp318 altered the expression of B cell gene products. As a primary screen, we analyzed the cKO splenic B cells for the expression of mature marker proteins such as CD35, CD21, B220, and CD19 and found expression levels of these proteins to be indistinguishable from those of WT (Fig. 3A–C). When assaying for the expression of IgM and IgD, the two Igs expressed by mature naive B cells, we were surprised to find the level of IgM elevated in the cKO animals compared with WT. As shown in Fig. 3D, cells from the cKO animal (gray filled plot) expressed significantly higher levels of IgM than did either WT or the Zfp318 cKO heterozygote animal in the T2 and FO populations. The analysis of IgD expression demonstrated the complete lack of IgD surface protein on cells of T1, T2, MZ, and FO populations compared with WT and heterozygote animals (Fig. 3E). The differences in expression levels of IgM and IgD were quantified by analysis of mean fluorescence intensity (MFI) in the WT and cKO populations (Fig. 3F). The elevated level of expression of IgM in the cKO cells was significantly different from that of WT in the T2, FO, and MZ populations, whereas the loss of IgD in the T1, T2, FO, and MZ populations was significantly different in the cKO samples.

Both IgM and IgD are encoded from a single heterogeneous nuclear RNA (hnRNA) transcript via alternative splicing of the VDJ sequences to those exons encoding the C region domains. To
determine if these differences in surface expression of IgD and IgM on the surface of Zfp318-deficient B cells were reflected in transcripts specific for the IgM and IgD products, quantitative RT-PCR was performed using RNA obtained from total spleen (WT and cKO) and analyzed using primers specific for the IgM (Ighm) and IgD (Ighd) constant regions. As shown in Fig. 3G, the level of IgM transcripts trended higher in the cKO animal, whereas those encoding IgD in the cKO animal were reduced >90% compared

FIGURE 4. RNA-Seq analysis of CD19+ splenic B cells from WT and Zfp318-deficient animals. (A) Transcriptome analysis of WT and cKO splenic CD19+ B cells. Three replicates per genotype were sequenced and analyzed (Supplemental Table III). On the left, log2 fold change per gene between WT and cKO samples is plotted against the p value. On the right, WT and cKO FPKM are plotted for all genes analyzed. Genes were considered significantly altered with a log2 fold change > 2 and a p value < 0.01. (B–E) Representative custom tracks (left) and RT-PCR validation (right) for selected genes of interest. (B) Zfp318 expression is lost in the knockout samples. (C) The alternatively spliced Cr2 locus demonstrates equivalent expression in WT and cKO CD19+ B cells. (D) The genomic region encompassing Sva shows global transcript enrichment. (E) Expression of the Ighm/Ighd locus is significantly impaired in the cKO specific to the regions for the IgD-encoding exons (Ighd) (>95% reduction). *p < 0.05.
The absence of Zfp318 blocks IgD production by transcriptional termination at the end of the IgM-encoding exons

The loss of IgD-encoding transcripts (and IgD protein) in the absence of Zfp318 suggested this protein alters transcript quantities. To determine if this effect was unique to that of IgD, we performed RNA-Seq using RNA obtained from purified CD19+ splenic B cells from three WT and three cKO animals (Supplemental Table III). Analysis of those data indicated that only three gene products showed significantly altered expression in the cKO animals: the Zfp318 gene itself; the region of the Igm/Ighd locus encoding the IgD protein; and Sva, a gene on mouse chromosome 6 that encodes a “seminal vesicle Ag” unique to mice (Fig. 4A). RNA-Seq data from three genes are shown (Fig. 4B-E), using one representative WT and cKO sample. As expected, Zfp318 transcripts are absent in the cDKO (Fig. 4B), the Cr2 gene products (which produce the Cr1 and Cr2 proteins via alternative splicing) are identical between the two samples (Fig. 4C), and the Igm and Ighd loci show IgM-specific transcripts in both WT and cKO, but absence of IgD-specific transcripts in the cKO animal (Fig. 4E). Therefore, in these B cell populations, Zfp318 uniquely influences only Ighd and Sva expression.

The loss of IgD-encoding transcripts in the cKO B cells could be due to at least two mechanisms: blocking the alternative splicing of the IgM- and IgD-encoding transcripts, or blocking the hnRNA transcription of the Igm locus to the sequences encoding the IgD protein. The Igm/Ighd locus contains at least three distinct transcriptional stop sites, including one just 3′ of the exons encoding secreted IgM, a second site allowing for the production of membrane bound IgM, and a third site 3′ of the last exon encoding the mature, membrane-bound IgD protein (16–19). To encode both IgM and IgD via alternative splicing of hnRNA, the transcriptional stop sites linked to the IgM-encoding exons must be ignored by the Pol II RNA polymerase complex. A close analysis of the RNA-Seq data obtained from the Igm/Ighd locus revealed a dramatic loss of virtually all RNA transcripts 3′ of the IgM-encoding exons (Fig. 5A), suggesting that production of hnRNA containing the IgD-encoding sequences was significantly reduced in the absence of Zfp318.

The production of Igm/Ighd hnRNA in the cKO animal was further quantified by RT-PCR analysis of intronic sequences from IgM-encoding and IgD-encoding regions of the locus. Two primer sets (Fig. 5A) were used to screen total RNA via RT-PCR. The first primer set (primer set A) included an IgM-specific intronic sequence and a turnaround within the last exon of the IgM region, and the Igm and Ighd loci show IgM-specific transcripts in both WT and cKO, but absence of IgD-specific transcripts in the cKO animal (Fig. 4E). Therefore, in these B cell populations, Zfp318 uniquely influences only Ighd and Sva expression.
whereas the second set of primers (primer set B) was 3′ of the Ighm transcriptional stop site to generate membrane-bound IgM. The relative products from these two sets of primers were quantified (Fig. 5B) by establishing a ratio of the IgD-intronic sequence (primer set B) versus the IgM-intronic sequence (primer set A). Clearly, there is a marked change in ratio of these intronic sequences comparing the WT with the cKO samples (70% reduction in the cKO). RT-PCR analyses of RNA samples can be clouded by the presence of genomic DNA. To control for this, triplicate WT and cKO RNA samples (and genomic DNA as control) were analyzed using primers specific for a region of the Cd3ε gene that is not transcribed, and using an oligo set within the final coding exon of the IgM protein (primer set C) (Fig. 5A). The cDNA samples obtained from the WT and cKO RNA samples did not demonstrate amplification of the Cd3ε-associated sequences but did show equal quantities of the Ighm gene product (Fig. 5B), indicating that amplification of genomic sequences from the IgM- and IgD-encoding regions of the Ighm locus was from hnRNA, not genomic DNA.

Discussion

Naive FO cells express both IgM and IgD, whereas MZ cells preferentially express IgM (Fig. 6 B, C). Expression of Ighd in naive maturing B cells closely parallels that of Zfp318 (Fig. 6A, C from Fig. 1A). Upon activation, FO B cells depress IgD expression and, with additional stimulation and T cell help, class switch to additional Ab isotypes. The historical view of IgM versus IgD production has been based upon an alternative splicing model in which the same VDJ coding sequences are alternatively spliced to exons encoding the constant regions of IgM or IgD. Clearly this requires an hnRNA that encompasses the entire Ighm/Ighd locus. Currently, very little is known about what regulates the differential expression of transcripts coding for IgM and IgD via alternative splicing of a common VDJ region to the IgM- or IgD-encoding exons. Indeed, the best parallel to this system may be in the analysis of the U1A-mediated regulation of membrane versus secreted IgM. Similar to Zfp318, U1A expression levels are regulated throughout B cell development/differentiation, with high expression being required for the repression of secretory IgM (20). U1A acts to block the expression of secretory IgM by impairing the binding and, hence, downstream function of the polyA cleavage complex, which includes CPSF (cleavage and polyadenylation specificity factor), Cstf, and polyA polymerase (20–23). U1A mediates this suppression by interacting with multiple nonconsensus U1A binding sites (AUGC-core) both upstream and downstream of the AAUAAA polyA signal. Owing to the nature of these binding sites, a mass action effect of U1A is required to achieve full repression. A similar scenario may be true for the function of Zfp318, as high Zfp318 gene expression levels correlate with high Ighd levels in various B cell populations (Fig. 6). Similar to the secretory IgM polyA site, three AUGC sites are clustered ∼500 bp upstream of the membrane IgM polyA signal. Whether these binding sites are functional for Zfp318 recognition remains to be determined. However, Zfp318 does possess multiple nonconsensus U1A binding sites, with high expression levels correlating with high Ighd levels in various B cell populations (Fig. 6).

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**FIGURE 6.** Model of Zfp318 transcriptional regulation throughout the Ighm locus. (A–C) Quantification of (A) Zfp318, (B) Ighm, and (C) Ighd coding transcripts within the indicated B cell subsets. (A) was reproduced from Fig. 1A. *p < 0.05, **p < 0.001. In (B), # represents comparisons between the indicated cell type and the T2 population, with #p < 0.05 and ###p < 0.001. In (C), $ represents comparisons between the indicated cell type and the FO population, with $$S p < 0.001. (D) Stylized depiction of transcription throughout the Ighm/Ighd locus (not drawn to scale). In the presence of Zfp318 (top), transcriptional termination is blocked, and transcription continues past the terminal Ighm-specific exon. This allows for splicing to Ighd-specific exons and generation of a mature IgD protein. Upon the loss of Zfp318 (bottom), transcription is terminated at the final Ighm-specific exon. As a result, the downstream Ighd-specific exons cannot be transcribed or spliced into a mature transcript.
the binding of U1A, leading to a more stable repression of polyA usage. This idea is partially supported by data in Fig. 5, showing the 70% loss of transcription downstream of the membrane IgM polyA signal rather than a complete block upon Zfp318 deletion. Our analysis of Zfp318-deficient B cells shows that the absence of IgD expression is linked to the preferential usage of a transcription stop site located within the final Ighm exon, thus blocking synthesis of the full Ighm/Ighd hnRNA, which is required for the production of IgD (Fig. 6D) (16, 18). This finding implicates the Zfp318 protein as functioning to block recognition of the IgM-specific transcriptional stop site, allowing for the RNA Pol II complex to proceed down the chromosome into the IgD-encoding region. This finding suggests that Zfp318, which possesses two putative nucleic acid–binding zinc finger domains (7), recognizes the IgM-specific transcriptional stop site and inhibits transcription termination at that site, possibly by inhibiting RNA pausing and transcription termination via the CPSF protein complexes or those required for cleavage (24–26). It also suggests that the regulation of Zfp318 is intimately tied to B cell differentiation (MZ B cells express low levels of both Zfp318 and IgD, whereas FO cells express much higher levels of both gene products) and that FO B cell activation, which results in the loss of IgD (but expression of IgM), may also regulate the expression of Zfp318.

We know of no other instance in mammalian cells in which a transcription stop site is blocked, in a similar fashion to what we see for the Ighm locus, to allow for the production of a longer hnRNA molecule and increased genetic capacity. The IgM-encoding region of Ighm also possesses a transcription stop site that generates the smaller, secreted form of the protein that must also be ignored by the Pol II complex in the naive B cell. This site is not blocked by Zfp318; if it were, we might have expected only secreted IgM in the absence of Zfp318. However, whether this second site is blocked by another protein functioning in an analogous fashion to Zfp318 is not known.

The expression of Zfp318 parallels that of other transcriptional regulators during the maturation and development of B cells. One such regulator, MeFC2, also demonstrates enhanced expression in FO cells compared with MZ cells. Our previously published analysis of MeFC2-deficient B cells demonstrated a dramatic reduction in expression of Zfp318 (27). This reduction in Zfp318 expression, however, was not to the same magnitude as a Zfp318 gene deficiency. Indeed, B cells lacking MeFC2 still express IgD (28), suggesting that sufficient Zfp318 was present to allow for Ighd transcription.

The analyses of our Zfp318 deficiency via Vav-Cre phenocopies the results described by Enders et al. (15), although a relative depression of T2 B cells of the spleen was not noted in their report. Enders et al. further suggested that Zfp318 served to regulate IgD production by controlling the alternative splicing of the hnRNA of the Ighm/Ighd locus, although no data were presented to support this model (15). Our conclusions regarding the proposed function of Zfp318 differ from those presented by Enders et al. because of our RNA-Seq data, which point to the regulation of transcription termination as the event that subsequently allows for the production of IgD via alternative splicing. Thus it is the primary production of the Ighm/Ighd hnRNA that is the key step regulated by Zfp318, not the alternative splicing choice of the VDJ sequence to either the IgM- or the IgD-encoding exons.

Acknowledgments

We thank the University of Utah Core facilities (FACS, RNA-Seq, Transgenic and Knockout Mouse, and Data Analysis), specifically Dr. Susan Tomowski, Dr. David Nix, and Dr. Tim Mosbruger, for assistance; Dr. Dean Tantin for the use of Hemavet; and members of the Weis laboratories for critique of this work and many useful suggestions.

Disclosures

The authors have no financial conflicts of interest.

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