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Blimp-1; Immunoglobulin Secretion and the Switch to Plasma Cells

R. Sciammas[1] (✉) · M. M. Davis[2]

[1] Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637, USA
rsciamma@uchicago.edu
[2] Department of Microbiology and Immunology and the Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305, USA

Abstract The transcription factor Blimp-1 governs the generation of plasma cells and immunoglobulin secretion. Recent microarray experiments indicate that Blimp-1 regulates a large set of genes that constitute a significant part of the plasma cell expression signature. The variety of differentially expressed genes indicates that Blimp-1 affects numerous aspects of plasma cell maturation, ranging from migration, adhesion, and homeostasis, to antibody secretion. In addition, Blimp-1 regulates immunoglobulin secretion by affecting the nuclear processing of the mRNA transcript and by affecting protein trafficking by regulating genes that impact on the activity of the endoplasmic reticulum. Interestingly, the differentiation events that Blimp-1 regulates appear to be modulated depending on the activation state of the B cell. This modulation may be due at least in part to distinct regions of Blimp-1 that regulate unique sets of genes independently of each other. These data hint at the complexity of Blimp-1 and the genetic program that it initiates to produce a pool of plasma cells necessary for specific immunity.

1 Plasma Cell Biology

Immunoglobulins, or antibodies, are serum proteins produced by the B cell arm of the adaptive immune system and serve as a specific barrier to primary and secondary infections. B cells clonally produce antibodies capable of binding thousands, if not millions, of different entities. The process by which a B cell becomes an antibody-secreting plasma cell is regulated at multiple checkpoints throughout the lifetime of the differentiating B cell, in order to secure both specific immunity and to prevent the production of autoreactive B cells.

B cell differentiation is managed by a coordinated and reiterative system of signaling and transcriptional regulators that act in response to extracellular cues with the purpose of driving gene expression programs that incrementally promote B cells through successive steps of lymphoid homeostasis and immune response (Glimcher and Singh 1999). The homeostatic steps include the production of rearranged antibody genes, the quality control of the antibody molecule, the lifespan, and the migration of B cells throughout the lymphatics. The immune response steps include responding to T-independent or T-dependent antigen, clonal expansion, processing foreign antigen, interacting with T cells and dendritic cells, and modifying the antibody genes to increase antigen affinity and effector function, specialized processing of the Ig transcript, and finally generation of a highly efficient secretory apparatus to facilitate antibody secretion. Upon termination of the immune response, namely through antigen clearance, a small pool of high-affinity memory or long-lived secretory B cells are retained in anticipation of secondary infections. The latter is termed a plasma cell by virtue of the high secretory capacity of these cells, which is necessary to maintain the protective levels of serum immunoglobulin. Whereas many of the signaling and transcription factors that control the early phases of both the homeostatic and immune response phases of B cell differentiation are steadily being characterized, the characterization of the factors that contribute to the homeostatic and immune response aspects of the late phases of B cell differentiation are in their infancy.

Plasma cell malignancy, or multiple myeloma, is a disease with a high incidence of morbidity where treatment is not yet curable (Hideshima et al. 2003). Knowledge of the properties of plasma cells may aid in the development of better therapy. Additionally, understanding the mechanisms of plasma cell generation and maintenance may aid in vaccine design where humoral immunity plays a critical role.

1.1 The Secretory Switch

The immunoglobulin locus extends over 1 Mb and is a highly complex unit involving multiple gene and regulatory elements (Ernst and Smale 1995). The primary transcript is subject to alternative splicing and poly-adenylation site usage that produces two mature transcripts differing in their 3′ ends (Alt et al. 1980; Early et al. 1980; Rogers et al. 1980). The longer transcript encodes the membrane bound form of the protein and the shorter transcript encodes the secreted protein. Production of the membrane transcript is dependent on a splicing event that removes a portion of the secreted transcript's exon and the secretory poly-A site, and production of the secreted transcript involves preferential usage of the secretory poly-A site and arrest of alternative splicing (Peterson et al. 1991). 3′ end processing is developmentally controlled such that mature B cells produce each form in equal amounts and plasma cells primarily produce the secreted form, approaching a ratio of 100:1 (Edwalds-Gilbert et al. 1997). The position and strength of the *cis*-acting splice and poly-A sites are integral to regulated processing; however, Ig gene-specific signals are not necessary (Peterson 1994). Strikingly, in plasma cells, it appears that the differentiation-specific event is accounted for by the enhanced activity, binding, and recruitment of the poly-adenylation complex to the secretory poly-A site (Edwalds-Gilbert and Milcarek 1995). Expression levels of the RNA-binding and specificity factor of the poly-adenylation complex, CstF64, is implicated in mediating the differentiation-specific switch (Takagaki et al. 1996); however, differential expression is not observed in all experimental systems (Martincic et al. 1998; Sciammas and Davis 2004). Nevertheless, CstF64 and the core poly-adenylation complex are necessary for proper processing (Takagaki and Manley 1998). In addition, it has been proposed that levels of hnRNP F can influence this decision (Veraldi et al. 2001). Recent developments indicate that general control of 3′ end processing is integrated during transcript initiation and the by nature of the polymerase II complex (Proudfoot et al. 2002). Therefore, elucidation of the differentiation-specific mechanism may require analysis beyond the secretory polyA site.

1.2 The History of Blimp-1

Blimp-1 is a five-zinc-finger transcription factor of the C2H2 family whose expression is induced during the immune response phase of B cell activation and is maintained in plasma cells (Turner et al. 1994). Independent studies of the *Blimp-1* human orthologue (*PRDFI-BF1*) identified a DNA-binding site in the β-interferon promoter, which is mediated by the first two zinc fingers (Keller and Maniatis 1991a, 1991b). This report also indicated that Blimp-1 expression is inducible during the antiviral response of the fibroblastoid cell line HeLa. In addition, *Blimp-1* expression is inducible in terminal-differentiating macrophage cell lines (Chang et al. 2000). Lastly, expression is restricted to a few cell types, as determined from the EST sequencing efforts, and includes the lymphoid system, head and brain, and embryo. Preliminary studies indicate that Blimp-1-null mice exhibit embryonic lethality (S. Vincent, R. Sciammas, M.M. Davis, and E. Robertson, unpublished observations). This is an exciting finding as the *X. laevis* orthologue, *xBlimp-1*, has roles in embryonic development and head formation (de Souza et al. 1999; Chang et al. 2002). Although these data indicate that Blimp-1 has roles in multiple tissues and aspects of gene regulation, this review will focus on recent work that has focused on B cell maturation.

Initial experiments demonstrated that Blimp-1, when ectopically expressed in mature B cell lines, can induce several hallmarks of the plasma cell phenotype, especially the switch to Ig secretion (Turner et al. 1994; Schliephake and Schimpl 1996). Thus the creation of the acronym Blimp-1 (B lymphocyte-induced maturation protein-1). This observation indicated that Blimp-1 was a potential "master regulator" of B cell terminal differentiation. Subsequently, Blimp-1 was found to repress the expression of *c-myc*, a key determinant of cells exiting the cell cycle and regulating their telomeres during the process of cellular senescence and terminal differentiation (Lin et al. 1997). Notably, this aspect of Blimp-1 activity is not sufficient to induce other hallmarks of plasma cell differentiation, including antibody secretion (Lin et al. 2000). Additionally, the gene *C2ta*, known to be silenced in plasma cells, was found to be directly repressed by Blimp-1, indicating that it has roles in controlling diverse aspects of plasma cell differentiation, in this instance restricting antigen presentation (Piskurich et al. 2000). Both of these genes contain functional Blimp-1 binding sites in their promoters.

The primary structure of Blimp-1 suggests the existence of multiple functional elements. In fact, the proline-rich region in the middle of the

sequence is necessary for repression of both the *c-myc* and *C2ta* promoters in reporter constructs (Yu et al. 2000; Ghosh et al. 2001). This is accomplished via recruitment of both the Groucho family of corepressors and members of the Histone deacetylase family of chromatin remodelers (Ren et al. 1999; Yu et al. 2000). Second, the roles of the additional zinc fingers that do not contribute to the known DNA-binding specificity are unknown. Third, the carboxy terminus, following the zinc finger region, is enriched for acidic residues, an attribute of many transcriptional transactivators. Fourth, the PR domain of Blimp-1 exhibits striking homology to the recently characterized catalytic SET domains, which have roles in chromatin remodeling by site-specific histone methylation (Huang et al. 1998; Jiang and Huang 2000; Jenuwein 2001). The roles of these regions in the master regulator activity of Blimp-1 during B cell maturation remain to be discovered.

This review will focus on four aspects of Blimp-1 and plasma cell biology. First, we present evidence that supports a role for Blimp-1 in transcriptional regulation of Ig secretion. Second, we discuss recent studies cataloguing the nature of the genetic program driven by Blimp-1 activity and the comparison of this program with the expression "signature" of a differentiated plasma cell. Third, we describe recent experiments that show that execution of the maturational program is regulated by distinct modules of Blimp-1. Lastly, we present evidence supporting the role of Blimp-1 in regulating Ig secretion at the post-translational level. These results impact on our basic knowledge of plasma cells and thus may contribute to better therapies for multiple myeloma and more efficient vaccine development.

2
Transcriptional Regulation of Ig Secretion

When expressed in a number of B cell lines or in stimulated primary B cells, Blimp-1 induces Ig secretion. We sought to determine whether Blimp-1 exerted its effect during 3′ end formation of the Ig transcript (Sciammas and Davis 2004). This can be discriminated by assessing the production of both the membrane and secretory transcripts using Northern analysis. Indeed, a 10:1 ratio of secretory to membrane transcripts is observed in Blimp-1-transduced M12 B cells compared to a 1:1 ratio in controls (Fig. 1A). Interestingly, the abundance of total Ig message is increased in parallel, indicating that Blimp-1 simultaneously influences the production or stability of the message. As with previous ex-

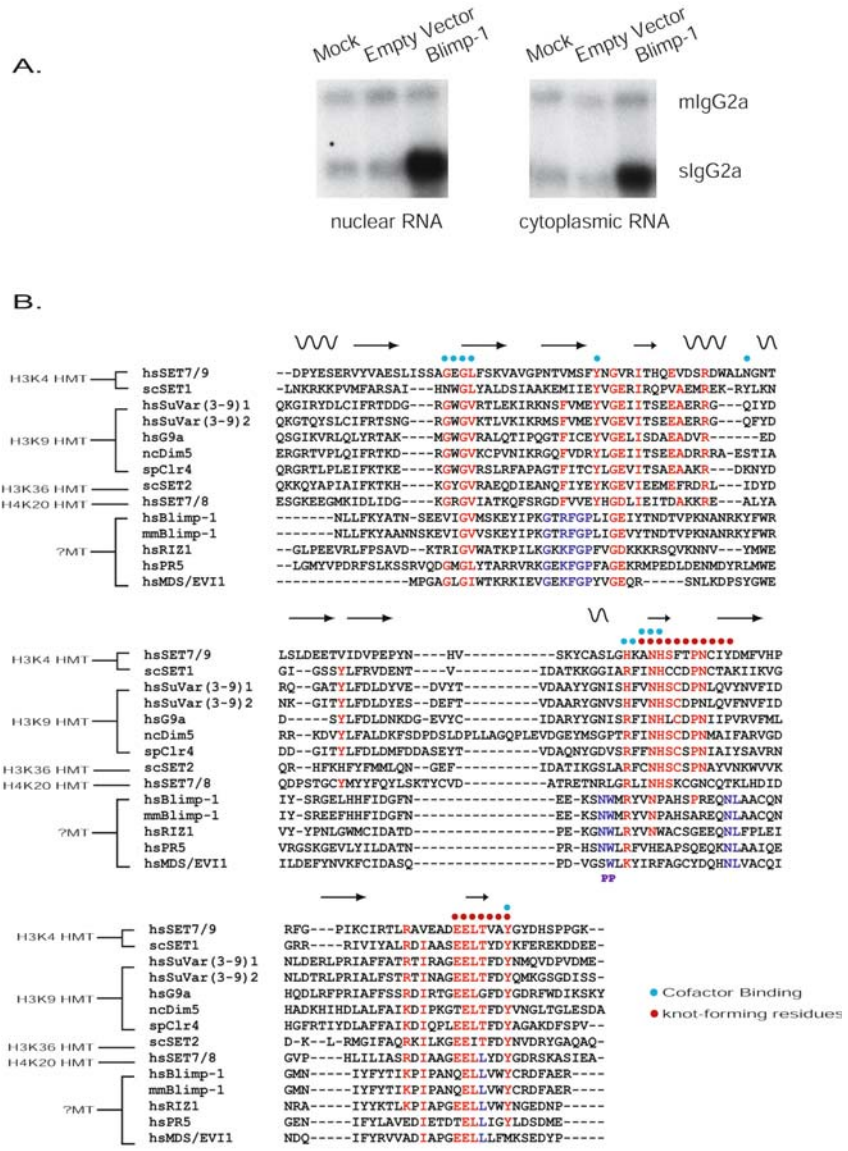


Fig. 1 **A** Blimp-1 regulates Ig secretion at the level of 3′ end processing of the Ig mRNA. Blimp-1 or control retroviruses were used to transduce the M12 lymphoma line. Nuclear or cytoplasmic RNA was prepared and used for Northern analysis with a probe for the IgG2a constant region. **B** Clustal W alignment of canonical SET domains and PR sequences. Sequences of canonical SET domains were used to align the PR domain proteins and the displayed layout is adapted from Jacobs et al.

periments, this step occurs in the nucleus since both the nuclear and cytoplasmic messages are similarly processed. This effect appears to be proximal to Blimp-1 activity since supernatant from Blimp-1-transduced cells does not stimulate Ig secretion in the heterologous B cell line, BCL_1 (R. Sciammas and M.M. Davis, unpublished observations). In addition, the effect is dependent on continued Blimp-1 expression, since transduced cells selected to discontinue Blimp-1 expression revert to unprocessed Ig production. Thus, Blimp-1 appears to induce Ig secretion by influencing the nuclear 3′ end processing events of the Ig message, the defining characteristic of plasma cell differentiation.

The mechanism of Blimp-1 induced Ig 3′ end processing and the relationship between Blimp-1 and CstF64-mediated processing was explored by using a dominant negative form of Blimp-1 consisting of the minimal zinc fingers. Interestingly, expression of this construct of Blimp-1 is able to attenuate cytokine-induced Ig secretion (Messika et al. 1998; Shaffer et al. 2002). Consistent with this, a mutant form of Blimp-1 lacking the first two zinc fingers fails to induce secretion when transduced in M12 cells (Sciammas and Davis 2004). This suggests that Blimp-1 regulates the expression of an intermediary gene that is involved in 3′ end processing. The expression levels of CstF64, an essential component of the poly-adenylation complex whose expression levels are reported to change upon acquisition of Ig secretory capacity (Takagaki et al. 1996), is static between control and Blimp-1-transduced M12 cells or between cytokine-treated and -untreated BCL_1 cells, negating it as a target of Blimp-1 regulation (Sciammas and Davis 2004). Secondly, CstF64 is not

(Jacobs et al. 2002). Annotations above the sequences represent tertiary structure configurations and dots above the sequences denote residues which were found to interact (*blue*) with the cofactor *S*-adenosyl-methionine or residues important for forming a unique knot structure (*red*) that are found in the SET 7/9 crystal. Residues in *red* are conserved in both the SET and PR families and residues in *blue* are conserved residues unique to the PR family. GenBank accession numbers for *Homo sapiens* (*hs*) SET7/9, P_040150; *hs* Su(Var)3–9H1, NP_003164; *hs* Su(Var)3–9H2, NP_078946; *hs* G9a, S30385; *Neurospora Crassa* (*nc*) Dim-5; AAL35215; Schizosaccharomyces pombe (*sp*) Clr4, O60016; *Saccharomyces cerevisiae* (*sc*) SET2, P46995; *hs* SET7/8, AAL40879; *hs* Blimp-1, NP_001189; *Mus musculus* (*mm*) Blimp-1, A53503; *hs* RIZ1, AAC50820; *hs* PR5, NP_061169; and *hs* translocation product MDS/EVI1, AAF04011 (MDS) spliced to AAB05840 (EVI1). The *PP below* the sequence denotes the residues in Blimp-1 that were mutated to proline-proline for functional characterization of this region in Ig secretion. (Sciammas and Davis 2004)

post-translationally modified by phosphorylation or proteolysis in Ig-secreting cells (Martincic et al. 1998). Since then, CstF64 has been shown to be genetically essential for secretory Ig mRNA processing (Takagaki and Manley 1998); perhaps Blimp-1 is regulating the transcription of a gene that functions to localize or augment the activity of CstF64. Recently, FY, an *Arabidopsis thaliana* protein that modulates the timing of flowering by affecting RNA processing, has been implicated as a poly-adenylation complex auxiliary factor. This observation establishes that differential poly-adenylation can be influenced by factors impacting on the core complex (Simpson et al. 2003), rather than by only the core complex itself, and that Blimp-1 may be regulating the plasma cell-specific expression poly-adenylation complex auxiliary factor.

The primary structure of Blimp-1 suggests the presence of multiple regulatory modules. To date the proline-rich region, which recruits transcriptional co-repressors, is the only one that has been extensively characterized. To determine the role of this or other regions of Blimp-1 in regulating Ig secretion, we transduced an allelic series of mutants in M12 cells (Sciammas and Davis 2004). Strikingly, in contrast to a deletion of the proline-rich or the carboxy-terminal regions of Blimp-1, the amino terminus deletion of 181 amino acids failed to induce secretion. Since this deletion removes half of the PR domain, a point mutant was employed to inactivate this domain. When compared to wild type Blimp-1, this point mutant failed to induce Ig secretion despite efficient protein expression. Therefore, the PR domain, in conjunction with the DNA binding domain, plays a critical role in Ig secretion.

The amino-terminus of Blimp-1 possesses a 130-amino acid region with strong homology to a growing family of genes (Jiang and Huang 2000). It was first noted by homology between the human genes *PRDI-BF1* (*Blimp-1)* and *RIZ* (Retinoblastoma-interacting zinc finger protein); hence the designation PR domain (Buyse et al. 1995). This region also exhibits striking similarity to SET domains (Su(var)39–1, Enhancer of Zeste, Trithorax), which are active sites for the catalysis of lysine methylation (Huang et al. 1998; Rea et al. 2000). Many, although not all, SET domains also contain pre- and post-regions of similarity, and these regions are thought to be important for histone substrate specificity (Jenuwein 2001). The PR domain, however, lacks these regions, suggesting a different specificity. In addition, the alignments of the PR domain with canonical SET domains are not perfect, throwing into question whether the PR domain also functions as a methyltransferase (Fig. 1B). However, recent X-ray crystallographic data of canonical SET domains has revealed both a unique knot structure and amino acid main- and side-

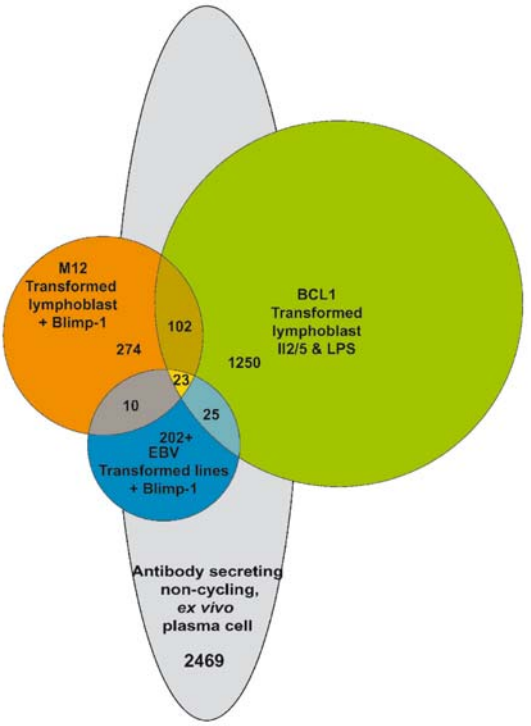
chain interactions with the obligate cofactor *S*-adenosyl-methionine (Jacobs et al. 2002; Trievel et al. 2002; Wilson et al. 2002; Zhang et al. 2002; Kwon et al. 2003; Xiao et al. 2003). It is these regions that are similar, suggesting, but not proving, that PR domains may also function as methyltransferases (Fig. 1B). To date, the SET type of methyltransferases are all lysine- and histone-specific and have roles in chromatin remodeling that, interestingly, affect transcription with both positive and negative outcomes (Zhang and Reinberg 2001). On the other hand, the six members of the PRMT class (protein arginine methyltransferase) are arginine-specific but have multiple protein targets and affect a range of functions, from roles in signaling to mRNA processing (Gary and Clarke 1998). PRMTs also bind the cofactor *S*-adenosyl-methionine; however, they use a different tertiary structure for catalysis (Fauman 1999). Therefore, post-translational modifications, via methionine, have multiple cellular activities and it is intriguing that Blimp-1 may regulate Ig secretion via a putative methyltransferase activity.

3
Blimp-1-Regulated Maturation Program

Two microarray studies have focused on the role of Blimp-1 in plasma cell differentiation and one has focused on the expression profile of terminally differentiated plasma cells (Shaffer et al. 2002; Underhill et al. 2003; Sciammas and Davis 2004). Strikingly, both similarities and substantial differences are seen between these studies, indicating that this maturation program is very plastic in nature (Fig. 2). Several different types of antibody-secreting cells exist in vivo that differ in proliferative capacity, migratory behavior or in the nature of the activating stimulus, and since all these cells appear to express Blimp-1, perhaps a differential expression program endows these cell types with specialized stage-specific properties (Turner et al. 1994; Angelin-Duclos et al. 2000; Martin and Kearney 2002). Interestingly, many of the differences are reconciled when analyzing the expression profile of terminal differentiated plasma cells.

With an interest in the mechanism of Ig secretion, we selected murine B cell lines with robust Ig secretory ability in order to track Blimp-1's effects on secretion (Sciammas and Davis 2004). Although the ability to secrete was consistently observed in different cell lines, the M12 cell line was the most dynamic. The M12 cell line was also pursued because of a lack of endogenous expression and a relatively tolerant phenotype in re-

A.



B.

M12 + Blimp-1 regulated genes compared to other plasma cell expression signatures

	Increased	Increased/ Decreased	Decreased/ Increased	Decreased	Number of genes in both sets / total
BCL1 + IL2/5	50	16	13	15	94 / 1400
BCL1 + LPS	49	19	12	21	101 / 1400
BCL1 + IL2/5 & LPS	35	13	9	11	68 / 1400
EBV Transformed cells + Blimp-1	4	19	1	9	33 / 260
Ex vivo Plasma cells	59	46	8	33	146 / 2469
Total genes validated in different cellular contexts	*92*			*36*	

Fig. 2 The Blimp-1 differentiation program is plastic in nature and constitutes a large fraction of the plasma cell expression signature. **A** Venn diagram of the microarray-based studies exploring the plasma cell genetic program induced by Blimp-1 compared to the expression signature of antibody secreting, nondividing, ex vivo murine plasma cells. The large quantity of nonoverlapping genes in the PC data is likely due to the contribution of the naïve B cell transcriptome used in the analysis

sponse to Blimp-1 toxicity. The retroviral expression strategy allowed a high enrichment of Blimp-1-expressing cells that could be analyzed at 72 h after transduction. When compared to control-transduced cells, a large number of differentially expressed genes are observed, 409, including both known and uncharacterized genes. Strikingly, the majority of these genes were induced, indicating an unanticipated ability of Blimp-1 in transcriptional upregulation. This possibility was tested in primary B cells transduced with Blimp-1-expressing retrovirus. Of 11 genes analyzed by real time PCR, eight were induced in both Blimp-1-transduced M12 and primary B cells, demonstrating Blimp-1's ability to activate gene expression cascades. In general, the differentially expressed genes have roles in B cell function, signaling, transcription, and homing, as well as genes involved in cell cycle control, as observed by Shaffer et al. (2002) (Fig. 3). However, a large set of genes involved in various aspects of secretion, including ER stress control, are upregulated in these cells, providing an unprecedented glimpse at the mechanism of Blimp-1 induced Ig secretion. The large number of differentially regulated genes combined with the transcriptional program associated with Ig secretion confirms the view of Blimp-1 as a master regulator of plasma cell differentiation.

In a separate study, the role of Blimp-1 was assessed by expressing it in various human EBV transformed cell lines (Shaffer et al. 2002). Calame and colleagues combined retroviral mediated transient expression of Blimp-1 with an inducible expression system. An inducible expression system was employed because they observed a high level of toxicity induced by Blimp-1 expression, as previously noted in B cell lines of an immature stage (Messika et al. 1998). Three cell lines were analyzed between these two systems and resulted in the differential regulation of

(Underhill et al. 2003). **B** Tabular display of the number of genes regulated in each condition shows the amount of both variability and overlap. The columns named Increased/Decreased or Decreased/Increased represent genes that, relative to M12 cells, are regulated in the opposite direction. The M12, BCL_1, and ex vivo plasma cell experiments were performed using Affymetrix MGU74 chips and can thus be easily compared. However, this comparison is not complete since the M12 and BCL_1 experiments include gene expression changes from all three chips of the MGU74 set and the ex vivo plasma cell experiments only include gene expression changes from the "A" chip. Comparisons with the EBV transformed cell experiments could only be estimated due to differences in the gene annotations between human and mouse

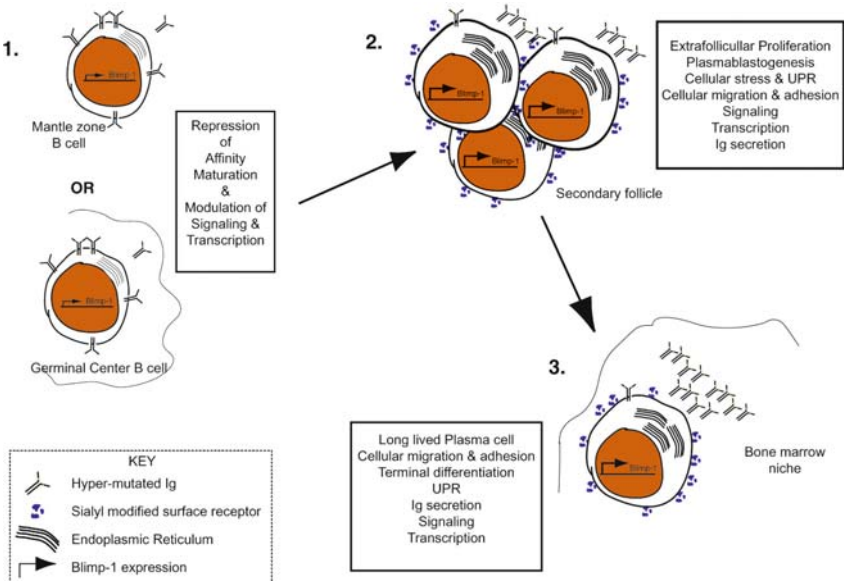


Fig. 3 Schematic of the transition of an activated B cell to a plasma cell and the functional categories of genes that Blimp-1 regulates at each step. Throughout the transition, signaling and transcription factors are differentially expressed as needed to respond to extracellular cues. *1* Activated mantle zone B cells (predominantly T-independent responders) and activated germinal center B cells (predominantly T-dependent responders) begin to express Blimp-1, which downregulates genes involved in affinity maturation. *2* Blimp-1-expressing cells migrate to secondary follicles where they expand by rapid proliferation as plasmablasts. Interactions with plasma cell-associated dendritic cells contribute to this process and thus the adhesion molecules regulated by Blimp-1 become candidates for this interaction. Transcriptional-coupled processing of the Ig transcript and high-rate Ig secretion is initiated by Blimp-1 and genes involved in the secretory pathway and in the UPR. *3* The Blimp-1 expressing plasmablasts, which survive in the secondary follicles at the termination of the immune response, migrate to specialized niches in the bone marrow where they initiate and maintain important contacts with stromal cells. These cells continue to secrete Ig and genes in the secretory pathway and the UPR are regulated. Lastly, these cells are terminally differentiated and are maintained for long periods but are arrested in the cell cycle

over 260 genes (the authors do not include a set of differentially expressed- and unannotated transcripts in this tally). Interestingly, the vast majority of the reported genes were repressed, consistent with the early description of Blimp-1 activity at specific target genes and Blimp-1's ability to interact with transcriptional repressors (Ren et al. 1999; Yu, et

al. 2000). The differentially expressed genes have roles in B cell function, signaling, transcription, and homing, as well as genes involved in cell cycle control (Fig. 3). Many of these genes have been documented to be silenced in plasma cells. Strikingly, Blimp-1 failed to induce Ig secretion in any of these cell lines, indicating that something about the nature of the cells or transformation status is blocking this function of Blimp-1. Still, the large number of genes differentially regulated by Blimp-1 as well as the identification of a large cohort of genes known to be silenced in plasma cells supports the notion of its role as a master regulator of terminal differentiation.

Functionally, the genes identified as regulated by Blimp-1 between Calame and colleagues (Shaffer et al. 2002) and ourselves (Sciammas and Davis 2004), are similar; however, significant differences exist. First is the set of genes involved in antibody secretion, which is only seen in our experiments. Second, Shaffer et al. (2002) observed the repression of a large number of genes that modulate B cell functions, including cell surface receptors, signaling, and transcription factors. In fact, the important B cell-specific transcription factors included in this set, *Ebf* (early B cell factor) and *Pax5,* may regulate many of these B cell-specific genes (Horcher et al. 2001). For reasons associated with activation or differentiation state, these two genes were not repressed by Blimp-1 in M12 cells. Third, the Blimp-1 maturational program in M12 cells is largely inductive in nature, indicating that a large fraction of activated genes is necessary for plasma cell differentiation. This conclusion is substantiated by the observation that many of the induced genes are also induced in Blimp-1-transduced primary B cells, fresh plasma cells (Underhill et al. 2003), and differentiating BCL_1 cells (Fig. 2 and described in Sect. 3.1). Whereas the M12 gene set is validated by the observed regulation of genes associated with antibody secretion (including the *Ig* genes themselves), the data of Shaffer et al. (2002) are validated by the repression of genes known to be silenced in plasma cells.

3.1
Differentiation-Specific Regulation

It is recognized that Blimp-1 expression peaks in rapidly cycling plasmablasts and is maintained throughout terminal differentiation, suggesting that the Blimp-1 transcriptional program is highly dependent on nuclear context. The reductionist approaches described by Shaffer et al. (2002) and Sciammas and Davis (2004) are very informative about the capacity of Blimp-1 as a differentiation agent; however, we still lack in-

sight into the nature of the complex regulatory networks that seem to impact on Blimp-1. To begin to address this issue, we analyzed the inducible B cell line, BCL_1, which, when stimulated with the cytokines IL2 and IL5 or by the B cell mitogen LPS, exhibits many of the physiological and genetic changes believed to occur during the transition of an activated B cell to a secreting plasmablast (Brooks et al. 1983; Blackman et al. 1986; Turner et al. 1994). These stimuli also induce Blimp-1 expression as an immediate early gene, indicating that the program (or subprogram) of differentiation is downstream of Blimp-1. A microarray-based, time-course experiment of gene expression changes following cytokine or LPS stimulation revealed a large set of genes with both stimulus-specific and temporal dynamics (Sciammas and Davis 2004). Genes with putative roles in differentiation, by virtue of first being differentially expressed during peak Blimp-1 protein expression (12 h) and second maintained at late time points when BCL_1 cells continue to express Blimp-1 and are secreting at a high rate (48 h), were identified from a large set of differentially expressed genes. Again, a large set of differentially expressed genes (606 at twofold and 1,400 at 1.5-fold) that affect B cell function, signaling, transcription, homing, mitogenesis, and secretion were identified.

Interestingly, a comparison of the BCL_1 data with the dataset derived from Blimp-1 expression in M12 cells revealed an overlap with genes in many of the functional categories associated with Blimp-1 activity. Indeed, these experiments validate the induction and repression of 64 and 25 genes, respectively (Fig. 2). Despite this functional overlap, the number of genes in common between the M12 and BCL_1 dataset is lower than expected (minimally 28%; a few genes that the microarrays failed to efficiently detect are shown by Northern analysis to be similarly regulated in both settings, suggesting that the microarray calculation is an underestimate). It is important to note that, in addition to differences between the cellular contexts of Blimp-1 expression in stimulated BCL_1 and genetically manipulated M12 cells, these two cell lines possess differences in transformation and lineage status that may also impact on Blimp-1 activity. Strikingly, however, these comparisons reveal that about one-third of the differentially expressed genes common between the BCL1 and M12 experiments exhibit unique stimulus- and temporal-expression characteristics. These results suggest that a subset of genes involved in the differentiation program can be influenced by extracellular cues and the nuclear milieu. Perhaps these genes are differentially required depending on the differentiation or activation state of the B cells. Indeed, different types of

antibody-secreting cells exist in vivo, providing a physiological context for these observations (Martin and Kearney 2002).

3.2 Blimp-1 as a Master Regulator

A very broad study has recently been published that describes the gene expression differences between murine resting B cells and nondividing, antibody-secreting plasma cells (Underhill et al. 2003). These plasma cells originate from a unique strain of mouse that presents with an unexplained pathology of hyper-gammaglobulinemia, cervical lymphadenopathy, and an inability to control skin infections. The cervical lymphadenopathy is in part contributed by plasma cells and therefore represents a tractable means to isolate a high number of "normal" plasma cells to perform microarray experiments. More than 2,400 genes were differentially expressed between these two cell types, hinting at the magnitude of gene expression changes associated with this transition. Strikingly, a share of these genes is downstream of Blimp-1 (Shaffer et al. 2002; Sciammas and Davis 2004), further supporting the concept of it as a master regulator. Importantly, many of the genes that are induced in the M12 expression system are represented in the untransformed plasma cell signature (Fig. 2), indicating that not all of Blimp-1's effects are repressive in nature. It is also important that many of the genes observed to change as reported by both Shaffer et al. (2002) and Sciammas and Davis (2004) are represented in the plasma cell expression profile. Many of the results of Shaffer et al. (2002) and Sciammas and Davis (2004) are represented in these experiments with "normal" plasma cells, which lends credence to the notion that different facets of the maturation program are differentially used in maturing B cells, but are ultimately resolved upon terminal differentiation. Indeed, this demonstrates that the set of differentially activated genes in the M12 cell experiments may be representative of different aspects or routes of the plasma cell state, and are thus not artifactual.

A multitude of the genes that are Blimp-1-responsive in both the EBV transformed lines and in the M12 lymphoblasts are components of the plasma cell expression signature as described by Underhill et al. (2003). This indicates that Blimp-1 plays a large part in managing this cellular transition. How large a part, however, cannot be fully discerned with these comparisons. It is likely that a large share of the 2,400 expression changes reflects on the nature of the naïve, resting B cell—of which Blimp-1 has no role. Lastly, the role of *Irf4,* a plasma cell expressed transcription factor with important roles in B cell maturation in this transition awaits analysis (Pernis 2002).

3.3 Modular Design

Preliminary experiments indicated that different mutants of Blimp-1 were both sufficient and deficient in regulating different sets of genes, suggesting a kind of autonomy of the different regulatory modules (Sciammas and Davis 2004). This notion was explored further by per-

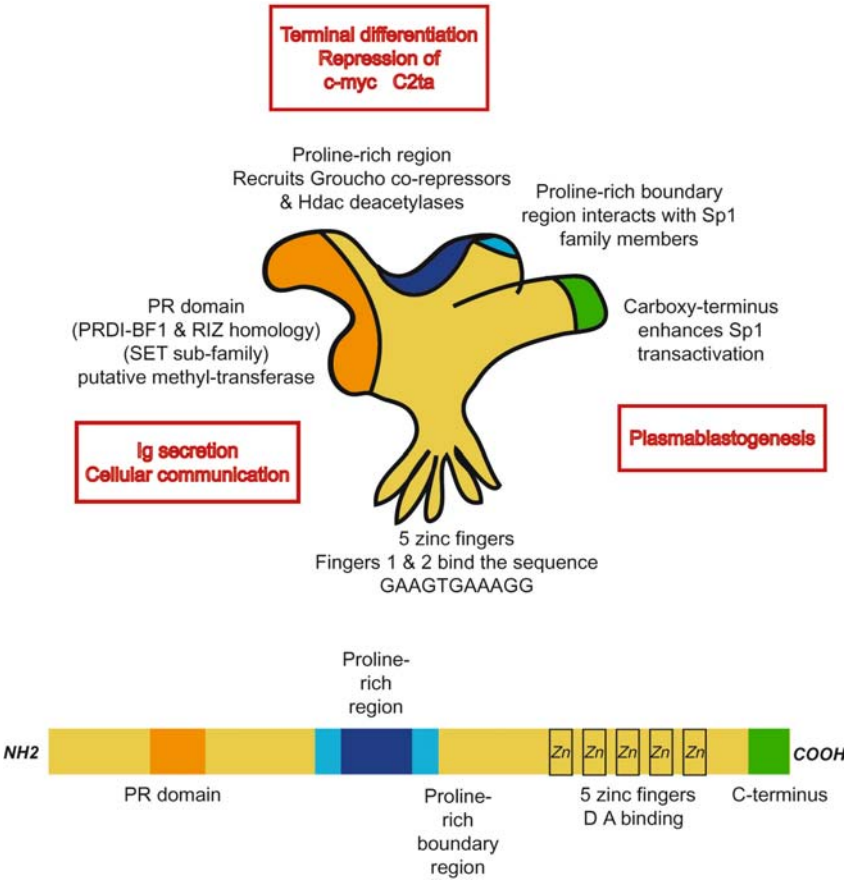


Fig. 4 Architecture of the primary and tertiary (imaginary) structure of Blimp-1 and the proposed functional roles of its modules. The linear arrangement of the color-coded modules from the primary structure is shown at the bottom and in the middle is the tertiary (imaginary) structure of Blimp-1. *Black text* describes the characteristics of the modules and the *red text* is the proposed function based on the types of types of differentially expressed genes dependent on that module

forming microarray experiments with select mutants of Blimp-1. Strikingly, when compared to either wild type or independent mutants of Blimp-1, two regions of Blimp-1 exhibit the ability to regulate a unique subset of genes (Fig. 4). Neither of these gene sets overlap, indicating that Blimp-1 operates at distinct regulatory regions or in mutually exclusive regulatory complexes to execute these changes. That the individual mutants did not alter the majority of genes indicates that the failure to observe differential expression is not a consequence of compromised stability, DNA binding, or expression level. The first mutant, disrupted by a double point mutation in the PR domain fails to induce Ig secretion as well as 30 other genes, the majority of which are destined for the extracellular space or are involved in the secretory pathway. The second mutant, a truncation of the carboxy terminus, fails to synergize with the Sp1 family of transcription factors and fails to regulate 13 genes (J-T Chi and MM Davis, manuscript in preparation) (Sciammas and Davis 2004). Interstingly, two of these genes have important roles in B cell activation. Mutation of the proline-rich region prevents repression at the *c-myc* and *C2ta* loci (Lin et al. 1997; Piskurich et al. 2000; Ghosh et al. 2001); however, information regarding the global role of this domain in Blimp-1 activity is lacking. Therefore, it appears that Blimp-1 diversifies the maturation program into separate pathways, presumably by interacting with different regulatory complexes. In addition, differential usage of the regulatory modules may contribute to stage-specific usage of the maturation program. To date, few transcription factors exhibit a diversification of its regulatory abilities, which may be a characteristic of proteins that sit at important regulatory junctions.

4 Post-translational Regulation of Ig Secretion

Subcellular analysis of plasma cells reveals a cytoplasm replete with a highly developed endoplasmic reticulum (ER), illuminating how plasma cells accommodate a high-level secretion of antibody (Wiest et al. 1990). These cytological changes occur following B cell activation; in parallel with the increase in production of secretory Ig transcript and the induction of Blimp-1 expression. Several recent observations suggest that staging of the plasma cell secretory machinery is a programmed pathway that involves the unfolded protein response (UPR). The UPR is a sensitive signaling pathway, conserved in all eukaryotic cells, which perceives malfolded proteins in the ER and signals to simultaneously arrest

protein translation and induce the expression of key chaperones and ER resident proteins that feedback to relieve the malfolded ER environment (Ma and Hendershot 2001).

Strikingly, it was found that B cells deficient in the transcription factor, Xbp1, fail to secrete Ig and, consequently, are severely immunocompromised (Reimold et al. 2001). Recently a connection between *Xbp1* and the UPR has been established, squarely implicating the UPR as a critical checkpoint in plasma cell development (Shen et al. 2001; Calfon et al. 2002). Ire1, an ER resident transmembrane protein, serves as an ER sensor. Interestingly, the cytoplasmic domain is an endonuclease that acts as an RNA-splicing enzyme whose target is the Xbp1 mRNA. Prior to stress, Xbp1 mRNA remains latent and poorly translated in the cytoplasm, and following ER stress, Ire1 splices a 26-bp intron, which converts the mRNA into a highly translatable form. Incidentally, the reading frame is altered and it produces an Xbp1 protein with both a different carboxy terminus and molecular weight. Experiments with Xbp1-deficient B cells suggest that UPR-dependent Xbp1 mRNA splicing is maintained throughout the life of the plasma cell and that nascent Ig continuously signals the UPR (Iwakoshi et al. 2003). Interestingly, regulation of the UPR and splicing of Xbp1 occur in two cell lines that are induced to secrete Ig by cytokines or LPS (R. Sciammas and M.M. Davis, unpublished observations) (Gass et al. 2002). Strikingly, UPR-dependent Xbp1 mRNA splicing leads to the transcription of IL6, a vital survival factor for plasma cells (Iwakoshi et al. 2003). This suggests an interesting feedback loop, which couples the quality control of secreted Ig with a survival signal. Importantly, *Xbp1*-deficient plasmablasts continue to express Blimp-1 and to produce high amounts of 3′ end processed Ig message, indicating that transcriptional and translational control of Ig secretion is regulated in parallel by Blimp-1 and Xbp1, respectively.

A broad-based proteomics approach has been applied to a temporal analysis of plasma cell differentiation and suggests that various aspects of the secretory apparatus and the UPR are regulated in two phases (van Anken et al. 2003). Several waves of protein changes were apparent following LPS stimulation, commencing with proteins involved in cellular metabolic competence and ER secretory capacity, which precedes the accumulation of Ig in the ER. In this analysis, the UPR—as defined by Xbp1 splicing—was initiated only coincident with the accumulation of Ig, indicating that the early phase of ER expansion occurs independently of a UPR signal.

The interface between Blimp-1, the ER, and the UPR is interesting. Shaffer et al. (2002) observed a slight increase in the total Xbp1 message

following ectopic Blimp-1 expression; however, the magnitude of this increase is small compared to plasmablasts, indicating that other factors are regulating Xbp1. On the other hand, in Blimp-1-expressing M12 cells that are induced to secrete Ig, a number of ER and UPR genes are induced. These include *Jaw1*, *Hsp70–1*, several DnaJs, *Erp44*, *Rer1*, several thioredoxin domain containing genes, several carbohydrate-modifying genes, and *Chop10*. This may in part be due to UPR-dependent processing of Xbp1, as two Xbp-1 target genes (*Edem* and *Dnajb9*) are upregulated in Blimp-1-transduced M12 cells. This processing is occurring at low levels since we fail to observe processed Xbp1 protein following Blimp-1 transduction (R. Sciammas and M.M. Davis, unpublished observations). Strikingly, upregulation of the two Xbp1 target genes is sensitive to the Blimp-1 PR mutation, indicating that the Xbp-1 arm of the UPR is downstream of Blimp-1 regulated 3′ end processing of the Ig mRNA (Sciammas and Davis 2004).

The overlap between the Blimp-1-induced ER genes and the LPS-induced ER proteins observed by van Anken et al. (2003) is not complete, indicating that Blimp-1 regulates a portion of the ER expansion that is pre-emptive of maximal Ig secretion. However, this is an imperfect comparison due to the limitations of drawing conclusions based on measuring different biochemicals, RNA and proteins, respectively. Nonetheless, these results suggest that Blimp-1 may be participating in the staging of the initial phase of ER expansion. Therefore, these data indicate that Blimp-1 regulates Ig secretion at the post-translational level, in addition to its role in regulating the post-transcriptional events that lead to antibody secretion.

5 Remarks and Future Directions

The identification of Blimp-1 as a major transcription factor involved in the transition from activated B cells to antibody-secreting plasma cells has opened new ways and insights towards understanding this critical differentiation phase. Additionally, the finding that Blimp-1, and specifically a putative methyltransferase domain in the amino-terminus (PR domain), plays a major role in regulating the mRNA processing events that affect efficient antibody secretion provides a new handle to tackling the mechanism of this long-standing question. Some of the issues regarding the molecular events involved during this critical stage of B cell maturation have been answered by analysis of the Blimp-1 regulated

genes. Differential expression of genes involved in affinity maturation, adhesion, migration, signaling, transcription, and secretion reveal mechanisms of how an activated B cell participates in the humoral response. Additionally, many of these genes are activated, indicating that the effect of Blimp-1 on differentiation is not strictly repressive in nature. Strikingly, these microarray experiments have identified a set of genes whose expression in the lymphoid system has not been previously noted, suggesting an unanticipated role in the immune system (Sciammas and Davis 2004). In addition, the large set of unannotated ESTs suggests the involvement of new players with roles in this maturation process.

Different aspects of this maturational program may be modulated by signaling- or activation-specific cues to endow different antibody-secreting cells with specialized functions. This plasticity is hinted at by the observation that a portion of the target genes observed in different Blimp-1-expressing cells, ranging from different transfected populations (M12 and Raji) and activated cells (BCL_1) to terminally differentiated plasma cells, can be regulated in different directions. These different contexts may be relating that plasma cell differentiation is more heterogeneous than previously anticipated.

Multiple domains of Blimp-1 subserve distinct functions and operate independently to execute different aspects of the maturation program. This property of Blimp-1 may also contribute to the plastic nature of differential gene expression observed under different conditions. The possibility that Blimp-1 may be a component of distinct protein complexes in these differentiation pathways is very interesting. It is intriguing to speculate that the different modules may regulate genes with related functional consequences. For instance, over half of the genes dependent on the PR domain are involved in secretion or traffic through the secretory pathway, and among the genes dependent on the carboxy-terminus are key regulators of plasmablastogenesis. The proline-rich region involved in repression of *c-myc* and *C2ta* suggests that this module may be important for maintaining the terminally differentiated phenotype (Fig. 4). Questions remain as to how these genetic programs are shuffled and/or regulated between different stages of B cell terminal differentiation.

As a whole, the differentiation program induced by Blimp-1 appears to be multilayered. Future work is needed to determine which of the genes are directly and indirectly regulated by Blimp-1. This is a high priority as it will provide information on the mechanisms of plasticity and will provide a blueprint for the networks of genetic regulation of B cell differentiation. Indeed, several transcription factors are required, up-

stream, downstream, and in parallel with Blimp-1. In this regard, there is accumulating evidence that there may be negative feedback regulation between different transcription factors to regulate the fate of B cell differentiation. First, Bcl6, an important transcription factor involved in affinity maturation, and Blimp-1 appear to antagonize the expression of each other (Shaffer et al. 2000; Shaffer et al. 2002; Sciammas and Davis 2004). Second, the observation that Il6 expression, an important plasma cell survival factor, may be tied into the integrity of the antibody-secreting cell, including Blimp-1 activity, since it is driving Ig secretion, indicates another checkpoint in this differentiation process (Iwakoshi et al. 2003). Finally, characterizing the role and potential interplay of Irf4 and these factors will be a stimulating area of investigation. Dissecting the regulatory network of and identifying the roles of the Blimp-1 regulated genes in plasma cell differentiation will surely provide a bounty of interesting surprises.

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