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Cellular and Chromatin Dynamics of Antibody-Secreting Plasma Cells

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Abstract

Plasma cells are terminally differentiated B cells responsible for maintaining protective serum antibody titers. Despite their clinical importance, our understanding of the linear genomic features and chromatin structure of plasma cells is incomplete. The plasma cell differentiation program can be triggered by different signals and in multiple, diverse peripheral B cell subsets. This heterogeneity raises questions about the gene regulatory circuits required for plasma cell specification. Recently, new regulators of plasma cell differentiation have been identified and the enhancer landscapes of naïve B cells have been described. Other studies have revealed that the bone marrow niche harbors heterogeneous plasma cell subsets. Still undefined are the minimal requirements to become a plasma cell and what molecular features make peripheral B cell subsets competent to become antibody-secreting plasma cells. New technologies promise to reveal underlying chromatin configurations that promote efficient antibody secretion.

Introduction

Antibody-secreting plasma cells undergo some of the most dramatic morphologic changes among all lymphocytes, even visible to early cytologists using simple microscopes and chemical stains. In 1890, Spanish neuroscientist Santiago Ramon y Cajal sketched the traces of cells he found in syphilis lesions and called them cyanophilic cells because of their propensity to stain blue. Notably, they also displayed dense chromatin condensation in a ring-like pattern at the nuclear periphery.^{1,2} Five years later, Hungarian pathologist Tamas Marschalko outlined the defining features of Cajal's isolates, which he called plasma cells: blocked chromatin, eccentric position of the nucleus, a perinuclear pale area (hof), and a spherical or irregular cytoplasm. Pathologists today still use these features to identify plasma cells (sometimes referred to as "Marschalko-type") and their malignant counterparts, myeloma cells, in blood smears and bone marrow aspirates.³⁻⁶

Textbook descriptions refer to a "clockface," "cartwheel," or "spoke-wheel" pattern of plasma cell chromatin, the same pattern that featured so prominently in Cajal's drawings.⁶⁻¹⁰

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conflicts of interest

no conflicts

How this distinct chromatin arrangement is established during plasma cell differentiation and its functional significance to antibody secretion is not known.

In general, the transformation of active chromatin regions, or euchromatin, into inactive regions, or heterochromatin, is considered part of the terminal differentiation of plasma cells, reticulocytes, and glial cells as a mechanism to lock in gene programs.^{11,12} Parallel with their morphologic changes, gene expression in plasma cells departs radically from other B cell lineage cells.^{6,13-15} Evidence suggests that the chromatin landscape of lymphocytes reflects both the vestiges of developmental origins in the form of bivalent marks that anticipate transcriptional activation^{6,11,12,16-18} as well as adaptations to local environments.^{6,13-15,19,20} By extension, plasma cells provide a compelling experimental system to study chromatin dynamics, as they are decedents of well-characterized B cell precursors that then become competent to secrete antibodies and enter unique survival niches. Plasma cells switch off the very transcription factors that direct their development and define their origins.¹⁵ They also survive for months to years in 'specialized' niches of the bone marrow^{21,22} and, for unspecified durations, in gut-associated lymphoid tissues.²³ Another adaptation visible by electron micrograph, the endoplasmic reticulum of plasma cells expands dramatically to process thousands of antibody molecules per second for secretion.^{24,25} How these changes happen in response to infection or immunization and stochastically across divisions is not well understood.²⁶

Lymphocytes develop from totipotent embryonic stem cells into mature blood cell lineages through a series of developmental intermediates. At each step, progenitor populations lose the capacity to adopt alternative fates due to increasingly restricted programs of lineage-specific gene expression, even as they acquire specialized effector functions. How fate choices are pruned and then take root have been extensively studied in the B cell lineage. New genes switch on, earlier genes are silenced, genomic elements become accessible or closed, and transcription factors find new partners. Underlying these transcriptional changes are modifications to the physical movement of chromosomes within the confined space of the nucleus. It is now well appreciated that the genome is organized not merely as strings of nucleotides but as intricate folded structures that permit long-range interactions between genetic segments. Chromatin architecture, or the organization of genomes into three-dimensional structures, is one tool to establish and maintain patterns of gene expression or repression as cells divide and differentiate.²⁷⁻²⁹

These multiple layers of gene regulation ensure tight control over the quantities and qualities of immune cells. Invading pathogens must be eliminated while minimizing damage to the host. The immune system has also evolved to respond more rapidly upon pathogen re-encounter by generating enduring populations of antigen-experienced cells. In the last decade, plasma cells have emerged as long-lived, constitutive secretors of immunoglobulin that maintain serum antibody titers, which provides the first line of defense against infection. Studies of primary B-cell immunodeficiencies in people have documented the importance of antibody in immune protection. Individuals lacking serum antibody exhibit increase susceptibility to bacterial infections and some viruses.³⁰ Studies using B cell deficient or secretory- μ deficient mice have further elucidated the importance of antibody: in the neutralization and clearance of toxins, bacteria, viruses, and parasites; enhanced phagocytic

function of innate cells; and the clearance of apoptotic cells.^{31,32} Surprisingly, despite their clinical importance, our understanding of the maintenance of serum antibody titers and the terminal differentiation of B cells into antibody-secreting effectors remains incomplete, particularly at the level of chromatin organization and structure. Recent advances in genome-wide technologies now make it possible to characterize the gene networks of these late stage B-lineage cells and identify chromatin modifiers involved in promoting terminal differentiation.

In addition to multiple layers of gene regulation, various peripheral B cell subsets generate plasma cells in response to diverse stimuli and at different rates. That varied peripheral B cell subsets are competent to become plasma cells raises questions about the minimal transcriptional and epigenetic requirements for plasma cell differentiation. Are there multiple pathways to become a plasma cell, or are they all intrinsically related? Once antibody secretion begins, are all plasma cells fundamentally the same, or do their origins really matter? In several recent studies, newly formed or low-affinity, “immature” plasma cells accessed bone marrow niches, once thought to harbor mainly high-affinity, “mature” plasma cells,³³ with near equal efficiency.³⁴⁻³⁶ This insight highlights the need to understand the early, intermediate, and late changes that occur after the initiation of the plasma cell fate.

As we will see in the following section, B cells take discrete steps on the path towards terminal differentiation. We review the conditions under which different peripheral B cells subsets generate plasma cells. In the second section, we will see that no single transcription factor controls all aspects of plasma cells; a core set of transcription factors cooperates to initiate and promote differentiation. Epigenetic modifications accompany the activation or silencing of these transcription factors, which in turn, recruit chromatin modifiers to regulate their own target genes. In the third and final section, we discuss recent advances in our understanding of the spatial organization of genes within the nucleus and what we know about activated B cells in this regard. We address how long-range chromatin interactions between promoters and regulatory regions support efficient antibody secretion and conclude with directions that would advance the field.

ALL ROADS LEAD TO ROME: PLASMA CELL DEVELOPMENT

B cells begin their development in the bone marrow or fetal liver where they undergo selection based on the signal strength of their unique antigen receptor. Newly formed B cells recirculate through peripheral lymphoid tissues scanning for invading pathogens. Unlike developing B cells that mature under prescribed conditions, peripheral B cells differentiate in response to diverse stimuli. Antibody generators, or antigens, are commonly grouped into two classes: Thymus-dependent (TD), usually proteins, and Thymus-independent (TI), usually mitogenic bacterial products or antigens with highly repetitive microbial structural patterns.³⁷ In parallel, most models depict two types of plasma cells: 1) a first wave of plasma cells that arise from extrafollicular foci and secrete low-affinity IgM antibodies, and 2) a second wave of plasma cells, which emanate chiefly from germinal centers and secrete high-affinity class-switched antibodies. Although exceptions exist, TI responses primarily generate the first wave of extrafollicular, unswitched cells that provide immediate, early

protection against pathogens whereas TD responses generate both unswitched and switched cells. Multiple B cell subsets can produce plasma cells: naïve follicular B cells, B1 B cells and marginal zone (MZ) B cells in primary responses, or antigen-experienced memory B cells in subsequent ones, as depicted in Fig. 1. Here, we begin to address the various extrinsic signals that drive plasma cell generation. In the following section, we discuss possible intrinsic differences of peripheral B cell subsets that may contribute to the character of their responses.

Plasma Cell Formation in TD Responses

Upon antigen receptor engagement, B and T cell clonal precursors migrate to the T:B border.³⁸ B cells with high antigen receptor affinity³⁹ form extrafollicular foci where they differentiate into early memory B cells and antibody-secreting cells. Some extrafollicular plasma cells gain the competence to seed the long-lived bone marrow pool but the majority undergo apoptosis within days.⁴⁰ Extrafollicular plasma cells make only transient contacts with T cells and do not express the cytokine receptor IL-21R.^{39,41} Consistent with this, B cell-specific loss of IL-21R does not affect extrafollicular responses.⁴² Shortly after this first wave of plasma cells, activated follicular B cells with lower antigen receptor affinity³⁹ enter germinal centers, microanatomical sites enriched for T follicular helper cells. Here, T cells deliver stimulatory signals in the form of cytokines (IL-21) and cell surface interactions (CD40-CD40L) to the B cells. B cells in germinal centers, and in some cases, outside of germinal centers, undergo antibody diversification processes. Class switch recombination from IgM to IgG, IgA, or IgE diversifies the effector functions of antibodies while somatic hypermutation further enhances the affinity of their receptors for antigen. Cycles of selection and clonal expansion give rise to memory B cells or to constitutively secreting plasma cells with B cell receptor affinities several-fold higher than their naïve counterparts. Finally, some plasma cells will migrate to specialized niches in the bone marrow enriched for limited survival factors. Depending on their anatomical distribution, activated B cells receive distinct cytokine and T cell-derived signals. Whether plasma cells derived from these various locations intrinsically differ is not known.

Memory B cells

Memory B cells are clonally expanded, antigen-experienced B cells that persist months to years after initial exposure to antigen. Once believed to be the sole source of persisting antibody via slow turnover or reactivation by polyclonal stimulation,⁴³ memory B cells do not secrete antibody but instead produce new plasma cells upon antigen reencounter. Recent studies have identified subsets of memory B cells with distinct cell surface markers, patterns of mutation, and dependence on survival factors.^{44,45} Memory B cells arise from both extrafollicular and germinal center responses, and vary in affinity and isotype.⁴⁶ Even in a TD response, typically associated with the formation of germinal centers, up to 35% of memory B cells were low affinity cells.^{47,48} Studies in both mice and people have reported less somatic hypermutation in IgM-bearing memory cells than plasma cells, suggesting that memory cells mature within germinal centers but exit “early” compared to plasma cells that exit “late.” For this reason, memory B cells have been described as more “naïve-like” than plasma cells. Several studies have found IgM+ memory cells are longer-lived than IgG+ memory B cells, and that the fate of unswitched and class-switched B cells also appears to

differ upon reactivation.^{49,50} In secondary infections, IgG+ memory gave rise to plasma cells whereas IgM+ memory B cells reinitiated germinal center reactions.^{49,50} Several groups have also shown that TI antigens promote memory B cells development despite the absence of typical germinal centers.⁵¹ Together, these observations emphasize the striking degree of heterogeneity among memory cells. It remains to be determined how select subsets of memory cells are ‘poised’ to rapidly differentiate into antibody secreting plasma cells.

Plasma Cell Formation in TI Responses

In contrast to follicular B cells that dominate TD responses, marginal zone (MZ) and B1 B cells are major sources of antibody (or antibody-producing cells) during TI immune responses, and can differentiate into plasma cells in response to TI agonists, alone or together with cytokines such as IL-4 and IL-5.^{52-54,55} MZ and B1 B cells together comprise only 5% of the splenic B cell compartment yet contribute disproportionately to the early wave of IgM antibody secreted in response to antigen encounter.⁵² MZ and B1 B cells localize near the marginal sinus where blood first enters the spleen. This situates MZ and B1 B cells for rapid responses to blood-borne pathogens. B1 and MZ B cells have an early advantage against invading pathogens owing to these special anatomical outposts but also due to their antigen-specificities.⁵⁶ Both pools also possess BCR specificities remarkably suited to recognize common bacterial antigens.⁵⁷

Recently, Chernova *et al.* identified three populations of antigen-specific bone marrow plasma cells with different turnover kinetics and cell surface marker expression. These data indicate that lasting antibody responses are mediated by both established plasma cells and newly formed plasma cells that are likely products of cycling memory cells.³⁵ This study and others⁵⁸ have elucidated markers to resolve plasma cells at different stages of maturity. Determining the epigenetic landscape of these subsets would elucidate how plasma cells derived from distinct precursors may differ. Phenotyping the precursor cells may also reveal unique ground-states of the various peripheral B cells that produce plasma cells.

TRANSCRIPTIONAL NETWORKS AND EPIGENETIC MODIFICATIONS

Transcription factors are thought to operate in a hierarchical manner with multiple “tiers” of functionality.⁵⁹⁻⁶¹ “Pioneer” transcription factors help establish the chromatin landscape by binding to specific sequences, evicting chromatin, and initiating gene transcription.⁶² Depending on the system, pioneers are either required or dispensable for continued gene expression. Pioneers cannot induce transcription alone, but their presence may facilitate more rapid responses. “Settler” transcription factors bind to DNA solely when their motifs appear in pre-established opened chromatin. “Migrant” transcription factors bind more ‘sporadically’ even when their motifs lie within open chromatin.^{61,63} Kinetics of lymphocyte differentiation depend on sequential transcription factor binding and subsequent recruitment of chromatin modifiers. In activated B cells, for example, Myc can only bind to regions that are already open, contain active marks⁶⁴ and have preloaded RNA polymerase. Mapping pre-existing and *de novo* binding requirements for transcription factors would help elucidate the fate decisions of activated B cells.

Early models of transcriptional regulation for terminal B cell differentiation depicted mutually antagonistic pairs of transcription factors favoring germinal center B cells or plasma cells (Blimp1:Bcl6, Pax5:Xbp1, Irf4:Bcl6). This widely accepted model has provided a powerful framework to study plasma cell differentiation, and recent studies continue to elucidate new plasma cell transcriptional regulators. Transcription factors from hematopoiesis, and even those important for alternative lineages, appear at this late stage in B cell differentiation but collaborate with different partners and regulate a set of targets different from those in early B cell progenitors. In addition, several studies have revealed heterogeneity among plasma cell transcriptional programs, raising questions about how cytokines and T cell signals may help shape the enhancer landscapes of newly formed plasma cells.

At the heart of the plasma cell program is the transcription factor Blimp-1, encoded by the gene *Prdm1*.⁶⁵ Transfection of mature B cells lines with Blimp-1 both silenced B cells genes and induced antibody secretion, situating Blimp-1 as ‘master regulator’ of the transcriptional network. Blimp-1 acts as a potent repressor. Microarray analysis showed that Blimp-1 repressed hundreds of genes and activated fewer than 50.¹⁵ Repression by Blimp-1 has also been described as “modular,” where different regions of the Blimp-1 protein exerted unique effects on defined clusters of genes.¹⁴ At least one study found that, depending on the model system, Blimp-1 sometimes exerted more positive than repressive effects on genes.^{13,14,15} However, most attention has been dedicated to understanding mechanisms of Blimp-1-mediated repression. Blimp-1 directly associated with histone deacetylases (HDACs)⁶⁶ and interacted with Groucho proteins.⁶⁷ Blimp-1 also directly interacted with the demethylase LSD1.⁶⁸ Depletion of LSD1 led to decreased plasma cell formation in human *in vitro* cultures and cell lines. Human Blimp-1 (PRDI-BF1) also interacted with G9a,⁶⁹ an H3K9 methyltransferase, increasing repressive H3K9me3 on the promoter of the B cell gene Pax5. H3K9me3 has been shown to provide binding sites for heterochromatin protein 1 (HP1) and promote gene silencing. In turn, HP1 recruits Su(Var)39 histone methyltransferase, promoting further H3K9 methylation and reinforcing heterochromatin. In T helper cells, this pathway helped ensure Th1 lineage silencing and Th2 stability.^{1,8,9} In response to a TI antigen, a modified H3K9me3 phosphorylated at serine 10 marked repressed genes, leading to displacement of HP1B in plasma cells. Surprisingly, HP1B was restored in bone marrow-derived plasma cells,⁷⁰ suggesting heterogeneity among plasma cells in the spleen versus bone marrow.

Isolating activated B cells prior to clonal expansion remains one of the challenges to studying the earliest events of plasma cell differentiation. Activated B cells show numerous changes in gene transcription within hours of stimulation *in vitro*. Well before proteins in the endoplasmic reticulum (ER) were induced, the metabolic demand and energy production of LPS-stimulated B cells increased.⁷¹ The plasma cell fate appears to be actively repressed in naïve B cells in part by Mitf⁷² and Pax-5. Downregulation of Pax-5 led to the subsequent loss of canonical B cell markers, including CD19, B220, and surface Ig, concomitant with the re-expression of Pax-5 repressed targets.^{73,74} Surprisingly, some recently generated plasma cells, even those that migrate to the bone marrow, retained B220, CD19, and MHC-II, indicative of their ‘immaturity.’^{34,35,75} Despite the loss of critical determinants of the B

cell lineage, plasma cells do not appear to trans-differentiate, perhaps because activated lymphocytes exhibit high methylation at embryonic stem cell-specific enhancers.⁷⁶ Calling in reinforcements, Pax-5 then induces Bach-2, which in turn cooperates with Bcl-6 to repress Blimp-1.⁷⁷ Mitf acts by repressing Irf-4 activity (perhaps indirectly), a positive regulator of both germinal center B cells and plasma cells. Together, Pax-5 and Mitf-mediated repression delays onset of plasma cell differentiation, extending the window for class switch recombination and somatic hypermutation. As expected, Bach-2 and Mitf-deficient B cells differentiated more rapidly into plasma cells and generated fewer class-switched B cells.

Despite the essential role for Blimp-1 in the maturation of plasma cells, the term ‘master regulator’ obscures important aspects of the plasma cell program. First, initiation of plasma cell identity does not require Blimp-1.⁷⁸ Blimp-1 deficient B cells displayed early features of plasma cell differentiation, including low level immunoglobulin secretion and downregulation of the germinal center B cell regulators Pax-5 and Bcl-6.⁷⁸ Second, Pax-5 downregulation appears to be critical for the plasma cell fate as forced Pax-5 overexpression halted Blimp-1 upregulation and prevented differentiation.^{79,80} In myeloma lines, inactivating histone methylation and deacetylation helped silence the Pax-5 locus.⁸¹ In turn, silencing of Pax-5 directly promoted de-repression of the immunoglobulin J chain⁸² and activation of the unfolded protein response gene Xbp-1.⁸³ Surprisingly, very early antibody secreting plasma cells expressed several genes repressed by Pax-5,⁷⁸ suggesting that the initiation of plasma cell differentiation occurs even prior to Pax-5 silencing. From this viewpoint, Blimp-1 appears to amplify the effects of Pax-5 repression or earlier activation events. Thus, despite its important role in completing and maintaining plasma cell identity, Blimp-1 cannot be considered a pioneer transcription factor in naïve follicular B cells.

Blimp-1 levels not only vary depending on the relative maturity of developing plasma cells, but peripheral B cells subsets also exhibit different basal Blimp-1 levels. B1 B cells may express lower levels of Blimp-1 and secretory μ transcripts than other plasma cells.^{3,5,6,12} For this reason, MZ and B1 B cells have been described as ‘transcriptionally-poised’ antibody-secreting cells^{6,7,10,13-15} with higher basal- and activation-induced levels of transcription factors like c-myc compared to follicular B cells.^{11,17} One group has suggested that B1s “violate” the current paradigm of plasma cell transcription.^{6,20} It is also unclear whether B1 B cells secrete constitutively, without dividing, or whether they exist as “partially” activated/differentiated cells.^{6,15,16,18} Constitutive secretion is supported by two findings: a fraction of B1 B cells in the peritoneal cavity express the plasma cell transcription factor Blimp-1,^{19,21,22} and B1 B cells can secrete antibody without division upon exposure to LPS.⁸⁴ Dissecting the epigenetic determinants or unique regulatory pathways that promote rapid differentiation in MZ and B1 B cells could be harnessed to “reprogram” slower responding B cells. Moreover, whether MZ or B1-derived plasma cells are intrinsically different from follicular B cell-derived plasma cells is not known. In sum, these specialized subsets of B cells rapidly respond to invading pathogens, not only because of their unique anatomical situation but also due to cell-intrinsic mechanisms that remain unresolved. One cell-intrinsic explanation for accelerated B1 B cell differentiation to antibody secreting cells is their high express levels of the transcription factor ZBTB20, a zinc finger (BTB-POZ) family member also highly expressed in germinal center B cells,

plasma cells and memory cells.⁸⁵ Surprisingly, MZ B cells did not express high levels of this factor, suggesting that the mechanisms underlying rapid differentiation of B1 and MZ subsets likely differs.⁸⁶

Heterogeneity within the plasma cell pool has important implications for regulatory networks and is most apparent in the disparate use of transcription factors. For example, Oct2-deficient B cells responded poorly to the TI antigen LPS whereas Obf-1 (Oca-B) deficient cells responded normally. Oct-2 directly regulated the alpha chain of the IL-5 receptor, leading to enhanced plasma cell differentiation in response to IL-5 and CD40.⁵⁵ In contrast, Obf-1 was critical for Blimp-1 induction and repression of genes associated with the mature B cell program.⁸⁷ Altogether, these results suggest that GC and extrafollicular responses are governed by different sets of transcription factors. Recent reports revealed an analogous example of divergent transcriptional requirements for plasma cell in response to alum- or LPS-based adjuvants. Intriguingly, after immunization with an alum-based adjuvant, antigen-specific plasma cells deficient in ZBTB20 waned dramatically, whereas adjuvants activating TLRs restored antibody production.⁸⁶⁸⁸ The reduction of plasma cells appeared primarily in the bone marrow pool, pointing to differential survival requirements for plasma cells in unique microenvironments. These findings raise the possibility that plasma cells utilize distinct transcriptional mechanisms to regulate antibody responses.

Whereas ZBTB20 promotes plasma cell differentiation, another BTB-POZ family member, Bcl-6 facilitates the proliferation and somatic hypermutation of GC B cells. Loss of one allele of Bcl-6, for example, impaired GC differentiation,⁸⁹ although full loss of Bcl-6 only increased plasma cell differentiation two-fold. Bcl-6 interacts with the chromatin remodeling complex Mi-2/NuRD in GC B cells to repress plasma cell genes.⁹⁰ Bcl-6 and Blimp-1 act as reciprocal antagonists in germinal centers.⁹¹ Bcl-6 and Bach2 directly bind and repress Blimp-1,^{91,9277} whereas Irf4 appears to inhibit Bcl-6, particularly at later stages of plasma cell differentiation, for example in GC-exiting cells.⁹³ Irf-4 was also found to activate Bcl-6 by binding to an upstream enhancer.⁹²

Irf-4 has emerged as an equally important regulator of plasma cell differentiation. Whether Blimp1 functions up or down stream of Irf-4 is unclear. In a study by Klein and colleagues, LPS-stimulated Irf-4^{-/-} B cells unregulated Blimp-1 protein, but did not take on a plasmacytic phenotype.⁹⁴ In contrast, Sciammas and colleagues found that Irf4 activated plasma cell differentiation by upregulating the expression of Blimp-1. More recently, Irf-4 has also been shown to be essential for GC B cells. At low levels of Irf4, Irf4 cobound with PU.1 or BATF whereas at higher concentrations IRF4 binding shifted to INF response motifs and promoted plasma cell differentiation.⁹² A potential feedback loop, Blimp-1 may reinforce Irf-4 expression, as Irf-4 is not upregulated in Blimp-deficient B cells. Retroviral overexpression of Blimp-1 into a Blimp-1-deficient cell line led to Irf-4 upregulation. However, Sciammas and workers found that restoring Blimp-1 in Irf-4-deficient cells rescued secretion, albeit at low levels, calling into the question the coordinated role of these two factors. A negative regulator of both Blimp-1 and Irf-4, Ezh-2, the main catalytic unit of polycomb repressive complex 2, is highly expressed in germinal center B cells.⁹⁵ Ezh-2 repressed Blimp-1 and Irf-4 and marked these loci with repressive H3K27me3. Stimulated Ezh-2-deficient cells *in vitro* increased differentiation into plasma cells.

Plasma cell differentiation also relies on PU.1, whose graded expression in early progenitors determines lymphoid versus myeloid fate, but here interacts with a different set of transcription factors to control plasma cell differentiation. PU.1 collaborates with IRF8 to negatively regulate plasma cell differentiation. Although conditional inactivation of PU.1 factor led to normal humoral responses and IRF8 deficient mice had mildly increased plasma cell numbers, mice deficient in both factors exhibited a dramatic increase in the rate of PC differentiation.⁹⁶⁻⁹⁸ IRF8/PU.1 activated Bcl6 and repressed Blimp-1. It has been suggested that Irf8 and PU.1 and perhaps SpiB prevent premature plasma cell differentiation by competing for binding sites to target sequences shared by Irf-4. In the absence of PU.1 and Irf-8, Irf-4 was bound to BCL6 and Blimp1. Irf-8/PU.1-deficient B cells *in vitro* generated plasma cells at earlier divisions and at higher frequencies with each subsequent division compared to their wildtype counterpart.⁹⁹

Studies in plasma cells have also demonstrated the profound effects of microRNAs on cell fate decisions. Although gene expression levels change no more than a two or threefold in response to mRNAs, in plasma cells, even small changes lead to substantial changes in outcome. PU.1 contains a highly conserved miR-155 binding site in its 3'UTR. PU.1 is highly expressed in miR-155-deficient B cells.¹⁰⁰ Overexpression of PU.1 in B cells leads to fewer IgG1 cells. Mir155 represses PU.1, which in turn represses Pax5. Releasing PU.1 repression led to increased levels of Pax5 in activated B cells and impaired terminal B cell differentiation. It was found that the most abundant mRNA in human and murine plasma cells, mir-148a, decreased Bach2 and Mitf abundance.¹⁰¹

Early studies suggested that plasma cells and secreting B1 B cells rely on the activation of inositol-requiring enzyme 1/X-box binding protein 1 (IRE1) and X-box binding protein-1 (XBP-1) to maintain a chronic (i.e., physiologic) unfolded protein response (UPR). Antibody-secreting plasma cells secrete on the order of 10,000 antibody molecules per second, and thus it was reasoned that some fraction of these proteins would misfold. Consistent with this, XBP-1-deficient B cells in chimeric animals upregulated Blimp-1 but failed to induce genes associated with the secretory pathway; loss of XBP-1 severely compromised circulating immunoglobulin levels.¹⁰² Later, in studies of B cell lines stimulated with LPS, XBP-1 was activated *before* an increase in antibody synthesis, suggesting that large quantities of antibody were not the initial trigger of XBP-1 activation.¹⁰³ Importantly, recent studies using B cell-specific inactivation of Xbp1 demonstrated that antibody-secreting cells formed relatively normally despite aberrant ER morphology.¹⁰⁶ Most notably, Xbp1 deficiency reduced Ig secretion, but transcription levels of key regulators were unaffected.¹⁰⁶ Interestingly, despite defects in ER morphology, the chromatin patterning in XBP-1 deficient plasma cells appeared relatively intact.¹⁰⁶

Another key question is how memory B cells give rise to plasma cells. Kometani *et al.* found that post-GC IgG1 memory B cells maintained lower levels of Bach2 than GC B cells, consistent with rapid differentiation into plasma cells. Human IgM memory B cells also differentiated to plasma cells with faster kinetics compared to naïve B cells¹⁰⁴ whereas mouse IgM memory B cells did not,⁵⁰ perhaps due to differences in B cell receptor signalling inherent to IgM.¹⁰⁵ Human IgM memory B cells were found to have lower expression of transcription factors important for cellular quiescence¹⁰⁴ and constitutive

expression of co-stimulatory molecules.¹⁰⁶ Memory B cells underwent more divisions than naïve B cells in response to the same stimuli,¹⁰⁴ and, like their entry into division, thresholds for cytokine stimulation were lower in memory cells than naïve B cells.¹⁰⁷ In general, memory B cells closely resemble naïve B cells,¹⁰⁸ although subsets of memory B cells do have distinct transcriptional profiles.⁴⁴ In human B cells, naïve and memory B cells shared similar gene expression profiles but vastly different methylomes. In contrast, memory B cells and plasma cells shared similar methylomes, but differed at genes whose expression levels changed in plasma cells.¹⁰⁹ ‘Molecular bookmarking’ in which histone acetyltransferase p300 and RNA polymerase II occupy the promoters of some early inducible genes, has been observed after T cell stimulation; similar mechanism may be at work in B cells.¹¹⁰ In this model, histone modifications may also be stably inherited across cell divisions,²⁸ poising activated B cells for plasma cell differentiation until concentrations of transcription factors reach optimal levels to trigger full differentiation.

From the standpoint of enhancers, naïve B cells are already poised to differentiate. Enhancer landscapes that make cells competent for differentiation are established well before B cells migrate to the periphery. In early bone marrow precursors (LSK), almost half of activated B cell enhancers are already devoid of repressive methylation marks, or demethylated.¹¹¹ As B cells progress through developmental stages, demethylation across B cell enhancers increased until, in resting B cells, 80% were fully demethylated.¹¹¹ Resting naïve B cells are associated with globally low levels of histone methylation but, as expected, exhibit active epigenetic markers for genes important for B cells such as Pax5 and Ebf1. In naïve B, approximately 90% of promoters for genes soon to be expressed are already poised with polymerase. Upon activation, B cells exhibit a 10-fold increase in total RNA and mRNA production as well as increase in acetylation at most histones over resting B cells.¹¹¹ Markers of activation like histone acetylation and H3K4 methylation across the V region of the immunoglobulin locus are similar in naïve and germinal center B cells.¹¹² These observations help explain how naïve B cells relatively rapidly give rise to differentiated daughter cells.

CHROMATIN DYNAMICS IN IMMUNE CELLS

The basic structural unit of chromatin is the nucleosome, an assembly of 147 bases of DNA wrapped around a histone octamer. Predictions about gene function and activity – for example, whether genes function as promoters or enhancers and are active, poised or silenced – can be made based on the post-translational modifications of histone tails. Apart from their modifications, clusters of histones, or nucleosomes, assemble like beads on a string, whose position chromatin remodelers can adjust. In this way, wrapped DNA may become exposed and open to binding by transcription factors. Chromatin may also associate with one of the more than 10 structures found in the nucleus, including the nuclear lamina and nuclear pores. These structures constitute nuclear ‘compartments,’ or ‘neighborhoods,’ and generally correlate with gene activity.¹¹³

Today, the chromatin patterns of plasma cells observed by early cytologists like Cajal and Marschalko can be explained in new terms. Large stretches of the genome are comprised of non-coding DNA elements that are essential for regulating gene expression. Enhancers in

particular have emerged as critical *cis*-regulatory elements that control gene transcription even at large distances from target promoters. Indeed, enhancers may reside thousands of base pairs away from the regions they regulate. Reflecting yet another layer of regulation, these three-dimensional structures may be organized in the nucleus within 1-10Mb transcriptionally permissive or repressive compartments, or neighborhoods. Interactions may also be restricted in space to “topological domains” (TADs) that constrain the spread of heterochromatin and demarcate boundaries of shared epigenetic features.¹¹⁴ Loops are the result of protein-protein interactions and are thus intimately tied to the evolving pattern of transcription factors that occur during differentiation in addition to the activity of housekeeping genes and binding patterns of broadly bound architectural proteins.¹¹⁵⁷⁶ Transcription at a given region appears to directly correlate with the number of regulatory domains regulating it.¹¹⁶

The visible heterochromatic changes in plasma cells correlate well with the general repressive nature of the plasma cell program. While large-scale folding and looping of chromatin within distinct nuclear territories helps coordinate complex transcriptional programs, the precise rules for this process are ill-defined.²⁷ Still unknown is the cause-effect relationship of these changes: whether chromosomal regions are situated in compartments reflective of their epigenetic modifications and transcriptional activity, or whether compartments favor and actively impart epigenetic modifications upon chromatin neighbors. Superimposing gene expression, epigenetic, and nuclear-positioning data will elucidate how cells differentiate from totipotent embryonic stem cells to terminally differentiated effectors and how sustained or transient adaptations to the environment regulate gene activity.¹¹⁷ New technologies will also illuminate these processes for rare, specialized subsets of immune cells.

Problems and Progress: Nuclear Features of Plasma Cells

Recent work using single cell analysis by FISH (fluorescent *in-situ* hybridization) has made an important step in understanding plasma cell chromatin dynamics. Park *et al.* found that the proximity of DNA elements to the endoplasmic reticulum prepared plasma cells for secretion of antibodies.¹¹⁸ In 1985, Gunther Blobel articulated the “gene gating theory” in which RNA transcripts aggregate closely to limited subsets of nuclear pores for optimal export.¹¹⁸ Park and coworkers formally demonstrated this proposal by examining the mechanisms underlying immunoglobulin transcription and export in primary mouse plasma cells. Their study revealed striking co-localization of the antibody *Igk*, *IgH*, and *IgJ* genes at clusters of RNA Polymerase II (PolII), known as transcription factories.¹¹⁹ In another study, approximately half of the genes with PolII binding in activated B cells were associated with long-range interactions.⁷⁶ Interestingly, the clusters observed by Park *et al.* often appeared at the nuclear periphery/nuclear lamina, a site typically associated with gene silencing. In plasma cells, gene localization to the nuclear periphery appeared to position transcripts in close proximity to nuclear pores adjacent to the endoplasmic reticulum. Positioning of active genes to nuclear pores has been described in other systems,¹²⁰ and in plasma cells, is proposed to facilitate efficient assembly and secretion of antibody molecules. This report was the first to connect directly nuclear organization to the gene expression changes and function uniquely achieved in plasma cells.

While Park *et al.* have helped elucidate how chromatin organization supports plasma cell function, no studies to date have addressed how activated B cells become plasma cells at the level of chromatin. If the nuclear position of chromatin correlates closely with transcription, one possibility is that the stochastic movements of single alleles into silenced compartments may determine cell fate decisions in activated B cells, as observed for olfactory neuron receptors and as we recently proposed for developing T cells.¹²¹ In other words, chromatin positioning prior to plasma cell commitment may determine whether activated B cells and self-renewing memory B cells produce plasma cells, generate memory B cells, or continue to divide. Another possibility is that extrinsic signals (i.e., cytokines, antigen affinity) tip the balance of fate-determining transcription factors in germinal center B cells to favor continued division, memory B cell or plasma cell production, or death. Alternatively, stochastic mechanisms may regulate the heterogeneous outcomes of dividing precursors, as observed for *in vitro* responses with division-linked changes in cell fate.^{26,122}

Conclusion

Progress towards defining the linear genomic features and three-dimensional chromatin dynamics of plasma cells has been tempered by the relative scarcity of these cells. Recent technologies like iChIP,¹⁰⁶ ATAC-Seq,^{123,124} and single cell Hi-C¹²⁵ promise to make plasma cell differentiation a pathway that can be studied. Understanding the gene circuitry that wires cells for antibody secretion will be pivotal to designing targeted therapies to temper autoreactive cells or bolster vaccine efficacy. Moreover, it is unclear how the signals instructing plasma cell differentiation shape their function and selectively enrich for desired features. Defining the plasma cell fate in terms of evolving gene networks and identifying novel factors that integrate to initiate and sustain plasma cell identity will be fundamental to selectively manipulating their activity. Establishing a tracking system to isolate early, activated B cells may help elucidate important gene networks obscured by analyses on bulk populations and at relatively late timepoints after activation. The advent of new technologies that permit genome-wide sequencing starting with small numbers of cells will elucidate some of these questions.

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Figure 1. Model depicting the multiple routes to terminal differentiation and heterogeneity within the plasma cell bone marrow niche

Recent data indicate that both T-dependent and T-independent responses yield plasma cells competent to enter the bone marrow. How these plasma cells differ is not known.

Additionally, switched and unswitched memory B cells give rise to plasma cells. It is uncertain whether plasma cells continue to mature once they reach the bone marrow, as depicted by dashed arrows. B memory cells (Bmem); Follicular B cells (FoB); Marginal zone (MZ); Germinal center B cells (GC B).