Gene control and remodeling during hematopoiesis
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CHAPTER 1

GENERAL INTRODUCTION
The immune system

The immune system protects the host against pathogens, like viruses and bacteria. Innate immunity will act as a first line of defense when pathogens manage to pass the epithelial barriers, such as the skin or the mucosal layers of the gastrointestinal and respiratory tracts. Key players in innate immunity are macrophages and neutrophils that recognize most common pathogens through pattern recognition receptors. Pattern recognition can initiate inflammatory responses, resulting in the release of cytokines by macrophages that increase the permeability of blood vessels, which allows fluid and other immune cells to enter the site of infection. In addition, they secrete chemokines that attract neutrophils, which destroy bacteria by phagocytosis.

When innate immunity is not capable of resolving an infection, it triggers and cooperates with the adaptive immune system. This system consists of T- and B lymphocytes that can recognize pathogens specifically and increase in number as a consequence. They also create immunological memory after infection, which provides protection against re-infection. T- and B cells are responsible for antigen specific cellular and humoral immune responses, respectively. Their specificity is based on a virtual infinite repertoire of antigen receptors. Adaptive immune responses can be initiated by dendritic cells (DCs) that ingest antigen at the site of infection. Subsequently, these cells become activated and start to present the antigen in the form of MHC antigen-peptide complexes at their surface. Activated DCs then migrate via the lymphatic system to the peripheral lymphoid organs, like the spleen and lymph nodes, where they present the processed antigen to T cells. When T cells recognize their cognate peptide-MHC complex via their clonotypic T cell antigen receptor (TCR) they differentiate, depending on cytokine- and costimulation, into cytotoxic T cells, which play a role in the cellular immune response, or into helper T cells.

In contrast to the TCR on T cells, the B cell antigen receptor (BCR) on B cells binds to the native form of its cognate antigen. This happens either in the blood or in the lymphatic organs. Upon binding, the antigen will be engulfed and processed, subsequently antigen-derived peptides will be presented by specific MHC molecules at the surface of the B cell. These are recognized by matching T helper cells, which induce B cell activation by co-stimulation, for instance through the CD40-CD40L interaction. Activated B cells proliferate extensively and form germinal centers (GCs) in the follicles of peripheral lymphoid organs. In these GCs, antigen-activated B cells further diversify their specific antigen receptor and subsequently differentiate into antibody producing plasma cells or memory B cells. These antibodies can opsonize their cognate antigen, thereby preventing it from damaging or entering host cells. In addition, antibody-antigen complexes can bind complement, or activate macrophages and other cells to destroy the antigen(-bearing cell).

Hematopoiesis

The developmental process that generates immune cells and all other cell types in the blood is called hematopoiesis. Hematopoietic stem cells (HSC) are the earliest progenitors that can self-renew and differentiate into all blood cell types (figure 1).
During early embryonic development, HSCs are found in the yolk-sac and aorta-gonad-mesonephros (AMG) region where they give rise to mainly erythroid cells. Later on hematopoietic activity is found in the spleen, liver and lymph nodes where besides the erythroid lineage also the lymphoid and myeloid lineages are generated. Finally, HSCs will migrate into the developing bone marrow where they will produce billions of blood cells during the entire lifetime. In some rare disorders, the spleen, thymus and liver resume their hematopoietic function, which is known as extramedullary hematopoiesis. As a consequence, these organs increase in size substantially.

HSC were first identified in the murine bone marrow, where they reside in the ‘LSK’ (Lineage-, ScaI+, c-Kit+) population of cells that lack the expression of lineage-affiliated markers but that do express high levels of ScaI and c-Kit (Spangrude et al., 1988). Reconstitution assays, where a single LSK cell was transplanted in an irradiated mouse, revealed that some of these cells were able to reconstitute the bone marrow for long-term (LT-HSC), while others could only reconstitute the bone marrow for short term (ST-HSC) (Osawa et al., 1996; Wagers et al., 2002).

According to the classical dichotomy model, ST-HSCs, also called multipotent progenitors (MPPs), can differentiate into lineage restricted common lymphoid progenitors (CLPs) or common myeloid progenitors (CMPs). CLPs have the potential to differentiate into B, T and NK cells, while CMPs have the potential to differentiate into myeloid cells, like monocytes and granulocytes, but also erythroid cells and megakaryocytes arise from CMPs (see figure 1). Kawamoto et al. proposed an alternative model for the first stages of hematopoiesis, the myeloid-based model. In this model, the MPPs can differentiate in either CMPs or common myeloid-lymphoid progenitors (CMLPs), which have both myeloid and lymphoid potential. The CMLPs can subsequently differentiate into myeloid-T cell progenitors (MTPs), which harbor myeloid- and T cell potential, or into myeloid-B cell progenitors (MBTs), which harbor myeloid and B cell potential (Kawamoto et al., 2009).

Entry into certain lymphoid sublineages depends on the tissue location of the common CLP. Precursors that migrate into the thymus will enter the T cell lineage, while a precursor in the bone marrow is more likely to enter the B cell lineage. These different niches provide different sets of growth factors, which induce signaling cascades and subsequent transcriptional reprogramming, resulting in lineage specific expression profiles. It has been shown by knock-out studies in mice that signaling by the IL-7 receptor and expression of the transcription factors E2A, Ebf1 and Pax5 are critical in driving lymphoid precursors into the B cell lineage (Cobaleda et al., 2007; Nutt et al., 2007). In contrast, T lymphopoiesis is triggered when lymphoid precursors are exposed to a high density of the Notch ligand Delta-like 4 in the thymus. Signaling through Notch receptors activates the T cell gene expression program (Hoflinger et al., 2004; Tanigaki et al., 2007). Myelopoiesis, which takes place in the bone marrow, is also dependent on a complex network of transcription factors. At the HSC stage, these factors are controlled by IL-3-, GM-CSF- and stem cell factor (SCF) signaling, which direct differentiation of the HSC towards the myeloid branch (Friedman et al., 2007).
Epigenetic regulation during hematopoiesis

Hematopoiesis is a highly regulated process in which pluripotent HSCs can differentiate into all lineages of the blood. At the basis of this process are signaling pathways, which can respond to extracellular stimuli by modulating gene expression, such that the HSC pool can react to the functional needs of the organism. Gene expression is controlled by various mechanisms such as sequence specific transcription factors that target gene promoters and enhancers. Multi-protein complexes that are recruited together with these transcription factors harbor enzymatic activities to modify chromatin, thereby marking a region to be transcribed.

Chromatin is a nucleoprotein complex built of nucleosome chains. A nucleosome consists of a histone octamer which packages 147 DNA base pairs (bp). Crystal structure studies revealed that these octamers are composed of two H3-H4 histone dimers bridged together as a stable tetramer that is flanked by two separate H2A-H2B dimers (Davey et al., 2002; Luger et al., 1997). Histones harbor amino- and carboxy-terminal tails that protrude from the nucleosomes. These tails are enriched in basic residues and are therefore a target for multiple post-translational modifications (PTMs), like phosphorylation, methylation, ubiquitination and acetylation. PTMs can influence the local chromatin structure and facilitate the recruitment of chromatin modifying factors. For example, when histone tails are hypoacetylated, tri-methylated on lysine residue 20 of H4 (H4K20me3) and tri-methylated on lysine residue 27 of H3 (H3K27me3), the
chromatin structure is compact and genes are silenced, in contrast hyperacetylation, tri-methylation on lysine residue 4 of H3 (H3K4me3) and tri-methylation on lysine residue 36 of H3 (H3K36me3) leads to a more open chromatin structure allowing gene transcription (Ruthenburg et al., 2007). Chromatin assembly is also regulated by DNA methylation, which is exclusively found on cytosine residues. Multiple studies have shown that methyl marks are associated with repressed chromatin and with silenced promoter activity (Bird et al., 1999). Furthermore, chromatin assembly is controlled by ATP dependent remodeling proteins. These proteins reposition nucleosomes, which can either lead to an open or a more compact chromatin structure, thereby affecting RNA transcription (Ho et al., 2010).

There is a variety of multi-protein complexes that regulate chromatin modifications. The Mi-2/nucleosome remodeling and deacetylase (Mi-2/NuRD) complex for example is a repressor complex that combines DNA methylation, histone deacetylation and ATPase chromatin remodeling activity. The core of this complex is formed by histone deacetylases (HDACs) 1 and 2, which harbor deacetylase activity, and are in complex with the ATPase activity of the Mi-2 protein (consisting of the subunits Mi2-α and Mi-2β) and the methyl CpG-binding domain (MBD) protein (Denslow et al., 2007). Studies in transgenic mice have shown that disruption of these multi-protein complexes can lead to severe hematopoietic phenotypes. To demonstrate the relevance of DNA methylation during hematopoiesis, a mouse model was constructed in which a deletion of the DNA methyltransferase 1 (Dnmt1) could be induced especially in hematopoietic cells. This resulted in complete ablation of HSCs and bone marrow progenitors by induction of cell-autonomous apoptosis. Moreover, hypomorphic Dnmt1 mice, which have reduced levels of Dnmt1 activity, already showed altered HSC differentiation, such that differentiation was restricted to the myeloid and erythroid lineages (Broske et al., 2009). Another example is a study where the conditional deletion of MI-2β, which is the ATPase remodeling activity in the NuRD complex, caused an increase in HSC cycling and an altered differentiation potential, which was restricted to the erythroid lineage (Yoshida et al., 2008).

Multi-protein complexes gain specificity by binding to transcription factors that can recruit the complex to a specific region in the genome. For instance, a recent study showed that the transcriptional co-factor friend of GATA-1 (FOG-1) interacts with the NuRD complex to recruit it to mast cell gene promoters (Hong et al., 2005). Furthermore, mice homozygous for a mutant form of Fog-1, which specifically disrupts the Fog-1/NuRD interaction showed severe hematopoietic abnormalities, indicating that proper targeting of the NuRD complex is crucial for normal hematopoiesis.

The next paragraph summarizes present knowledge on the role of HDACs, which are the core enzymes in a variety of multi-protein gene repressor complexes.

**Histone deacetylases**

HDACs deacetylate lysine (Lys) residues in histones, thereby changing the charge of Lys from neutral to positive resulting in chromatin compaction, which is associated with repression of gene transcription. Besides histones, HDACs deacetylate also
other protein substrates. For example, the activity of tumor suppressor p53 is partly regulated by Lys acetylation, while HDACs were shown to deacetylate p53 (Brooks et al., 2003; Zeng et al., 2006). Moreover, the transcription factor Stat3 is acetylated upon cytokine induction, which allows dimerisation required for DNA binding. HDACs were shown to reverse Stat3 dimerisation by deacetylation of the acetylated Lys residue (Yuan et al., 2005). Recently, Choudhary et al. identified 1750 proteins that harbored acetylated Lys using high-resolution mass spectrometry, demonstrating that acetylation is a global protein modification important in a variety of major cellular processes (Choudhary et al., 2009).

Phylogenetic analysis revealed that HDACs can be subdivided into four different classes that are based on domain homology and sequence similarity to yeast prototypes (Gregoretti et al., 2004). Class I HDACs are related to Saccharomyces cerevisiae Rpd3 and consist of HDAC1, HDAC2, HDAC3 and HDAC8. Of note, HDAC1 and HDAC2 exhibit an 82% amino acid sequence overlap, suggesting that they originate from gene duplication. Class II HDACs are subdivided in two subclasses: class Ila (HDAC4, HDAC5, HDAC7 and HDAC9) and class Iib (HDAC6 and HDAC10), which are homologues of Saccharomyces cerevisiae Hda1. Class III consists of NADH+ dependent HDACs, which are homologous to the yeast sirtuins and class IV consists of one member HDAC11, which is related to both class I and class II HDACs.

Class I members HDAC1, HDAC2 and HDAC3 are predominantly localized in the nucleus and can function as catalytic subunits of multi-protein complexes that repress transcription by specific interactions with DNA-sequence specific transcription factors. For instance, in mammals the Sin3, Mi-2/NuRD and the corepressor of RE1-silencing transcription factor (CoREST) complex have a core that consists out of the heterodimer HDAC1-HDAC2 (Yang et al., 2008). The class Ila HDACs contain nuclear import and export sequences, which allows shuttling between the nucleus and the cytoplasm suggesting a role as signal transducers (Khochbin et al., 2001; Verdin et al., 2003; Han et al., 2005). In contrast, HDAC6 which belongs to class Iib is predominantly localized in the cytoplasm (Boyault et al., 2007). It was demonstrated that HDAC6 plays a role in major cellular processes like cell motility and cell adhesion by deacetylation of α-tubulin and cortactin (Hubbert et al., 2002). The relatively unknown class IV HDAC11 was shown to regulate IL-10, which is an interleukin involved in immune tolerance (Villagra et al., 2009). Another study revealed a role for HDAC11 in gene expression of oligodendrocytes, specific myelin-producing cells in the central nervous system (Liu et al., 2009).

Genetically engineered mouse models demonstrated crucial roles for Hdacs in development. Deletion of Hdac1 results in embryonic lethality at E 9.5 due to proliferation defects in embryonic stem cells (Lagger et al., 2002; Montgomery et al., 2007). In contrast, mice with a homozygous deletion of Hdac2 are viable, although their body weight is severely reduced. Furthermore, these mice show enhanced memory and learning capacity due to increased synaptogenesis (Trivedi et al., 2007; Zimmermann et al., 2007; Guan et al., 2009; Zimmermann et al., 2007). Conditional deletion of both Hdac1 and Hdac2 in cardiomyocytes resulted in premature death
due to cardiac arhythmias and left ventricular dilation, indicating a crucial role for Hdac1 and Hdac2 in heart development (Montgomery et al., 2007).

Hdac1 and Hdac2 have also been implied as regulators of hematopoietic development, as it has been shown that the NuRD complex interacts with IKAROS, a key regulator that enables HSC differentiation along the lymphoid pathway (Kim et al., 1999). Moreover, studies in chicken B cells revealed that HDAC2 controls the expression of AIOLOS, PAX5 and EFB-1, transcription factors that regulate immunoglobulin heavy chain (IgH) transcription (Nakayama et al., 2007). Furthermore, it was shown that HDAC2 is required for Ig gene conversion, a diversification process active in chicken B cells (Lin et al., 2008). Studies in mice that lack Hdac1 and Hdac2 specifically in B cells revealed an early block in differentiation. In contrast, depletion of Hdac1 and Hdac2 in mature B cells had no negative effects. However, in vitro activation indicated that these cells lost the ability to proliferate, providing a role for Hdac1 and Hdac2 in cell cycle regulation (Yamaguchi et al., 2010).

Ablation of both Hdac1 and Hdac2 in the complete murine hematopoietic compartment

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**Figure 2.** Schematic representation of B cell development. Upon functional VDJ recombination of the IgH chain, pro-B cells differentiate into large pre-B cells, which express the pre-BCR. Subsequently, these cells undergo cycles of proliferation. Hereafter they differentiate into small pre-B cells. At this stage, IgL chain VJ recombination takes place. Functional pairing of IgH and IgL proteins leads to presentation of the BCR at the cellular surface and differentiation into immature B cells. Self tolerant immature B cells leave the bone marrow and further mature in the periphery where they start to express both IgM and IgD at their surface. When B cells encounter their cognate antigen they get activated with the help of T cells and undergo secondary diversification processes known as somatic hypermutation (SHM) and class switch recombination (CSR), which leads to high affinity antibodies with differential isotypes (for instance IgG). Finally, these activated B cells differentiate into memory B cells and antibody producing plasma cells.
for Hdac1 and Hdac2 in cell cycle regulation (Yamaguchi et al., 2010). Ablation of both Hdac1 and Hdac2 in the complete murine hematopoietic compartment causes anemia and thrombocytopenia associated with bone marrow cytopenia (Wilting et al., 2010). Interestingly, deletion of Hdac1 or Hdac2 alone in hematopoietic cells did not have any severe effect on hematopoiesis, indicating redundant functions of Hdac1 and Hdac2 in the hematopoietic system (Wilting et al., 2010; Zimmermann et al., 2007).

**The B cell lineage**

In mammals, B cell development begins in the bone marrow. During highly controlled differentiation steps, defined by the recombination state of Ig genes, a BCR is generated and expressed at the surface of the immature B cell. This developmental process generates the primary Ig gene repertoire. Immature B cells which are tolerant for self antigens migrate via the blood stream to the spleen where they differentiate into mature, naïve B cells. As part of immuno-surveillance, mature B cells circulate throughout the body. When a B cell encounters its cognate antigen, it may be triggered to undergo secondary Ig gene diversification processes. Subsequently, it differentiates into an antibody secreting plasma cell or long-lived memory B cell, which protects against future attacks by the same pathogen (figure 2).

**Generation of the primary Ig gene repertoire in precursor B cells: Ig loci and the mechanism of V(D)J recombination**

An antibody or Ig consists of two identical heavy chains and two identical light chains. The N-terminal part of the heavy and light chains harbors a variable domain, which mediates specific binding to antigen. In contrast, the C-terminal part consists out of constant domains, which determine the effector function of the antibody. Antibodies are encoded by the IgH and Ig light chain (IgL) genes; the latter can be either κ or λ. In the murine system, the IgH locus spans approximately 3Mb and is located near the telomeric end of chromosome 12. The locus is composed of arrays of variable (V), diversity (D) and joining (J) gene segments followed by an array of constant gene segments.

The variable portion of the antibody is encoded by V, D and J gene segments. In precursor B cells, these segments are joined somatically by a site specific V(D)J recombination (figure 3) (Tonegawa et al., 1983). This reaction requires expression of the recombination-activating genes 1 and 2 (RAG1 and RAG2), which together form the RAG endonuclease (Oettinger et al., 1990). To assemble V, D and J gene segments, RAG aligns two RSS sites and introduces two double stranded breaks (DSBs) at the border between recombination signal sequences (RSS) and coding segments. The RSS consists of a highly conserved heptamer, which is followed by a non-conserved spacer sequence of either 12 or 23 nucleotides and a conserved nonamer. Both V and J heavy chain segments (V<sub>H</sub> and J<sub>H</sub>) harbor a 23 spacer-RSS, which are located 3’ of V<sub>H</sub> and the 5’ of J<sub>H</sub>. The D heavy chain segments (D<sub>H</sub>) are flanked by 12 spacer-RSSs.
RAG activity requires one segment with a 23 spacer-RSS and one segment with a 12-spacer RSS, a specificity which is known as the 12/23 rule (Eastman et al., 1996). As a consequence, the majority of VDJ rearrangements in the IgH locus will result in a D segment sandwiched between a V and J segment. The κ and λ light chain loci are composed of only V and J segments and lack D segments. Also in these loci, the RSSs are in agreement with the 12/23 rule, Vκ segments are followed by a 12-spacer RSS and Jκ segments are preceded by 23-RSS. In contrast, Vλ light chain segments are followed by a 23-spacer RSS and Jλ segments by a 12-spacer RSS.

Figure 3. Schematic representation of VDJ recombination in the IgH locus. RAG initiates recombination between one D and one J segment at the germline (GL) IgH locus. Subsequently, RAG initiates recombination between the DJ join and one V-segment. Finally, the VDJ join will be transcribed and spliced into mRNA.

After RAG mediated cleavage, two hairpins are formed at the coding ends, which together with the two signal ends remain bound to RAG, a molecular ensemble known as the postcleavage synaptic complex (PSC) (Fugmann et al., 2000). Subsequently, the non-homologous end-joining (NHEJ) machinery ligates the joining ends together, while the intervening DNA, containing the signal ends, is looped out. This process starts with the Ku70 subunit, which binds to the coding ends thereby attracting Ku80. Ku80 in turn attracts the DNA-dependent protein kinase subunit (DNA-PKcs), which is required for the nuclease activity of Artemis, which generates junctional diversity by opening the hairpin of the coding ends decentrally. These open coding ends can be processed by different activities 1) the terminal deoxynucleotidyl transferase (TdT) or its relative polymerase μ (pol μ), which add non-templated nucleotides (N-regions) to coding ends 2) exonuclease activities that can introduce small deletions 3) polymerases that can extend the coding end, which lead to palindromic duplications (P-segments). Finally, the two coding ends are joined by Ligase IV, whose activity is enhanced by the X-ray repair cross complementing protein 4 (XRCC4) (VDJ recombination is reviewed by Jung et al., 2006).
Ordered Ig rearrangements dictates early B cell development

Early B cell development begins in the bone marrow when lymphoid precursors, which start to express the Rag1 and Rag2 genes, differentiate into pro-B cells through signaling via c-Kit, FLT3 and IL-7 receptors. At this stage, the IgH and IgL loci are relocated to the active environment of the nuclear center (Kosak et al., 2002). Here the chromatin, surrounding D and Jₜ gene segments and the IgH constant genes, becomes demethylated and hyperacetylated, thereby losing its closed formation. As a consequence, RAG can access the DNA and initiate Dₜ to Jₜ recombination on both alleles. Upon DJₜ rearrangement, the Vₜ region becomes activated and accessible to RAG, which then initiates recombination of a single Vₜ gene segment to the preexisting DJₜ joint (Cherry et al., 1999; Chowdhury et al., 2001; Hsieh et al., 1992). At this stage, the Vₜ region is contracted into DNA loops in a flower like structure. It was suggested that this structure places proximal and distal Vₜ gene segments in a similar distance to the DJₜ joint thereby equalizing the chance to recombine (Roldan et al., 2005; Sayegh et al., 2005; Jhunjhunwala et al., 2008).

An in-frame Vₜ, DJₜ joint will result in stable IgH mRNA, which is subsequently translated into IgH protein. The quality of membrane bound IgH is tested by its capacity to interact with the surrogate light chain (SLC) proteins VpreB1 and λ5. Recently Ubelhart et al., demonstrated that a conserved asparagine (N)-linked

![Figure 4](image-url)  
**Figure 4.** Schematic representation of allelic exclusion at the IgH locus. At the pro-B cell stage, both IgH alleles recombine a D to J segment. Subsequently one DJ join will recombine with a V segment. This can be either productive (1/3) or non-productive (2/3). If it is non-productive, the other DJ join gets a chance to recombine with a V segment, in case of a productive VDJ join, IgH protein is expressed and the pro-B cell differentiates into the pre-B cell stage. If this rearrangement is again non-productive the pro-B cell will die. In case the first V to DJ recombination is functional, IgH protein expressed at the surface will signal for silencing of V to DJ recombination at the other allele (this figure is adapted from Jung et al., 2006).
A proper interaction will lead to presentation of the pre-BCR–signaling complex at the surface of the cell which comprises the pre-BCR and the transmembrane signaling proteins Igα and Igβ. Signaling through the pre-BCR is essential, as it prevents the cell from undergoing apoptosis. Pro-B cells that fail to produce a functional pre-BCR are excluded from further differentiation and die. It was shown that signaling through the pre-BCR results in transient downregulation of RAG, thereby preventing further IgH recombination events (Grawunder et al., 1995). Hereafter, pre-B cells undergo two to seven cell divisions, thereby expanding the pre-B cell pool that succeeded in generating a functional IgH.

Gene deletion studies demonstrated that a functional pre-BCR complex, together with IL-7 receptor signaling are pivotal for driving this clonal expansion as deletion of IL-7Rα, the transmembrane portion of IgH, VpreB, λ5, Igα, Igβ, but also of downstream signaling components such as Syk and Zap70 abolishes pre-B cell expansion (Kitamura et al., 1992; Mundt et al., 2001; Shimizu et al., 2002; Gong et al., 1996; Kitamura et al., 1992; Schweighoffer et al., 2003). It was proposed that pre-BCR signaling downregulates SLCs expression, resulting in a gradual decrease of pre-BCR expression and signaling during subsequent cell doublings. As a consequence, RAG is no longer suppressed and its re-expression enables VJ rearrangement at the IgL loci (Herzog et al., 2009; Schlissel et al., 2003; Grawunder et al., 1995; Herzog et al., 2009). Upon functional light chain rearrangement, the pre-B cell differentiates to the immature B cell stage, which is characterized by surface expression of the BCR.

At the immature stage, B cells are tested for reactivity against self antigens. B cells that harbor a BCR that does not bind to self antigens are positively selected and leave the bone marrow immediately to continue maturation in the periphery. Immature B cells that harbor an autoreactive BCR can reactivate RAG and attempt to generate a new non-autoreactive BCR in a process known as receptor editing. Receptor editing involves the replacement of an already rearranged V\text{H} or V\text{L} segment by one that is still available. If V gene replacement results in a non-autoreactive BCR, the B cell continues maturation in the periphery. B cells that do not succeed to generate a non-autoreactive BCR will be deleted by apoptosis (Nemazee et al., 2006).

**Allelic exclusion**

In 1957, thus long before the identification of IgH and IgL loci, Burnet proposed his clonal selection theory, which states that each antibody producing cell should have a unique antibody specificity. Interestingly, not much later, experiments performed by Pernis and Cebra et al. using rabbit antisera against allotypic IgH and IgL demonstrated that B cells isolated from F1 rabbits express only one of the two possible allotypic IgH or IgL at their surface (Pernis et al., 1965; Cebra et al., 1966). Thus despite the presence of two heavy and two light chain alleles (both κ and λ), only a single allele appears to be expressed. The phenomenon that B cells are monospecific, i.e. usually express only a single IgH and IgL chain allele is known as allelic exclusion.
About twenty years later, studies by Alt et al. proposed the regulated model for allelic exclusion (Alt et al., 1980; Alt et al., 1984). This model was based on the observation that 40% of Igκ-expressing B cells rearrange both κ alleles, while 60% rearrange only one allele leaving the other allele in germline configuration (Coleclough et al., 1981; Coleclough et al., 1983). These findings suggested that a functional VJ rearrangement in one allele leads to silencing of further V to J rearrangements at the other allele. Subsequent studies revealed that, similar to the Igλ rearrangements, 40% of B cells carry VDJ-rearrangements on both heavy chain alleles, while 60% have only one allele that harbors a VDJ-rearrangement (figure 4) (Alt et al., 1984).

The virtual absence of germline configured IgH loci suggested an ordered rearrangement where D and J segments are rearranged on both alleles and subsequently a V segment is rearranged to the preexisting DJ join. According to these results, a DJ rearrangement at the IgH locus was considered as an equivalent of a germline Igκ locus. Therefore allelic exclusion of both IgH and Igλ rearrangements is controlled at the level of V gene rearrangements. The regulated model for allelic exclusion proposes that V to DJ rearrangement is ordered and that a functional VDJ rearrangement prohibits secondary V to DJ rearrangements. The chance that a V to DJ rearrangement will be in frame and lead to a functional mRNA is 1/3. In this case, IgH protein is produced and thought to inhibit V to DJ recombination at the other IgH allele via a feedback signal mediated by the surface exposed clonotypic pre-BCR. Accordingly, two thirds of the initial VDJ rearrangements is out of frame, as a consequence the transcribed RNA will be unstable and quickly degraded by the nonsense mediated decay machinery (Buhler et al., 2006). In this scenario the other allele gets a chance to recombine. Again the chance for a functional VDJ rearrangement is 1/3. A functional rearrangement will result in the generation of a pre-BCR and subsequent signalling for survival and further differentiation. Precursor B cells that fail the second attempt to generate a functional VDJ rearrangement will not be able to form a pre-BCR and die. However, B cells may take advantage of cryptic RSS sites within rearranged V_{H} segments to replace it with an upstream V_{H} element.

Strong evidence that supported feedback inhibition by IgH came from multiple transgenic mouse studies showing that expression of a functional IgH partially blocked rearrangement of the endogenous loci (Ritchie et al., 1984; Rusconi et al., 1985; Weaver et al., 1985). Two years later, the membrane-bound form of IgH was demonstrated to be essential in establishing allelic exclusion. While expression of a membrane bound IgH in precursor B cells prohibited V to DJ rearrangements of endogenous IgH alleles, the secretory version had no effect (Nussenzweig et al., 1987). The same conclusion was reached by studying IgH knock in mice that lack the membrane coding exons of IgH (Kitamura et al., 1992). Moreover, it was shown that the interaction of membrane bound IgH with the membrane signaling molecules Igα and Igβ appears to be essential as mutations blocking IgH chain association, as well as mutations in the cytoplasmic part of these proteins results in disruption of allelic exclusion and progression to the pre-B cell stage (for review Herzog et al., 2009 and
Papavasiliou et al., 1997). In agreement with these observations, knockout mouse models that harbor a deletion of Syk or Zap70, which are downstream signaling proteins of Igα and Igβ, show disrupted allelic exclusion (Schweighoffer et al., 2003).

The regulated model for allelic exclusion requires that V to DJ recombination is initiated on one allele while the other is actively prohibited from further rearrangements. An important question resulting from this concept is: How do cells that initiate V to DJ rearrangements on one allele inhibit V to DJ rearrangement on the other allele? Suggestions have been made that differential localization, locus contraction, allelic marking associated with early replication and demethylation may play a role in regulating monoallelic V to DJ rearrangements (Alt et al., 1980; Alt et al., 1992; Gorman et al., 1998; Mostoslavsky et al., 1998; Roldan et al., 2005). Recent studies by Hewitt et al. revealed that RAG and ATM together control monoallelic recombination and localization of the Ig loci. Their study indicated that the Ig alleles pair during the pro-B stage where V to DJ recombination takes place. In the absence of RAG, pairing of Ig alleles was reduced suggesting a role for RAG in Ig gene pairing. Moreover, the introduction of DNA breaks by RAG in one allele appears to induce ATM dependent repositioning of the other allele to pericentric heterochromatin (Hewitt et al., 2009).

Secondary Ig gene diversification in B cells: Somatic hypermutation and class switch recombination

The primary antibody repertoire generated by V(D)J recombination and combinatorial IgH and IgL chain pairing, is thought to provide around $10^5$-$10^6$ different antibody specificities. However, given the fact that two out of the three complementary determining regions (CDR1 and CDR2) are germline-encoded, i.e. within the V gene segments, the primary repertoire is despite its high complexity generally of low affinity (Davis et al., 1988). To further improve antigen binding, i.e. generate high affinity antibodies, antigen specific B cells have the unique capacity to initiate a mutation process that introduces single nucleotide substitutions into the variable region of Ig genes. This process of somatic hypermutation (SHM) occurs at an extraordinary rate of about $10^{-3}$ bp per generation, a million-fold higher as compared to the overall spontaneous mutations in our genome.

Besides SHM, antigen-specific B cells can also undergo class switch recombination (CSR), a deletional recombination event that alters the effector function of the antibody. During CSR, the constant μ region is replaced by one of the other downstream constant regions, resulting in an isotype switch from IgM/IgD to IgG, IgE, or IgA. Interestingly, both SHM and CSR are initiated by the activation induced cytidine deaminase (AID), an essential molecular component of secondary Ig gene diversification.

The germinal center reaction

Naïve B cells express a BCR of the IgM/D isotype that usually has a low affinity for its cognate antigen. When these cells migrate through the T cell rich areas of secondary
lymphoid organs, such as spleen, lymph nodes, Peyer’s patches and tonsils, they may get activated by antigen presenting follicular dendritic cells (FDC) and antigen specific T helper cells (Garside et al., 1998; MacLennan et al., 1997; Okada et al., 2005). Antigen-activated B cells undergo rapid proliferation and can have different developmental fates. Some may develop directly into antibody secreting plasmablasts, which reside in specialized extrafollicular areas like the medullary cords of lymph nodes, while others mature into GC-precursor B cells and move to the primary follicle, where they trigger a process known as the GC reaction (Liu et al., 1991; Jacob et al., 1991).

The primary follicle is a structure where initially recirculating naïve B cells reside in a network of FDCs. When GC-precursors start their clonal expansion and form a GC, they push naïve B cells to the outside of the follicle where they form the so-called mantle zone. The GC can be divided in a dark zone, which consists of densely packed proliferating GC B cells known as centroblasts, and a light zone where non-dividing centrocytes reside together with FDCs, specific T helper cells and macrophages. Centroblasts further diversify their IgH genes by SHM and CSR and subsequently express the newly modified BCR. These cells migrate to the light zone, and differentiate into small centrocytes, which are selected for improved affinity by FDCs. Selected centrocytes are stimulated to further differentiate into long-lived memory B cells or terminally differentiated plasmablasts (the GC reaction is reviewed by Klein et al., 2008).

**Somatic hypermutation**

SHM is a process that enables antigen-specific B cells of the GC to introduce point mutations into the V regions of rearranged IgH and IgL chain loci at a very high mutation rate of $10^{-3}$ bp per generation. As a consequence, the antigen binding sites encoded by the CDRs can be modified, which may result in Ig variants with an increased affinity for the cognate antigen. The hypermutation domain is restricted to a 2 kb window starting approximately 150 bp downstream of the IgH promoter (Rada et al., 2001). This observation suggested that transcription plays a role in SHM. Indeed, studies by Fukita et al. showed that replacing the strong endogenous IgH promoter by a weak truncated DQ52 promoter results in diminished SHM (Fukita et al., 1998). Moreover, it was shown in a SHM competent cell line that the mutation rate is proportional to the rate of transcription, confirming the importance of transcription for SHM (Bachl et al., 2001).

Although transcription of the IgH locus is crucial for SHM, other factors must be involved, as other highly transcribed genes in GC B cells are not mutated, at least not at a rate comparable to Ig genes. Remarkably, as shown in Ung/Msh2 double mutant mice, 25% of the 120 most highly transcribed genes do get mutated in GC B cells, albeit normally at a frequency that is 100 fold lower as compared to Ig genes (Liu et al., 2008). The mutation spectra of hypermutated Ig genes revealed that all four bases can be mutated, with transitions predominating transversions (Golding et al., 1987). Additionally, mutated Cs were often seen in conjunction with a RGYW/WRCY hotspot motif (R is a purine base A or G, Y a pyrimidine base C or T, and W
weak hydrogen bond A or T) (Rogozin et al., 1992). Furthermore, it was proposed that the E2A (CAGGTG) binding motif might play a role in the recruitment of AID, as insertions of this motif in the variable region of the IgH enhances SHM without altering transcription (Michael et al., 2003). Interestingly, the primary sequence of the hypermutation domain turned out not to be crucial, as replacement by a heterologous gene showed no difference in mutation rate (Yoshikawa et al., 2002). Mouse IgL transgenes revealed that deletion of the intronic or 3′ Igκ enhancer strongly reduced SHM, suggesting an important role of transcription and transcriptional enhancers in SHM (Betz et al., 1994; Goyenechea et al., 1997; Klix et al., 1998).

While these studies provided critical insights regarding the role of cis-acting elements in controlling the outcome of SHM, the molecular mechanism of SHM remained unknown, since its discovery more than 40 years ago. A major break through concerning the initiation of SHM was provided by the Honjo lab in 1999. Using cDNA subtraction they identified AID as the initiator of CSR and interestingly also of SHM (Muramatsu et al., 1999; Muramatsu et al., 2000). A series of independent studies indicated that AID acts as a cytidine deaminase on ssDNA. AID binds and deaminates ssDNA but not dsDNA in vitro or in E. coli (Bransteitter et al., 2003; Chaudhuri et al., 2003; Dickerson et al., 2003; Pham et al., 2003; Ramiro et al., 2003). The binding of AID to ssDNA is facilitated by its interaction with replication protein A (RPA), a protein that forms DNA/protein filaments to stabilize ssDNA (Basu et al., 2005). Deamination of a deoxy-cytidine (dC) generates a deoxy-uracil (dU), the primary lesion thought to be responsible for all mutations associated with SHM.

How can deamination of a cytidine result in mutations in all of the four different bases? G:C to A:T transition mutations are easily explained by replication over dU (dU instructs a template dT), but this cannot explain G:C transversions and A:T mutations. Evidence that the initiating U:G lesion attracts natural DNA repair processes for mutagenic purposes came from different studies in E. coli, mouse and human, in which the base excision repair (BER) component Uracil-N-glycosylase (UNG) was inhibited or lacking. UNG removes dU from the genome resulting in an abasic site. Lack of UNG causes a significant increase in C to T and G to A transition mutations; this increase is paired with a significant decrease in G and C transversion mutations (Imai et al., 2003; Petersen-Mahrt et al., 2002; Rada et al., 2002). Moreover, mice deficient in MSH2, MSH6, or Exo1, three key factors involved in mismatch repair (MMR), displayed alterations in the spectrum of point mutations in GC B cells. In particular, a significant reduction of point mutations at template A and T was observed (Martomo et al., 2004; Phung et al., 1998; Rada et al., 1998; Wiesendanger et al., 2000).

These observations led to the broadly accepted DNA deamination model of SHM. The model proposes that upon deamination of a dC, which results in a dU, three mutagenic pathways can process this initial lesion: 1) Direct replication across the dU instructs a template T to generate predominantly G:C to A:T transitions, a finding consistent with SHM analysis in Ung/Msh2 double mutant mice. 2) The dU can be removed by UNG to generate a non-instructive abasic site. During replication, specific
translesion synthesis (TLS) polymerases can get activated to continue error-prone replication across the non-instructive abasic site, generating both G:C transitions as well as transversions. 3) Alternatively, the U/G mismatch can be recognized by the MSH2/MSH6 heterodimer resulting in excision of the mismatch and a single stranded gap. An error-prone fill in reaction by TLS polymerases results in A/T mutations surrounding the initial U/G mismatch.

Evidence supporting the existence of individual mutator pathways was provided in mice and patients carrying defined mutations. XPV patients suffer from a variant form of xeroderma pigmentosum (XP) that does not relate to global nucleotide excision repair, but rather defective DNA damage tolerance in response to UV lesions, caused by the lack of an active TLS Pol η. Intriguingly, SHM analysis of these patients revealed a selective decrease of A/T mutations, a finding that was later confirmed in Pol η deficient mice (Delbos et al., 2005). In fact, albeit that Pol κ can replace to some extent Pol η in generating somatic mutations at template A or T (Faiili et al., 2009), Pol η has been identified as the sole contributor to A/T mutations in hypermutating Ig genes. Furthermore, as shown in PCNA<sup>K164R</sup> mutant mice, the recruitment and activation of the Pol η strongly depends on site specific ubiquitination of PCNA (Langerak et al., 2007). PCNA-Ub activates Pol η to generate the vast majority of A/T mutations downstream of MSH2/MSH6 as well as UNG (Krijger et al., 2009). In contrast, mutations at template G/C appear independent of modification of PCNA-Ub. For example, G to C transversions, which are normally generated by the related TLS polymerase Rev1, are generated normally in PCNA<sup>K164R</sup> mutant mice.

In summary, the mutation outcome depends on the activity of AID, the initiation of canonical BER (UNG) or MMR pathways (MSH2, MSH6, or Exo1), post-translational modifications of the DNA sliding clamp PCNA and selective recruitment and activation of error prone TLS polymerases, each of which displays its characteristic error signature.

**Class switch recombination**

CSR is an intrachromosomal deletional recombination event, which can replace the constant μ gene segment for one of the other downstream constant gene segments encoded by μ, γ, ε and α, resulting in an isotype switch from IgM/IgD to IgG, IgE, or IgA. Each of the different isotypes is involved in specific antigen removal processes. For instance, IgM antibodies can activate the complement system, while the most abundant isotype in the serum, IgG, can activate phagocytic cells by binding to specific Fc receptors at their surface. (Torres et al., 2008). CSR takes place anywhere within or near switch (S) regions, which are specific regions that precede the constant genes (Dunnick et al., 1993; Min et al., 2005). S regions consist of tandem repeats of short G-rich sequences (20-80 bp) with an overall length varying from 1 kb to 12 kb. Interestingly, like SHM, also CSR is initiated by AID, which converts cytosines in S regions to uracils by deamination (Chaudhuri et al., 2003; Dickerson et al., 2003; Muramatsu et al., 2000; Petersen-Mahrt et al., 2002; Revy et al., 2000).
It was shown that CSR sites correlate with RGYW/WRCY hotspot motifs, known to be preferentially targeted in SHM (Chaudhuri et al., 2004; Min et al., 2005). In addition, it was demonstrated that ssDNA, the substrate of AID, is stabilized in the G-rich S regions. The G-richness of the S regions allows the formation of stable RNA-DNA complexes after S region transcription. In these complexes, known as R-loops, the G-rich RNA transcript stably pairs with the C-rich DNA template. This causes the G-rich DNA strand to be displaced as a single strand (Daniels et al., 1995; Ramiro et al., 2003; Reaban et al., 1990; Yu et al., 2003). Maizels et al. showed that this G-rich ssDNA could be stabilized by the formation of characteristic structures known as G-loops, thereby facilitating the binding of AID to the S regions (Duquette et al., 2004; Yu et al., 2005). AID action on these ssDNA substrates results in dC deamination leading to dU at the position of the original dC. Different studies have shown that these dUs are required for generation of S region DSBs, the necessary intermediates for the recombination process (Catalan et al., 2003; Rush et al., 2004). Evidence that the BER machinery is involved in processing these dU lesions came from studies using Ung deficient mice. These studies revealed that CSR is reduced in Ung−/− splenic B cells induced to undergo CSR by approximately 95%. This reduction was associated with a significant decrease in DSBs (Imai et al., 2003; Rada et al., 2002; Schrader et al., 2005). Accordingly, it was proposed that UNG excises the dU in the S regions created by AID. In the BER pathway apurinic/apyrimidinic endonuclease 1 (APE1) subsequently repairs the abasic sites left by UNG by incising the phosphate backbone of DNA abasic sites. As APE1 is an essential gene, studies using splenocytes from Ape1−/− mice confirmed a role for APE1 in CSR as a 30-40% reduction in switching and a reduction in DSBs in the S regions was observed (Meira et al., 2001; Raffoul et al., 2004). In the canonical BER pathway DNA Pol β would close the single nucleotide gap that is generated by the combined action of UNG and APE1. A study by Wu et al. showed that pol β−/− splenocytes, isolated from fetal liver, have an increase in CSR varying from 1.5-1.7 fold (Wu et al., 2007). This observation suggests that Pol β attempts to faithfully repair the S region lesions, but that its activity is not sufficient to repair all lesions downstream of APE1.

An alternative pathway that could explain the generation of staggered DSBs downstream of AID is MMR. Evidence for a role of MMR in CSR came from studies of mice lacking MMR genes, like Msh2, Msh6, Mlh1, Pms2 and Exo1, showing a reduction in CSR activity varying from two to seven fold depending on the gene and Ig isotype (Bardwell et al., 2004; Ehrenstein et al., 1999; Ehrenstein et al., 2001; Kadyrov et al., 2006; Li et al., 2004; Martin et al., 2003; Martomo et al., 2004; Schrader et al., 1999). According to a model described by Stavnezer et al. (Stavnezer et al., 2006) Msh2-Msh6 can recognize and bind U:G mismatches created by AID. Subsequently, Mlh1-Pms2 is recruited to the lesion and Exo1 excises from the nearest 5′SSB created by AID-UNG-APE activity towards the mismatch. Exo1 continues past the mismatch until it reaches a SSB on the other strand, thereby creating a DSB.

DSBs generated by the combined action of BER and MMR are repaired by the classical NHEJ pathway. This pathway is triggered by the phosphoinositide kinase
ataxia-telangiectasia mutated (ATM), which plays a central role in orchestrating a network of cellular responses like cell cycle control, DNA repair and apoptosis. Patients deficient for ATM, show besides chromosomal instability and cancer predisposition also immunological defects such as IgA and IgG deficiency, suggesting a defect in CSR (Lavin et al., 1997). Moreover, studies in ATM deficient mice revealed similar findings as splenic B cells derived from these mice cannot switch efficiently to IgG and IgA as compared to WT B cells (Lumsden et al., 2004; Reina-San-Martin et al., 2004).

γH2AX is known to be phosphorylated by ATM upon DNA damage, and was shown to be localized in foci near DNA break sites (Burma et al., 2001). In B cells undergoing CSR, it was shown that these foci including also NBS1 co-localize at the Ig constant genes during the G1 phase of the cell cycle, suggesting a role in the response to CSR-induced DSBs (Petersen et al., 2001). 53BP1, which is another protein phosphorylated by ATM upon DNA damage, also participates in CSR as deletion of 53BP1 in mice results in decreased serum levels of IgG and IgA. In vitro CSR-assays using 53BP1-deficient B cells revealed a severe reduction in CSR to less than 10% of the WT levels (Manis et al., 2004; Ward et al., 2004).

After the action of the damage signaling proteins, Ku70-Ku80 comes into play and mediates synapses of the two DNA ends, thereby positioning the ends to allow end processing and direct end-to-end joining (Lieber et al., 2003; Meek et al., 2004). Ku70-Ku80 binds to the DNA ends and serves as a tool belt for the end joining reaction by recruiting enzymes that affect the recombination process. After binding, Ku slides away from the ends, allowing the catalytic subunit, the DNA-PKcs, to bind to each end (Walker et al., 2001). DNA-PKcs bind and phosphorylate Artemis, thereby activating its DNA end processing activities. Subsequently, the DNA ends are ligated by the two protein ligase complex XRCC4-Ligase IV (Casellas et al., 1998; Manis et al., 1998; Pan-Hammarstrom et al., 2005; Reina-San-Martin et al., 2003). Additionally, Ku70-80 improves the binding of XRCC4-ligase IV to DNA ends. Interestingly, XRCC4 and ligase IV deficient mice showed 25% of the normal level of CSR, indicating that these enzymes are important for CSR, but also that there must be another alternative pathway involved in CSR besides the classical-NHEJ (Yan et al., 2007).

**Memory B cells and terminally differentiated plasma cells**

Selected high affinity BCR-variant GC-B cells can differentiate in either terminally differentiated antibody secreting plasma cells or into memory B cells. Plasma cells can develop from marginal-zone- and follicular B cells, and from memory B cells. Terminally differentiated plasma cells migrate from the spleen to the bone marrow, where stromal cells provide survival signals. These plasma cells can survive long term, providing a source of high affinity antibody, well after antigen clearance. When an activated GC-B cell differentiates into a memory B cell, it retains its high affinity BCR, but in contrast to plasma cells it does not secrete antibody. Memory B cells are long-lived cells that will respond rapidly, by an extensive proliferative burst, upon secondary antigen encounter (Tangye et al., 2003). Subsequently, the memory B cells can differentiate into antibody producing plasma cells.
Oncogenic risks of Ig gene diversification

The hallmark of many types of B cell lymphoma is a reciprocal translocation between the Ig locus and an oncogene. As a consequence, the oncogene is placed into close proximity of Ig regulatory elements, resulting in deregulation of its expression. For example, translocations between MYC and IgH [t(8;14)] are associated invariably with the sporadic form of Burkitt lymphoma, BCL6 t[(3;14)] and less frequent BCL2 t[(14;18)] with diffuse large B cell lymphoma (DLBCL), fibroblast growth factor receptor 3 (cyclinD3) t[(4;14)], c-MAF t[(14;16)] and MUM1/IRF4 t[(6;14)] with multiple myeloma, and PAX5 t[(9;14)] with lymphoplasmacytic lymphoma (Kuppers et al., 2001). As DSBs are the necessary intermediates of these translocations, it was proposed that deregulation of Ig gene remodeling processes like V(D)J recombination, SHM and CSR might be involved in the generation of these breaks. Indeed, translocation breakpoints were identified near RSS sites, in the V region and in the S regions.

Evidence for the oncogenic potential of RAG came from studies showing that truncated versions of RAG were capable of integrating signal end flanked DNA fragments into plasmid substrates through a transposition-type reaction (Fugmann et al., 2000). More recently, studies revealed that RAG transposition can also occur in vivo. This was shown by approaches that permit selection of cells in which DNA fragments flanked by signal ends have integrated ectopically into the genome (Messier et al., 2003; Reddy et al., 2006). The oncogenic potential of RAG could also be explained by miss-joining of two DNA ends generated by RAG. This would include one end at an RSS site in the Ig locus and one end at a cryptic RSS in a non-Ig gene. The latter does not necessarily involve the action of RAG and these ends might be generated by alternative mechanisms. Interestingly, a study by Tsai et al. showed that the majority of translocation breakpoints in pro- and pre B cells occur at CpG islands. These translocations require the combined action of AID and RAG (Tsai et al., 2008). A model was proposed where AID deaminates methylated cytosines resulting in T:G mismatches. Subsequently, these mismatches are recognized by RAG before or during attempted repair. The resulting DNA ends now join to a V(D)J recombination signal end or an end of another break, leading to a chromosomal translocation.

Evidence for a critical role of AID in the generation of t(8;14) translocations came from in vitro studies showing that AID promotes chromosomal translocations involving the proto-oncogene c-Myc and the Ig S regions in mouse splenic B cells stimulated with LPS and IL-4 (Ramiro et al., 2006). Moreover, studies using a IL-6 transgenic mouse model showed that AID is required for the generation of IL-6 induced c-Myc-IgH translocations in hyperplastic lymph nodes, which can lead to plasmacytomas (Ramiro et al., 2004). Robbiani et al. were the first to provide direct evidence demonstrating that AID is required for the c-Myc DNA breaks required for IgH-c-Myc translocations by performing gene targeting experiments (Robbiani et al., 2008). More direct evidence proving a role for AID in B cell lymphomagenesis came from a study where different B cell lymphoma mouse models were crossed with mice deficient for AID. It was convincingly shown that only Bcl-6 transgenic mice, which
normally develop GC derived lymphoma, were prevented from GC derived lymphoma development. (Pasqualucci et al., 2008).

Apart from specific translocations, the malignant transformation of B cells requires additional genetic alterations, some of which might relate to aberrant SHM. There is strong evidence that SHM and aberrant targeting of AID occurs in (onco)-genes, like BCL6, FAS, IGA, IGB, RHOD, PIM1, PAX5, MYC and others (Gordon et al., 2003; Liu et al., 2008; Odegard et al., 2006; Pasqualucci et al., 1998; Shen et al., 1998). In addition, employing SHM reporter genes two independent studies demonstrated that AID triggers mutagenesis of predefined SHM substrates at random insertion sites in the genome (Parsa et al., 2007; Wang et al., 2004). Further evidence for the mutagenic potential and aberrant targeting of AID has been provided recently for myeloid cells. During the B lymphoid blast crisis of chronic myeloid leukemia (CML), AID confers drug resistance by mutating the BCR-ABL1 fusion (Klemm et al., 2009).

OUTLINE OF THIS THESIS

This thesis covers research concerning epigenetic and genetic remodeling during hematopoiesis. Chapters 2 and 3 present findings about the critical role of Hdac1 and Hdac2 in proliferation and hematopoiesis. Chapter 4 provides the first evidence on a novel role of IgH gene transcription in controlling primary antibody diversification, while chapter 5 tackles a central issue in secondary antibody diversification processes, the specificity of AID targeting during SHM and CSR in B cells.

HDACs control a variety of cellular processes by deacetylation of lysine residues in histone and non-histone substrates. HDAC inhibitors (HDACi) are proposed to have a great potential in cancer treatment and are presently applied in the clinic. However, the precise mechanism on how HDAC inhibition leads to tumor suppression remains largely unknown. To learn more about HDAC function, we focused on the class I HDACs HDAC1 and HDAC2, which are frequently upregulated in hematological neoplasms and are efficiently targeted by HDACi. Our studies made use of specific mouse models that enable conditional ablation of Hdac1 and Hdac2 in vivo. Studies in MEFs indicated a critical role for Hdac1 and Hdac2 in cell proliferation. Moreover, studies in mice revealed that specific ablation of Hdac1 and Hdac2 in the hematopoietic compartment resulted in anemia and thrombocytopenia, which was found to be caused by a severe bone marrow cytopenia (chapter 2). Further studies indicated an intrinsic role for Hdac1 and Hdac2 in maintaining HSCs. In addition, these studies provided further insights into the role of Hdac1 and Hdac2 in lymphocyte and erythrocyte development. Suprisingly, low levels of Hdac1 and Hdac2 caused cancer, which provides a cautionary note regarding the application of HDACi in cancer treatment (chapter 3).

The second half of this thesis specifically focuses on Ig gene remodeling during B cell development. Chapter 4 focuses on the role of IgH transcription in regulating allelic exclusion during V(D)J recombination. Using specific IgH knock-in
mouse models, in which \( IgH \) transcription can be dissected from \( IgH \) translation, we demonstrate a novel role for \( IgH \) transcription in the control of V(D)J recombination and allelic exclusion. After functional VDJ recombination at one \( IgH \) chain allele, the transcript itself is able to inhibit rearrangements of the other allele, a finding that changes the 30 year old dogma stating that allelic exclusion is regulated exclusively at the level of \( IgH \) protein.

In chapter 5, we investigate the specificity of secondary Ig gene diversification in GC B cells. In contrast to primary diversification which is mediated by site specific recombination, secondary Ig gene diversification, i.e. SHM and CSR, is not site-specific but targeted to transcribed promoter proximal Ig regions. Both, SHM and CSR critically depend on the mutation inducer protein AID. As mistargeting of AID has been implicated in B cell lymphomagenesis, we here addressed the genome wide targeting specificity of AID using the DamID approach. Our data indicate that AID binding is not restricted to the \( IgH \) loci but scattered throughout the genome. Moreover, AID binding favors active genomic regions, which are enriched in GC content.

Finally, the highlights of this thesis are discussed and reflected to present literature (chapter 6).
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**1**

**INTRODUCTION & OUTLINE**


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