TRANSCRIPTIONAL NETWORKS IN DEVELOPING AND MATURE B CELLS

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Abstract | The development of B cells from haematopoietic stem cells proceeds along a highly ordered, yet flexible, pathway. At multiple steps along this pathway, cells are instructed by transcription factors on how to further differentiate, and several check-points have been identified. These check-points are initial commitment to lymphocytic progenitors, specification of pre-B cells, entry to the peripheral B-cell pool, maturation of B cells and differentiation into plasma cells. At each of these regulatory nodes, there are transcriptional networks that control the outcome, and much progress has recently been made in dissecting these networks. This article reviews our current understanding of this exciting field.

ALLELIC EXCLUSION
In theory, every B cell has the potential to produce two immunoglobulin heavy chains and two immunoglobulin light chains. In practice, however, a B cell produces only one immunoglobulin heavy chain and one immunoglobulin light chain. The process by which production of two different chains is prevented is known as allelic exclusion.

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doi:10.1038/nri1633

The haematopoietic system constantly generates a large number of specialized cell types from pluripotent haematopoietic stem cells (PHSCs), which have self-renewal potential and give rise to different progenitors with a more restricted differentiation capacity. Each developmental step is a binary commitment switch, so at each of these steps, the cell can take alternative directions (FIG. 1).

One of the earliest differentiated precursors is the multipotential progenitor (MPP), which is at the bifurcation between the myeloid and lymphoid lineages: MPPs can differentiate into common myeloid progenitors (CMPs) or into the recently identified early lymphoid progenitors (ELPs)[1]. The latter start to express recombination-activating gene 1 (*Rag1*) and *Rag2* (REF. 2) and initiate rearrangement at the immunoglobulin heavy chain (IgH) locus (TABLE 1). ELPs can further differentiate into thymic precursors of the T-cell lineage (early T-cell-lineage progenitors, ETPs)[3] or into bone-marrow common lymphoid progenitors (CLPs), which are lymphoid restricted and can generate B cells, T cells, dendritic cells (DCs) and natural killer (NK) cells. Expression of the B-cell marker B220 by a subset of CLPs (known as pro-B cells or CLP-2s) coincides with their entry into the B-cell-differentiation pathway. The next step can be identified by expression of CD19 and completion of IgH diversity (D_H)-to-joining (J_H) gene-segment rearrangement by pre-BI cells. (However, pro-B cells still show plasticity and can efficiently develop into T cells under certain conditions[4].) The IgH locus then continues to rearrange its variable (V)-region gene segments until productive V_H–DJ_H alleles are generated in large pre-BII cells. These cells cease to express *Rag1* and *Rag2*, and they display the product of the rearranged IgH gene at the cell surface; there, it assembles with the surrogate immunoglobulin light chains (IgLs) VpreB and λ5, together with the signalling molecules Igα (which is encoded by the *MB-1* gene) and Igβ (which is encoded by the *B29* gene) to form the pre-B-cell receptor (pre-BCR).

Expression of the pre-BCR is a crucial check-point in early B-cell development, at which the functionality of the heavy chain is monitored. Signalling through the pre-BCR allows for ALLELIC EXCLUSION of the IgH locus[5] and stimulates a burst of proliferative clonal expansion of large pre-BII cells, which is followed by re-expression of RAGs and rearrangement at the IgL locus in small pre-BII cells. Allelic exclusion of the IgH locus might also be achieved through an alternative pathway that does not require pre-BCR function, as indicated by mice that show IgH allelic exclusion despite lacking expression of the surrogate IgLs[6].

During normal development, appearance of the assembled BCR at the cell surface defines the immature B-cell stage, at which cells are tested for

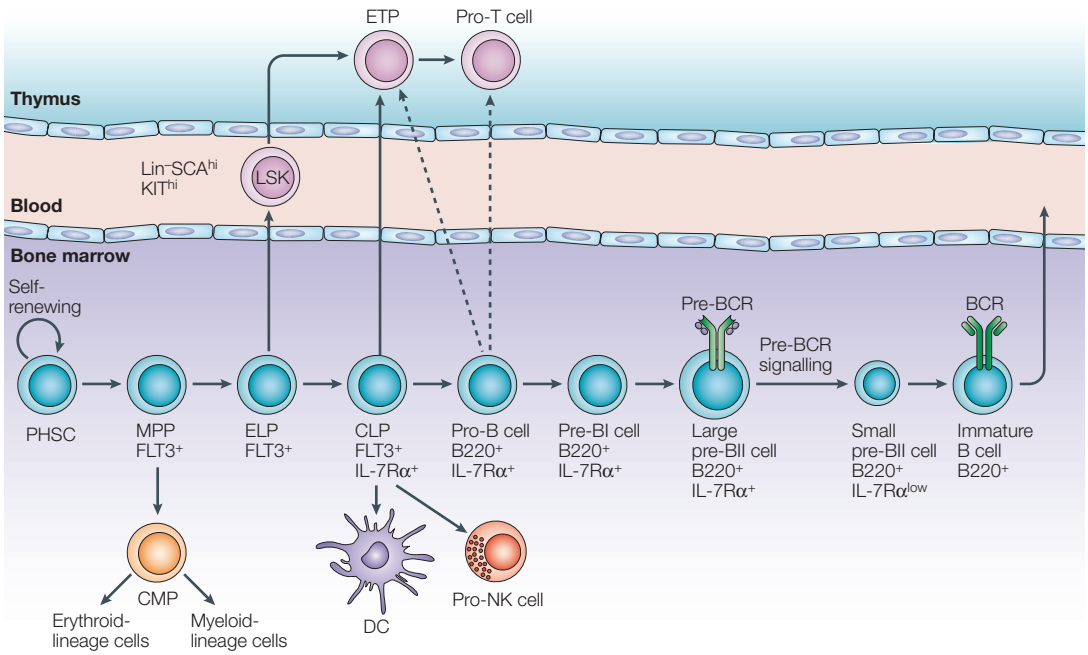

Figure 1 | **B cells develop from early haematopoietic progenitors.** This diagram summarizes adult haematopoietic-cell development, with an emphasis on B cells. The main compartments — bone marrow, thymus and blood — are shown. The various developmental stages that have been defined are indicated, as well as their relative order. Dashed arrows show pathways that are not yet firmly established. Pluripotent haematopoietic stem cells (PHSCs), multipotential progenitors (MPPs), common myeloid progenitors (CMPs), early lymphoid progenitors (ELPs) and common lymphoid progenitors (CLPs) are known as lineage (Lin)$^-$ cells; these cells lack detectable expression of any of the markers that are associated with cells of the mature blood lineages or their committed progenitors: that is, CD3, CD8, B220, CD11b, CD19, GR1 and TER119. Cells that are defined as LSK are Lin$^-$ stem-cell antigen (SCA)^{hi}KIT^{hi}, which is a possible precursor stage to early T-cell-lineage progenitors (ETPs)[112]. Using the Hardy classification[113], pro-B cells (also known as fraction B/C) are defined as B220$^+$CD43$^+$, and pre-B cells (also known as fraction C′/D) are defined as B220$^+$CD43$^-$ surface IgM$^-$. BCR, B-cell receptor; DC, dendritic cell; FLT3, fms-related tyrosine kinase 3; NK, natural killer; IL-7Rα, α-chain of the interleukin-7 receptor.

autoreactivity. B cells that are autoreactive can be rescued by a secondary immunoglobulin gene rearrangement, which is known as receptor editing[7], otherwise they are eliminated or inactivated by apoptosis or anergy, respectively. After they have successfully passed this examination, immature B cells leave the bone marrow and enter the periphery, where they seed the spleen and further differentiate through several transitional stages[8]. They eventually become mature B cells, which can be categorized into two main groups — FOLLICULAR B CELLS and MARGINAL-ZONE B CELLS — that have specific anatomical locations and distinct properties (BOX 1; TABLE 2). In addition, transitional B cells can be negatively selected in the periphery, so this is a crucial check-point for the generation of mature B cells.

Our understanding of the different stages of B-cell development is based on results from complementary analyses: definition of discriminating cell-surface markers (TABLE 1), analysis of the status of immunoglobulin gene rearrangement and examination of the capacity of cellular fractions to differentiate *in vitro* or *in vivo* into cells with defined properties. In addition, gene-targeting studies of many regulatory molecules in mice have now identified several check-points along the B-cell-differentiation pathway and have shown the importance of specific factors, thereby beginning to reveal the underlying genetic control of this process. From these studies, a picture has emerged that B-cell development, maturation and function are coordinated by a 'battery' of transcription factors and signal-transduction molecules that regulate the sequential execution of the different steps. In some cases, alternative differentiation routes that challenge the more common view have been described. An example of this is provided by a rare population of B220$^-$CD19$^+$ mouse cells: these cells — which, on the basis of CD19 expression, would classically be considered to be committed B cells (that is, pre-B cells) — can nevertheless efficiently differentiate *in vitro* into macrophages[9]. Such observations underscore the great flexibility of the haematopoietic system. This article focuses on the role of transcription factors in the control of B-cell development and maturation, an area in which considerable progress has been made in the past few years.

From stem cells to early B-cell progenitors

PU.1 and MPPs. PU.1 — a transcription factor of the ETS FAMILY (FIG. 2) — is a protein that is crucial in the early stages of haematopoietic-cell lineage specification. *Pu.1*$^{-/-}$ mice die during embryonic development at day 18.5 (E18.5), and they lack B cells, T cells, monocytes, and granulocytes[10,11] (FIG. 3). In *Pu.1*$^{-/-}$ fetal livers, there is also a reduction in the number of MPPs (which are defined as fms-related tyrosine

FOLLICULAR B CELLS
A recirculating, mature B-cell subset that populates the follicles of the spleen and lymph nodes.

MARGINAL-ZONE B CELLS
A static, mature B-cell subset that is enriched mainly in the marginal zone of the spleen, which is located at the border of the white pulp.

ETS FAMILY
A family of transcription factors that have a DNA-binding domain with homology to the avian leukosis virus E26.

ZINC-FINGER TRANSCRIPTION FACTORS
A class of transcription factors that contain a DNA-binding domain in which cysteine and histidine residues are coordinated by zinc atoms and thereby form 'fingers' that bind DNA.

kinase 3 (FLT3)$^+$lineage (Lin)$^-$AA4.1$^+$), and those that can be detected are impaired in their capacity to enter the B-cell-differentiation pathway in response to interleukin-7 (IL-7) and stromal-cell contact[12]. In addition, these mutant progenitors also fail to respond to myeloid cytokines such as granulocyte colony-stimulating factor (G-CSF) and macrophage colony-stimulating factor (M-CSF), as the genes that encode the corresponding receptors depend on PU.1 for their expression; however, the mutant progenitors can proliferate *in vitro* in the presence of stem-cell factor (also known as KIT ligand), IL-3 and IL-6 (REFS 13,14). *Pu.1*$^{-/-}$ MPPs also show reduced expression of the cytokine receptors FLT3 and the α-chain of the IL-7 receptor (IL-7Rα), and this can explain the previously mentioned proliferative defects. Expression of *Il-7rα* in *Pu.1*$^{-/-}$ progenitors rescues the development of CD19$^+$ B cells, but only partially, indicating that PU.1 has additional important targets at this stage of B-cell development[14]. PU.1 has been suggested to control *Il-7rα* gene expression through direct promoter binding, so it is crucial in supporting early B-cell development[14]. By contrast, the highly related ETS protein SPI-B, which is expressed at all stages of B-cell development[15] (similar to PU.1), does not seem to be required for IL-7Rα expression[16], and it cannot compensate for the absence of PU.1. Furthermore, expression of SPI-B from the *Pu.1* locus in mice was found to rescue myeloid differentiation but not the defect in lymphopoiesis, showing that these two proteins have specific roles in haematopoietic-cell development[17].

Intriguingly, the levels of PU.1 seem to be crucial for the decision to enter either the B-cell pathway or the myeloid-cell pathway: low PU.1 levels correlate with B-cell lymphopoiesis, whereas high PU.1 levels suppress the B-cell fate and promote myeloid-cell development[18]. Consistent with this, it was found that mice expressing ~20% of normal PU.1 levels, as a result of deletion of an upstream regulatory element of the *Pu.1* gene, show a block in early myeloid-cell differentiation and an accumulation of immature cells, which eventually leads to the development of acute myeloid leukaemia[19].

Ikaros and CLPs. The ZINC-FINGER TRANSCRIPTION FACTORS of the Ikaros, Aiolos and Helios family have been found to be crucial for cell-fate specification at very early stages of haematopoietic-cell differentiation. Ikaros has numerous splice variants and is expressed by all haematopoietic-cell lineages but mainly by T cells[20], whereas Helios is restricted to PHSCs and T-cell subsets[21]. By contrast, Aiolos is expressed by pre-B cells and pre-T cells, but its expression is maintained mainly by mature B cells[22]. These factors have a similar organization: they have an amino (N)-terminal DNA-binding domain and a carboxy (C)-terminal dimerization domain through which they form homodimers and

Table 1 | **Expression markers defining B-cell developmental stages in the bone marrow**

Marker	PHSC	MPP	ELP	CLP	Pro-B cell (CLP-2)	Pre-BI cell	Pre-BII cell (large)	Pre-BII cell (small)	Immature B cell
Lineage	–	–	–	–	+	+	+	+	+
FLT3	–	+	+	+	(+)	–	–	–	–
KIT	+	+	+	(+)	(+)/–	(+)	–	–	–
CD25	–	–	–	–	–	–	+	+	(+)
IL-7Rα	–	–	–	+	+	+	+	(+)	–
CD43	–	–	–	–	+	+	+/–	–	–
B220	–	–	–	–	+	+	+	+	+
CD19	–	–	–	–	–	+	+	+	+
RAG1	–	–	+	+	+	+	–	+	+
RAG2	–	–	+	+	+	+	–	+	+
TDT	–	–	+	+	+	+	–	–	–
D_H–J_H	–	–	(+)	(+)	(+)	+	+	+	+
V_H–DJ_H	–	–	–	–	–	–	+	+	+
V_L–J_L	–	–	–	–	–	–	–	+	+
IgM	–	–	–	–	–	–	+	+	+
IgL	–	–	–	–	–	–	–	+	+

High expression is indicated by +, low expression by (+) and absence of detectable expression by –. When a B-cell subset is not homogeneous for expression of a given marker, alternative expression patterns are shown separated by a slash. The presence or absence of detectable immunoglobulin gene-segment rearrangements are indicated by + or –, respectively. DNA rearrangements that are weakly detectable, and therefore correspond to a proportion of the cellular subset, are indicated by (+). This table was compiled on the basis of data from several independent studies[1–6]. CLP, common lymphoid progenitor; D_H, diversity gene segment of the immunoglobulin heavy chain; ELP, early lymphoid progenitor; FLT3, fms-related tyrosine kinase 3; IgL, immunoglobulin light chain; IL-7Rα, α-chain of the interleukin-7 receptor; J_H, joining gene segment of the immunoglobulin heavy chain; J_L, joining gene segment of the IgL; MPP, multipotential progenitor; PHSC, pluripotent haematopoietic stem cell; RAG, recombination-activating gene; TDT, terminal deoxynucleotidyltransferase; V_H, variable gene segment of the immunoglobulin heavy chain; V_L, variable gene segment of IgL.

Box 1 | **Peripheral B cells**

Immature B cells are produced in the bone marrow and migrate into the spleen through the terminal branches of central arterioles. After entering the spleen, at first, these B cells remain immature. On the basis of expression of various cell-surface markers (TABLE 2)**, immature B cells can be subdivided into three transitional B-cell fractions: T1, T2 and T3. Immature splenic B cells can be distinguished from their mature counterparts by their cell-surface marker expression, their short half-life (2–4 days) and their sensitivity to apoptosis induced by antibodies specific for IgM. Transitional B cells can be negatively selected in the periphery, which is therefore a crucial check-point for the generation of mature B cells. The tumour-necrosis-factor family member B-cell-activating factor (BAFF; also known as BLYS) and its receptor have a crucial role in regulating the transition of immature B cells to mature B cells.**

About 10% of mature splenic B cells are marginal-zone B cells. Marginal-zone B cells are found only in the spleen. They are strategically positioned at the blood–lymphoid-tissue interface, where they can initiate a fast and vigorous antibody response to blood-borne pathogens. Transitional, follicular and even memory B cells can give rise to marginal-zone B cells and/or be recruited into this compartment. In mice, most mature B cells are follicular B cells. These cells are mainly responsible for generating humoral immune responses to protein antigens. With the help of T cells, they form germinal centres. Germinal-centre B cells proliferate rapidly, undergo somatic hypermutation of their immunoglobulin variable gene segments and undergo isotype-switch recombination of immunoglobulin genes. At about day ten after immunization, the germinal-centre reaction reaches its peak. Subsequently, germinal centres slowly vanish, and memory B cells and effector plasma cells are generated. The mechanisms and factors that guide some germinal-centre B cells to become memory B cells and others to become plasma cells are not yet clear.

heterodimers[20] (FIG. 2). Ikaros, and probably Aiolos and Helios, activate or repress genes by recruiting CHROMATIN-REMODELLING COMPLEXES — such as SWI–SNF (switching-defective–sucrose non-fermenting) or NURD (nucleosome remodelling and disruption) — or chromatin-repressing complexes[23,24].

Several different mutant alleles of *Ikaros* have been generated in mice. One *Ikaros* allele encodes a DOMINANT-NEGATIVE PROTEIN, which presumably also interferes with the function of Aiolos and Helios, and mice that express this allele fail to generate B cells, T cells, NK cells and DCs[25]. Another mutant allele, considered to be a null allele, yields a milder phenotype: mice that express this allele lack B cells and NK cells but can develop T cells postnatally[26]. In addition, cells from these mice also show a strongly reduced capacity for long-term reconstitution of PHSCs in cell-transfer experiments[27]. A HYPOMORPHIC allele has also been made. Mice that express this hypomorphic allele have reduced levels of functional Ikaros protein; in addition, their B-cell development is impaired at the transition from pro-B cells to pre-B cells, and the numbers of mature B cells are reduced[28]. Finally, using a forward-mutagenesis screen, a mouse strain has been identified that has a single point mutation in the DNA-binding domain of Ikaros[29]. In mice that express this mutant, known as Plastic (*Plstc*), the Ikaros protein is produced at normal levels and assembles normally in different molecular complexes, but it cannot bind DNA. This results in the most severe phenotype of all of the *Ikaros*-mutant mice, presumably because all Ikaros-containing complexes are inactivated and because no related factor can substitute for Ikaros function. In these mice, B cells and T cells are completely absent, and myeloid-cell and erythroid-cell development are severely impaired. Consequently, *Plstc/Plstc* mutant embryos suffer from severe anaemia and die before birth. From these studies, it is clear that Ikaros function is required for the development of CLPs. So far, only a few genes — such as those encoding terminal deoxynucleotidyltransferase (TDT)[30], λ5 (REF. 31) and CD8α[32] — have been identified to be direct targets of Ikaros.

Although both Ikaros and PU.1 are essential for the generation of ELPs (FIG. 3), the phenotypes of mice that lack these two factors are clearly distinct, and Ikaros expression is normal in *Pu.1*$^{-/-}$ cells[12,14]. Therefore, the simplest model is that these two transcription factors function in parallel pathways that are both required for early lymphocyte differentiation.

CHROMATIN-REMODELLING COMPLEXES
Multiprotein complexes that are recruited to the regulatory elements of genes and remodel chromatin in an ATP-dependent manner, thereby increasing the accessibility of chromatin to molecules that regulate transcription.

DOMINANT-NEGATIVE PROTEIN
A defective protein that retains interaction capabilities and thereby distorts or competes with normal proteins.

Table 2 | **Markers defining B-cell stages in the periphery**

Marker	T1 B cell	T2 B cell	T3 B cell	MZ B cell	Follicular B cell
CD93	+++	++	+	–	–
IgM	+++	+++	++	+++	++
IgD	(+)	+	++	(+)	++
CD21	–	++	++	+++	++
CD23	–	++	++	(+)	++

The relative expression of the different markers used to distinguish the various peripheral B-cell subsets is indicated; the lowest level of detectable expression is indicated by (+). Because the data were obtained from side-by-side comparisons[8], the relative intensities of the markers can be directly compared. MZ, marginal zone, T, transitional.

From CLPs to immature B cells

Three transcription factors have been found to be essential for the differentiation of CLPs into specified pro-B cells: transcription factor E2A (E2A), early B-cell factor (EBF; also known as OLF1) and paired box protein 5 (PAX5; also known as BSAP). Absence of any one of these factors leads to an early block in B-cell development at the pro-B-cell or pre-B-cell stage. These three factors seem to work in collaboration, and together, they form a master control switch for engaging B-cell differentiation (FIG. 3).

E-box factors. The gene that encodes E2A (FIG. 2) is a founding member of the BASIC HELIX–LOOP–HELIX (bHLH) GENE FAMILY, and it encodes the broadly expressed E12 and E47 proteins (collectively known as E2A), which are generated by alternative splicing. HEB (HeLa E-box-binding protein) and E2-2 are encoded by two further members of this gene family, and these factors are also expressed by B cells. Because these proteins bind the E BOX — CANNTG (where N denotes any nucleotide), a motif that was initially identified in the mouse IgH enhancer — they are known as E-box factors or E proteins. Although the *E2a* gene is widely expressed, its mutation in mice leads to defects that are most marked in the B-cell lineage[33,34], but other cell lineages (for example, T cells) are also affected[35]. The mainly B-cell-specific function of E2A can be explained by the B-cell-specific formation and/or presence of E2A homodimers, whereas in other cells, E2A heterodimerizes with other E-box factors. B-cell-specific

HYPOMORPHIC
A type of mutation in which either the altered gene product has a decreased level of activity or the wild-type gene product is expressed at a decreased level.

BASIC HELIX–LOOP–HELIX GENE FAMILY
(bHLH gene family). A family that encodes transcription factors with a conserved DNA-binding domain that consists of an HLH motif, which mediates dimerization, adjacent to a basic region, which is responsible for binding to DNA.

E BOX
A DNA element with a conserved sequence that was initially found in the IgH enhancer on the basis of *in vivo* footprinting assays. E boxes have the consensus sequence CANNTG (where N denotes any nucleotide) and are binding sites for basic helix–loop–helix proteins.

STERILE IGH RNA
(Sterile immunoglobulin heavy-chain RNA). A transcript of an unrearranged IgH locus, which does not result in functional protein. Its presence is thought to reflect the accessibility of the locus for recombination.

homodimerization of E2A seems to be caused by two factors: increased expression of E2A by pro-B cells, followed by a gradual further increase throughout differentiation[36]; and hypophosphorylation of E2A. In the absence of E2A, B cells are blocked at the earliest stage of B-cell development, the pro-B-cell stage, and their IgH gene segments are not rearranged[33,37]. In these cells, several B-cell-lineage-specific transcripts — such as those encoding Igα and PAX5 — as well as STERILE IgH RNA, are absent. Mechanistically, E2A has been shown to interact, through a motif in its N-terminal activation domain, with the SAGA (Spt–Ada–Gcn5

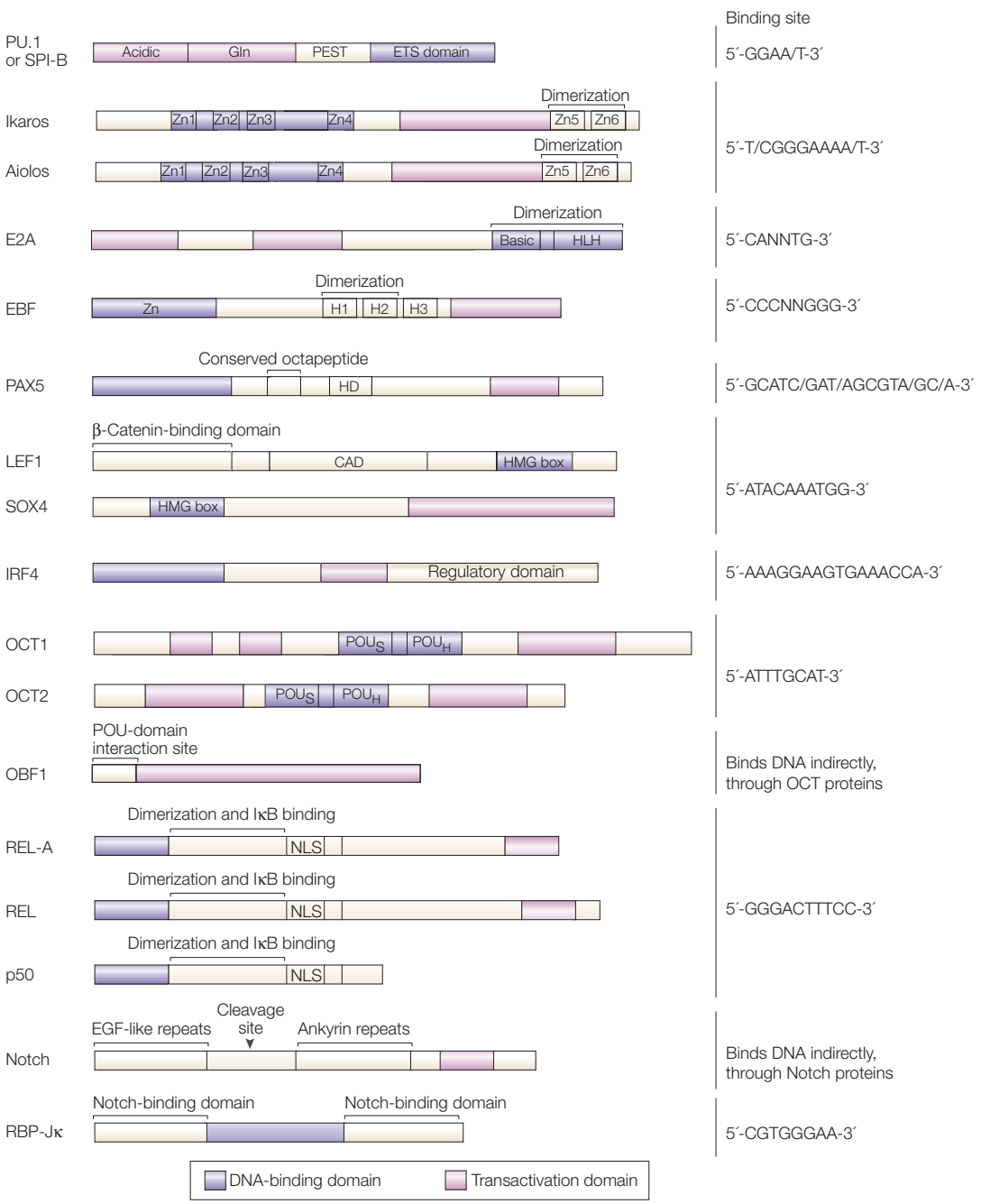

Figure 2 | **Transcription factors important for B-cell development and function.** This diagram shows a schematic representation of the various transcription factors that are discussed in this article, as well as their cognate binding sequences. CAD, context-dependent activation domain; E2A, transcription factor E2A; EBF, early B-cell factor; EGF, epidermal growth factor; Gln, glutamine-rich domain; H, conserved helical domain; HD, homeodomain; HLH, helix–loop–helix; HMG, high-mobility group; IκB, inhibitor of nuclear factor-κB; IRF4, interferon-regulatory factor 4; LEF1, lymphoid-enhancer-binding factor 1; N, any nucleotide; NLS, nuclear-localization sequence; OBF1, OCT-binding factor 1; OCT, octamer-binding transcription factor; PAX5, paired box protein 5; PEST, proline-, glutamic-acid-, serine- and threonine-rich; POU_H, POU homeodomain; POU_S, POU specific; RBP-Jκ, immunoglobulin κ light-chain J-region recombination-signal-binding protein 1; SOX4, sex-determining region Y (SRY) box 4; Zn, zinc finger.

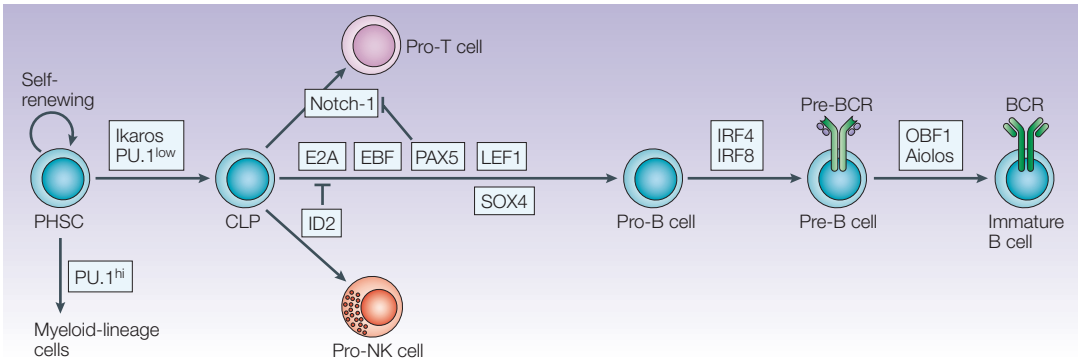

Figure 3 | **The genetic control of B-cell specification and commitment in the bone marrow.** This diagram shows a scheme of the genetic switch that regulates early B-cell specification. For simplicity, only some of the differentiation stages are shown. The developmental steps that are affected by the mutation of specific transcription factors are indicated. In some cases, the simultaneous mutation of two factors is required for a developmental phenotype to be observed. ID2 (inhibitor of DNA binding 2)-deficient mice have impaired natural killer (NK)-cell development and increased E2A activity, which correlates with an increased frequency of class switching to IgE[114]. For further details, see main text. BCR, B-cell receptor; EBF, early B-cell factor; CLP, common lymphoid progenitor; IRF, interferon-regulatory factor; LEF1, lymphoid-enhancer-binding factor 1; OBF1, OCT (octamer-binding transcription factor)-binding factor 1; PAX5, paired box protein 5; PHSC, pluripotent haematopoietic stem cell; SOX4, sex-determining region Y (SRY) box 4.

acetyltransferase) histone-acetyltransferase complex[38], a multiprotein complex that is involved in chromatin remodelling and transcriptional activation. This indicates that one of the mechanisms by which E2A can induce recombination at the IgH locus and activation of target genes is by promoting chromatin remodelling through recruitment of SAGA.

Although E2A is essential for B-cell differentiation, other E-box factors also have a role in this process, as shown by the observation that mice lacking HEB or E2-2 have about half the number of pro-B cells[39]. In addition, it was shown that the combined level of E-box factors present in B cells is crucial for differentiation of the B-cell lineage: mice that are heterozygous for mutations in any two of these three genes show B-cell defects, in contrast to mice that are heterozygous for mutations in a single gene, which are unaffected[39]. This indicates that the precise expression profile of E2A is an essential part of the regulatory puzzle of B-cell development; accordingly, it was found that expression of *Heb* cDNA from the *E2a* locus mostly rescues the B-cell defect in E2A-deficient mice[40].

Further complexity is added by the existence of several inhibitor of DNA binding (ID) proteins (ID1, ID2, ID3 and ID4), which are HLH proteins lacking the basic region that is required for DNA binding. These factors can heterodimerize with E2A or with other bHLH proteins, thereby inhibiting the capacity of these proteins to bind their target sequences. Several ID proteins are expressed in lymphoid tissues in a regulated manner. For example, ID1 is expressed by B cells up to the pro-B-cell stage, so it could inactivate E2A function before that stage. Indeed, forced expression of *Id1*, in B cells of *Id1*-transgenic mice, leads to a block in B-cell differentiation at the pro-B-cell stage, which is similar to the block observed in the absence of E2A[41].

EBF. EBF is a transcription factor that is expressed specifically by pro-B cells, pre-B cells and mature B cells, and it has been implicated in the regulation of the B-cell-specific gene that encodes the Igα subunit of the BCR (that is, *MB-1*). EBF contains a region with three putative helices, which shows homology to an HLH domain, but it lacks an adjacent basic region; the first two of these helices are essential for homodimerization and EBF function (J. Hagman, personal communication). The EBF DNA-binding domain is in the N-terminal part of the protein and contains a novel cysteine-rich zinc-coordination motif, which defines a unique class of DNA-binding domains[42]. Ablation of EBF in mice leads to a very early block in B-cell differentiation, similar to the block that is observed in the absence of E2A[43]: *Ebf*$^{-/-}$ B cells are B220^{+}CD19^{-} CD43^{+} and thereby correspond to pro-B cells (TABLE 1). These cells fail to express, or have reduced expression of, B-cell-specific genes, such as those encoding Igα, Igβ, VpreB and PAX5, but they express normal levels of IL-7Rα and IgH sterile transcripts. Similar to E2A-deficient pro-B cells, they lack D_H–J_H rearrangements at the IgH locus.

In *Ebf*$^{-/-}$ B cells, the *E2a* gene is transcribed at normal levels; by contrast, EBF is not expressed by *E2a*$^{-/-}$pro-B cells, indicating that EBF is downstream of E2A[44]. Consistent with this, the *Ebf* promoter contains a functionally relevant E2A-binding site[45]. Whereas *E2a*$^{+/-}$ or *Ebf*$^{+/-}$ mutant mice have half the number of pro-B cells present in wild-type mice, *E2a*$^{+/-}$*Ebf*$^{+/-}$ mutant mice have one-tenth the number of pro-B cells, further showing that these factors collaborate[46]. Forced expression of both E2A and EBF in Ba/F3 pro-B cells leads to synergistic activation of the surrogate light-chain genes *λ5* and *VpreB*[47]. Strikingly, ectopic expression of either E2A or EBF together with RAG1 or RAG2 by human embryonic kidney cells is sufficient to induce IgH gene recombination and lead to D_H–J_H rearrangement[48]. However, the absence of V_H–DJ_H recombination in these cells indicates that additional factors are required to complete IgH gene rearrangement. The block in B-cell development observed in

E2a$^{-/-}$ mice can be rescued by expression of EBF but not by expression of another B-cell-specific transcription factor, PAX5, which is itself a downstream target of EBF (discussed later). This shows that expression of EBF — which is encoded by *Ebf*, an essential target gene of E2A — is required for the expression of crucial B-cell-specific downstream genes other than *Pax5* (REF. 44). Consistent with this, forced expression of EBF by *Ebf*$^{-/-}$ progenitors is sufficient to restore expression of PAX5 and early B-cell lineage genes, such as those encoding Igα, Igβ and VpreB, whereas expression of neither PAX5 nor PU.1 is sufficient to overcome the B-cell developmental block in *Ebf*$^{-/-}$ pro-B cells[49].

Expression of the *Mb-1* gene is regulated throughout B-cell development, and several transcription factors — such as EBF, E2A and PAX5 — have been implicated in its control at different stages of development[50]. It was recently shown that the *Mb-1* promoter is fully methylated in PHSCs, then starts to be demethylated in CLPs and is completely demethylated at the pre-BI-cell stage, when the gene is active. These epigenetic changes are mediated by EBF in cooperation with RUNX1 (runt-related transcription factor 1)[51], and they are required for subsequent *Mb-1* gene activation by PAX5.

PAX5. PAX5 is a paired HOMEODOMAIN PROTEIN that is expressed by B cells and in the nervous system at the midbrain–hindbrain boundary. This factor has been implicated in the direct transcriptional regulation of several B-cell-specific genes, such as those encoding CD19, Igα and lymphoid-enhancer-binding factor 1 (LEF1)[52]. Mutation of the *Pax5* gene leads to a complete block in B-cell differentiation, which is downstream of the block that is seen in the absence of E2A or EBF: in *Pax5*$^{-/-}$ B cells, expression of E2A and EBF is normal, and rearrangement at the IgH locus has already been initiated. Although DJ gene segments are rearranged normally in these cells, rearrangement of V_H gene segments (in particular, distal segments) is severely impaired[53]. It was recently found that, in haematopoietic progenitors and pro-T cells, the IgH locus and the Igκ locus (one of the two IgL loci) are located near the nuclear periphery; as committed B-cell precursors proceed through the pro-B-cell stage, the IgH locus is repositioned in the centre of the nucleus and becomes compacted[54]. In PAX5-deficient pro-B cells, IgH gene segments are correctly repositioned from the nuclear periphery to the centre of the nucleus; however, the IgH locus fails to contract and juxtapose distal V_H gene segments in proximity to D gene segments[55].

PAX5 is required not only for rearrangement of V_H gene segments and for expression of genes that are required for progression to the pre-B-cell stage but also for commitment to and maintenance of the B-cell-differentiation pathway. Remarkably, in the absence of PAX5, pro-B cells show an amazing degree of plasticity and can differentiate *in vitro*, after stimulation with the appropriate cytokines, into macrophages, osteoclasts, DCs, granulocytes or NK cells[56]. Furthermore, after transfer to RAG2-deficient mice, which lack B cells and T cells, *Pax5*$^{-/-}$ pro-B cells provide long-term reconstitution of the thymus and can generate mature T cells[57]. *Pax5*$^{-/-}$ pro-B cells can also generate other blood-cell types *in vivo*, but with reduced efficiency: whereas T cells are detected within 1 week of cell transfer, macrophages and granulocytes require 2–3 months, and erythrocytes even longer[58]. So, *Pax5*$^{-/-}$ pro-B cells are pluripotent but are poised towards the lymphoid lineage.

These findings show that, although E2A and EBF are absolutely essential for the formation of pro-B cells, these two factors are not sufficient for true commitment to B-cell differentiation, which is only achieved after expression of PAX5 is secured. Further analysis has shown that, to preserve B-cell identity, PAX5 activity needs to be continuously maintained. Using conditional mutagenesis, PAX5 has been selectively removed from either pro-B cells[59] or mature B cells[60]: in both cases, B-cell commitment was shown to be lost when PAX5 was absent. PAX5-deleted pro-B cells regained plasticity and acquired the capacity to differentiate *in vitro* into macrophages or to reconstitute the T-cell compartment of *Rag2*$^{-/-}$ mice *in vivo*[59]. At a molecular level, PAX5-deleted mature B cells downregulated expression of *Cd19*, *Mb-1* and B-cell linker (*Blnk*) (three genes that are known to be direct targets of PAX5), and these cells were strongly depleted in the periphery[60]. This loss of mature peripheral B cells might reflect, in part, the impaired BCR signalling that results from BLNK downregulation in *Pax5*$^{-/-}$ B cells.

At a molecular level, PAX5 not only activates genes but also represses the expression of genes that are inappropriate to the B-cell lineage, such as those encoding the M-CSF receptor and Notch-1 (REF. 61). PAX5 exerts its repressive function by recruiting co-repressors of the GROUCHO FAMILY, such as groucho-related gene 4 (GRG4)[62]. Another of the established targets of repression by PAX5 is the IgH HS1,2 enhancer (which is located at the 3′ end of the IgH locus); in this case, repression by PAX5 also requires interaction with PU.1, which itself also recruits GRG4 (REF. 63). Signalling through Notch has emerged as one of the most crucial pathways promoting T-cell specification and development[64]. Therefore, the upregulation of Notch-1 that is observed for *Pax5*$^{-/-}$ pro-B cells provides an explanation for the ability of these cells to differentiate into T cells. Two sets of experiments support this model. First, pan-haematopoietic expression of PAX5 from the *Ikaros* locus did not interfere with myeloid or erythroid lineage development, but it did suppress T-cell development[61]. Second, *in vitro* culture of *Pax5*$^{-/-}$ pro-B cells on stromal cells expressing a Notch ligand confirmed that Notch signalling is essential for the differentiation of these cells into T cells[65].

In apparent conflict with some of the previously mentioned observations indicating that PAX5 expression is synonymous with B-cell lineage commitment, it was recently reported that retroviral expression of the basic-leucine-zipper-family transcription factors CCAAT/enhancer-binding protein-α (C/EBP-α) and C/EBP-β by wild-type ($CD19^+PAX5^+$) bone-marrow B cells leads to their efficient reprogramming into

HOMEODOMAIN PROTEIN
A member of a class of transcription factors that contains a DNA-binding domain with homology to the *Drosophila melanogaster* homeodomain regulatory proteins. This DNA-binding domain contains a helix–turn–helix motif, which was initially found in bacterial repressor proteins.

GROUCHO FAMILY
A family of transcriptional repressors that were named after the neurogenic *Drosophila melanogaster* gene *groucho*. These repressors interact with various transcription factors — such as members of the basic helix–loop–helix family, T-cell factors (TCFs) and lymphoid-enhancer-binding factor 1 (LEF1) and paired box protein 5 (PAX5) — thereby repressing the expression of target genes of these factors.

CD11b$^+$GR1$^+$ macrophages *in vitro* and *in vivo*[66]. This surprising outcome occurs together with inhibition of PAX5 activity but not PAX5 expression (through an unidentified mechanism), consequently leading to downregulation of PAX5 target genes. In this case, C/EBP-α and C/EBP-β synergize with PU.1 to activate the macrophage gene-expression programme. In addition, it was recently found that B-lineage cell cultures can be established from *Pu.1*$^{-/-}$ fetal livers. These cells express all early B-cell markers that have been tested, including EBF and IL-7Rα (but not B220), and they have DJ and VDJ IgH gene-segment rearrangements and can be induced to produce IgM (M. Ye and T. Graf, personal communication). The existence of these *Pu.1*$^{-/-}$ B cells challenges the blueprint for lymphopoiesis commitment that is presented here (FIG. 3), and it indicates that the current models describing the genetic control of haematopoietic-cell development are still incomplete.

SOX4 and LEF1. The transcription factors SOX4 (sex-determining region Y (SRY) box 4) and LEF1 are members of the HIGH-MOBILITY GROUP (HMG)-BOX FAMILY (FIG. 2) and have a crucial role at an early stage of B-cell development. In adult mice, *Sox4* is expressed mainly by immature T and B cells, whereas during embryogenesis, expression is broader. Mice lacking SOX4 die at E14 due to a defect in cardiac formation. However, when *Sox4*$^{-/-}$ fetal liver cells were used to reconstitute the haematopoietic system of lethally irradiated mice, a very strong block was observed at the pro-B-cell-to pre-B-cell transition[67]. The few pro-B cells that were present showed a reduced proliferative capacity after stimulation with IL-7; however, whether this reflects reduced expression of the *Il-7rα* gene, as is the case for PU.1-deficient mice, is not known. Because only limited studies of this factor have been carried out, it is not yet clear how SOX4 integrates with the rest of the regulatory circuitry that has so far been identified.

LEF1 is also expressed by developing B and T cells, as well as at many sites of organ formation during embryogenesis. LEF1 is highly related to T-cell factor 1 (TCF1), TCF2 and TCF3, which have been implicated in control of transcription in T cells[68]. LEF1 and TCFs interact with β-catenin and are the downstream integrators of the WNT-signalling pathway[69]. By itself, LEF1 does not activate transcription, but it can function as an architectural protein — promoting the formation of multiprotein complexes on enhancer DNA sequences by inducing DNA bending — and can also recruit a transcriptional co-activator, ALY. In B-lineage cells, LEF1 is expressed during the early stages of B-cell development in the fetal liver and adult bone marrow; by contrast, IgM$^+$ cells do not express LEF1. In *Lef1*$^{-/-}$ mice, the size of the B-cell compartment is reduced; however, no obvious differentiation defects have been observed, and normal rearrangement of immunoglobulin gene segments occurs. Furthermore, no impairment of proliferation is seen following stimulation with IL-7. Instead, *Lef1*$^{-/-}$ pro-B cells have a survival defect and show increased apoptosis. In addition, these LEF1-deficient pro-B cells have a proliferation defect in response to WNT signalling (initiated by WNT3A) *in vitro*, showing that this pathway is important during early B-cell development and that LEF1 is its mediator[70].

Other transcription factors: IRF4, IRF8, Aiolos and OBF1. Interferon-regulatory factor 4 (IRF4) (FIG. 2) and IRF8 are highly related factors that are expressed by both lymphoid-lineage cells and myeloid-lineage cells. They belong to the IRF family[71] but bind only weakly to cognate sites on their own; instead, they are recruited to composite binding sites through interactions with other transcription factors. In particular, it has been shown that PU.1 or SPI-B can recruit IRF4 and IRF8 to binding sites in the Igκ or Igλ 3′ enhancers. Mice lacking IRF4 have normal B-cell development but greatly impaired B- and T-cell activation, resulting in a strongly impaired immune response[72]. By contrast, mice lacking IRF8 show defects in macrophage development and have a chronic-myeloid-leukaemia-like phenotype[73]. These specific and complementary defects led to the hypothesis that IRF4 and IRF8 could be redundant in B-cell development. Indeed, mice deficient in both IRF4 and IRF8 showed a block in early B-cell development at the pro-B-cell-to-pre-B-cell transition, with a strongly reduced number of immature B cells in the bone marrow[74]. In these pre-B cells, the Igμ chain was expressed normally, but the levels of Igκ or Igλ sterile and mature transcripts were reduced; in addition, DNA rearrangement at these two loci was blocked. Moreover, an increased proportion of the mutant pre-B cells were found to be cycling, and this correlated with more cells expressing the pre-BCR at the cell surface. Strikingly, in these cells, expression of the *VpreB* and *λ5* genes was found to be upregulated; however, whether IRF4 and IRF8 directly regulate transcription of these genes has not yet been determined. Because downregulation of surrogate light-chain expression is thought to be an essential requirement for progression through the pre-B-cell stage, it has been proposed that IRF4 and IRF8 (by controlling the downregulation of expression of *VpreB* and *λ5*) function as a crucial switch at this developmental stage.

Aiolos and OBF1 (OCT (octamer-binding transcription factor)-binding factor 1; also known as OCA-B and BOB1) are transcription factors that are crucial for the function of mature B cells in the periphery (discussed later). Mice that simultaneously lack both factors also have a developmental block at the pre-B-cell stage, and IgM$^+$ immature B cells are almost absent[75]. However, unlike *Irf4*$^{-/-}$*Irf8*$^{-/-}$ mice, *Aiolos*$^{-/-}$*Obf1*$^{-/-}$ mice have normal levels of sterile and mature Igκ transcripts. It is not yet clear whether IRF4, IRF8, Aiolos and OBF1 are all part of a common pathway, but several genes have been identified that are dysregulated specifically in *Aiolos*$^{-/-}$*Obf1*$^{-/-}$ B cells, which could explain the developmental block that has been observed (A. Karnowski, J. Sun, C. Cao, G. Matthias and P.M., unpublished observations).

Peripheral B cells

Several transcription factors have been shown to be important for either the generation or the function of

HIGH-MOBILITY GROUP BOX FAMILY (HMG-box family). A class of transcription factors with a DNA-binding domain that has homology to a motif found in the HMG of DNA-binding proteins. Unlike most transcription factors, HMG-box proteins bind in the minor groove of DNA.

Box 2 | **Plasma cells**

Terminally differentiated B cells are known as plasma cells. These cells are essential for protective immunity. In plasma cells, the production of immunoglobulin shifts from a membrane-bound form, as found in mature B cells, to a secreted form. Plasma cells are non-dividing cells, and they can be classified into short- and long-lived populations. The former arise after primary immunization and are found at extrafollicular sites, where they persist for only a few days. The latter arise from germinal-centre B cells and reside mainly in the bone marrow.

Several transcription factors — including PAX5 (paired box protein 5), BCL-6 (B-cell lymphoma 6), IRF4 (interferon-regulatory factor 4), BLIMP1 (B-lymphocyte-induced maturation protein 1) and XBP1 (X-box-binding protein 1) — are involved in the terminal differentiation of B cells into plasma cells. Transcriptional control of plasma-cell generation is complex and is reviewed in REF. 76. In brief, PAX5 and BCL-6 are expressed at high levels by germinal-centre B cells. Maintenance of expression of either protein results in inhibition of plasma-cell differentiation. BCL-6 is a transcriptional repressor that downregulates the expression of genes that are involved in the inhibition of cell proliferation, and *BLIMP1* is a target gene of BCL-6. PAX5 is the B-cell 'identity' gene. In germinal-centre B cells, PAX5 represses the expression of XBP1, a ubiquitously expressed transcription factor that is required for plasma-cell differentiation. In B cells that are deficient in BLIMP1, XBP1 or IRF4, the plasma-cell differentiation pathway is severely impaired. BLIMP1 represses the transcription of genes that are associated with cell proliferation, such as *MYC*. Moreover, BLIMP1 represses PAX5 expression. In plasma-cell differentiation, XBP1 seems to function downstream of BLIMP1. The target genes of XBP1 that are involved in plasma-cell differentiation have not yet been identified.

specific peripheral B-cell subsets: transitional B cells, marginal-zone B cells and germinal-centre B cells (BOX 1; TABLE 2). We discuss the most important of these factors in this section. The factors involved in plasma-cell differentiation are only briefly considered (BOX 2), as they are the subject of a recent review[76].

OCTs and their co-activator OBF1. The conserved octamer motif ATGCAAAT has been associated with B-cell-specific transcription of immunoglobulin genes and various other genes. OCT1 and OCT2 are POU-DOMAIN-containing transcription factors (FIG. 2) that specifically bind this conserved octamer motif, on which they can then form a ternary complex with the co-activator OBF1. Whereas OCT1 is ubiquitous, OCT2 and OBF1 are mainly B-cell-specific proteins, although transient expression of OBF1 can be detected in T cells after their activation. In B-lineage cells, the highest OBF1 levels are found in germinal-centre B cells, and this is mainly due to post-transcriptional regulation[77,78]. In addition, there are several isoforms of OBF1, one of which is myristoylated and anchored to the cell membrane[79]; the roles of the different OBF1 isoforms are not yet known.

Because of their B-cell-specific expression, it had been assumed that OCT2 and/or OBF1 mediate transcriptional activation in B cells through the octamer motif and therefore that they should be crucial for activation of immunoglobulin-gene promoters. However, mice that are deficient in OCT2 (REF. 80) or OBF1 (REFS 81–83) do not indicate a clear role for these factors in the regulation of immunoglobulin genes, although the role of the octamer site in immunoglobulin-gene promoter activity has been extensively documented[84]. Strikingly, even B cells that lack both OBF1 and OCT2 have been shown to develop normally *in vivo* and to have normal transcription of IgM[85]. In other studies, the absence of OBF1 correlated with moderately reduced expression of some isotype-switched genes and with impaired transcription and editing of a subset of Igκ genes[86]. So, OBF1 and OCT2 are not stringently required for immunoglobulin-gene transcription, but they have other crucial functions in B cells.

Mice that are deficient in OBF1 show almost normal early B-cell development, but they have a reduction in the total number of peripheral B cells and in the number of recirculating B cells in the bone marrow. The most marked phenotype of these mice is the severely impaired T-cell-dependent immune response, which occurs together with a lack of germinal-centre formation (FIG. 4). This reflects the diminished capacity of OBF1-deficient B cells to respond to activation signals. This essential role of OBF1 for germinal-centre formation was further shown by studies of mice that are deficient in both Aiolos and OBF1: whereas Aiolos-deficient mice spontaneously form germinal centres[87], the double-deficient mice fail to do so[75].

OBF1 also has a crucial role in the transition of B cells from the bone-marrow to the spleen. In radiation chimeras that were reconstituted with a mixture of wild-type and OBF1-deficient bone-marrow cells, it was found that *Obf1*$^{-/-}$ cells can efficiently compete with wild-type cells up to the stage of immature B cells in the bone-marrow but that they cannot markedly contribute to the peripheral B-cell compartment[88]. Consistent with this, mice lacking OBF1 and carrying the X-linked immune deficiency (*xid*) mutation (a mutation of the gene encoding Bruton's tyrosine kinase) also have a severely reduced number of peripheral B cells, further showing the importance of OBF1 at the bone-marrow-to-spleen transition[89]. Furthermore, whereas two OBF1-deficient mouse strains show normal development of marginal-zone B cells, a third strain (on a C57/BL6 background) fails to develop marginal-zone B cells[90]. So, in conjunction with appropriate genetic modifiers, OBF1 is required for development of this B-cell subset.

Surprisingly, mice that lack OCT2 are not viable, and they also show a complex phenotype that affects mature B cells[80]. In particular, OCT2 is required for *in vitro* proliferation of B cells following stimulation with T-cell-independent antigens[91], as well as for maintenance of the B-cell pool in the peritoneal cavity (which comprises B1 cells)[92]. At a molecular level, few of the OBF1 or OCT2 target genes that have been identified can explain the observed phenotypes. Finally, mice with a hypomorphic mutation[93] or a conditional deletion (S. Massa, K. Schubart, G. Matthias, A. Mizeracki, A.G.R. and P.M., unpublished observations) of *Oct1* in B cells show normal B-cell development, indicating that OCT1 is not essential for this process.

NF-κB and its associates. Nuclear factor-κB (NF-κB) is an inducible transcription factor comprising related proteins[94] (FIG. 2) that dimerize and activate the transcription of many target genes, the products

POU DOMAIN
A class of bipartite DNA-binding domains that is characterized by a carboxy-terminal variant homeodomain separated by a flexible linker from an amino-terminal POU (Pit1, Oct1, Unc86)-specific domain. Because of this unique structure, the POU domain can bind DNA when the domain itself is in many different configurations.

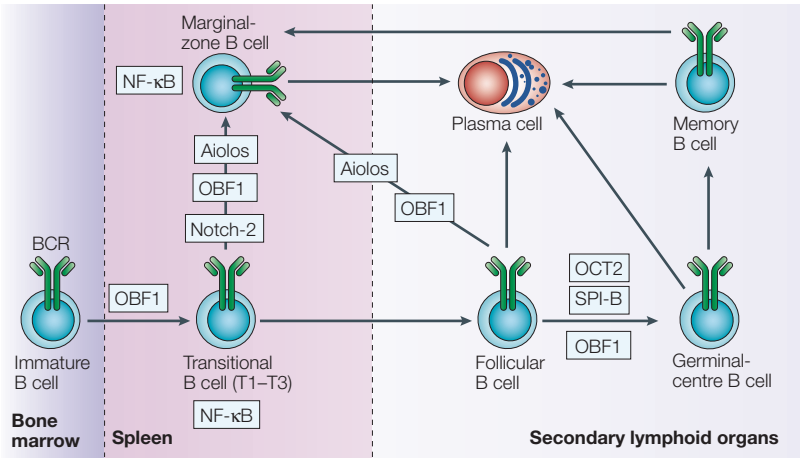

Figure 4 | **The genetic control of B-cell maturation and function in the periphery.** B cells that exit the bone marrow as immature B cells are first found in the spleen as transitional cells (T1–T3) and are then found in secondary lymphoid organs, such as lymph nodes and Peyer's patches. The routes of transit between these anatomical locations are not yet completely understood, but some of the mechanisms of genetic control have been identified. The developmental steps that are affected by the mutation of specific transcription factors are indicated (for further details, see main text). Note that there is no evidence for a hierarchy of transcription factors at any of the stages of B-cell development that are depicted here. BCR, B-cell receptor; NF-κB, nuclear factor-κB; OBF1, OCT-binding factor 1; OCT2, octamer-binding transcription factor 2; T, transitional.

of which are implicated in triggering and coordinating the adaptive and innate immune responses[95,96]. Ablation of various NF-κB subunits in mice revealed that early B-cell development and immunoglobulin-gene transcription do not require this factor. Instead, several aspects of late B-cell differentiation and/or maturation are affected by the absence of NF-κB, and the different components of the NF-κB system have distinct functions, as well as redundant functions. For example, B cells from mice that are deficient in the p50 (also known as NF-κB1) subunit of NF-κB fail to proliferate *in vitro* in response to stimulation with lipopolysaccharide (LPS)[97]. In addition, p50-deficient mice lack marginal-zone B cells; by contrast, mice that are defective in REL (also known as cREL) or REL-A (also known as p65) have marginal-zone B cells, but they are present in reduced numbers[98]. Interestingly, survival genes such as B-cell lymphoma X (*Bcl-x*) and *A1* (also known as *Bfl1*) were found to be direct targets of NF-κB in maturing marginal-zone B cells[99,100]. Furthermore, in contrast to p50-deficient mice but similar to REL-A-deficient mice, REL-B-deficient mice cannot form germinal centres and follicular dendritic-cell networks in the spleen after antigenic challenge. REL-B is also required for normal organization of the marginal zone and for its population by macrophages and B cells[101]. Moreover, in mice that are deficient in both p50 and p52 (also known as NF-κB2), B-cell development is blocked at the immature transitional stage[102]. A similar block is observed for irradiated mice that are reconstituted with fetal liver cells deficient in both REL-A and REL[103]. So, NF-κB activation also has an important role in the transition of immature B cells to mature B cells.

Other transcription factors: Aiolos, RBP-Jκ, Notch and SPI-B. Aiolos is a zinc-finger transcription factor of the *Ikaros* gene family (FIG. 2), and it is expressed at low levels during early B-cell differentiation and at high levels by mature and recirculating B cells. In Aiolos-deficient mice, early B-cell development is normal; however, mature B cells have a reduced activation threshold in response to BCR ligation, and germinal centres develop spontaneously[87]. Moreover, ageing Aiolos-deficient mice produce nuclear autoantibodies[87] and show symptoms of systemic lupus erythematosus[75]. Absence of Aiolos also blocks the development of marginal-zone B cells, which is consistent with the idea that the intensity of BCR signalling regulates entry into the follicular or marginal-zone compartment[104].

Notch signalling occurs in vertebrates and invertebrates in developmental processes that involve binary cell-fate determination, and it has crucial roles in T- and B-cell lineage commitment. This complex system has been the subject of several recent reviews (see REF. 64), so here, we discuss some of the findings that are relevant to B-cell development. Notch is crucial for suppression of B-cell fate in the thymus: induced deletion of the gene encoding Notch-1 in adult bone marrow leads to ectopic generation of B cells in the thymus[105]. By contrast, in the spleen, Notch-2 is essential for the decision to enter the marginal-zone or follicular B-cell pathway (FIG. 4): mice with B cells that lack the transcription factor RBP-Jκ (Igκ J-region recombination-signal-binding protein 1) (FIG. 2), which is essential for Notch-mediated transcription, have normal early B-cell differentiation but lack marginal-zone B cells[106]. Conversely, mice with B cells that lack the Notch suppressor MINT (MSH homeobox homologue 2 (MSX2)-interacting nuclear target) have threefold more marginal-zone B cells[107]. In addition, mice that are deficient in Notch-2, which is expressed preferentially by mature B cells, lack marginal-zone B cells[108].

SPI-B is a transcription factor of the ETS family, and it is highly homologous to PU.1. SPI-B-deficient mice are viable, unlike PU.1-deficient mice, and they have normal numbers of mature B and T cells, indicating that early lymphoid development is unaffected. However, SPI-B-deficient mice have several defects in the B-cell compartment: B cells that lack SPI-B proliferate normally following stimulation with LPS, but they proliferate poorly in response to BCR crosslinking and die. In addition, SPI-B-deficient mice show impaired T-cell-dependent immune responses *in vivo* and form only small and transient germinal centres[16]. When the level of PU.1 in SPI-B-deficient mice is reduced by half, novel phenotypes appear (such as reduced numbers of immature and mature B cells), and the functional defects of B cells are exacerbated[109]. In these *Pu.1*$^{+/-}$*Spi-b*$^{-/-}$ splenic B cells, expression of the NF-κB subunit REL was shown to be strongly down-regulated. The *Rel* promoter was subsequently found to contain three essential PU.1 and/or SPI-B binding sites. Importantly, retroviral expression of REL was sufficient to restore wild-type B-cell numbers in mice that were reconstituted with *Pu.1*$^{+/-}$*Spi-b*$^{-/-}$ bone-marrow cells[110].

Concluding remarks

As we have discussed, many transcription factors that control various stages of B-cell development or function have been identified, but it is probable that other important factors have yet to be discovered. From the studies described here, it has become clear that transcription factors (can) function in collaboration to form transcriptional regulatory networks. The best studied network of this type is the E2A–EBF–PAX5 circuit. Other networks — such as the PU.1–SPI-B, OBF1–OCT2, IRF4–IRF8 and OBF1–Aiolos networks — have recently been identified; however, how these factors work together is still largely unknown. Because many transcription factors are essential for embryonic development or postnatal survival, analysis of their function in B cells requires conditional gene inactivation. By this approach, the important question of whether one transcription factor operates at several stages of B-cell development can also be addressed. For most of the transcription factors that are involved in B-cell development, the crucial target genes have not yet been identified. It also remains to be established how the different transcription factors mediate their functions in B cells and how they 'communicate' with the many newly discovered factors that modify and remodel chromatin. In addition to the impact of nuclear dynamics on immunoglobulin gene-segment rearrangement (discussed earlier), recent studies of immunoglobulin allelic exclusion have highlighted the importance of epigenetic mechanisms[111]. The crucial factors that mediate this regulation have not yet been defined by genetic experiments. But there is no doubt that the coming years will continue to be rich in surprising findings.

1. Medina, K. L. *et al*. Identification of very early lymphoid precursors in bone marrow and their regulation by estrogen. *Nature Immunol.* **2**, 718–724 (2001).
2. Igarashi, H., Gregory, S. C., Yokota, T., Sakaguchi, N. & Kincade, P. W. Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. *Immunity* **17**, 117–130 (2002).
3. Allman, D. *et al*. Thymopoiesis independent of common lymphoid progenitors. *Nature Immunol.* **4**, 168–174 (2003).
4. Martin, C. H. *et al*. Efficient thymic immigration of B220$^+$ lymphoid-restricted bone marrow cells with T precursor potential. *Nature Immunol.* **4**, 866–873 (2003).
5. Loffert, D., Ehlich, A., Muller, W. & Rajewsky, K. Surrogate light chain expression is required to establish immunoglobulin heavy chain allelic exclusion during early B cell development. *Immunity* **4**, 133–144 (1996).
6. Shimizu, T., Mundt, C., Licence, S., Melchers, F. & Martensson, I. L. VpreB1/VpreB2/λ5 triple-deficient mice show impaired B cell development but functional allelic exclusion of the IgH locus. *J. Immunol.* **168**, 6286–6293 (2002).
7. Nemazee, D. & Weigert, M. Revising B cell receptors. *J. Exp. Med.* **191**, 1813–1817 (2000).
8. Loder, F. *et al*. B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals. *J. Exp. Med.* **190**, 75–89 (1999).
9. Montecino-Rodriguez, E., Leathers, H. & Dorshkind, K. Bipotential B-macrophage progenitors are present in adult bone marrow. *Nature Immunol.* **2**, 83–88 (2001).
10. McKercher, S. R. *et al*. Targeted disruption of the *PU.1* gene results in multiple hematopoietic abnormalities. *EMBO J.* **15**, 5647–5658 (1996).
11. Scott, E. W., Simon, M. C., Anastasi, J. & Singh, H. Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. *Science* **265**, 1573–1577 (1994).
This was the first study to show that PU.1 is required for the earliest stages of lymphocyte development.
12. Scott, E. W. *et al*. PU.1 functions in a cell-autonomous manner to control the differentiation of multipotential lymphoid-myeloid progenitors. *Immunity* **6**, 437–447 (1997).
13. DeKoter, R. P., Walsh, J. C. & Singh, H. PU.1 regulates both cytokine-dependent proliferation and differentiation of granulocyte/macrophage progenitors. *EMBO J.* **17**, 4456–4468 (1998).
14. DeKoter, R. P., Lee, H. J. & Singh, H. PU.1 regulates expression of the interleukin-7 receptor in lymphoid progenitors. *Immunity* **16**, 297–309 (2002).
15. Schweitzer, B. L. & DeKoter, R. P. Analysis of gene expression and Ig transcription in PU.1/Spi-B-deficient progenitor B cell lines. *J. Immunol.* **172**, 144–154 (2004).
16. Su, G. H. *et al*. Defective B cell receptor-mediated responses in mice lacking the Ets protein, Spi-B. *EMBO J.* **16**, 7118–7129 (1997).
17. Dahl, R., Ramirez-Bergeron, D. L., Rao, S. & Simon, M. C. Spi-B can functionally replace PU.1 in myeloid but not lymphoid development. *EMBO J.* **21**, 2220–2230 (2002).
18. DeKoter, R. P. & Singh, H. Regulation of B lymphocyte and macrophage development by graded expression of PU.1. *Science* **288**, 1439–1441 (2000).
19. Rosenbauer, F. *et al*. Acute myeloid leukemia induced by graded reduction of a lineage-specific transcription factor, PU.1. *Nature Genet.* **36**, 624–630 (2004).
20. Georgopoulos, K., Winandy, S. & Avitahl, N. The role of the *Ikaros* gene in lymphocyte development and homeostasis. *Annu. Rev. Immunol.* **15**, 155–176 (1997).
21. Kelley, C. M. *et al*. Helios, a novel dimerization partner of Ikaros expressed in the earliest hematopoietic progenitors. *Curr. Biol.* **8**, 508–515 (1998).
22. Morgan, B. *et al*. Aiolos, a lymphoid restricted transcription factor that interacts with Ikaros to regulate lymphocyte differentiation. *EMBO J.* **16**, 2004–2013 (1997).
23. Koipally, J., Renold, A., Kim, J. & Georgopoulos, K. Repression by Ikaros and Aiolos is mediated through histone deacetylase complexes. *EMBO J.* **18**, 3090–3100 (1999).
24. Kim, J. *et al*. Ikaros DNA-binding proteins direct formation of chromatin remodeling complexes in lymphocytes. *Immunity* **10**, 345–355 (1999).
25. Georgopoulos, K. *et al*. The *Ikaros* gene is required for the development of all lymphoid lineages. *Cell* **79**, 143–156 (1994).
This work showed the crucial role of Ikaros for the generation of all lymphocytic lineages.
26. Wang, J. H. *et al*. Selective defects in the development of the fetal and adult lymphoid system in mice with an Ikaros null mutation. *Immunity* **5**, 537–549 (1996).
27. Nichogiannopoulou, A., Trevisan, M., Neben, S., Friedrich, C. & Georgopoulos, K. Defects in hemopoietic stem cell activity in *Ikaros* mutant mice. *J. Exp. Med.* **190**, 1201–1214 (1999).
28. Kirstetter, P., Thomas, M., Dierich, A., Kastner, P. & Chan, S. Ikaros is critical for B cell differentiation and function. *Eur. J. Immunol.* **32**, 720–730 (2002).
29. Papathanasiou, P. *et al*. Widespread failure of hematolymphoid differentiation caused by a recessive niche-filling allele of the Ikaros transcription factor. *Immunity* **19**, 131–144 (2003).
This interesting paper showed that an *Ikaros* gene with a point mutation in the region encoding the DNA-binding domain has a stronger phenotype in mice than a null mutant, presumably because it inactivates several Ikaros-containing complexes.
30. Trinh, L. A. *et al*. Down-regulation of *TDT* transcription in CD4$^+$CD8$^+$ thymocytes by Ikaros proteins in direct competition with an Ets activator. *Genes Dev.* **15**, 1817–1832 (2001).
31. Sabbattini, P. *et al*. Binding of Ikaros to the *λ5* promoter silences transcription through a mechanism that does not require heterochromatin formation. *EMBO J.* **20**, 2812–2822 (2001).
32. Harker, N. *et al*. The *CD8α* gene locus is regulated by the Ikaros family of proteins. *Mol. Cell* **10**, 1403–1415 (2002).
33. Bain, G. *et al*. E2A proteins are required for proper B cell development and initiation of immunoglobulin gene rearrangements. *Cell* **79**, 885–892 (1994).
This paper, together with references 37 and 41, was the first to identify the crucial role of E2A proteins in the earliest stages of B-cell development.
34. Kee, B. L., Quong, M. W. & Murre, C. E2A proteins: essential regulators at multiple stages of B-cell development. *Immunol. Rev.* **175**, 138–149 (2000).
35. Bain, G. *et al*. E2A deficiency leads to abnormalities in αβ T-cell development and to rapid development of T-cell lymphomas. *Mol. Cell. Biol.* **17**, 4782–4791 (1997).
36. Zhuang, Y., Jackson, A., Pan, L., Shen, K. & Dai, M. Regulation of *E2A* gene expression in B-lymphocyte development. *Mol. Immunol.* **40**, 1165–1177 (2004).
37. Zhuang, Y., Soriano, P. & Weintraub, H. The helix–loop–helix gene *E2A* is required for B cell formation. *Cell* **79**, 875–884 (1994).
38. Massari, M. E. *et al*. A conserved motif present in a class of helix–loop–helix proteins activates transcription by direct recruitment of the SAGA complex. *Mol. Cell* **4**, 63–73 (1999).
39. Zhuang, Y., Cheng, P. & Weintraub, H. B-lymphocyte development is regulated by the combined dosage of three basic helix–loop–helix genes, *E2A*, *E2-2*, and *HEB*. *Mol. Cell. Biol.* **16**, 2898–2905 (1996).
40. Zhuang, Y., Barndt, R. J., Pan, L., Kelley, R. & Dai, M. Functional replacement of the mouse *E2A* gene with a human *HEB* cDNA. *Mol. Cell. Biol.* **18**, 3340–3349 (1998).
41. Sun, X. H. Constitutive expression of the *Id1* gene impairs mouse B cell development. *Cell* **79**, 893–900 (1994).
42. Hagman, J., Gutch, M. J., Lin, H. & Grosschedl, R. EBF contains a novel zinc coordination motif and multiple dimerization and transcriptional activation domains. *EMBO J.* **14**, 2907–2916 (1995).
43. Lin, H. & Grosschedl, R. Failure of B-cell differentiation in mice lacking the transcription factor EBF. *Nature* **376**, 263–267 (1995).
The was the first report to identify the crucial role of EBF at the pro-B-cell stage of development.
44. Seet, C. S., Brumbaugh, R. L. & Kee, B. L. Early B cell factor promotes B lymphopoiesis with reduced interleukin 7 responsiveness in the absence of E2A. *J. Exp. Med.* **199**, 1689–1700 (2004).
45. Smith, E. M., Gisler, R. & Sigvardsson, M. Cloning and characterization of a promoter flanking the early B cell factor (*EBF*) gene indicates roles for E-proteins and autoregulation in the control of EBF expression. *J. Immunol.* **169**, 261–270 (2002).
46. O'Riordan, M. & Grosschedl, R. Coordinate regulation of B cell differentiation by the transcription factors EBF and E2A. *Immunity* **11**, 21–31 (1999).
47. Sigvardsson, M., O'Riordan, M. & Grosschedl, R. EBF and E47 collaborate to induce expression of the endogenous immunoglobulin surrogate light chain genes. *Immunity* **7**, 25–36 (1997).
48. Romanow, W. J. *et al*. E2A and EBF act in synergy with the V(D)J recombinase to generate a diverse immunoglobulin repertoire in nonlymphoid cells. *Mol. Cell* **5**, 343–353 (2000).
49. Medina, K. L. *et al*. Assembling a gene regulatory network for specification of the B cell fate. *Dev. Cell* **7**, 607–617 (2004).
50. Sigvardsson, M. *et al*. Early B-cell factor, E2A, and Pax-5 cooperate to activate the early B cell-specific *mb-1* promoter. *Mol. Cell. Biol.* **22**, 8539–8551 (2002).

51. Maier, H. *et al*. Early B cell factor cooperates with Runx1 and mediates epigenetic changes associated with *mb-1* transcription. *Nature Immunol.* **5**, 1069–1077 (2004).
This interesting paper describes the hierarchical involvement of RUNX1 and EBF in activation of the *Mb-1* promoter, allowing PAX5, in turn, to activate the *Mb-1* gene. The data presented agree with the model proposed in reference 49, which describes the genetic control of pre-B-cell specification.
52. Nutt, S. L., Morrison, A. M., Dorfler, P., Rolink, A. & Busslinger, M. Identification of BSAP (Pax-5) target genes in early B-cell development by loss- and gain-of-function experiments. *EMBO J.* **17**, 2319–2333 (1998).
53. Urbanek, P., Wang, Z. Q., Fetka, I., Wagner, E. F. & Busslinger, M. Complete block of early B cell differentiation and altered patterning of the posterior midbrain in mice lacking Pax5/BSAP. *Cell* **79**, 901–912 (1994).
54. Kosak, S. T. *et al*. Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. *Science* **296**, 158–162 (2002).
55. Fuxa, M. *et al*. Pax5 induces V-to-DJ rearrangements and locus contraction of the immunoglobulin heavy-chain gene. *Genes Dev.* **18**, 411–422 (2004).
56. Nutt, S. L., Heavey, B., Rolink, A. G. & Busslinger, M. Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature* **401**, 556–562 (1999).
57. Rolink, A. G., Nutt, S. L., Melchers, F. & Busslinger, M. Long-term *in vivo* reconstitution of T-cell development by Pax5-deficient B-cell progenitors. *Nature* **401**, 603–606 (1999).
References 56 and 57 were the first to identify the essential role of PAX5 in B-cell identity. It was shown that *Pax5*$^{-/-}$ B cells can have alternative cell fates, such as development into T cells or macrophages.
58. Schaniel, C., Bruno, L., Melchers, F. & Rolink, A. G. Multiple hematopoietic cell lineages develop *in vivo* from transplanted *Pax5*-deficient pre-BI-cell clones. *Blood* **99**, 472–478 (2002).
59. Mikkola, I., Heavey, B., Horcher, M. & Busslinger, M. Reversion of B cell commitment upon loss of *Pax5* expression. *Science* **297**, 110–113 (2002).
This paper elegantly showed that ablation of PAX5 in already committed pro-B cells allows these cells to regain plasticity and differentiate into diverse cell types, such as T cells or macrophages.
60. Horcher, M., Souabni, A. & Busslinger, M. Pax5/BSAP maintains the identity of B cells in late B lymphopoiesis. *Immunity* **14**, 779–790 (2001).
61. Souabni, A., Cobaleda, C., Schebesta, M. & Busslinger, M. Pax5 promotes B lymphopoiesis and blocks T cell development by repressing *Notch1*. *Immunity* **17**, 781–793 (2002).
62. Eberhard, D., Jimenez, G., Heavey, B. & Busslinger, M. Transcriptional repression by Pax5 (BSAP) through interaction with corepressors of the Groucho family. *EMBO J.* **19**, 2292–2303 (2000).
63. Linderson, Y. *et al*. Corecruitment of the Grg4 repressor by PU.1 is critical for Pax5-mediated repression of B-cell-specific genes. *EMBO Rep.* **5**, 291–296 (2004).
64. Radtke, F., Wilson, A., Mancini, S. J. & MacDonald, H. R. Notch regulation of lymphocyte development and function. *Nature Immunol.* **5**, 247–253 (2004).
65. Hoflinger, S. *et al*. Analysis of Notch1 function by *in vitro* T cell differentiation of *Pax5* mutant lymphoid progenitors. *J. Immunol.* **173**, 3935–3944 (2004).
66. Xie, H., Ye, M., Feng, R. & Graf, T. Stepwise reprogramming of B cells into macrophages. *Cell* **117**, 663–676 (2004).
67. Schilham, M. W. *et al*. Defects in cardiac outflow tract formation and pro-B-lymphocyte expansion in mice lacking Sox-4. *Nature* **380**, 711–714 (1996).
68. Schilham, M. W. & Clevers, H. HMG box containing transcription factors in lymphocyte differentiation. *Semin. Immunol.* **10**, 127–132 (1998).
69. Staal, F. J. & Clevers, H. C. WNT signalling and haematopoiesis: a WNT–WNT situation. *Nature Rev. Immunol.* **5**, 21–30 (2005).
70. Reya, T. *et al*. Wnt signaling regulates B lymphocyte proliferation through a LEF-1 dependent mechanism. *Immunity* **13**, 15–24 (2000).
71. Taniguchi, T., Ogasawara, K., Takaoka, A. & Tanaka, N. IRF family of transcription factors as regulators of host defense. *Annu. Rev. Immunol.* **19**, 623–655 (2001).
72. Mittrucker, H. W. *et al*. Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. *Science* **275**, 540–543 (1997).
73. Holtschke, T. *et al*. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the *ICSBP* gene. *Cell* **87**, 307–317 (1996).
74. Lu, R., Medina, K. L., Lancki, D. W. & Singh, H. IRF-4,8 orchestrate the pre-B-to-B transition in lymphocyte development. *Genes Dev.* **17**, 1703–1708 (2003).
75. Sun, J., Matthias, G., Mihatsch, M. J., Georgopoulos, K. & Matthias, P. Lack of the transcriptional coactivator OBF-1 prevents the development of systemic lupus erythematosus-like phenotypes in *Aiolos* mutant mice. *J. Immunol.* **170**, 1699–1706 (2003).
76. Shapiro-Shelef, M. & Calame, K. Regulation of plasma-cell development. *Nature Rev. Immunol.* **5**, 230–242 (2005).
77. Boehm, J., He, Y., Greiner, A., Staudt, L. & Wirth, T. Regulation of BOB.1/OBF.1 stability by SIAH. *EMBO J.* **20**, 4153–4162 (2001).
78. Tiedt, R., Bartholdy, B. A., Matthias, G., Newell, J. W. & Matthias, P. The RING finger protein Siah-1 regulates the level of the transcriptional coactivator OBF-1. *EMBO J.* **20**, 4143–4152 (2001).
79. Yu, X., Wang, L., Luo, Y. & Roeder, R. G. Identification and characterization of a novel OCA-B isoform: implications for a role in B cell signaling pathways. *Immunity* **14**, 157–167 (2001).
80. Corcoran, L. M. *et al*. Oct-2, although not required for early B-cell development, is critical for later B-cell maturation and for postnatal survival. *Genes Dev.* **7**, 570–582 (1993).
81. Kim, U. *et al*. The B-cell-specific transcription coactivator OCA-B/OBF-1/Bob-1 is essential for normal production of immunoglobulin isotypes. *Nature* **383**, 542–547 (1996).
82. Nielsen, P. J., Georgiev, O., Lorenz, B. & Schaffner, W. B lymphocytes are impaired in mice lacking the transcriptional co-activator Bob1/OCA-B/OBF1. *Eur. J. Immunol.* **26**, 3214–3218 (1996).
83. Schubart, D. B., Rolink, A., Kosco-Vilbois, M. H., Botteri, F. & Matthias, P. B-cell-specific coactivator OBF-1/OCA-B/Bob1 required for immune response and germinal centre formation. *Nature* **383**, 538–542 (1996).
References 81–83 showed that the co-activator OBF1 is not essential for immunoglobulin gene transcription but rather for robust humoral immune responses and germinal-centre formation. In addition, reference 85 showed that immunoglobulin gene transcription is normal even when OBF1 and OCT2 are both absent.
84. Matthias, P. Lymphoid-specific transcription mediated by the conserved octamer site: who is doing what? *Semin. Immunol.* **10**, 155–163 (1998).
85. Schubart, K. *et al*. B cell development and immunoglobulin gene transcription in the absence of Oct-2 and OBF-1. *Nature Immunol.* **2**, 69–74 (2001).
86. Casellas, R. *et al*. OcaB is required for normal transcription and V(D)J recombination of a subset of immunoglobulin κ genes. *Cell* **110**, 575–585 (2002).
87. Wang, J. H. *et al*. Aiolos regulates B cell activation and maturation to effector state. *Immunity* **9**, 543–553 (1998).
88. Hess, J., Nielsen, P. J., Fischer, K. D., Bujard, H. & Wirth, T. The B lymphocyte-specific coactivator BOB.1/OBF.1 is required at multiple stages of B-cell development. *Mol. Cell. Biol.* **21**, 1531–1539 (2001).
89. Schubart, D. B., Rolink, A., Schubart, K. & Matthias, P. Lack of peripheral B cells and severe agammaglobulinemia in mice simultaneously lacking Bruton's tyrosine kinase and the B cell-specific transcriptional coactivator OBF-1. *J. Immunol.* **164**, 18–22 (2000).
90. Samardzic, T., Marinkovic, D., Nielsen, P. J., Nitschke, L. & Wirth, T. BOB.1/OBF.1 deficiency affects marginal-zone B-cell compartment. *Mol. Cell. Biol.* **22**, 8320–8331 (2002).
91. Corcoran, L. M. & Karvelas, M. Oct-2 is required early in T cell-independent B cell activation for G1 progression and for proliferation. *Immunity* **1**, 635–645 (1994).
92. Humbert, P. O. & Corcoran, L. M. *Oct-2* gene disruption eliminates the peritoneal B-1 lymphocyte lineage and attenuates B-2 cell maturation and function. *J. Immunol.* **159**, 5273–5284 (1997).
93. Wang, V. E., Tantin, D., Chen, J. & Sharp, P. A. B cell development and immunoglobulin transcription in Oct-1-deficient mice. *Proc. Natl Acad. Sci. USA* **101**, 2005–2010 (2004).
94. Ghosh, S., May, M. J. & Kopp, E. B. NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* **16**, 225–260 (1998).
95. Ghosh, S. & Karin, M. Missing pieces in the NF-κB puzzle. *Cell* **109**, S81–S96 (2002).
96. Hayden, M. S. & Ghosh, S. Signaling to NF-κB. *Genes Dev.* **18**, 2195–2224 (2004).
97. Sha, W. C., Liou, H. C., Tuomanen, E. I. & Baltimore, D. Targeted disruption of the p50 subunit of NF-κB leads to multifocal defects in immune responses. *Cell* **80**, 321–330 (1995).
This was the first study to identify the multiple *in vivo* roles of NF-κB. This paper also revealed that NF-κB is not required for Igκ light-chain rearrangement and transcription.
98. Cariappa, A., Liou, H. C., Horwitz, B. H. & Pillai, S. Nuclear factor κB is required for the development of marginal zone B lymphocytes. *J. Exp. Med.* **192**, 1175–1182 (2000).
99. Chen, C., Edelstein, L. C. & Gelinas, C. The Rel/NF-κB family directly activates expression of the apoptosis inhibitor Bcl-x_L. *Mol. Cell. Biol.* **20**, 2687–2695 (2000).
100. Hsu, B. L., Harless, S. M., Lindsley, R. C., Hilbert, D. M. & Cancro, M. P. BLyS enables survival of transitional and mature B cells through distinct mediators. *J. Immunol.* **168**, 5993–5996 (2002).
101. Weih, D. S., Yilmaz, Z. B. & Weih, F. Essential role of RelB in germinal center and marginal zone formation and proper expression of homing chemokines. *J. Immunol.* **167**, 1909–1919 (2001).
102. Franzoso, G. *et al*. Requirement for NF-κB in osteoclast and B-cell development. *Genes Dev.* **11**, 3482–3496 (1997).
103. Grossmann, M. *et al*. The anti-apoptotic activities of Rel and RelA required during B-cell maturation involve the regulation of Bcl-2 expression. *EMBO J.* **19**, 6351–6360 (2000).
104. Cariappa, A. *et al*. The follicular versus marginal zone B lymphocyte cell fate decision is regulated by Aiolos, Btk, and CD21. *Immunity* **14**, 603–615 (2001).
This paper proposed a model in which the level of BCR signalling is a key determinant of differentiation into marginal-zone B cells, and it identified Aiolos as one of the essential factors in this process.
105. Radtke, F. *et al*. Deficient T cell fate specification in mice with an induced inactivation of *Notch1*. *Immunity* **10**, 547–558 (1999).
106. Tanigaki, K. *et al*. Notch–RBP-J signaling is involved in cell fate determination of marginal zone B cells. *Nature Immunol.* **3**, 443–450 (2002).
107. Kuroda, K. *et al*. Regulation of marginal zone B cell development by MINT, a suppressor of Notch/RBP-J signaling pathway. *Immunity* **18**, 301–312 (2003).
108. Saito, T. *et al*. Notch2 is preferentially expressed in mature B cells and indispensable for marginal zone B lineage development. *Immunity* **18**, 675–685 (2003).
109. Garrett-Sinha, L. A. *et al*. PU.1 and Spi-B are required for normal B cell receptor-mediated signal transduction. *Immunity* **10**, 399–408 (1999).
110. Hu, C. J. *et al*. PU.1/Spi-B regulation of *c-rel* is essential for mature B cell survival. *Immunity* **15**, 545–555 (2001).
111. Bergman, Y. & Cedar, H. A stepwise epigenetic process controls immunoglobulin allelic exclusion. *Nature Rev. Immunol.* **4**, 753–761 (2004).
112. Schwarz, B. A. & Bhandoola, A. Circulating hematopoietic progenitors with T lineage potential. *Nature Immunol.* **5**, 953–960 (2004).
113. Hardy, R. R. & Hayakawa, K. B cell development pathways. *Annu. Rev. Immunol.* **19**, 595–621 (2001).
114. Sugai, M. *et al*. Essential role of Id2 in negative regulation of IgE class switching. *Nature Immunol.* **4**, 25–30 (2002).

Acknowledgements

Work in the laboratory of P.M. is supported by Novartis Research Foundation. A.G.R. is holder of the Roche Chair of Immunology, endowed by F. Hoffman-La Roche Ltd. Work in the laboratory of A.G.R. is supported by grants from the Swiss National Science Foundation. We apologize to colleagues whose articles could not be cited because of space limitations.

Competing interests statement

The authors declare no competing financial interests.

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