BACH transcription factors in innate and adaptive immunity

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Abstract | BTB and CNC homology (BACH) proteins are transcriptional repressors of the basic region leucine zipper (bZIP) transcription factor family. Recent studies indicate widespread roles of BACH proteins in controlling the development and function of the innate and adaptive immune systems, including the differentiation of effector and memory cells of the B and T cell lineages, $CD4^+$ regulatory T cells and macrophages. Here, we emphasize similarities at a molecular level in the cell-type-specific activities of BACH factors, proposing that competitive interactions of BACH proteins with transcriptional activators of the bZIP family form a common mechanistic theme underlying their diverse actions. The findings contribute to a general understanding of how transcriptional repressors shape lineage commitment and cell-type-specific functions through repression of alternative lineage programmes.

Cells of the innate and adaptive immune systems undergo dynamic changes in population size, differentiation state and function to counteract diverse and temporally stochastic threats to organismal integrity. These dynamic changes in cellular character are in part driven by transcription factors, which bind to regulatory DNA elements and programme cell-type-specific gene expression.

Transcription factors can be categorized on the basis of their effect on gene expression. Transcriptional activators promote gene expression and have constitutive or signal-dependent activity. Transcriptional repressors inhibit gene expression driven by transcriptional activators and therefore function to oppose or shape their functional outcome. Therefore, to understand the function of transcriptional repressors, it is essential to consider the transcriptional activators that they antagonize. Moreover, as the process of cellular differentiation requires both activation of lineage-specific gene expression programmes and suppression of alternative lineage gene expression programmes, it is important to consider the functions of transcriptional repressors in the context of the alternative lineage programmes that they antagonize[1,2].

Here, we review our emerging understanding of the widespread functions of the transcriptional repressors BTB and CNC homology 1 (BACH1) and BACH2 in the innate and adaptive immune systems. These functions lead to their involvement in diverse immunological processes, including tolerance, memory and immunosuppression. We propose that the functional reciprocity of BACH factors with transcriptional activators of the basic region leucine zipper (bZIP) family forms a common mechanistic theme underlying the diverse actions of BACH1 and BACH2, and we consider these actions in the context of the alternative lineage programmes that they repress.

Transcriptional repressors of the bZIP family

BACH proteins belong to the bZIP transcription factor family, members of which contain bZIP domains[3]. The bZIP family forms a large network of dimeric transcription factors, which in humans contains 53 members comprising 21 subfamilies, including the activator protein 1 (AP-1), nuclear factor erythroid 2 (NFE2), cAMP response element-binding (CREB), MAF and BACH transcription factor families[4]. bZIP transcription factors form dimeric interactions at DNA binding sites containing core sequences known as TPA response elements (TREs) or cAMP response elements (CREs)[4]. bZIP domains have highly conserved basic regions that make contact with DNA, and short amphipathic leucine zipper domains that are less conserved and form dimeric coiled-coil interactions with the bZIP domains of other bZIP monomers[5,6]. Through their leucine zipper interactions, bZIP transcription factors dimerize around DNA, with the basic regions of each monomer binding to similar sequences on opposing strands of DNA (FIG. 1a), such that the TREs and CREs to which they bind form the palindromic sequences TGA(G/C)TCA and TGACGTCA, respectively.

Dimerization makes important contributions to the size and regulatory flexibility of transcription factor repertoires[7]. First, it enables various homo- and

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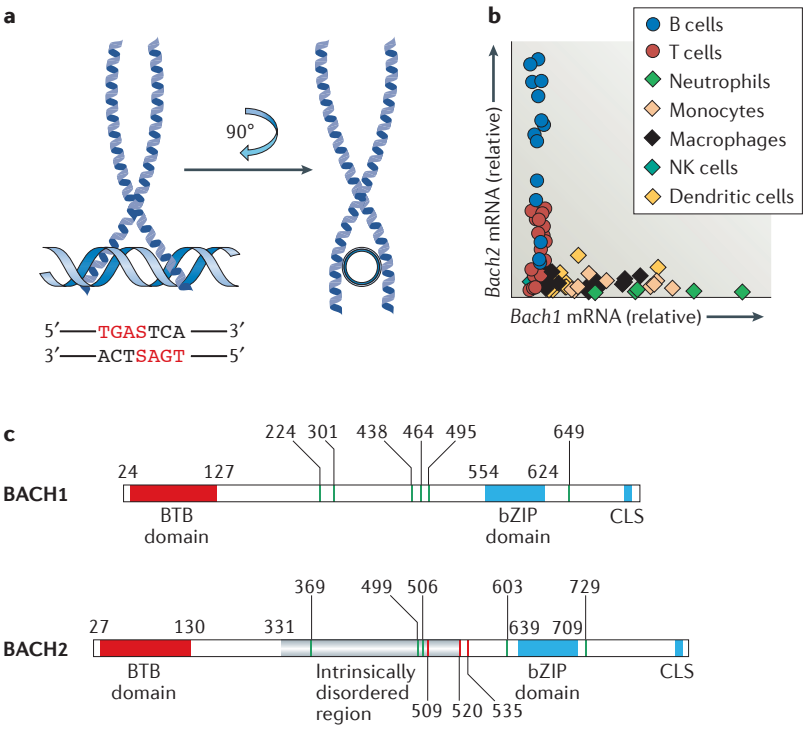

Figure 1 | **BACH proteins belong to the bZIP dimeric transcription factor family.** **a** | Prototypic dimeric interaction of basic region leucine zipper (bZIP) transcription factors at DNA exemplified by the interaction of JUN and FOS. The basic regions of each bZIP domain bind to similar DNA sequences running in a 5′ to 3′ direction (as highlighted in red; S represents the presence of C or G) on opposing strands of DNA, resulting in palindromic binding sites of bZIP dimers. **b** | Reciprocal pattern of BTB and CNC homology 1 (*Bach1*) and *Bach2* expression in mature myeloid and lymphoid lineage cells in mice. *Bach1* and *Bach2* mRNA expression among indicated mature myeloid and lymphoid cell types was analysed by the Immunological Genome Consortium (v1 data sets)[109]. *Bach1* mRNA is highly expressed in subsets of monocytes, macrophages, neutrophils and dendritic cells, whereas *Bach2* mRNA is highly expressed in subsets of B cells and T cells. **c** | Schematic representation of the domain structures and post-translational modifications of mouse BACH1 and BACH2. Green lines indicate cysteine–proline residues; numbers indicate the amino acid position of the cysteine. Red lines indicate selected identified phospho-residues. BTB domain, bric-a-brac–tramtrack–broad complex domain; CLS, cytoplasmic localization signal; NK, natural killer. Part **a** adapted from REF. 6, Macmillan Publishing.

heterodimeric transcription factor complexes with distinct functions to assemble at regulatory DNA, depending on the concentration of available monomers. Second, distinct heterodimers can have differing DNA sequence specificities, which enables different dimer combinations to form at distinct regulatory sequences in the presence of similar concentrations of available monomers. Last, sharp temporal changes in dimer composition can be induced by signal-driven changes to monomer concentration or allostery, which enables a common dimerization partner to rapidly alternate functions through partner exchange. bZIP transcription factors make extensive use of these properties. Despite substantial overall homology of bZIP domains, there is considerable partner selectivity in the dimeric interactions of bZIP transcription factors[4], and different dimeric complexes can have distinct sequence specificity[7]. Moreover, the dimeric nature of the bZIP family enables a common bZIP monomer, which is often functionally inert, to dimerize with either repressors or activators.

BACH factors form well-characterized heterodimeric complexes with the small MAF bZIP transcription factors MAFF, MAFG and MAFK[3]. Moreover, BACH2 also engages in heterodimeric interactions with the AP-1 bZIP transcription factor BATF (bZIP transcription factor ATF-like)[8] and is capable of homodimerizing at DNA *in vitro*[3]. The DNA sequences recognized by BACH transcription factor complexes contain TREs. As a result, their binding sites form recognition sequences for other bZIP factors, including transcriptional activators of the NFE2, NFE2-related factor (NRF) and AP-1 families[3,8,9]. This feature enables BACH factors to regulate gene expression by competing with NFE2, NRF and AP-1 transcriptional activators for binding to the same DNA sequences. Such competitive interactions are emerging as a core aspect of the functions of BACH factors in both the innate and adaptive immune systems, as we discuss below.

Evolutionary and biochemical features

The bZIP family probably evolved from a single ancestral eukaryotic gene through multiple independent genome- and gene-duplication events occurring over a billion years of metazoan evolution[10]. The ascidian *Ciona intestinalis* is the most primitive organism known to have a *BACH*-like gene[11]. This indicates that a common ancestor of BACH transcription factors existed before the vertebrate split and before the emergence of known forms of adaptive immunity that depend on variable antigen receptors. A single gene with similarity (approximately 66%) to human *BACH2* is found in the sea lamprey[12], which is the most primitive organism known to possess adaptive immunity[13]. It is not until jawed vertebrates, however, that two distinct genes with homology to *BACH1* and *BACH2* are found, which are likely to have arisen as a result of gene duplication after the gnathostome–lamprey split[14]. The early emergence of *BACH1* in ascidians suggests that its function was unrelated to adaptive immunity, whereas the later emergence of *BACH2* in the sea lamprey coincides with the evolution of adaptive immunity. This evolutionary distinction between *BACH1* and *BACH2* is reflected in the distinct functions of the two transcription factors in innate and adaptive immunity, respectively.

Bach1 and *Bach2* have important, non-redundant functions in mature myeloid and lymphoid lineages. This functional non-redundancy may in part be explained by partial mutual exclusivity in their patterns of expression in mature myeloid and lymphoid lineages. In particular, *Bach2* mRNA is predominantly expressed in mature lymphoid lineages, whereas *Bach1* mRNA is most highly expressed in myeloid lineages (FIG. 1b). However, there are important similarities between BACH1 and BACH2 at a protein level (FIG. 1c), and the two transcription factors exhibit functional redundancy during lymphoid development[15]. It will therefore be important to test whether BACH1 and BACH2 are redundant at a protein level through cross-complementation experiments.

TPA response elements (TREs). DNA sequences (with similarity to 5′-TGA(G/C)TCA-3′) that promote gene expression induced by the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and that are the canonical recognition sequence of the activator protein 1 (AP-1) transcription factor complexes formed by dimers of JUN and FOS.

cAMP response elements (CREs). DNA sequences (with similarity to 5′-TGACGTCA-3′) that promote gene expression induced by the cyclic AMP (cAMP) pathway and that are the canonical recognition sequence of CRE-binding protein (CREB) complexes.

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BTB domain. The BTB (bric-a-brac–tramtrack–broad complex) domain, which is sometimes known as the POZ (pox virus and zinc finger) domain, is commonly present in transcription factors with zinc finger domains[16]. BACH1 and BACH2 are the only two examples of BTB proteins with a bZIP DNA-binding domain[3,17]. Biochemical and crystallographic analyses indicate that the BTB domain of BACH1 can mediate homodimer formation *in vitro*, enabling BACH1–small MAF heterodimers to interact with each other and form a divalent DNA-binding complex[18]. Such a complex can generate a looped DNA structure between two distant binding sites *in vitro*[19]. BTB domains are also known to coordinate interactions with co-repressor complexes that post-translationally modify histones to repress transcription. In particular, BACH2 interacts with histone deacetylase 3 (HDAC3) to repress its B cell target genes, including PR domain containing 1, with ZNF domain (*Prdm1*, which encodes BLIMP1)[20].

Cytoplasmic localization signal. Another well-conserved feature of the primary structures of BACH1 and BACH2 is the cytoplasmic localization signal (CLS). Overexpression studies of BACH1 and BACH2 indicate that they tend to accumulate in the cytoplasmic region of transfected cells. When a short carboxy-terminal sequence containing the CLS is removed from BACH1 and BACH2, the mutant proteins accumulate in the nuclear region of transfected fibroblasts, which indicates that the CLS directs cytoplasmic accumulation of these proteins[21]. Although the CLS is not related to the nuclear export signal (NES) that is found in other proteins, the activity of the CLS does depend on the nuclear exporter chromosome maintenance 1 (CRM1; also known as exportin 1)[21].

Cysteine–proline motifs. A unique feature of BACH proteins among lymphoid transcription factors is that they directly bind to haem, resulting in their inactivation[17]. Haem binding is in part mediated by cysteine–proline motifs present in both proteins. BACH1 has six cysteine–proline motifs, whereas BACH2 has five of these motifs[17]. Both factors possess two modes of haem binding — six coordination and five coordination modes[22,23] — but the amino acid residues that mediate these distinct coordination modes are yet to be determined. Indeed, multiple mutations of cysteine–proline motifs in BACH2 do not abolish six coordination haem binding[24], which indicates that other cysteine and/or histidine residues may also be involved. As we discuss below, the activity of BACH factors is tightly regulated by haem levels.

Intrinsically disordered region. Recently, intrinsically disordered regions (IDRs) have been shown to have crucial roles for proteins involved in wide-ranging functions, such as DNA binding and signal transduction[25]. IDRs form protein–protein and protein–ligand binding surfaces by adopting specific structures on encountering their binding partners or by modification, including phosphorylation. Interestingly, at least four of the cysteine–proline motifs of BACH2 are found in an IDR[24]. Haem binding to the BACH2 IDR does not induce a specific tertiary structure *in vitro*. However, small-angle X-ray scattering suggests that the spatial distribution of the IDR is limited on haem binding (in other words, the randomness of its structure is reduced)[24]. In addition, an analysis of charge-state distributions using electrospray ionization mass spectrometry showed that haem induces a more compact conformation of BACH2 on binding to its IDR[26]. Such subtle structural changes may be sufficient to affect protein–protein interactions and/or domain–domain interactions of BACH2, resulting in inhibition of DNA binding or protein degradation.

Competition with bZIP transcriptional activators

BACH transcription factors possess bZIP domains, which enables them to bind to similar DNA sequences as bZIP factors of the NFE2, NRF and AP-1 families[3,8,9]. In some instances, common, transcriptionally 'inert' binding partners exchange between BACH factors and transcriptional activators, facilitating rapid functional switching between the repression and activation of gene expression. This is exemplified by a well-characterized regulatory relationship between BACH1 and NRF2 (FIG. 2a). In mouse macrophages, heterodimers of NRF2 and small MAF proteins activate haem oxygenase 1 (*Hmox1*; which encodes HO1)[27]. Activation of *Hmox1* by NRF2 is blocked by heterodimers of BACH1 and MAFK at low haem concentrations[28,29]. At high haem concentrations, BACH1 dissociates from DNA, is exported from the nucleus and is degraded[28,30–32], which enables MAFK to bind to NRF2 and form an activation complex that drives expression of *Hmox1*. Inhibition of basal polyubiquitylation and proteasomal degradation of NRF2 through haem-mediated disruption of NRF2–Kelch-like ECH-associated protein 1 (KEAP1) complexes further contributes to the haem sensitivity of this regulatory system[33]. This allows for rapid switching between the repressor and activator functions of MAF-containing transcription factor complexes in response to haem levels, which has implications for the control of macrophage-dependent iron homeostasis (discussed below).

A similar functional relationship between BACH2 and members of the AP-1 family is emerging[8,9] (FIG. 2b). AP-1 transcription factors include members of the FOS family (CFOS, FOSB, FOS-like 1 (FOSL1) and FOSL2), JUN family (CJUN, JUND and JUNB) and BATF family (BATF1, BATF2 and BATF3), and they are important activators of lymphocyte gene expression programmes[34,35]. AP-1 heterodimers can form ternary complexes with nuclear factor of activated T cells (NFAT) and interferon regulatory factor (IRF) transcription factors at AP-1–NFAT and AP-1–IRF composite elements (AICEs), respectively, such that the DNA sequences surrounding the core TRE can specify the composition and function of associated transcription factor complexes[34,35]. BACH2 is an inhibitor of TRE-driven gene expression in experiments with non-chromatinized reporter DNA[36], which implies that BACH2 can repress AP-1-driven gene expression in a manner independent of chromatin regulation.

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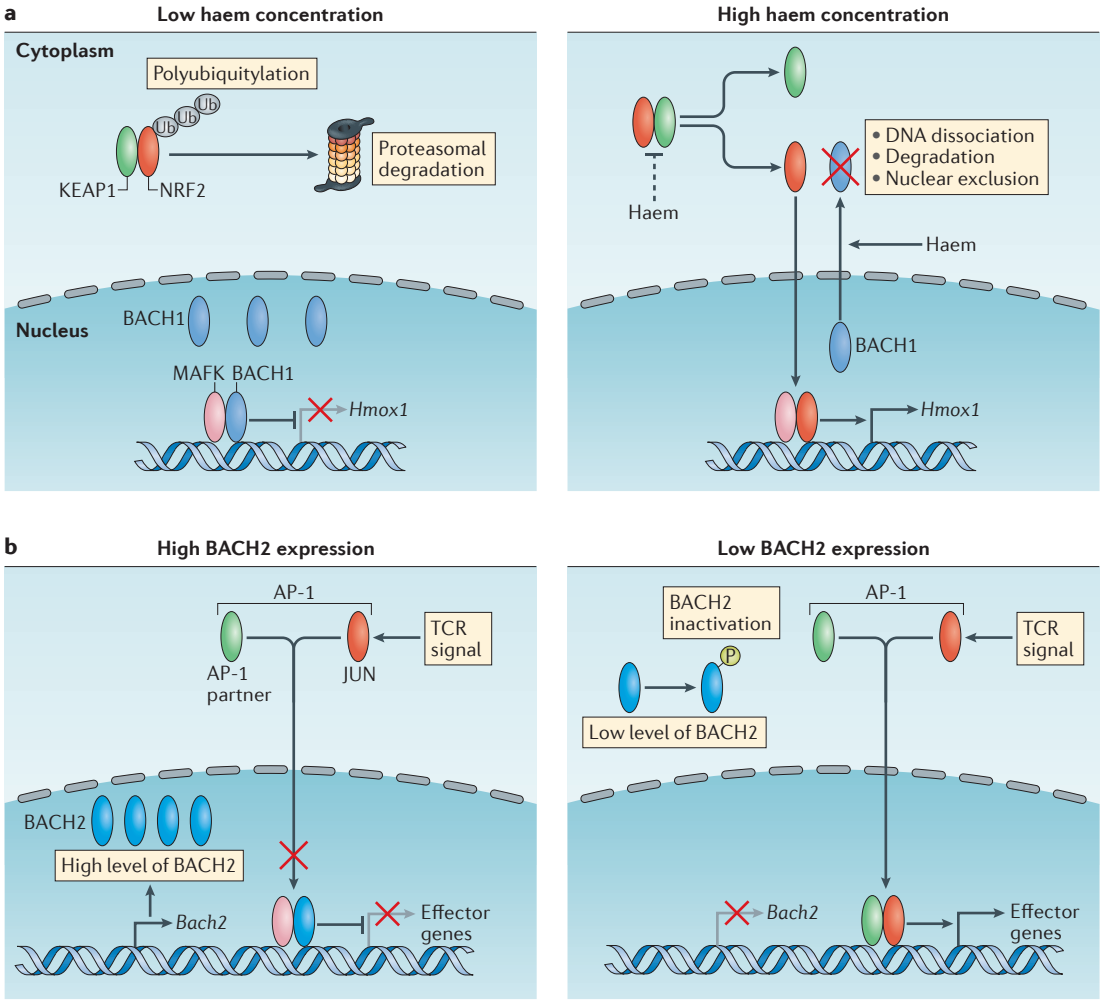

Figure 2 | **Repressor–activator relationships of BACH family transcription factors. a** | Relationship between BTB and CNC homology 1 (BACH1) and NFE2-related factor 2 (NRF2) in the regulation of haem oxygenase 1 (*Hmox1*) expression. Heterodimers of NRF2 and small MAF proteins, such as MAFK, activate *Hmox1* expression. Activation of *Hmox1* expression by NRF2 is blocked by heterodimers of BACH1 and MAFK at low haem concentrations[28,29,44] (left). Also at low haem concentrations, NRF2 undergoes polyubiquitylation and proteasomal degradation through its interaction with Kelch-like ECH-associated protein 1 (KEAP1). At high haem concentrations, BACH1 dissociates from DNA, is exported from the nucleus and is inactivated, which enables MAFK to bind to NRF2 and form an activation complex for *Hmox1* expression (right). Inhibition of basal polyubiquitylation and proteasomal degradation of NRF2 through haem-mediated disruption of NRF2–KEAP1 complexes further contributes to the haem sensitivity of this regulatory system. **b** | Relationship between BACH2 and activator protein 1 (AP-1) transcription factors in $CD8^+$ T cells. In cells with high levels of BACH2 expression, BACH2 limits binding of JUN family AP-1 transcription factors at regulatory elements of genes associated with effector differentiation, which prevents inappropriate T cell receptor (TCR)-driven induction of effector programmes in cells destined for a memory cell fate (left). *Bach2* gene expression is reduced in effector T cells and BACH2 is inactivated through phosphorylation to enable TCR-driven, AP-1-mediated induction of effector programmes (right).

Enhancers
Regulatory elements that function together with promoters to control gene expression. Enhancers can lie within intronic and intergenic regions and form looping interactions to bring enhancer-bound transcription factor complexes into contact with general transcription factors assembled at promoters. Distinct repertoires of enhancers function in different cell types, allowing for cell-type-specific regulation of gene expression.

In $CD8^+$ T cells, BACH2 occupies the DNA binding sites of multiple AP-1 transcription factors, including the enhancers of genes involved in effector differentiation, such as interferon-γ (*Ifng*) and *Prdm1* (REF. 9). At these sites, BACH2 restrains the binding of JUN family AP-1 transcription factors, which prevents T cell receptor-driven expression of genes involved in terminal effector differentiation. However, it was unclear whether BACH2 shares a common inert binding partner with JUN factors to enable repressor–activator switching through partner exchange. Evidence of a common partner shared between BACH2 and AP-1 factors has been provided by recent studies of T helper 2 (T_H2)-type cytokine regulation in $CD4^+$ T cells[8]. Expression of genes encoding the T_H2 cell cytokines interleukin-4 (IL-4), IL-5 and IL-13 is controlled by multiple enhancers, including a distal enhancer in the *Rad50* gene[37]. At these enhancers, BACH2 heterodimerizes with the AP-1 factor BATF to form a repressor complex. Loss of BACH2 causes BATF to form a heterotrimeric complex with JUND and IRF4, forming a transcriptional activator that drives expression of *Il4*, *Il5* and *Il13*. Consequently, similar to observations in $CD8^+$ T cells, loss of BACH2 in $CD4^+$ T cells results in increased binding of JUN factors

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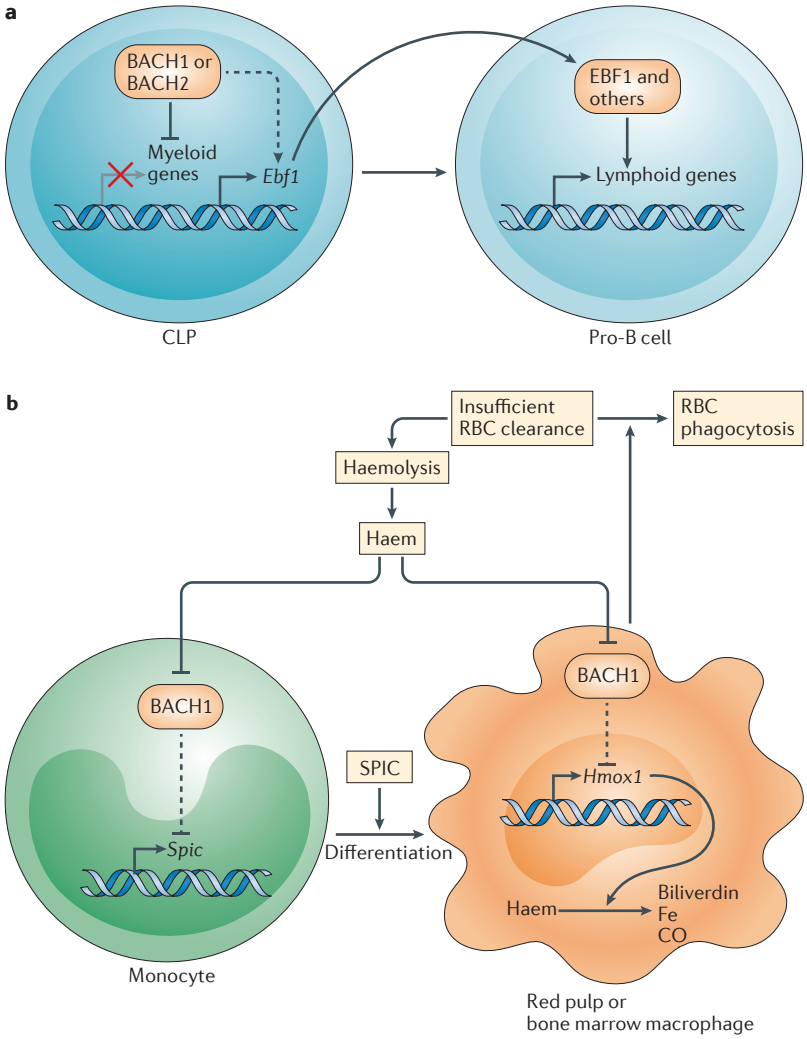

Figure 3 | **Functions of BACH factors in myeloid differentiation. a** | BTB and CNC homology (BACH) factors function at an early stage in B cell development (in common lymphoid progenitors (CLPs)) to repress the expression of myeloid genes and to indirectly promote early B cell factor 1 (*Ebf1*) expression. These functions enable the consolidation of early B cell identity. **b** | Haem-sensitive regulation of the differentiation of red pulp and bone marrow macrophages by BACH1. Insufficient clearance of red blood cells (RBCs) drives increased rates of haemolysis and, consequently, increased levels of haem. Haem drives inactivation of BACH1, which results in de-repression of *Spic* expression in monocytes and thereby promotes SPIC-driven red pulp and bone marrow macrophage differentiation. Haem-mediated inhibition of BACH1 also drives de-repression of haem oxygenase 1 (*Hmox1*) in macrophages (FIG. 2a), which mitigates the toxic effect of haem through HO1-mediated degradation of haem into biliverdin, ferrous iron (Fe) and carbon monoxide (CO). Whereas substantial evidence supports the direct repression of *Hmox1* by BACH1, it is unclear whether BACH1-dependent inhibition of *Spic* is direct or indirect.

at a specific set of loci[38]. These findings provide a mechanism of repressor–activator switching at AICEs involving the common partner BATF, but it is unclear whether a common subunit allows switching between BACH2 and JUN factors at other loci.

Redundant functions in lymphoid development

During haematopoiesis, the development of lymphoid potential occurs through the sequential differentiation of multipotent progenitors (MPPs), which have both lymphoid and myeloid potential, into lymphoid-primed MPPs and then common lymphoid progenitors (CLPs)[39,40]. This process is associated with loss of myeloid potential. Mice with a combined deficiency of *Bach1* and *Bach2* have reduced frequencies of pro-B cells, which is not the case for mice lacking functional copies of either gene alone[15]. This indicates that there is functional redundancy between BACH factors in early development of the B cell lineage. *Bach1* and *Bach2* double-deficient CLPs undergo defective B cell differentiation under specific conditions *in vitro*. This defect is associated with aberrant induction of myeloid differentiation. Overexpression experiments indicate that BACH2 directly represses genes involved in myeloid differentiation, including CCAAT/enhancer-binding protein beta (*Cebpb*), aryl hydrocarbon receptor (*Ahr*), lymphocyte antigen 96 (*Ly96*), *Spic,* and cytokine inducible SH2-containing protein (*Cish*), whereas BACH1 directly represses only *Cebpb* among the genes examined[15]. *Bach1* and *Bach2* double-deficient animals have increased frequencies of CD11b$^+$ and/or Gr1$^+$ (LY6C$^+$/LY6G$^+$) cells, but it is unclear whether this is a consequence of aberrant myelopoiesis or defective regulatory T (T_{reg}) cell-mediated immunoregulation caused by BACH2 deficiency (see later). Early B cell factor 1 (EBF1; also known as COE1) is similarly proposed to promote B cell lineage commitment and suppression of myeloid characteristics. CLPs lacking EBF1 fail to differentiate into B cells and exhibit increased myeloid potential, aberrantly acquiring macrophage characteristics under specific conditions *in vitro*[41]. *Ebf1* mRNA levels are decreased in *Bach1* and *Bach2* double-deficient CLPs, and overexpression of EBF1 is sufficient to promote pre-B cell differentiation at equivalent levels in both wild-type and *Bach1* and *Bach2* double-deficient MPPs *in vitro*[15]. Thus, it is probable that BACH factors function at an early stage in B cell development, repressing myeloid lineage genes and indirectly promoting *Ebf1* expression to consolidate early B cell identity (FIG. 3a). It will be important to assess the extent to which indirect induction of *Ebf1* expression by BACH factors accounts for their ability to promote pro-B cell differentiation *in vivo* and to resolve the precise mechanisms by which BACH factors promote *Ebf1* expression.

Functions of BACH1 in macrophages

Erythrophagocytic macrophage differentiation. Macrophages are functionally diverse and have central roles in development, immunity, homeostasis and tissue repair[42]. Bone marrow macrophages and red pulp macrophages, which are dependent on the transcription factor SPIC, are localized in the splenic red pulp and contribute to blood homeostasis by phagocytosing injured and senescent red blood cells[43]. Approximately 2×10^{11} red blood cells are recycled each day by phagocytosis, releasing iron for re-use in erythropoiesis. This phagocytic system is regulated by haem levels, which allows the rate of haemolysis to direct the size of red pulp and bone marrow macrophage populations.

BACH1 has an important function in the haem-sensitive regulation of red pulp and bone marrow macrophage differentiation[27]. Haem attenuates

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BACH1-mediated repression of *Spic*, thereby promoting SPIC expression and the differentiation of monocytes into red pulp and bone marrow macrophages. Attenuation of BACH1 function by haem also promotes the ability of macrophages to mitigate the toxic effects of haem, by de-repressing the BACH1 target gene *Hmox1,* encoding HO1, which catalyses the breakdown of haem into biliverdin, ferrous iron and carbon monoxide[43] (FIG. 3b). Whereas substantial evidence supports the direct repression of *Hmox1* by BACH1, it is unclear whether BACH1-dependent inhibition of *Spic* is direct or indirect.

Haem directly inhibits the DNA-binding activity of BACH1 *in vitro*[28,30] and in erythroid cells *in vivo*[44]; induces its nuclear export[31]; and promotes its polyubiquitylation and proteasomal degradation[32]. Haem-oxidized IRP2 ubiquitin ligase 1 (HOIL1; also known as RBCK1) promotes the polyubiquitylation and proteasomal degradation of BACH1 in response to haem levels, although it is unclear whether the interaction between BACH1 and HOIL1 is modulated by haem levels[32]. Expression of BACH1 is also fine-tuned through basal turnover induced by a suppressor of the kinetochore protein 1–cullin 1–F-box/LRR-repeat protein 17 (SKP1–CUL1–FBXL17) E3 ubiquitin ligase complex, thereby influencing the relative availability of BACH1 and NRF2 and biasing the system towards haem sensitivity[45]. This allows macrophage-mediated iron homeostasis to be guided in a manner that is exquisitely sensitive to haem levels. Post-transcriptional regulation of *Bach1* mRNA by the microRNA miR-155 may also contribute to the control of BACH1 expression, although the significance of this to iron homeostasis has not been determined[46,47].

Inflammatory macrophage differentiation. A BACH1–HO1 regulatory circuit also seems to control the differentiation of inflammatory macrophages. BACH1-deficient mice survive to a similar age to wild-type mice[48] but exhibit resistance to inflammation in various contexts, including ischaemia–reperfusion myocardial injury[49], spinal cord injury[50,51], lipopolysaccharide-induced hepatic injury[52], hyperoxic lung injury[53], atherosclerosis[54] and colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS)[55]. Macrophage populations can be classified into classically activated M1 macrophages and alternatively activated M2 macrophages with pro- and anti-inflammatory functions, respectively, although in reality a much greater functional spectrum exists[56]. Peritoneal macrophages from *Bach1*-deficient animals express higher levels of genes associated with M2 macrophage differentiation, including those encoding arginase 1, FIZZ1 (also known as resistin-like-α), CD206 and YM1 (also known as chitinase-3-like protein 3), which suggests that BACH1 suppresses M2 macrophage differentiation[55]. *Bach1*-deficient mice also exhibit partial resistance to the induction of experimental autoimmune encephalomyelitis (EAE)[57]. This phenotype was attributed to reduced antigen-presentation capacity in these animals resulting from reduced frequencies of MHC class II-expressing macrophages and dendritic cells, although qualitative differences in these cells may also have contributed to the phenotype observed.

HO1 promotes the differentiation of M2 macrophages, although the precise mechanism by which it does so is unclear[58]. Suppression of HO1 expression in macrophages is proposed as a mechanism by which BACH1 restrains anti-inflammatory macrophage differentiation. Expression of HO1 is increased in various *Bach1*-deficient tissues either before or after injury, and this has been attributed to its dysregulated expression in macrophages[49–51,53]. The resistance of *Bach1*-deficient mice to TNBS-induced colitis[55] and to atherosclerosis caused by apolipoprotein E deficiency[54] is lost upon treatment of mice with tin protoporphyrin IX (SnPP), which inhibits HO1. BACH1-mediated suppression of NRF2-driven gene expression also promotes osteoclast differentiation from macrophage precursors in response to receptor activator of nuclear factor-κB ligand (RANKL; also known as TNFSF11) signalling, which indicates that BACH1 has a role in bone homeostasis[38,59]. Thus, emerging evidence indicates that BACH1 controls the differentiation of various macrophage lineages, but its precise functions in macrophages and their precursors require further careful evaluation.

BACH2 is required for peripheral tolerance

Differentiation of FOXP3$^+$ T_{reg} cells. Large genome-wide association studies associate genetic polymorphisms in the human *BACH2* locus with susceptibility to diverse autoimmune and allergic diseases (BOX 1), which suggests that BACH2 has a role in immunological tolerance. Mice bearing a germline disruption of the first coding exon of *Bach2* develop lethal inflammation and pulmonary alveolar proteinosis, which limit median survival to 4.5–6 months[60–62]. *Bach2*-deficient animals develop profound lung inflammation, systemic autoantibody responses and incompletely penetrant inflammation of the small intestine and stomach[9]. Eosinophils, macrophages and CD4$^+$ T cells bearing an effector phenotype and expressing the T_H2 cell transcription factor GATA3 and the T_H2-type cytokines IL-4 and IL-13 are found at higher frequencies in the lungs and secondary lymphoid organs of *Bach2*-deficient mice compared with wild-type mice[60,61]. Macrophages in the lungs of *Bach2*-deficient mice acquire an M2 phenotype and exhibit an impaired capacity to clear pulmonary surfactant[62]. A histopathologically similar lung inflammation is present in mice bearing a T cell-specific conditional deletion of *Bach2*, which indicates that T cell-intrinsic BACH2 expression is required to prevent immunopathology arising from *Bach2* deficiency[8].

CD4$^+$ T_{reg} cells, which depend on the transcription factor forkhead box protein P3 (FOXP3)[63–65], suppress effector T cells and prevent lethal inflammation directed against both self-antigens and innocuous foreign antigens[66–68]. Reduced frequencies of T_{reg} cells are observed in the thymi, spleens, inguinal lymph nodes and mesenteric lymph nodes of *Bach2*-deficient mice[60,61]. There is a more profound, near-complete absence of thymic

Genetic polymorphisms
Genetic variations occurring in a specific population at such a frequency that the rarest of them cannot be maintained by recurrent mutation or immigration alone: therefore, they most frequently occur through inheritance.

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Thymus-derived T_{reg} cells (tT_{reg} cells). Most of these cells develop at the $CD4^+$ single-positive stage of thymic T cell maturation as a result of the intermediate affinity of their T cell receptors for self-antigens. These cells contribute to peripheral tolerance against self-antigens.

Peripherally derived T_{reg} cells (pT_{reg} cells). These cells develop from mature $CD4^+FOXP3^-$ T cells in peripheral tissues. pT_{reg} cells are frequently induced at mucosal sites, such as the lungs and gut, where they contribute to tolerance against innocuous foreign antigens.

and splenic T_{reg} cells in the *Bach2*-deficient compartment of bone marrow chimaeras reconstituted with mixtures of wild-type and *Bach2*-deficient bone marrow cells[60]. This indicates an absolute cell-intrinsic requirement for BACH2 in the differentiation of both thymus-derived T_{reg} cells (tT_{reg} cells) and peripherally derived T_{reg} cells (pT_{reg} cells) (also known as induced T_{reg} (iT_{reg}) cells) under non-inflamed or competitive conditions. Lethal inflammation resulting from BACH2 deficiency can be experimentally attributed to defective T_{reg} cell differentiation: inflammation and increased effector differentiation of $CD4^+$ T cells are recapitulated upon reconstitution of lymphocyte-deficient hosts with *Bach2*-deficient bone marrow cells, and this phenotype is prevented by the co-transfer of wild-type, but not FOXP3-insufficient, bone marrow cells, or by co-transfer of purified wild-type $CD4^+$ T_{reg} cells[60]. Thus, the provision of wild-type T_{reg} cells rescues the inflammatory defect that occurs in the absence of BACH2. This demonstrates that a primary means by which BACH2 prevents lethal inflammation is by promoting $FOXP3^+$ T_{reg} cell differentiation (FIG. 4).

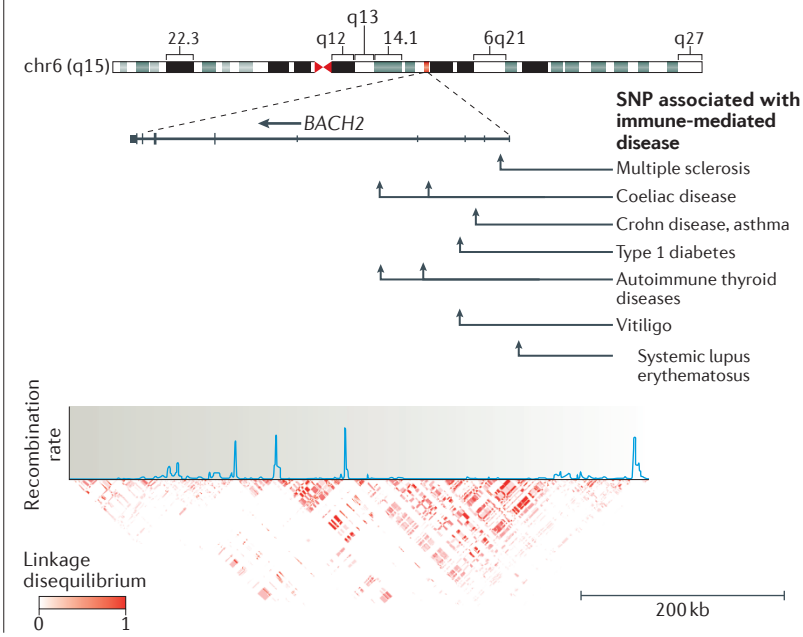
Although T_{reg} cells have a fundamental role in immune homeostasis, they also contribute to immunosuppression in tumours by restraining the function of $CD4^+$ and $CD8^+$ effector T cells[69,70]. Growth of intradermal B16 melanoma and EL-4 lymphoma tumours is impaired in *Bach2*-deficient animals, but not in *Bach2* and recombination-activating gene 1 (*Rag1*) double-deficient animals, which lack B and T cells[71]. The observation of impaired tumour growth in *Bach2*-deficient animals has also been recapitulated in a large *in vivo* B16 pulmonary metastasis screen[72]. Reduced tumour growth in *Bach2*-deficient animals is accompanied by reduced frequencies of $FOXP3^+$ T_{reg} cells and increased frequencies of $CD4^+$ and $CD8^+$ effector T cells expressing the effector cytokine IFNγ in tumours. Impaired tumour growth is also observed in *Rag1*-deficient mice reconstituted with *Bach2*-deficient bone marrow cells, but is reversed on provision of wild-type T_{reg} cells[71]. This indicates a role for BACH2 in promoting T_{reg} cell-mediated tumour immunosuppression. In this context, it is interesting to note that individuals with tumours that express high levels of *BACH2* mRNA have better clinical responses to therapy with an immune checkpoint inhibitor targeting programmed cell death protein 1 (PD1)[73], although the intratumoural cell types that contribute to the *BACH2* mRNA signal and the precise significance of this observation have yet to be determined.

Restraint of effector programmes in conventional CD4⁺ and CD8⁺ T cells. In addition to its role in thymic T_{reg} cell differentiation, BACH2 intrinsically regulates the differentiation and function of multiple conventional T cell lineages (FIG. 4). BACH2 restrains excessive induction of genes encoding the T_H cell cytokines IFNγ, IL-13, IL-4, IL-5 and IL-17A when naive $CD4^+$ T cells are stimulated under neutral or T_H1 cell-, T_H2 cell- or T_H17 cell-inducing culture conditions[8,60,74,75]. Inappropriate induction of a programme of gene expression associated with T_H cell differentiation is observed following stimulation of *Bach2*-deficient naive $CD4^+$ T cells under pT_{reg} cell-inducing conditions *in vitro*[60]. Genome-wide mapping of BACH binding sites in both $CD4^+$ and $CD8^+$ T cells indicates that BACH2 binds to enhancers associated with genes involved in effector T cell differentiation. Prominent among these are enhancers of genes encoding the effector cytokines IFNγ, IL-4, IL-5 and IL-13 (REFS 8,9).

Tight regulation of genes encoding cytokines and their autocrine and/or paracrine feedforward effects seems to be relevant for appropriate control of $CD4^+$ T cell differentiation by BACH2. For example, antibody-mediated blockade of IFNγ and IL-4 partially reverses the defective pT_{reg} cell induction and increased expression of the T_H1- and T_H2-type lineage transcription factors T-bet and GATA3 that occur when *Bach2*-deficient cells are stimulated under pT_{reg} cell-inducing conditions *in vitro*[60]. Similarly, IL-4 blockade or signal transducer and activator of transcription 6

Box 1 | **Association of *BACH2* with autoimmune and allergic disease**

Mammalian genomes are interspersed with recombination hotspots that are separated by regions of low haplotype diversity, wherein the rate of recombination is much lower and sets of allelic variations, or haplotypes, are inherited together at a frequency higher than would have been expected based on the intervening genomic distance (linkage disequilibrium)[118]. Large genome-wide association studies have identified single-nucleotide polymorphisms (SNPs) in the BTB and CNC homology 2 (*BACH2*) locus that are associated with susceptibility to diverse autoimmune and allergic diseases, including multiple sclerosis[112], asthma[115], type 1 diabetes[113], coeliac disease[114], Crohn disease[116,119], generalized vitiligo[117] and the autoimmune thyroid diseases Graves disease and Hashimoto thyroiditis[120] (see the figure). These polymorphisms occur, in populations of European descent, within a region of high linkage disequilibrium that is bounded by recombination hotspots upstream of the transcriptional start site and within intron 4 of *BACH2*. This suggests that single or limited numbers of haplotypes in this region may contribute to susceptibility to diverse immune-mediated diseases. The identity and effect of such risk haplotypes on *BACH2* expression and splicing require elucidation. However, these results suggest a common involvement of *BACH2* in immune disorders in which responses against either self-antigens or innocuous foreign antigens are inappropriately regulated.



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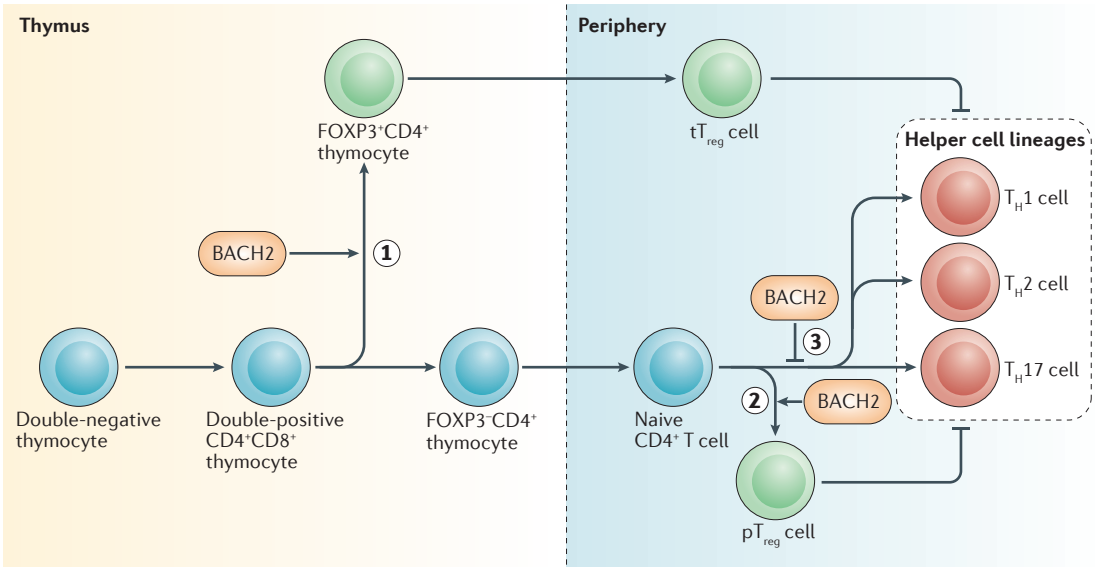

Figure 4 | **BACH2 restrains immune activation by controlling CD4⁺ T cell differentiation.** BTB and CNC homology 2 (BACH2) functions at multiple stages of $CD4^+$ T cell differentiation to restrain immune activation. BACH2 promotes the differentiation of forkhead box protein P3 $(FOXP3)^+$ thymus-derived regulatory T (tT_{reg}) cells (**1**). BACH2 restrains effector programmes in conventional $FOXP3^-CD4^+$ T cells, which results in stabilization of peripherally derived regulatory T (pT_{reg}) cell differentiation from naive $CD4^+$ T cell precursors (**2**) and cell-intrinsic suppression of T helper 1 (T_H1), T_H2 and T_H17 cell differentiation (**3**). The absence of BACH2 leads to defective tT_{reg} and pT_{reg} cell differentiation and de-repression of T_H cell programmes, resulting in inappropriate immune activation.

(*Stat6*) deletion reduces aberrant T_H2-type cytokine expression and induction of GATA3 upon stimulation of *Bach2*-deficient naive $CD4^+$ T cells under neutral conditions *in vitro,* instead causing *Bach2*-deficient cells to produce T_H1-type cytokines[8]. The function of BACH2 as an AP-1 repressor is relevant to its role in the suppression of T_H-type cytokine production, particularly given the observation that BACH2–BATF heterodimers restrain expression of the T_H2-type cytokine genes *Il4* and *Il13* by blocking the recruitment of activating BATF–JUND–IRF4 heterotrimers to a distal enhancer of these genes in the *Rad50* locus[8]. Moreover, *Bach2* and *Stat6* double-deficient animals develop reduced pulmonary immunopathology compared with *Bach2*-deficient mice, which indicates the importance of cytokine signalling in reinforcing the T_H2 cell-mediated pathology that occurs in the absence of adequate T_{reg} cell-mediated immunosuppression in *Bach2*-deficient animals[8].

BACH2 in immunological memory

T cell memory. T cell receptor (TCR) signalling is required for the initiation and diversification of $CD8^+$ T cell responses. In response to cognate antigens, naive $CD8^+$ T cells proliferate and differentiate to form a heterogeneous population of memory precursor cells and effector cells[76–80]. According to the progressive differentiation model, it is proposed that strong or repeated TCR signalling drives progressive changes in gene expression that result in loss of lymphoid homing potential, acquisition of effector cell functions, terminal effector differentiation and apoptosis[81,82]. Naive T cells that have received weak antigen signals fail to undergo full effector differentiation, retain lymphoid homing characteristics, remain functionally quiescent and differentiate into memory precursor cells that persist to form long-lived memory populations[76,77,82,83].

Restraint of T cell effector programmes by BACH2 has an important role in the generation of immunological memory. Adoptive transfer experiments indicate that BACH2 is required for the differentiation of long-lived memory $CD8^+$ T cell responses following viral infection[9,84]. Increased proportions of *Bach2*-deficient $CD8^+$ T cells responding to viral infection undergo terminal effector differentiation and apoptosis compared with wild-type T cells, and this is accompanied by a reduction in the differentiation of memory precursor cells and cells with a central memory phenotype[9] (FIG. 5). Importantly, increased effector differentiation of naive BACH2-deficient T cells requires antigen and is not spontaneous. However, naive *Bach2*-deficient T cells acquire effector characteristics and undergo increased apoptosis when stimulated with much lower levels of antigen than do wild-type T cells[9].

Bach2 mRNA is highly expressed in naive $CD8^+$ T cells, intermediately expressed in central memory T cells and weakly expressed in effector T cells[9] (FIG. 5). *Bach2*-deficient naive T cells exhibit inappropriate TCR-driven induction of genes associated with effector and terminal effector T cell differentiation. This effect of BACH2 in limiting TCR-driven effector programmes is diminished in effector T cells, in which low expression levels of BACH2 correlate with unrestrained induction of TCR-driven genes[9]. As discussed above, the repressor–activator relationship between BACH2 and AP-1 factors of the JUN family

Progressive differentiation model
Proposes that naive T cells differentiate into a heterogeneous pool of memory precursor and effector T cells early during infection. Effector cells are short-lived and die following the withdrawal of antigen. Memory precursor cells survive after contraction of the effector population and give rise to memory cells. The alternative linear differentiation model proposes that naive $CD8^+$ T cells differentiate uniformly into effector cells early during immune responses, and a subset of these differentiate into memory cells on the withdrawal of antigen.

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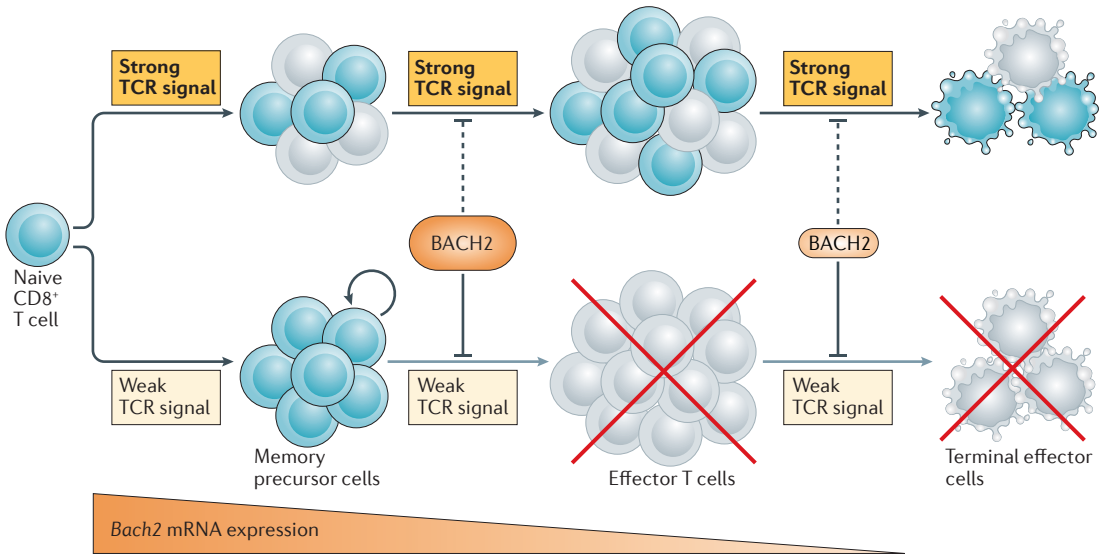

Figure 5 | **BACH2 restrains terminal effector programmes to promote memory CD8⁺ T cell differentiation.** A model to account for the function of BTB and CNC homology 2 (BACH2) in CD8⁺ T cells. *Bach2* mRNA is highly expressed in naive CD8⁺ T cells, intermediately expressed in central memory cells and weakly expressed in effector cells. According to the progressive differentiation model, strong or repeated T cell receptor (TCR) signalling drives progressive acquisition of effector cell characteristics. However, in cells that have received weak antigen signals, BACH2 blocks the induction of effector programmes, allowing them to retain memory cell characteristics, remain functionally quiescent and persist in contributing to memory.

is important for BACH2-mediated restraint of TCR-driven transcriptional programmes. This mechanism therefore enables the transcriptional consequences of TCR signalling to be shaped by the differentiation state and prior stimulation history of T cells.

Bach2-deficient T cells undergo increased apoptosis following antigen stimulation *in vitro* and *in vivo*. Consistent with their apoptotic phenotype, *Bach2*-deficient cells stimulated *in vitro* are depleted of the anti-apoptotic B cell leukaemia/lymphoma 2 (BCL-2) family members BCL-X_L and MCL1, similarly to terminally differentiated wild-type CD8⁺ T cells[85–87]. Intriguingly, *Bach2*-deficient CD4⁺ T cells stimulated *in vitro* also exhibit features of cellular senescence, including increased expression of cyclin-dependent kinase inhibitor 2A (*Cdkn2a*) mRNA, senescence-associated β-galactosidase staining and expression of IL-6 (REF. 75). Overexpression of BACH2 in CD8⁺ T cells is also reported to promote cell proliferation[84]. Although we have observed increased expression of *Cdkn2a* mRNA and senescence-associated β-galactosidase staining among *Bach2*-deficient CD8⁺ T cells following stimulation *in vitro* (R.R., unpublished observations), the consequence of this is unclear. In particular, *Bach2*-deficient CD8⁺ T cells do not exhibit increased growth arrest during antiviral responses *in vivo* and instead proliferate at a higher rate. The failure of *Bach2*-deficient CD8⁺ T cell populations to expand *in vivo* is instead associated with increased rates of apoptosis[9]. *Cdkn2a* encodes both $p16^{INK4A}$ and $p19^{ARF}$, which have anti-proliferative and pro-apoptotic functions, respectively. It is possible that predominant expression and/or function of $p19^{ARF}$ results in features of senescence associated with apoptosis rather than growth arrest in *Bach2*-deficient T cells. It will be important to determine the level of epistasis between *Bach2* and *Cdkn2a* in T cells.

The involvement of BACH2 in processes related to cellular senescence and survival may in part explain its role in cancers of the haematopoietic system (BOX 2). In addition, the *BACH2* locus is a frequently detected site of proviral integration in CD4⁺ T cells that are persistently infected with HIV-1 during antiretroviral therapy (ART)[88–90]. It is plausible that high levels of *BACH2* expression and associated chromatin states favour proviral integration in the *BACH2* locus in memory T cells, thereby marking cells before ART that tend to persist following the initiation of therapy. Alternatively, proviral integration may interfere with *BACH2* transcription, resulting in an effect on cellular differentiation or survival.

Humoral immune responses. B cells differentiate into plasma cells or memory B cells to exert their effector or memory functions, respectively. In responses against T cell-dependent antigens, the choice between memory and plasma cell fates takes place at two distinct stages[91]. At the T cell–B cell border of a lymphoid follicle, recently proliferating B cells enter the germinal centre or differentiate into either short-lived plasma cells or germinal centre-independent memory B cells. Germinal centres are composed of a dark zone and a light zone. In the dark zone, germinal centre B cells proliferate and undergo somatic hypermutation (SHM). B cells bearing hypermutated B cell receptors (BCRs) transit to the light zone, where competition for antigen displayed on the surface of follicular dendritic

Cellular senescence
A form of irreversible growth arrest that limits the replicative lifespan of cells. Replicative senescence is induced by telomere shortening that occurs as a result of DNA replication during mitosis. Premature cellular senescence occurs in the absence of telomere shortening and results in growth arrest and apoptosis through the action of gene products of the INK4/ARF locus.

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Box 2 | **Involvement of BACH2 in cancers of the haematopoietic system**

Several lines of observation suggest that BTB and CNC homology 2 (BACH2) is involved in the development of haematopoietic cancers. However, BACH2 seems to either inhibit or promote cancer depending on its cellular context. In B cell acute lymphoblastic leukaemia (ALL), BACH2 functions as a tumour suppressor. *BACH2* is located at chromosome 6q15, which is a region of frequent minimal deletion in individuals with B cell ALL with 6q mutations[121]. In addition, loss-of-function mutations of its upstream activator paired box 5 (PAX5) lead to lower levels of expression of *BACH2* mRNA, which is associated with poor clinical outcome. Lower levels of expression of *BACH2* in ALL cells negate the pre-B cell receptor (pre-BCR) checkpoint, which allows the expansion of abnormal clones. The BCR–ABL oncoprotein also directly suppresses BACH2 expression in chronic myeloid leukaemia cells by inactivating its upstream transcriptional activator PAX5 (REF. 122) and drives its inactivation by phosphorylating BACH2 at S521 (REF. 108). In a subset of diffuse large B-cell lymphoma (DLBCL), *BACH2* expression is frequently lost[123]. However, there is contradictory evidence as to the positive or negative prognostic significance of *BACH2* expression in individuals with DLBCL[124,125], possibly as a result of confounding mutations that affect the contribution of BACH2 to DLBCL. Tumour-promoting functions of BACH2 in leukaemia are further supported by a genetic screen to identify cooperating pathways promoting leukaemia initiation and progression on a genetically susceptible background[126].

cells (FDCs) and for survival signals provided by T cells drives the selection of B cells with the highest affinity for antigen. Cells recycle between dark and light zones or exit the germinal centre, differentiating into either long-lived plasma cells or memory B cells. During this process, class-switch recombination (CSR) enables functionally diversified antibody responses to be generated.

BACH2 is required for the proliferation of antigen-stimulated B cells, germinal centre B cell differentiation, CSR and SHM[92,93] (FIG. 6). Repression of plasma cell differentiation programmes by BACH2 seems to be important in promoting these events. BACH2 is known to repress expression of *Prdm1*, encoding the transcription factor BLIMP1, which promotes differentiation of plasma cells[92,94]. BACH2 is also required for appropriate expression of BCL-6 and activation-induced cytidine deaminase (AID; also known as AICDA), which drive germinal centre differentiation, and CSR and SHM, respectively. *In vitro* experiments show that impaired expression of both AID and BCL-6 in *Bach2*-deficient B cells is restored by concomitant deficiency of *Prdm1* (REF. 95), which suggests that repression of BLIMP1 expression by BACH2 is important for promoting CSR, SHM and germinal centre development. *In vivo* experiments support this[96]. Defective proliferation of antigen-stimulated *Bach2*-deficient B cells is similarly restored by concomitant loss of BLIMP1 (REF. 96). This finding is consistent with the hypothesis that aberrantly expressed BLIMP1 resulting from the absence of BACH2 functions as an anti-proliferative factor[93]. By contrast, BACH2-dependent memory B cell differentiation is independent of repression of BLIMP1 (REF. 93), which suggests that there are other mechanisms by which BACH2 promotes memory B cell differentiation. An interaction between BACH2 and BCL-6 also seems to be important for the maintenance of germinal centres[97,98].

Stochastic and instructive models have been proposed to account for the selective differentiation of a subset of germinal centre B cells into memory cells[99–101]. Light zone germinal centre B cells of relatively low affinity are favoured for selection into the memory compartment, and these cells have higher expression levels of BACH2. Haploinsufficiency of BACH2 results in reduced generation of memory B cells. These results suggest that weak antigen signals and low levels of T cell help result in the maintenance of relatively high levels of expression of *Bach2* in germinal centre B cells, which favour their entry into the memory pool[93]. By contrast, strong signals from the BCR and T cells reduce the level of expression of *Bach2* and promote plasmablast differentiation[93]. High levels of expression of IRF4 and RELA (also known as p65) also contribute to plasma cell differentiation[102,103]. BACH2 participates not only in memory B cell differentiation but also in the reactivation of memory B cells by secondary infection. For example, reduced expression of BACH2 in antigen-specific IgG1 memory B cells compared with IgM memory B cells could explain why IgG1 memory B cells have a greater propensity to differentiate into plasma cells[96].

Control of BACH2 expression in B and T cells

The ability of wild-type B and T cells to undergo plasma cell and effector cell differentiation, respectively, implies that the expression or function of BACH2 is attenuated in these subsets of cells. In $CD8^+$ T cells, *Bach2* transcription is progressively downregulated with repetitive antigen stimulation[82], and its expression level progressively decreases from naive T cells to central memory, effector memory and terminally differentiated effector T cells[9,83,104]. Decreased *Bach2* expression in effector T cells coincides with its reduced inhibitory effect on TCR-driven gene expression[9]. Decreased expression of *Bach2* mRNA in effector $CD8^+$ T cells is associated with decreased trimethylation of promoter-associated histone H3 at K4 (REF. 9). In $CD4^+$ T cells, the tumour suppressor menin promotes *Bach2* expression by acetylating promoter-associated histone H3 (REF. 75). Regulation of *Bach2* transcription also probably involves multiple regulatory regions outside the promoter. Putative enhancers bound by p300 are grouped with unusually high density in a ~200 kb region in the *Bach2* locus, forming a 'super-enhancer' in $CD4^+$ T_H1, T_H2 and T_H17 cells[105]. The functional significance of this is yet to be determined, but these findings suggest that *Bach2* is subject to complex gene regulation in $CD4^+$ T cells. The presence of large 5′ and 3′ untranslated regions of *Bach2* suggests that *Bach2* may be subject to post-transcriptional regulation. BACH2 is also sensitive to regulation by haem[23,106]. Haem promotes the degradation of BACH2 (REF. 23) and associates with an IDR of BACH2, which directly affects its DNA binding activity[24,26]. As a result, the repression of plasma cell differentiation by BACH2 is susceptible to inhibition by haem.

Emerging evidence indicates that the AKT–mechanistic target of rapamycin (mTOR) pathway regulates the expression and function of BACH2 at multiple levels. Stimulation-induced downregulation of

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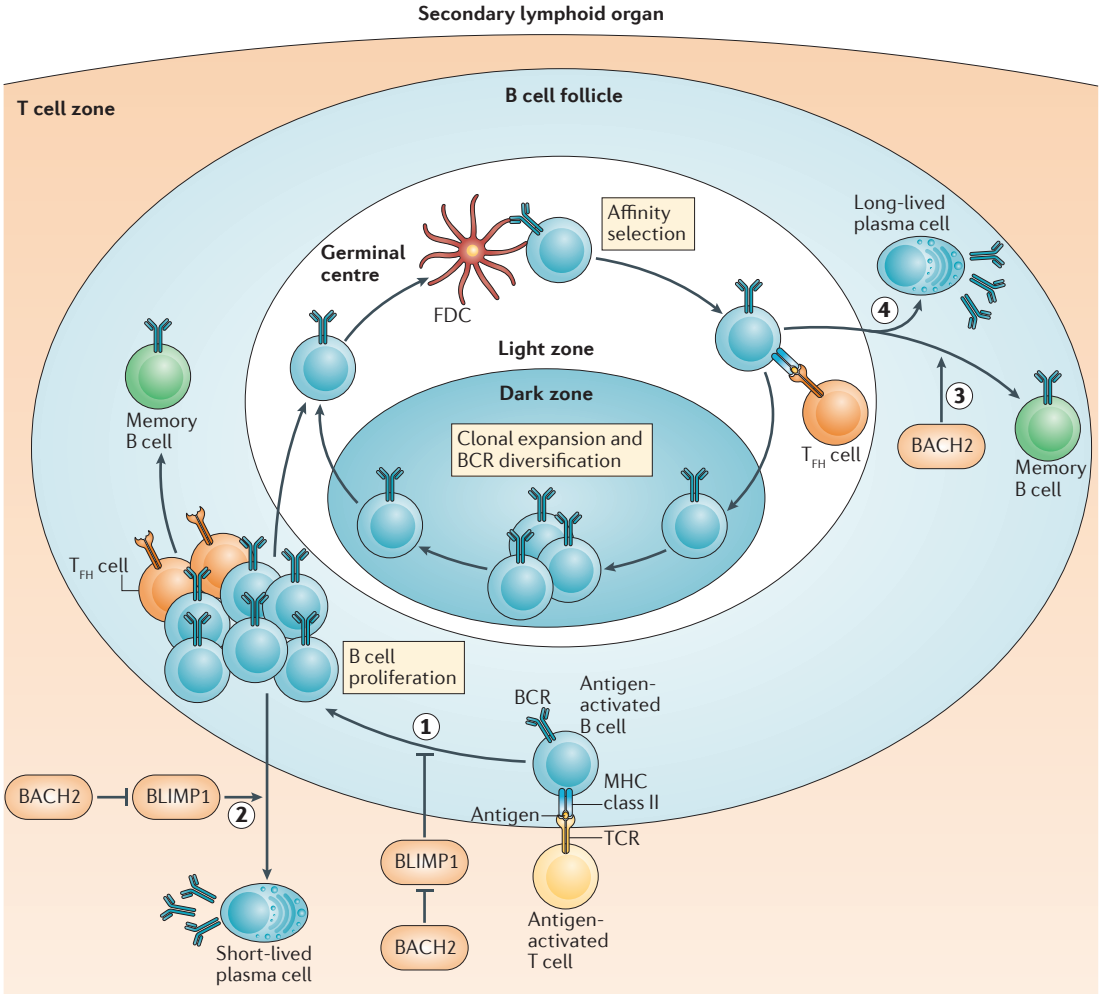

Figure 6 | **BACH2 promotes B cell proliferation and memory cell differentiation.** Antigen-activated B and T cells migrate towards T cell–B cell borders of lymphoid follicles, where they establish stable T cell–B cell interactions. This enables B cells to obtain signals from cognate T cells. Activated B cells and T cells migrate to the outer follicles, where B cells undergo proliferation (**1**). At this stage, BTB and CNC homology 2 (BACH2) is required for the proliferation of B cells in a manner dependent on suppression of BLIMP1 expression. Following proliferation, B cells either differentiate into short-lived plasma cells (**2**) or memory B cells, or alternatively enter the germinal centre. In the dark zone of the germinal centre, clonal expansion of antigen-specific B cells is accompanied by B cell receptor (BCR) diversification through somatic hypermutation. B cells that exit the cell cycle relocate to the light zone, where affinity selection takes place through interaction with immune complex-coated follicular dendritic cells (FDCs) and T follicular helper (T_{FH}) cells. Affinity-matured light zone germinal centre B cells can re-enter the dark zone, allowing cyclic proliferation and mutation. Alternatively, these cells exit the germinal centre, as either memory B cells (**3**) or long-lived plasma cells (**4**). BACH2 is required for the differentiation of germinal centre cells into memory B cells in a manner independent of the suppression of BLIMP1 expression. Modified from REF. 91, Macmillan Publishing. TCR, T cell receptor.

Bach2 mRNA expression, which is an instructive event in the differentiation of plasma cells, can be prevented by pharmacological inhibition of AKT and mTOR complex 1 (mTORC1)[96]. *Bach2* mRNA expression is also reduced in phosphatase and tensin homologue (*Pten*)-deficient primary B cells, which have hyperactive AKT–mTOR signalling[107]. These findings indicate that *Bach2* expression is regulated by the AKT–mTOR pathway. BACH2 is also subject to post-translational regulation by the AKT–mTOR pathway. Phosphoproteomic analysis of B cell extracts using mass spectrometry identified 72 highly likely phosphorylation sites in BACH2 (REF. 107). Among these sites, S509 and S535 phosphorylation were found to promote nuclear exclusion of BACH2 in pre-B cell lines[107]. Phosphorylation of the S520 residue of murine BACH2, and the corresponding S521 residue of human BACH2, also promotes nuclear exclusion and functional inactivation of BACH2 in $CD8^+$ T cells[9] and chronic myeloid leukaemia cells, respectively[108]. BACH2 phosphorylation in B cells and $CD8^+$ T cells is sensitive to pharmacological inhibition of AKT but insensitive to mTORC1 inhibition[9,107]. Together, these findings indicate that the AKT–mTOR pathway negatively regulates BACH2 at both the transcriptional

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level and the post-translational level. The functional relationship between and redundancy of these two modes of regulation in the context of endogenous immune responses has yet to be determined.

Conclusions

Common mechanistic themes underlying the diverse functions of BACH family transcription factors have emerged. BACH factors stabilize lineage commitment and promote cell-type-specific functions through the repression of alternative lineage programmes. Frequently, these alternative programmes are driven by transcriptional activators of the bZIP family. This is exemplified by the activities of BACH factors in both B and T cells, in which the restraint of effector programmes by BACH2 is a key mechanism underlying several of its important functions. Suppression of plasma cell programmes by BACH2 in B cells enables CSR, SHM and germinal centre differentiation to proceed in a subset of cells[92,93,95]. Analogously, within T cells, restraint of effector programmes enables BACH2 to stabilize the development of FOXP3$^+$ T_{reg} cells and promote the establishment of CD8$^+$ T cell memory, which has implications for immune homeostasis, tumour immunosuppression and immunological memory[9,60,61,109]. In these cases, plasma cell differentiation and CD4$^+$ and CD8$^+$ T cell effector differentiation are the alternative fates that are repressed by BACH2. In T cells, BACH2 functions in part through the restraint of gene expression programmes driven by transcriptional activators of the AP-1 family[8,9]. This functional reciprocity has parallels with the relationship between BACH1 and transcriptional activators of the NRF family in myeloid cells[27,28,44]. Further work is required to understand the complex relationships between activating and repressive members of the bZIP transcription factor family at their shared DNA binding sites, and the factors that determine their differential recruitment.

Another important observation relates to the function of BACH factors at enhancers. Most BACH2 binding sites are detected in intergenic and intronic regions of T cell genomes[8,9], and BACH2 is enriched at super-enhancer regions in the genomes of CD4$^+$ T cells[105] and multipotent haematopoietic progenitor cells[110]. Enhancers enable cell-type-specific and stage-dependent transcriptional outputs to be generated in response to extrinsic signals[111]. The availability of distinct enhancer repertoires in distinct cell types and in cells of different differentiation states enables similar signal transduction cascades to induce different transcriptional outcomes. Competitive interactions of BACH factors with transcriptional activators at enhancers provide a basis by which cell-type-specific and stage-dependent transcriptional outputs are further fine-tuned.

Finally, emerging evidence indicates the involvement of BACH factors in human immune function: genetic polymorphisms in the *BACH2* locus are associated with autoimmune and allergic disease risk[112–117], and expression levels of *BACH2* in tumours are associated with the response to immunotherapy[73]. The precise functions of BACH factors in human immunity are unclear, but they may, in the future, be valuable therapeutic targets for the manipulation of immune function in individuals with autoimmune diseases, allergies, chronic infections or cancer.

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