

# To be red or white: lineage commitment and maintenance of the hematopoietic system by the “inner myeloid”

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## ABSTRACT

Differentiation of hematopoietic stem and progenitor cells is tightly regulated depending on environmental changes in order to maintain homeostasis. Transcription factors direct the development of hematopoietic cells, such as GATA-1 for erythropoiesis and PU.1 for myelopoiesis. However, recent findings obtained from single-cell analyses raise the question of whether these transcription factors are “initiators” or just “executors” of differentiation, leaving the initiation of hematopoietic stem and progenitor cell differentiation (i.e. lineage commitment) unclear. While a stochastic process is likely involved in commitment, it cannot fully explain the homeostasis of hematopoiesis nor “on-demand” hematopoiesis in response to environmental changes. Transcription factors BACH1 and BACH2 may regulate both commitment and on-demand hematopoiesis because they control erythroid-myeloid and lymphoid-myeloid differentiation by repressing the myeloid program, and their activities are repressed in response to infectious and inflammatory conditions. We summarize possible mechanisms of lineage commitment of hematopoietic stem and progenitor cells suggested by recent findings and discuss the erythroid and lymphoid commitment of hematopoietic stem and progenitor cells, focusing on the gene regulatory network composed of genes encoding key transcription factors. Surprising similarity exists between commitment to erythroid and lymphoid lineages, including repression of the myeloid program by BACH factors. The suggested gene regulatory network of BACH factors sheds light on the myeloid-based model of hematopoiesis. This model will help to understand the tuning of hematopoiesis in higher eukaryotes in the steady-state condition as well as in emergency conditions, the evolutionary history of the system, aging and hematopoietic disorders.

## Introduction

Hematopoietic stem cells (HSC) possess the abilities of self-renewal and multi-lineage differentiation, including that to red and white blood cells and platelets (i.e., erythrocytes, megakaryocytes, innate immune cells and acquired immune cells).<sup>1</sup> Salient aspects of the hematopoietic system include its potential to produce huge numbers of cells with distinct functions throughout the life span of a human and its tunability, by which the output is balanced in response to environmental changes, such as from the steady state to an infectious state.

Erythrocytes are the most abundant cells in the human body, accounting for around 70% of the total cell number<sup>2</sup> and  $200 \times 10^9$  erythrocytes are produced daily.<sup>3</sup> Although the estimated number of white blood cells is much lower than that of erythrocytes,<sup>2</sup> the short life span of myeloid cells necessitates the production of a huge number of these cells as well. For instance, the circulating half-life of neutrophils is 6-8 h, and their estimated production rate is  $50-100 \times 10^9$  cells per day.<sup>4</sup> In line with this, label tracing analyses of HSC have revealed that the production rate of erythroid-myeloid progenitors is about 180 times higher than that of lymphoid progenitors in unperturbed hematopoiesis.<sup>5</sup> Thus, hematopoietic

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stem and progenitor cells (HSPC) have an exceptionally vigorous ability to produce huge numbers of cells constitutively. To maintain its homeostasis, the production pace of each mature cell lineage must be tightly regulated according to environmental changes (“on-demand” hematopoiesis).

Infection is one of the most common challenges facing hematopoiesis and evokes the induction of myelopoiesis as well as the suppression of erythropoiesis.<sup>6</sup> Induced myelopoiesis during an infection is an effective way of eliminating pathogens, whereas the repression of erythropoiesis may help by limiting the availability of nutritional iron supply to pathogens and/or red blood cells as a target of infection, such as in malaria infection.<sup>7</sup> However, infection and prolonged inflammation can cause anemia of inflammation, which is the second-most prevalent type of anemia after iron-deficiency anemia.<sup>8</sup>

As with infection, the activity of HSPC is also altered with aging and in various disease conditions. The production of erythrocytes is often reduced in elderly people, leading to anemia,<sup>9</sup> and acquired immunity becomes less effective with aging, which can result in increased susceptibility to infectious diseases and malignancy in the elderly.<sup>10,11</sup> In contrast, the production of myeloid cells often increases with aging.<sup>11,12</sup> This skewed trajectory selection of HSPC induced by aging might be related to the development of aging-related hematopoietic disorders, such as myelodysplastic syndrome (MDS). Although the molecular mechanisms by which the function and differentiation of HSPC are altered by aging are still largely unknown, emerging evidence suggests contributions of inflammation and/or inflammatory signaling to aging of HSPC.<sup>15</sup>

In order to facilitate the treatment of infection-associated and aging-associated diseases, it is important to understand the mechanisms by which the differentiation trajectory of HSPC and their commitment are defined at steady state and how these mechanisms are altered in inflammatory conditions. Although accumulating knowledge has shown that transcription factors (TF) play central roles in the differentiation of HSPC, the precise mechanisms underlying the initial lineage commitment and “on-demand” hematopoiesis are still unclear and cannot be wholly attributed to TF. Complicating matters further is the fact that HSPC are substantially heterogeneous and many appear to be already committed to certain differentiation fates.<sup>14,16</sup> It is, therefore, important to distinguish the roles of TF in initiating the commitment of uncommitted progenitors from that of their executive roles in the progression of differentiation toward a particular fate. Thus, the actual point of differentiation commitment may need to be reconsidered.

We recently demonstrated the roles of BTB and CNC homology (BACH) TF, BACH1 and BACH2 (BACH factors), in instructing erythroid-myeloid progenitors and lymphoid-myeloid progenitors to respond to environmental changes.<sup>17–19</sup> BACH factors form heterodimers with small Maf proteins to bind to the Maf recognition element (MARE), which contains an AP-1 site.<sup>20</sup> Importantly, AP-1 sites play central roles in hematopoietic cell immune reactions.<sup>20,21</sup> BACH1 plays important roles in the maturation of erythrocytes by balancing heme and globin proportions, especially in the condition of iron deficiency,<sup>22</sup> whereas BACH2 plays important roles in the development of plasma cells, memory B cells,

regulatory T cells and memory T cells.<sup>23–30</sup> These findings suggest ubiquitous roles for BACH factors in the maintenance of homeostasis in both steady-state and inflammatory-state hematopoiesis, as described below.

In this review, we summarize the latest findings concerning the mechanisms underlying lineage commitment of HSPC and potential questions to be addressed. We also discuss gene regulatory networks composed of genes encoding key TF which compete for lineage identities and downstream genes encoding effector molecules, focusing particularly on erythroid-myeloid and lymphoid-myeloid differentiation, two major points of commitment in HSPC differentiation. In addition, we review the roles of BACH factors in the myeloid-based model of hematopoiesis, which may provide a new concept of the fundamental mechanism in HSPC differentiation, and its meaning in an evolutionary perspective. We also discuss the diverse functions of BACH factors in mature hematopoietic cells as a strategy to cope with environmental changes through the maintenance of hematopoiesis. Finally, we describe how changes in lineage commitment can lead to diseases, such as anemia of inflammation and MDS.

## Lineage commitment of hematopoietic stem and progenitor cells

The multipotency of HSC has been demonstrated by single-cell transplantation into irradiated mice.<sup>31</sup> This led to vigorous investigations into how HSC differentiate into diverse lineages of cells with distinct functions. The isolation and characterization of progenitor cells led to the idea that HSC gradually and systematically lose multipotency, generating progenitor cells with limited differentiation trajectories, such as common myeloid progenitors (CMP),<sup>32</sup> which can generate myeloid cells and erythroid cells but not lymphoid cells. On the other hand, although all blood cells derive from a FLT3<sup>+</sup> multipotent progenitor stage,<sup>33</sup> lymphoid-primed multipotent progenitors (LMPP) preferentially differentiate into lymphoid cells and myeloid cells with a low differentiation potential to erythroid cells.<sup>34–36</sup> This led to the recognition that HSC eventually lose their ability to differentiate to erythroid or lymphoid cells, leaving erythroid-myeloid bifurcation and lymphoid-myeloid bifurcation as the two major subsequent points of branching.

Such subpopulations of progenitors have been defined based on the presence or absence of a limited number of cell surface markers, leaving the potential impurity of these subpopulations as a limitation. Indeed, recent comprehensive, single-cell transcriptomic analyses have shown that the known subpopulations of HSPC are composed of heterogeneous cells in terms of gene expression.<sup>14,16</sup> In addition, *in vitro* and *in vivo* single-cell differentiation analyses have shown that only a limited number of cells in progenitor cell populations can produce multilineage mature cells and that a majority of the cells in these populations are already committed to become unilineage mature cells.<sup>14,15</sup> Furthermore, an *in vivo* HSC chasing system using endogenous fluorescent tagging revealed that the differentiation trajectory of HSC is already oriented to specific lineage outputs by epigenetic memory.<sup>37</sup> These observations raise two possibilities: (i) HSPC can be further divided into subpopula-

tions representing pure differentiation bifurcation points; or (ii) lineage commitments occur only in HSC, whereas each progenitor population is a mixture of committed cells sharing the same cell surface markers at the time of isolation. Since single-cell differentiation analysis from CMP and LMPP showed that a minor part of these populations can produce multilineage mature cells,<sup>14,15</sup> there might be further subpopulations that represent actual bifurcation points of erythroid-myeloid or lymphoid-myeloid differentiation. However, whether or not these hypothetical subpopulations can be defined using additional cell surface markers remains unclear. Before addressing these two possibilities, we need to stop and consider the potential limitations of recent studies using single-cell analyses. A single-cell transcriptomic analysis is a ‘snapshot’ observation. Therefore, if a set of genes shows dynamic fluctuations in expression with coherent patterns in cells of a specific subpopulation, these cells might be considered heterogeneous. However, these subpopulations can be homogenous when time-dependent fluctuations are considered, like those observed in neural progenitors.<sup>58</sup> In addition, despite the importance of the microenvironment for HSPC biology,<sup>59</sup> an *ex vivo* single-cell transcriptomic analysis is devoid of anatomical information. Furthermore, single-cell *in vivo* or *in vitro* differentiation analyses can only examine the differentiation potential under stress and/or artificial conditions (i.e., cell sorting, culture and transplantation into irradiated mice), which can skew the original differentiation trajectory of progenitor cells,<sup>40</sup> possibly by altering activities of critical TF whose expression is thought to be maintained to some extent for multilineage priming,<sup>41</sup> a state in which multiple, conflicting lineage-affiliated genes can be induced or co-expressed. In other words, there is a chance that progenitor cells with unilineage output potential in perturbed conditions still possess multilineage output potential in an unperturbed condition. Recent studies using single-cell analyses may, therefore, lack information about the dynamics (time and three-dimensional information) of lineage commitment,<sup>42</sup> especially regarding unperturbed hematopoiesis. Potential effects of circadian rhythm in HSC differentiation might also have to be considered.<sup>43</sup> The analysis of entropy in gene expression within single cells<sup>44</sup> and the three-dimensional detection of transcriptomics<sup>45</sup> might be helpful. Remarkably, recent *in vivo* barcoding analyses give new support to the existence of a hierarchical development model in hematopoiesis.<sup>46,47</sup>

We must therefore reconsider the actual point at which lineage commitment occurs. An alternative approach to define such a point involves using the regulatory mechanisms of the differentiation of HSPC. To this end, the precise understanding of gene regulatory networks governed by TF may provide a dynamic view of lineage commitment.

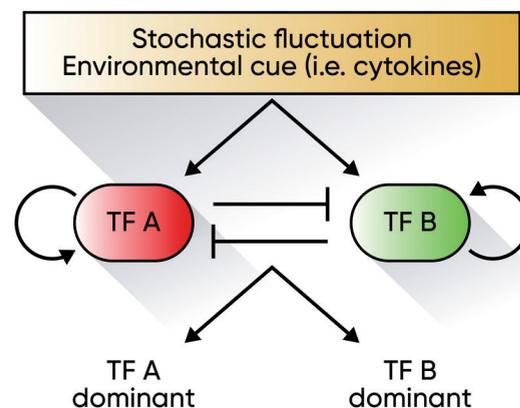
This leads us to the second point that should be considered: how are the differentiation trajectories shaped and restricted along the path of differentiation? Several models of lineage commitment have been proposed, showing that TF are critical to shaping and resolving the patterns of lineage-affiliated gene expression.<sup>48-50</sup> One model features a network of two TF, each promoting differentiation into a specific lineage. If the expression of these two TF is inhibited in a mutual manner and thus they induce their own expression, they can define two

cell types with distinct expression patterns of the two TF and thus their downstream target genes (Figure 1).<sup>48</sup> Machine-learning methods using single-cell transcriptomic data support the notion that gradual, stochastic changes in a few TF have a strong influence on the lineage commitment of progenitor cells.<sup>50</sup> Such a gene regulatory network may therefore dictate lineage commitment.

However, it has been unclear how one or the other of these TF are initially upregulated or downregulated upon lineage commitment. Stochastic fluctuation in these TF may be involved,<sup>48</sup> but the output of hematopoiesis should be dynamically tuned in response to diverse stressors, as HSPC produce huge numbers of mature cells daily in a fine balance, as noted above. This property of the hematopoietic system may not therefore be fully explained merely by the stochastic fluctuation of TF. The differentiation trajectory of HSPC must be tightly controlled by responding to environmental changes in order to maintain homeostasis. This means that environmental factors, including pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP),<sup>51</sup> may affect the cell-intrinsic TF of gene regulatory networks that control the differentiation trajectory. It is therefore important to understand how cell-intrinsic systems of TF are connected to extrinsic signals.

### The gene regulatory network for erythroid lineage commitment

Erythroid cells are derived from progenitor cells that possess the ability to differentiate into erythroid or myeloid cells.<sup>1,52</sup> CMP have long been considered to represent a bifurcation point of erythroid-myeloid differentiation.<sup>32</sup> However, single-cell analyses have challenged this notion. A single-cell RNA sequencing analysis of c-kit<sup>+</sup>Sca1<sup>+</sup> lineage<sup>+</sup> bone marrow cells revealed at least seven different subpopulations with lineage priming at the transcriptomic level.<sup>16</sup> Importantly, no subpopulations with multilineage priming were observed in that



**Figure 1. Stochastic model of lineage commitment.** Transcription factors (TF) A and TF B play important roles in determining cell differentiation. If these two TF activate themselves and work in a mutually exclusive manner, slight stochastic fluctuations that alter the ratio of TF A to B can affect cell fate.

study. In addition, a barcoded progenitor cell transplantation analysis revealed that the majority of CMP can differentiate into only erythroid or myeloid cells after transplantation.<sup>15</sup> Therefore, CMP are a highly heterogeneous population of progenitor cells, and the dominant populations in CMP are already committed to erythroid or myeloid differentiation. However, it should be noted that these findings were obtained from a “snapshot” analysis, which may have overlooked the plasticity of differentiation potential or gene expression patterns in CMP. Indeed, the introduction of specific TF (such as GATA-1 and DDIT3) into myeloid lineage progenitors can switch the lineage output to the erythroid lineage, suggesting the existence of plasticity under the control of TF in erythroid-myeloid progenitors.<sup>53,54</sup> This idea is not surprising when we consider the fact that TF often alter the epigenetic modifications for lineage commitment (e.g., via pioneer TF<sup>55</sup>) and that epigenetic changes *per se* are reversible.<sup>56,57</sup> Therefore, the observed subpopulations of CMP may show plasticity under physiological conditions, which can be masked during transplantation. In this context, it is still too early to conclude that CMP are heterogeneous populations of already committed progenitors. Further investigations combining single-cell chasing with the comprehensive measurement of epigenomes and transcriptomes in unperturbed conditions will be needed.

In the view of the gene regulatory networks at the erythroid-myeloid bifurcation, key TF, including the CCAAT-enhancer-binding protein (C/EBP) family,<sup>58,59</sup> PU.1<sup>60</sup> and GATA-1,<sup>61</sup> play essential roles in erythroid or myeloid differentiation, which might operate the erythroid-myeloid bifurcation at the level of CMP or multipotent progenitors. Given that GATA-1 and PU.1 show mutually exclusive expression patterns during erythroid and myeloid differentiation and repress each other and activate themselves, the gene regulatory network of *GATA1* and *SPI1* (encoding PU.1) may determine the bifurcation of myeloid and erythroid cells.<sup>48</sup> In this model, stochastic alterations in the ratio of GATA1 to PU.1 activity might initiate the differentiation. However, a recent study found that *GATA1* and *SPI1* are not co-expressed in CMP.<sup>62</sup> Upon erythroid differentiation, the expression of *GATA1* commences with a substantial lag after the cessation of *SPI1* expression. In contrast, upon myeloid differentiation, no progenitor cells showed a period with *GATA1* expression.<sup>62</sup> Thus, GATA-1 may not be the initiator of erythroid differentiation but just the executor of the erythroid development from progenitor cells whose erythroid commitment has already been defined by unknown factors. Recent single-cell proteomic analyses additionally revealed that the TF KLF1 and FLI1 play important roles in the bifurcation of erythroid and megakaryocytes.<sup>63</sup>

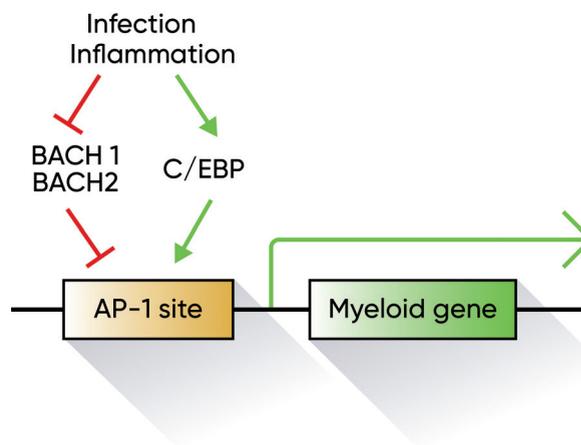
Since erythroid cells and myeloid cells are rigorously produced from progenitor cells every day, as described above, there should exist a mechanism to fine tune the differentiation trajectory shift of erythroid-myeloid common progenitor cells depending on the demand, which can vary with environmental changes. For instance, infections and inflammation induce myeloid differentiation and reduce erythroid differentiation, which can lead to anemia of inflammation. It has long been accepted that the major cause of this form of anemia is a disorder of iron utility for erythroid maturation caused by the

induction of hepcidin, which inhibits iron uptake and recycling.<sup>8</sup> However, since iron supplementation for the treatment of anemia of inflammation is still controversial<sup>8,64</sup> and infections as well as inflammation can induce a shift in the differentiation trajectory at the level of erythroid-myeloid progenitors,<sup>65</sup> there may be other factors that modulate the differentiation trajectory of progenitors, depending on environmental changes.

We recently reported that BACH factors are required for the efficient commitment of HSPC to an erythroid fate.<sup>19</sup> BACH factors inhibit the expression of *Cebpb*, the gene encoding the TF C/EBP $\beta$ , which plays an indispensable role in emergency myelopoiesis.<sup>59</sup> Importantly, BACH factors and C/EBP $\beta$  exert opposite effects on their downstream target genes: BACH factors repress a set of myeloid-affiliated genes, whereas C/EBP $\beta$  activates these genes at the same genomic loci. Since both BACH factors and the C/EBP family can bind to AP-1 motifs, the balance between repression and activation via the AP-1 motif appears to be critical for determining myeloid fate<sup>21,66</sup> (Figure 2). Since infectious stimuli repress the expression of BACH factors and induce C/EBP $\beta$  expression,<sup>18,19</sup> the gene regulatory network of these TF genes can fluctuate in response to environmental input between two states, which correspond to erythroid and myeloid fates.

### The gene regulatory network for lymphoid lineage commitment

Lymphoid cells are also derived from common progenitor cells that possess the ability to differentiate into lymphoid or myeloid cells. LMPP are now considered such common progenitors.<sup>1,34,52</sup> Similar to the single-cell transcriptomic observations in CMP, a single-cell analysis of LMPP also showed that LMPP are a heterogeneous population.<sup>14</sup> For instance, a single-cell *in vitro* differentiation assay showed that most LMPP were only able to differentiate into either myeloid or lymphoid cells.<sup>14</sup> Therefore, most LMPP may be cells whose differentia-



**Figure 2. Control of myeloid gene expression by BACH and C/EBP transcription factors in a state of infection.** The BACH and C/EBP transcription factors (TF) repress and activate, respectively, myeloid gene expression by binding the same genomic loci. Infection/inflammation-induced alteration of these TF can affect myeloid gene expression, depending on the environment.

tion commitment has already been decided. However, there may be pitfalls associated with these observations, similar to those regarding erythroid-myeloid bifurcation. The presence of myeloid-lymphoid progenitors is also supported by the findings of an analysis of lymphopoiesis in human embryos. This progenitor population first emerges as a myeloid progenitor and later acquires myeloid-lymphoid bipotential, co-expressing genes affiliated with the two lineages in single cells.<sup>67</sup>

A number of key TF have been identified as important factors for the development of lymphoid cells, including the C/EBP family, PU.1, E2A, IKAROS and FOXO1. An analysis combining RNA sequencing and chromatin immunoprecipitation sequencing has suggested the existence of gene regulatory networks that are important for lymphoid-myeloid bifurcation<sup>49</sup> (Figure 3). However, which component in the gene regulatory networks of these TF define lineage commitments and how the expression of the TF is altered in response to environmental changes remain unclear. The initiators of the lymphoid or myeloid lineage commitment also have yet to be clarified, and there may in fact be multiple entry points for commitment.

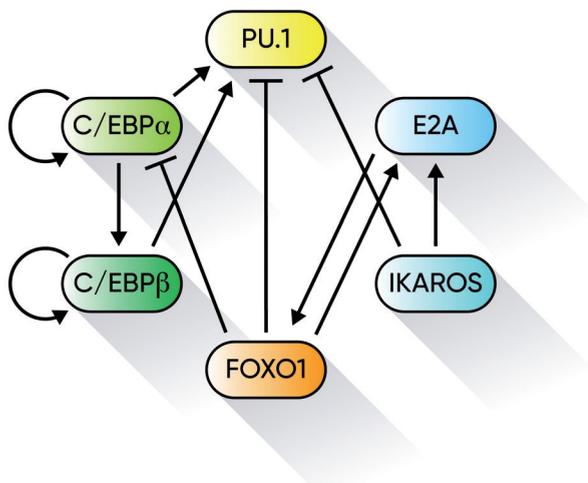
We previously reported that BACH factors are required for efficient commitment of multipotent progenitors and common lymphoid progenitors to the lymphoid fate.<sup>17,18</sup> BACH factors repress the expression of C/EBP, and C/EBP repress the expression of BACH factors. The gene regulatory networks of these TF therefore define lymphoid or myeloid lineage commitment depending on fluctuations of the expression of C/EBP and BACH TF (Figure 4). Since the expression of these TF is affected by environmental changes,<sup>18,19,59</sup> these TF may be initiators of lymphoid or myeloid lineage commitment responding to environmental changes. The development of fetal myeloid-lymphoid progenitors mentioned above<sup>67</sup> may reflect changes in the interplay between BACH factors and C/EBP. Since steady-state hematopoiesis and emer-

gency hematopoiesis are contrasting, it is still unclear to what extent the altered expression in response to extracellular signals would affect lineage commitment. Further understanding of these issues will help to clarify the mechanisms of lineage commitment in steady-state and emergency conditions. To this end, taking advantage of using TF reporter mice exposed or not to stress might be helpful to expand the TF-based analysis further. Identification of surrogate marker genes whose expression reports activity of particular TF will also be important.

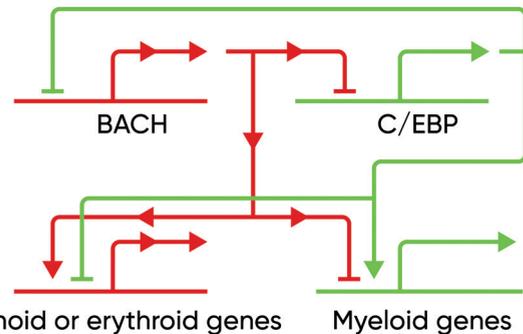
### Myeloid cells as the default and evolutionary prototype pathway of hematopoietic stem and progenitor cells

The lineage commitment of HSPC is the process in which these cells lose their multipotency. For erythroid cell differentiation, the progenitor cells first lose their capacity for lymphoid differentiation, resulting in erythroid-myeloid common progenitors,<sup>32,35</sup> at which point the decision of erythroid or myeloid lineage commitment is made. In contrast, for lymphoid cell differentiation, the progenitor cells first lose their capacity for erythroid differentiation, resulting in lymphoid-myeloid common progenitors,<sup>34,35</sup> at which point the decision of lymphoid or myeloid lineage commitment is made.

Interestingly, it has been reported that, even after lymphoid commitment, T-cell or B-cell progenitors retain the capacity to differentiate into myeloid cells.<sup>68-70</sup> Indeed, myeloid differentiation potential might remain until just before terminal differentiation. Results from studies using five blood-lineage marking, which is a precise method of detecting erythroid cells and platelets in addition to myeloid, B and T cells after transplantation, also support the notion that myeloid differentiation potential is retained after losing either erythroid or lymphoid differentiation potential.<sup>71</sup> In addition, at least some platelets are derived from HSC possessing myeloid lineage potential.<sup>72,73</sup> Myeloid cell differentiation might, therefore, be a default and/or prototypical pathway of



**Figure 3. Gene regulatory networks controlling lymphoid cell differentiation.** Several factors have been identified as important regulators of lymphoid cell differentiation commitment.<sup>49</sup> Each factor works as an activator and/or repressor of other factors forming complex gene regulatory networks, suggesting the existence of a precise mechanism underlying lymphoid cell differentiation. However, how the activities of these factors are controlled at the initial point of lineage commitment remains unclear.



**Figure 4. Gene regulatory networks of BACH and C/EBP transcription factors for myeloid and non-myeloid gene expression.** The transcription factor (TF) BACH represses C/EBP and myeloid genes and induces lymphoid/erythroid genes. In contrast, the TF C/EBP represses BACH and lymphoid/erythroid genes and induces myeloid genes. Therefore, both stochastic fluctuation and environment-derived changes in the expression of BACH and C/EBP can induce differentiation commitment in progenitor cells.

HSPC, originally described as the “myeloid-based model”,<sup>74</sup> and repression of the myeloid differentiation program appears important for lineage commitment in HSPC. In line with this, recent reports suggest the importance of myeloid-biased HSC in emergency myelopoiesis.<sup>75-77</sup> Further analysis is still needed to clarify how the gene regulatory networks are altered in myeloid-biased HSC.

In addition to the repression of the myeloid program during progenitor cell differentiation, re-activation of the myeloid program is observed in some mature hematopoietic cells. During maturation of erythroid and lymphoid cells, the function and expression of BACH factors are repressed, which can induce part of the myeloid program. For instance, *Prdm1* (encoding TF BLIMP-1) is a repressed target of BACH2, and its repression is necessary for the proper development of B cells.<sup>26</sup> BLIMP-1 *per se* is necessary for the proper development of plasma cells and T cells.<sup>78</sup> Since BLIMP-1 is important for myeloid cell development as well,<sup>79</sup> BLIMP-1 can be considered as a part of the myeloid program deployed during plasma-cell and T-cell development. To support this notion, some of the myeloid genes are expressed in plasma cells.<sup>17</sup> From the perspective of erythropoiesis, the expression of *Hmox1* (encoding heme oxygenase-1) is induced to avoid the toxic activity of free heme during erythroblast maturation.<sup>80</sup> On the other hand, heme oxygenase-1 is also important for the proper function of myeloid cells.<sup>81</sup> Therefore, heme oxygenase-1 can be considered as a part of the myeloid program deployed during the development of erythroid cells. Both mature myeloid cells and non-myeloid cells (erythroid and lymphoid cells) must cope with conditions of stress (such as oxidative stress during oxygen transportation or at the site of inflammation). We therefore assume that mature hematopoietic cells may reactivate a part of the myeloid program, such as heme oxygenase-1, to protect themselves from stresses, irrespective of their lineages. The myeloid program, which is temporarily repressed upon lineage commitment, is thus referred to as the “inner myeloid”,<sup>82</sup> because part of it can be re-activated in mature cells. Given these findings, we propose “an extended myeloid-based model” of hematopoiesis, which posits that the myeloid program possesses important roles not only in hematopoietic cell differentiation but also in mature cell function.

The extended myeloid-based model with the “inner myeloid” is well understandable when the history of biological evolution is considered. Lower organisms, such as insects, possess phagocytic cells but lack erythroid and lymphoid cells.<sup>83</sup> A human-like HSC system was recently found in the chordate *Botryllus schlosseri*, with stem cells generating solely cells of myeloid lineage, such as phagocytic cells and granulocytes.<sup>84</sup> It should be noted that a BACH-like TF is present in chordates and vertebrates<sup>85</sup> but not in lower organisms. The prototype BACH TF may restrict myeloid differentiation of HSC. Since erythroid cells and lymphoid cells arose in the hematopoietic system during the evolution of higher organisms, repressing the myeloid program in progenitor cells (“inner myeloid”) might be necessary to make non-myeloid cells (erythroid and lymphoid cells). The findings regarding the function of BACH factors as repressors of the “inner myeloid” may constitute the molecular foundation of the myeloid-based model of

hematopoiesis and lineage commitment. The hematopoietic system in higher eukaryotes is therefore evidence of our ancient history, just like our other body systems.<sup>86</sup>

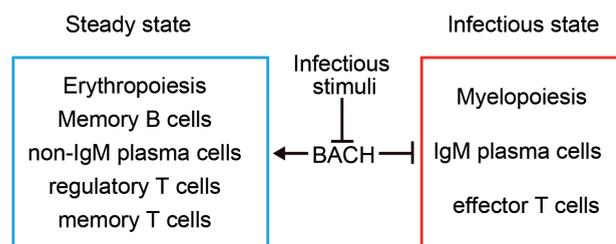
### Fortifying roles of BACH factors in blood homeostasis

BACH factors play not only repressive roles in the myeloid program in progenitor cells but also several indispensable roles in the operation of the hematopoietic system. For instance, BACH1 works as a balancer of globins and heme during erythroid cell maturation.<sup>22</sup> BACH2 is required for the development of non-IgM type plasma cells, memory B cells, regulatory T cells and memory T cells.<sup>23-30,87,88</sup> and therefore works as a regulator of lymphocyte effector *versus* non-effector differentiation. Remarkably, these functions of BACH2 in lymphoid cells might be explained by its binding to the AP-1 site as a transcription repressor, which is in contrast to the other TF (Fos, Jun, etc.) targeting the AP-1 site, many of which work as transcription activators.<sup>89</sup>

These functions of BACH factors in erythroid and lymphoid cells can be interpreted as indicative of their role as ‘fortifying factors’, since they shape steady-state hematopoiesis to prepare for infection at multiple points, as described below. With regard to erythropoiesis, the hemoglobin concentration in human blood is kept around 14 g/dL in the steady state whereas, in general, a hemoglobin concentration <7 g/dL is life-threatening. There is therefore sufficient capacity for erythropoiesis to endure emergency conditions. For instance, progenitor cell differentiation can be shifted toward myelopoiesis, thus promoting the innate immune defense at the expense of erythropoiesis during a state of infection. This means that BACH factors support erythropoiesis by suppressing myelopoiesis in the steady state, fortifying the system for infection. With regard to the B-cell response, IgM-secreting plasma cells work as the first line of defense against pathogens, whereas non-IgM-type plasma cells and memory B cells are produced at a later phase or after the infection as a more effective second-line defense.<sup>90</sup> With regard to the T-cell response, effector T cells provide the first line of defense against pathogens whereas regulatory T cells and memory T cells work to repress an excess immune response and/or to return the state to the steady condition in preparation for the next infection.<sup>91</sup> These responses may be coordinated by the expression of BACH factors. When their expression is reduced in response to infection, IgM-secreting plasma cells and effector T cells are preferentially generated. Conversely, resumption of the expression of BACH factors leads to the generation of non-IgM plasma cells, memory B cells, regulatory T cells and memory T cells. Therefore, BACH factors are required to fortify the hematopoietic system as a whole, in preparation for future infections (Figure 5).

### Hematologic disorders as failures of BACH gene regulatory networks

The gene regulatory networks of HSPC may explain why pathological alterations in one lineage often accom-



**Figure 5. The fortifying role of BACH for the next emergency.** BACH factors support erythropoiesis and repress myelopoiesis in steady-state conditions. BACH2 in particular supports the development of memory B cells, non-IgM plasma cells, regulatory T cells and memory T cells in the steady state while repressing the development of IgM plasma cells and effector T cells. Since BACH factors support the development of the cells needed for the suppression of a previous emergency reaction and the preparation for the next emergency, BACH factors can be considered “fortifying factors”.

pany changes in other lineages in the opposite direction. Infection and inflammation cause anemia of inflammation, which is frequently observed in chronic infections and autoimmune diseases.<sup>8</sup> Inflammatory cytokines, such as interleukin-6, induce the expression of hepcidin, resulting in the inhibition of ferroportin.<sup>92</sup> This regulatory axis is the mechanism by which the iron supply for erythroblast maturation is limited, resulting in anemia.<sup>8</sup> However, this mechanism may not explain how the differentiation trajectory is modulated at the erythroid-myeloid bifurcation point during an infection and in inflammatory conditions.<sup>6</sup> In those circumstances, the expression of BACH factors is repressed in HSPC,<sup>19,93</sup> leading to increased myelopoiesis at the expense of erythropoiesis. Therefore, the reduced activity of BACH factors might be a novel mechanism underlying anemia of inflammation.

MDS is a major hematopoietic malignancy and is caused by a clonal disorder in HSC.<sup>94</sup> The phenotypic features of MDS, such as anemia, autoimmune reactions and transformation into acute myeloid leukemia, may also be attributed to alterations in the state of gene regulatory networks. The expression of *BACH2* is repressed in MDS.<sup>19</sup> Since the loss of *BACH2* is expected to induce anemia, an inflammatory reaction and myeloid skewing of progenitors, the repression of *BACH2* that has been observed in MDS patients might be one of the causes of the characteristic symptoms of MDS. A recent genome-wide analysis showed that MDS clones frequently have mutations in epigenetic modifiers and splicing factors,<sup>95</sup> suggesting that such genetic alterations may lead to a reduction in *BACH2* expression. *BACH2* repression is also observed in lymphocytes on aging, with *PRDM1*

induction in humans.<sup>96</sup> Since the loss of *BACH2* causes autoimmune-like disorders,<sup>28</sup> *BACH2* repression (and the induction of the “inner myeloid”) during aging may be one of the causes of aging-related inflammation. Interestingly, HSC in mice become restricted to a myeloid fate upon aging.<sup>97</sup> This may be due to a reduction in *BACH2* expression. Moreover, mutations of epigenetic modifiers, such as those observed in clonal hematopoiesis with aging,<sup>98</sup> may cause dysregulation of the repression of the “inner myeloid”, resulting in myeloid skewing and inflammation. If this is the case, aging-related dysregulation of the “inner myeloid” is part of a vicious circle since inflammation *per se* can cause DNA mutations.<sup>99</sup> *BACH2* haploinsufficiency in humans has been reported to cause *BACH2*-related immunodeficiency and autoimmunity (BRIDA),<sup>100</sup> a finding that may further support these possibilities. In contrast, *BACH2* overexpression in progenitor cells induces erythropoiesis by repressing myelopoiesis.<sup>19</sup> Therefore, BACH factors might be new therapeutic targets of refractory anemia induced by inflammation and MDS. Further investigations will be needed in order to determine whether or not *BACH2* re-activation (or “inner myeloid” repression) in aged HSC can rescue the phenotype related to aging.

## Conclusions

In this review, we have highlighted recent findings concerning the differentiation of HSPC and their limitations. Novel findings from single-cell analyses suggest the need to reconsider the canonical hierarchical differentiation model of the hematopoietic system. However, we should also consider the limitations associated with these single-cell analyses, as discussed above. The myeloid-based model involving the gene regulatory networks of BACH factors may provide a further molecular basis for understanding lineage commitment, evolutionary perspectives and pathological processes of the hematopoietic system. Understanding the roles of BACH factors as repressors of the “inner myeloid” and “fortifying factors” in preparation for future emergency situations will help us to develop a more comprehensive model of the hematopoietic system.

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