Manipulating hematopoietic stem cell amplification with Wnt

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The mechanisms that regulate self-renewal of hematopoietic stem cells have remained elusive. Recent papers in *Nature* point to an important role of the Wnt–β-catenin signaling pathway in promoting this process.

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Fifty years ago, the discovery that a deficient blood-forming system can be permanently rescued and replaced by intravenously injected bone marrow cells launched a new era of stem cell biology and regenerative medicine. Ever since, the dream has been to characterize the cells responsible and define the molecular pathways that regulate their turnover and differentiation in the hope of developing new methods to direct these processes both *in vitro* and *in vivo*. Systematic analysis of the long-term multilineage reconstituting abilities of different subsets of bone marrow cells has gone a long way toward addressing the first goal and, in the mouse, such functionally defined hematopoietic stem cells (HSCs) can now be routinely isolated at purities of 10–20%. The use of such HSC-enriched populations has allowed extensive information to be obtained about the transcriptome of these cells[1,2] and their short-term responses *in vitro* to different combinations and concentrations of 'hematopoietic' growth factors[3–6]. However, thus far it has not been possible to translate these findings into a strategy that will produce a net amplification of HSC numbers *in vitro* of more than a few fold.

A pair of studies published recently in *Nature*[7,8] now provide evidence that Wnt activation of the β-catenin signaling pathway may be the magic touch required. The authors of these studies used a variety of complementary approaches to develop this concept. Their *in vitro* studies included the forced overexpression in an HSC-enriched population of an activated form of β-catenin, and exposure of these cells to a purified source of bioactive Wnt3A. In addition, the authors went on to show that the β-catenin signaling pathway is activated upon stimulation of these cells by hematopoietic growth factors, suggesting that these induce an autocrine Wnt mechanism. Experimental support for a role of Wnt–β-catenin signaling in the control of HSC self-renewal *in vivo* was provided by the demonstration that HSC-enriched cells from repopulated hosts had LEF and TCF activity (LEF and TCF being downstream transcription factors activated by the Wnt–β-catenin pathway) and that HSC activity was abrogated in recipients of cells transduced with Axin (Axin being an antagonist of β-catenin signaling).

In trying to connect these observations to the biological responses that HSCs must regulate, four important outcomes need to be considered: life versus death, proliferation versus quiescence, self-renewal versus differentiation, and maintenance versus loss of marrow homing activity. In theory, each pair might be controlled by completely distinct signaling pathways, without any overlap or intersection. However, available evidence already suggests that the same extrinsic cues may influence each of these responses, although they may have different sensitivities to the intensity of receptor activation initially obtained or whether other receptors are coactivated (for example, ref. 6). In this regard, the observation by Reya *et al.* of activated Wnt signaling in HSC-enriched populations, even when these were stimulated *in vitro* by standard growth factors in the absence of exogenous Wnt addition, is of interest because these findings would support a common pathway promoting HSC self-renewal divisions.

However, it is also important to emphasize the potential hazards in making retrospective independent assignments of survival, mitogenic and self-renewal responses to input HSCs if these were not predominant in the original cell population manipulated. It has been widely believed that operational purities of quiescent HSCs greater than 10–20% may not be achievable because of inefficiencies in their ability to home successfully to appropriate niches within the bone marrow after intravenous injection, as recently documented[9]. However, this assumption is now subject to question, because 40–50% pure HSC populations have recently been reported (Uchida, N., Dykstra, B., Lyons, K. J., Leung, F.Y.K. & Eaves, C.J., manuscript submitted)[10]. Such issues are relevant to the calculations of HSC amplification undertaken by Reya *et al.*[7] They are also pertinent to the

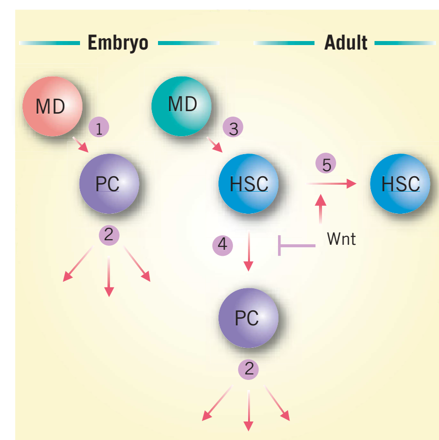

Figure 1 A simplified model of HSC control. Early in embryogenesis, cells with pluripotent hematopoietic differentiation potential arise (step 1) from mesodermal precursors (MDs). These pluripotent cells (PCs) proliferate and undergo lineage restriction before generating different types of mature blood cell progeny (step 2). However, these cells do not have long-term self-maintaining ability and do not reconstitute irradiated mice. At later stages, mesodermal precursors generate a different type of cell with pluripotent hematopoietic differentiation potential (step 3). These cells are referred to as hematopoietic stem cells (HSCs) because they now also have a mechanism to block expression of their acquired competence for multilineage hematopoietic differentiation. According to the external signals they receive, these cells can thus either give rise directly to PCs (step 4), which then terminally differentiate, or they can divide many times without beginning to differentiate (step 5). A role of the Wnt–β-catenin pathway may be to activate or maintain the expression of genes that serve as a master go–no-go switch allowing differentiation to proceed or not.

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data of Willert *et al.*[8] indicating that Wnt3 exposure increased the frequency of clones with HSC activity as compared to the frequency of HSCs in the starting population. It might thus be speculated that reinstatement of sufficient levels of activated β-catenin conferred HSC status again on later types of hematopoietic cells that were still pluripotent but already molecularly destined to complete the changes that establish lineage restriction within a few cell cycles. An intermediate population of pluripotent hematopoietic cells has been well recognized for many years, and these cells are known to differ from HSCs in terms of their phenotype and regulation as well as their engrafting durability.

A model of HSC regulation might be envisaged in which the HSC state includes the acquisition of competence to differentiate into all of the blood cell lineages as well as a mechanism to block the activation of events that initiate these differentiation processes (**Fig. 1**). It is interesting to note that, during embryogenesis, the first hematopoietic cells to appear arise directly from mesodermal precursors without passing through a state that would meet the functional definition of a transplantable HSC with self-renewal activity. In fact, such cells develop relatively late and become detectable only after pluripotent hematopoietic cells can already be identified. These findings are consistent with the concept that HSC development represents a molecularly distinct process requiring the activation of a mechanism for blocking the expression of a pre-established differentiation potential during successive cell cycles—a property that would then be detected functionally as self-renewal.

A number of transcription factors thought to be involved in the initial establishment of hematopoietic competence and in the final control of lineage programming have been defined. Transcription factors that might have comparable roles in regulating HSC self-renewal are newer to our thinking. As recognized in the study by Reya *et al.*, however, enhanced expression of *Hoxb4*, previously shown to characterize HSC-enriched populations, and the profound ability of forced overexpression of *Hoxb4* to stimulate HSC amplification *in vitro* and *in vivo*[11–13] strongly point to this gene as a primary candidate. It was therefore provocative to observe that the increased HSC activity resulting from forced overexpression of activated β-catenin in HSC-enriched populations was paralleled by a specific enhancement in *Hoxb4* expression.

Identification of a new method for isolating bioactive Wnt proteins and inducing downstream activation of the Wnt–β-catenin pathway in HSCs raises many exciting possibilities for future exploitation in the genetic manipulation and transplantation of patient HSC populations. At the same time, the association of Wnt1 overexpression with human leukemia suggests caution in how these are pursued and highlights the need for a better understanding of the fine line between normal and neoplastic stem cell behavior.

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Bcl11: sibling rivalry in lymphoid development

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Lymphocyte development is a complex process that involves the coordinated action of many transcription factors. The puzzle of B cell and T cell development gains an additional clue with the discovery of two critical factors.

Kruppel-like transcription factors[1], such as the Ikaros family of proteins and the lung Kruppel-like transcription factor, are essential for lymphoid development[2] and T cell homeostasis[3]. Two Kruppel-like transcription factors, Bcl11a and Bcl11b, have now been shown to be required in lymphoid development based on the phenotype that results from knockout of the corresponding genes, as described by Liu *et al.*[4] and Wakabayashi *et al.*[5], respectively, in this issue of *Nature Immunology*. The block in lymphoid development is marked in these mice and introduces two new key players and a quest to find out how they work.

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Bcl11a and Bcl11b are similar in sequence and physical properties. Both proteins can act as transcriptional repressors in transient transfection assays and both bind the same consensus sequence and repress transcription of a reporter gene[6]. Both Bcl11a and Bcl11b also bind to a type of orphan nuclear receptor (COUP-TF1) that itself represses transcription. But transcriptional repression may not be their key function, because other C_2H_2 zinc finger proteins, like Ikaros and YY1, can also function as transcriptional activators in some promoters. Mice in which either Bcl11a or Bcl11b has been knocked out die shortly after birth of unknown causes, and both types of knockout mice have defects in early lymphoid development[4,5]. Despite the structural similarity of Bcl11a and Bcl11b, however, the lymphoid defects that result from knocking them out are distinct (**Fig. 1**). Whereas Bcl11a is required for B cell development, Bcl11b is required for αβ cell development.

Bcl11a was first discovered in the mouse as a site of retroviral integration and termed Evi9. It was activated by viral integration, it induced myeloid leukemias and it was shown to transform NIH 3T3 cells[7,8]. The mouse gene was also independently identified as a protein that interacted with the chicken ovalbumin upstream promoter