binding affinity toward biotin was decreased by roughly 11 orders of magnitude but which retained its tetrameric structure. Mixing the denatured mutant monomer with denatured wild-type monomer in a 3:1 ratio, followed by refolding and purification, yielded a stable tetramer that was composed of three subunits of mutant and one subunit of wild-type streptavidin (**Fig. 1a**). Notably, this chimeric streptavidin had a single functional biotin-binding subunit that had an affinity for biotin almost identical to that of the wild type.

Although there is still the problem of introducing bulk when it binds to biotinylated proteins, monovalent streptavidin avoids the danger of unwanted cross-linking (**Fig 1b**). To demonstrate the advantage of monovalent streptavidin over the wild-type tetravalent protein, the authors examined the labeling of the postsynaptic adhesion protein neuroligin-1 on the surface of dissociated hippocampal neurons. In these experiments, they expressed neuroligin-1 as a fusion protein with an acceptor peptide and site-specifically biotinylated it. Notably, adding monovalent streptavidin allowed stable labeling of the protein without inducing substantial cross-linking of the receptor, whereas the addition of wild-type streptavidin lead to clustering of neuroligin-1 and seemingly disrupted the formation of presynaptic contacts.

Which research areas will benefit the most from the use of monovalent streptavidin? Concerning the labeling of cell-surface proteins, it should be mentioned that alternative approaches now exist for the specific labeling of recombinant cell-surface proteins that allow a specific and covalent labeling with diverse synthetic probes[4,5]. These methods avoid both the potential cross-linking of cell-surface proteins and minimize the addition of extra bulk. Another aspect of the work by Howarth *et al.*, however, could turn out to be quite interesting for the research on cell surface proteins: refolding mixtures of mutant and wild-type streptavidins not only yields monovalent tetramer but inevitably also di- and trivalent tetrameric streptavidins. These streptavidins with different valencies might then be used for defined cross-linking of receptor proteins on cell surfaces.

Similar applications might be found in bottom-up nanotechnology, where tetravalent streptavidin has been used in (four-way) junctions to build up multimolecular assemblies. The availability of mono-, di- and trivalent streptavidin could, therefore, enlarge the toolkit of the nanotechnologist. Furthermore, another important field of use of monovalent streptavidin might be the immobilization of biotinylated proteins on biosensors such as the chips used for surface plasmon resonance experiments. This cross-linking–free measurement of affinities and binding constants should be superior to measurements based on wild-type streptavidin. In conclusion, the existing plentitude of applications of the streptavidin-biotin interaction provides an enormous playground for streptavidins with reduced but defined valencies.

1. Howarth, M. *et al. Nat. Methods* **3**, 267–273 (2006).
2. Howarth, M., Takao, K., Hayashi, Y. & Ting, A.Y. *Proc. Natl. Acad. Sci. USA* **102**, 7583–7588 (2005).
3. Campbell, R.E. *et al. Proc. Natl. Acad. Sci. USA* **99**, 7877–7882 (2002).
4. George, N., Pick, H., Vogel, H., Johnsson, N. & Johnsson, K. *J. Am. Chem. Soc.* **126**, 8896–8897 (2004).
5. Yin, J. *et al. Proc. Natl. Acad. Sci. USA* **102**, 15815–15820 (2005).

Myeloid progenitors on demand

Dan A Liebermann & Barbara Hoffman

A new approach to generating large quantities of myeloid progenitor cells *ex vivo* will facilitate detailed studies of normal white blood cell differentiation and of abnormalities leading to blood disorders such as leukemia.

Hematopoiesis, the formation of blood cells, is a widely studied and informative example of a cellular differentiation process. A hierarchy of multipotent and more-restricted hematopoietic progenitor cells in the bone marrow proliferate and terminally differentiate along multiple, distinct cell lineages, including the myeloid lineage that gives rise to white blood cells[1,2]. In this issue of *Nature Methods*, Wang *et al.*[3] describe an interesting approach for generating large quantities of intermediate progenitor cells *ex vivo* that retain proliferation and self-renewal capacity, but are committed to the myeloid lineage and can be induced to terminally differentiate.

Myeloid progenitors differentiate into peripheral blood leukocytes: lymphocytes, eosinophils, basophils, neutrophils and monocytes. Monocytes in turn differentiate into macrophages, which together with neutrophils constitute the most important phagocytes essential for normal effective host defense in states of acute inflammation[4]. Unfortunately, access to normal myeloid progenitors at homogenous stages of development and in quantities sufficient for biochemical analysis is not generally feasible, so a large body of information about myeloid differentiation has been obtained by studies of leukemia cells arrested at various developmental stages[5]. As leukemia cells have aberrant features of normal blood cell differentiation, findings gleaned from them need to be corroborated by using normal bone marrow–derived progenitors.

The cultivation of large amounts of myeloid progenitors will allow a more detailed analysis of steps involved in their normal differentiation. Furthermore, these cells represent an interesting model to study abnormalities of myeloid development that lead to myeloidysplasia, dysplasia and leukemia[5]. Finally, they will constitute a renewable source of differentiated cells for functional studies at the cellular, biochemical and molecular levels. Elucidating the molecular mechanisms by which, for example, phagocytes respond to chemokines and cytokines, activate proinflammatory pathways, modulate lymphocyte expansion and function, and affect microbial killing, is of utmost importance.

To generate myeloid progenitors *ex vivo*, Wang *et al.* worked with the so-called Hox homeodomain transcription factors that are

Dan A. Liebermann and Barbara Hoffman are at the Fels Institute of Cancer Research & Molecular Biology, and the Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania, USA.
e-mail: lieberma@temple.edu

known to promote the expansion of hematopoietic progenitors[6]. Enforced ectopic expression of Hoxb8 or Hoxa9 is known to block differentiation of myeloid progenitors[7,8]. Taking advantage of this knowledge, Wang *et al.* generated retroviral vectors that encode estrogen-dependent forms of Hoxb8 or Hoxa9, termed ER-Hoxb8 and ER-Hoxa9, respectively. Infection of bone marrow cells with these vectors led to generation of myeloid progenitor cells that can be expanded *in vitro* in the presence of hematopoietic cytokines such as stem cell factor (SCF) or granulocyte-macrophage colony stimulating factor (GM-CSF) and estrogen. Removal of estrogen from the culture medium, thereby inactivating the ectopically expressed gene encoding Hox, resulted in synchronous differentiation of SCF-dependent Hoxb8 progenitors into neutrophils and GM-CSF dependent Hoxb8 progenitors into macrophages. ER-Hoxa9 progenitors exhibited biphenotypic neutrophil and macrophage differentiation regardless of the cytokine that was used during their derivation (**Fig. 1**).

The authors show that this *ex vivo* differentiation system models the normal transcriptional mechanisms that take place during differentiation of progenitors into neutrophils and macrophages. For example, they observed downregulation of promyelocytic genes such as neutrophil elastase and upregulation of the terminal differentiation genes such as lactoferrin. Such processes are difficult to reproduce by treating immortalized progenitors with cytokines and chemicals. Previously investigators have used a dominant-negative retinoic acid receptor to immortalize normal bone marrow progenitors to generate progenitor cell lines, termed MPRO EML, which can be induced to differentiate into mature myeloid cells by adding excess concentrations of all-*trans* retinoic acid to release the cells from the differentiation block imparted by the a dominant-negative retinoic acid receptor[9]. The advantage of the current approach over these previous systems is that the cells undergo synchronous rapid differentiation that is driven by physiological cytokines.

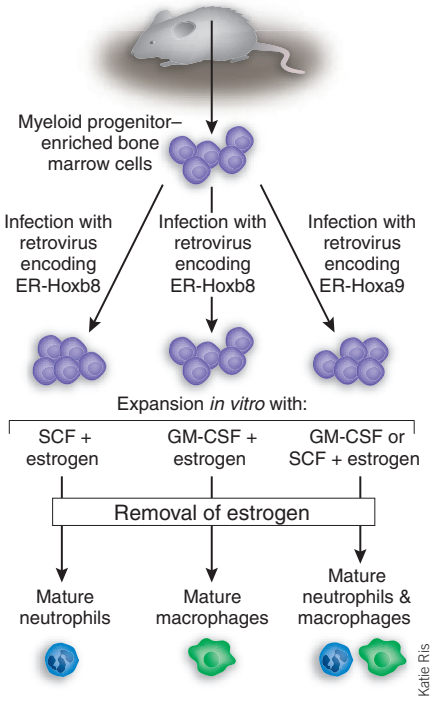

Figure 1 | A schematic of the immortalization and differentiation of myeloid progenitor cells.

Moreover, in the mature phagocytes, pattern-recognition inflammation-specific receptors were upregulated during differentiation and triggered established genetic programs of inflammation, underscoring that this differentiation protocol followed the steps of endogenous differentiation.

In addition, Wang *et al.* demonstrate that progenitors can be immortalized not only from adult marrow but also from day-13 fetal liver that does not yield enough progenitor cells for differentiation studies by biochemical methods. This permitted the derivation of Traf3$^{-/-}$ knockout progenitor cell lines from embryonic lethal phenotypes, whose normal innate immune functions could be rescued by restoration of Traf3 expression, underscoring the pivotal role of this gene in the process.

There are several interesting applications of the ER-Hox approach to expand and study development and function of myeloid progenitors. First, it can be used to expand multipotent bone marrow progenitors to study differentiation of other myeloid lineages, lymphoid lineages or even epithelial stem cell lineages, depending on the exposure to different growth factors. Second, as demonstrated in this study, this approach can be used to generate conditionally immortalized myeloid progenitors from knockout progenitors including those from mice with embryonic lethal phenotypes, which will facilitate dissection of the function of genes involved in orchestrating hematopoietic cell development and function. Third, it should allow investigation into how Hox oncoproteins block differentiation in myeloid leukemia. Fourth, it should facilitate the study of the transcriptional mechanisms that dictate lineage-specific myeloid differentiation. Fifth, it should aid identification of new genes controlling phagocyte innate immune functions.

In conclusion, the ER-Hox approach provides a useful tool to study differentiation, innate immune function and inflammatory functions of neutrophils and macrophages. Nevertheless, it must be kept in mind that ultimately knowledge acquired using this *ex vivo* system must be tested with primary hematopoietic cells *in vivo* and *in vitro*. We hope that the use of ER-Hox progenitors will pave the way for such demanding endeavors.

1. Liebermann, D.A. & Hoffman, B. *Stem cells* **12**, 352–369 (1994).
2. Rosmarin, A.G., Yang, Z. & Resendes, K.K. *Exp. Hematology* **33**, 131–143 (2005).
3. Wang G., *et al. Nat. Methods* **3**, 287–293 (2006).
4. Seely, A.J., Pascual, J.L. & Christou, N.V. *Crit. Care* **7**, 291–307 (2003).
5. Tenen, D.G. *Nat. Rev. Cancer* **3**, 89–101 (2003).
6. Pineault, N. *et al. Exp. Hematol.* **30**, 49–57 (2002).
7. Calvo, K.R. *et al. Mol. Cell. Biol.* **20**, 3274–3285 (2000).
8. Knoepfler, P.S. *et al. Oncogene* **20**, 5440–5448 (2001).
9. Lawson, N.D., Krause, D.S. & Berliner, N. *Exp. Hematol.* **26**, 1178–1185 (1998).