TECHNOLOGY

A deeper peek into living organisms

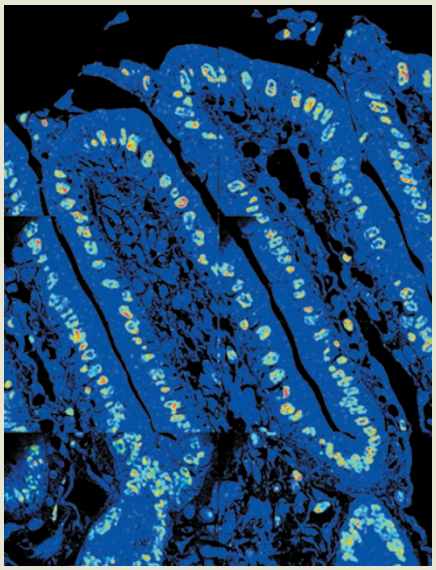
In two papers in this issue, Lechene and colleagues[1,2] report the first use of an approach called multi-isotope imaging mass spectrometry (MIMS) in living organisms (see pages 516 and 520). This technique has outstanding resolution: it provides data in the sub-micrometre range, allowing analysis of structures as small as cellular regions.

MIMS involves labelling living tissues with stable isotopes. The isolated sample surface is then bombarded with a beam of ions, and the ejected 'secondary' ions are measured with a mass spectrometer to determine the sample's molecular composition. The technique can distinguish between ions of very similar mass, providing a precise measurement of isotope labels, which can be imaged simultaneously.

Lechene and co-workers used MIMS to test the immortal-strand hypothesis, which proposes that asymmetrically dividing stem cells also segregate their DNA asymmetrically. That is, the daughter cells that will remain stem cells retain the older DNA template, whereas those that are committed to differentiation inherit newly synthesized DNA strands. The authors disprove this proposal, showing that DNA strands segregate randomly in proliferating crypt cells of the mouse small intestine (pictured). This finding should further our understanding of tissue homeostasis.

The researchers also analysed protein turnover in the mechanosensory hair cells in the inner ear of frogs and mice. During most vertebrates' lifetime, hair cells are not replaced, but their degraded proteins are. One kind of structure within these cells is the stereocilia, each of which is made up of hundreds of filaments of the protein actin. Lechene and collaborators quantified actin turnover in both adult and neonatal hair cells and report that, with the exception of the filaments' tips, this protein's turnover is particularly slow throughout stereocilia. This observation differs from previous findings[3] that stereocilium actin has a rapid turnover time. According to Lechene and co-authors, this discrepancy may be due to differences in experimental conditions between the two studies.

The team also demonstrates successful use of MIMS for human studies. They thus not only further prove the broad applicability of their technique, but also open the door to its use for investigations of metabolism and cell-lineage tracking in humans. **Francesca Cesari and Deepa Nath**



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1. Steinhauser, M. L. *et al. Nature* **481,** 516–519 (2012).
2. Zhang, D.-S. *et al. Nature* **481,** 520–524 (2012).
3. Rzadzinska, A. K. *et al. J. Cell Biol.* **164,** 887–897 (2004).

changes has been difficult to ascertain.

To establish the relative importance of the various SCF-producing cells in HSC growth, Ding *et al.*[1] developed a mouse model in which the *Scf* gene could be replaced with a gene encoding green fluorescent protein, in all or in selected cell types, at different times during the animal's life. In this way, cells that would normally express *Scf* became fluorescent instead. Using this mouse model, Ding *et al.* found that, when they abolished SCF production in all cell types, HSCs disappeared from the bone marrow. The authors then removed *Scf* from specific cell types in the HSC niche, and found that loss of *Scf* from blood cells, osteoblasts and nestin-expressing mesenchymal cells did not alter HSC abundance in the bone marrow. Mice with *Scf*-lacking endothelial cells, however, had fewer HSCs during embryonic development, and only some HSCs remained in these mice during adulthood.

The researchers went on to identify a type of mesenchymal cell that surrounds sinusoids in adult bone marrow and that, in contrast to other niche cells, expresses the gene *Lepr*, which encodes the leptin receptor. The LEPR protein regulates fat metabolism in some cell types, but its function in bone-marrow mesenchymal cells is unknown. Ding *et al.* observed that loss of SCF from *Lepr*-expressing mesenchymal cells reduced HSC abundance in adult mice. Moreover, when the authors deleted the *Scf* gene from both endothelial and *Lepr*-expressing cells, nearly all HSCs disappeared from adult mice. These results indicate that, in the bone marrow of adult mice, SCF comes primarily from *Lepr*-expressing mesenchymal cells that envelop sinusoids, with a smaller contribution of SCF expression from sinusoid endothelial cells.

Future studies are needed to characterize the functions of the *Lepr*-expressing mesenchymal cells in the HSC niche. According to Ding and colleagues' gene-expression data[1], these cells produce another HSC-regulating signal, stromal-cell-derived factor 1 (SDF1), along with the enzyme alkaline phosphatase, which makes them similar to previously described mesenchymal cells that also surround blood vessels in the bone marrow[8]. But the *Lepr*-expressing cells do not produce nestin, which underscores the diversity among mesenchymal stromal cells. Because several genetic tools are available to control gene expression of specific cells in mice, a next logical step should be determining whether *Lepr*-expressing mesenchymal cells are one of the main sources of other HSC-regulating signals.

Different vertebrates have HSC niches in different tissues, and the location of the HSC niche can change during an organism's lifetime[9]. In mammals, HSCs are found first in the aorta, then in the liver and, finally, in the bone marrow. In fish, HSCs also start out in the aorta, but then go on to occupy tissue in the tail and, eventually, the kidneys. All of these seemingly unrelated vertebrate tissues have vascular endothelial cells surrounded by mesenchymal cells, which — as Ding *et al.*[1] now highlight — are required for regulating HSCs in mice. It remains unclear, however, whether different tissue-specific cell types that make up the bone marrow, liver and kidneys provide similar signals to regulate HSC activity.

At present, clinically useful HSCs cannot be efficiently cultured in the lab. The presence of mesenchymal cells in HSC cultures has occasionally been linked to enhanced HSC growth[10], but which mesenchymal cell types are responsible for this effect has been difficult to determine. Robust HSC growth could perhaps be achieved by culturing them on top of mesenchymal 'feeder' cells that express specific markers such as LEPR or nestin. According to Ding and colleagues' results[1], only one in 8,000 bone-marrow cells expresses LEPR, yet these rare cells are the primary source of the SCF needed to support HSCs. Unfortunately, culturing LEPR- or nestin-expressing cells is not an easy task, so more robust conditions should be developed to culture such cells.