Myosin forces in haematopoiesis

Stem cells maintain stemness and produce differentiated progeny by undergoing asymmetric cell divisions that differentially segregate cell-fate-determining factors to the two daughter cells. Discher and colleagues now investigate the roles of non-muscle myosin (MII) isoforms in regulating asymmetric cell division and differentiation of haematopoietic cells (*Cell Stem Cell* **14,** 81–93; 2014).

The authors used mass-spectrometry-calibrated intracellular flow cytometry to demonstrate that human adult haematopoiesis involves a switch from the expression of both MIIB and MIIA isoforms to MIIA alone. They showed that MIIB is more membrane-polarized in $CD34^+$ haematopoietic stem and progenitor cells (HSPCs) than MIIA, and localizes at the cleavage furrow during asymmetric cell division. MIIB segregated asymmetrically with the $CD34^+$ progeny, and partial MIIB depletion blocked both MIIB asymmetric segregation and asymmetric cell division. MIIA did not exhibit asymmetric distribution, but was instead present in its phosphorylated, inactive form, and was activated by differentiation-inducing cytokines in a matrix-stiffness-dependent manner. Injection of MIIB-depleted $CD34^+$ cells into the bone marrow of mice led to reduced peripheral blood cell levels, whereas genetic loss of MIIA suppressed the numbers of differentiated cells. Short-term blebbistatin-mediated inhibition of both isoforms enriched for HSPCs, whereas long-term inhibition blocked cell division and resulted in apoptosis of dividing $CD34^+$ cells.

These findings highlight the distinct roles of MII isoforms in regulating haematopoiesis. AIZ

Opposing enzymes cooperate in ERAD

Misfolded proteins are extracted from the endoplasmic reticulum (ER) and eliminated through ER-associated degradation (ERAD), in which the E3 ubiquitin ligase gp78 and the p97/VCP ATPase play central roles. The chaperone Bag6 helps maintain substrate solubility and may hand over substrates from p97 to the proteasome. Ye and colleagues find that the de-ubiquitylase USP13 cooperates with gp78 in ERAD by maintaining a functional Bag6 complex (*eLife* **3,** e01369; 2014).

Several de-ubiquitylation enzymes interact with p97 but their role in ERAD remains unclear. In a screen for p97-interacting proteins that increase the p97–USP13 interaction, the authors find gp78 and demonstrate that it directly interacts with USP13. Depletion of USP13 prevents ERAD of a model substrate, and further analyses indicate that USP13 acts at a step downstream of retrotranslocation. Specifically, the authors find that USP13 also interacts with the Bag6 complex and prevents accumulation of ubiquitylated Ubl4A, a Bag6 cofactor. Hyper-ubiquitylation of Ubl14 is associated with cleavage of a fraction of the Bag6 pool and with inhibition of ERAD. Lys48 is identified as the residue in Ubl4A targeted by ubiquitylation (which is mediated by gp78 and other ligases). The authors had shown previously that Ubl4A promotes the interaction of Bag6 with the co-chaperone SGTA, and they now discover that USP13 knockdown reduces this interaction. They suggest that USP13 is required to antagonize promiscuous gp78-mediated ubiquitylation of Ubl4A, to prevent Bag6 cleavage and promote the interaction between Bag6 and SGTA. CKR

A scaffold for phagophore membranes

Autophagosome membrane size correlates with levels of Atg8 conjugated to phosphatidylethanolamine (Atg8-PE). Conjugation and association of Atg8-PE with autophagic membranes is mediated by Atg5-modified Atg12 (Atg12-Atg5), which also associates with Atg16. The Atg12-Atg5–Atg16 complex localizes to the convex face of the sequestering membrane, called the phagophore, and is required for autophagosome biogenesis. Kaufmann *et al.* reconstitute these interactions *in vitro* and reveal that the Atg12-Atg5–Atg16 complexes form a mesh-like scaffold on phagophore membranes (*Cell* **156,** 469–481; 2014).

The authors found that Atg12-Atg5 and Atg16 associated with giant unilamellar vesicles (GUVs) containing Atg8-PE, suggesting that lipidated Atg8 recruits and retains Atg12-Atg5 at membranes. Intriguingly, Atg16 caused deformation of GUV membranes reminiscent of protein clustering, raising the possibility that Atg16 immobilizes Atg12-Atg5–Atg8-PE complexes on GUVs. Further analyses revealed that Atg16 acts as a scaffold for generating a flat meshwork of Atg12-Atg5–Atg8-PE complexes. The addition of the cargo adaptor Atg32 caused the meshwork to dissemble by displacing Atg12-Atg5 from Atg8-PE. Lipidated Atg8 was then cleaved in an Atg4-dependent manner, and dissociated from the membrane. Importantly, mutations in Atg16 or Atg12 that blocked scaffold formation but did not affect Atg8 lipidation inhibited autophagic flux *in vivo*. EJC

Endothelial cells drive epithelial repair in lung

Lung epithelial repair depends on progenitor cell populations located in specific niches, but little is known about the signalling crosstalk occurring between the progenitors and their niches in homeostasis and post-injury repair. Kim and colleagues have set up a 3D co-culture system of bronchioalveolar stem cells (BASCs) and lung-derived endothelial cells to obtain colonies growing from single BASC clones (*Cell* **156,** 440–455; 2014). These clones expand to differentiate into three types of colonies: bronchiolar, bronchioalveolar or alveolar structures. They find that the multipotentiality of the bronchioalveolar structures obtained was conserved following multiple passages or subcutaneous transplantations. They noticed that secretion of thrombospondin-1 (Tsp1) from the endothelial cells increased after injury, and that the lung endothelial cells defective for Tsp1 lost their capacity to induce alveolar differentiation. They also observed impaired alveolar repair in Tsp1-null mice. Further analysis led the authors to delineate that the growth factor BMP4 produced after injury activates NFAT-dependent Tsp1 production in endothelial cells to drive alveolar differentiation and repair. This 3D co-culture system will help in the analysis of maintenance and repair of lung stem cells in homeostasis and repair. NLB

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