STEM CELLS

Getting a replacement

The molecular mechanisms that underlie stem-cell maintenance and differentiation are poorly understood. Brawley and Matunis now show, in a report in *Science*, that male germline stem cells (GSCs) in the *Drosophila melanogaster* testis can be replaced by dedifferentiated spermatogonia, which then repopulate the stem-cell microenvironment (or 'stem-cell niche').

Stem cells in the *D. melanogaster* male germline are maintained by local activation of the JAK (Janus kinase)–STAT (signal transducer and activator of transcription) pathway. In the absence of JAK–STAT signalling, GSCs differentiate into spermatogonia and spermatocytes without self-renewal. Indeed, this is what happened when the authors used a temperature-sensitive allele of the *D. melanogaster* STAT homologue *stat92E* (*stat92E^{F}*) under non-permissive conditions.

But, what happens when *stat92E* function is restored by shifting the temperature to permissive conditions? Cells that were negative for a spermatogonial differentiation marker returned to the hub (a cluster of somatic cells that forms the stem-cell niche) in most testes. And, the number of testes that contained GSCs increased from 22.5% to 75.8%. This implies that testes that completely lacked GSCs regained them by some kind of dedifferentiation process.

Male flies that had been allowed to differentiate at the non-permissive temperature until they no longer contained spermatogonia, but only spermatocytes, never regained GSCs after signalling was restored. This indicates that it is spermatogonia, but not spermatocytes, that dedifferentiate to replace the GSCs.

Spermatogonia exist as multicellular cysts, which must somehow break away to become GSCs when JAK–STAT signalling is restored. Brawley and Matunis showed this by labelling spermatogonia with bromodeoxyuridine before reactivating the signalling pathway. Labelled cysts were detected near the hub in 6% of testes before recovery. And after signalling was reactivated, again, 6% of testes contained labelled GSCs. This indicates that GSCs must be breakdown products of labelled cysts.

The authors concluded that the partially differentiated spermatogonia must have remarkable plasticity by dedifferentiating into stem cells in response to signals from the stem-cell niche. This dedifferentiation mechanism contributes to stem-cell maintenance and renewal.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Brawley, C. & Matunis, E. Regeneration of male germline stem cells by spermatogonial dedifferentiation *in vivo*. *Science* **304**, 1331–1334 (2004)

CELL ADHESION

Spread the word

Using a mass-spectrometry-based technique to uncover proteins that are present in focal adhesions, Matthias Mann and colleagues have identified an early cell-spreading structure — the spreading initiation centre (SIC) — that contains RNA and RNA-binding proteins. Their findings are reported in *Cell*.

The technique — stable isotope labelling by amino acids in culture (SILAC) — 'labels' proteins by culturing them in medium that contains deuterium-substituted leucine or ^{13}C-substituted arginine. These proteins can then be distinguished by mass spectrometry from non-labelled proteins that have been isolated from cells grown in normal medium. The authors applied the technique to immunoprecipitates from lysates of adherent and non-adherent fibroblasts using antibodies against the important focal-adhesion proteins talin, paxillin or vinculin. Many other known focal-adhesion proteins were identified, but some new candidates emerged.

During their characterization of one differentially expressed protein, RACK1 (receptor for activated C kinase-1), Mann and colleagues found that cells underwent at least three different stages of spreading after initial attachment. Circular patches — which the authors called 'spreading initiation centres' — were present in the early stages but became less abundant as cells spread and focal adhesions formed, and disappeared when the focal adhesions were established. Surrounding these SICs was a sheath of actin, which disappeared as the mature focal adhesion became an attachment point for actin stress fibres. Interestingly, SICs were only observed in fibroblasts and non-transformed cells, and on physiological substrates such as fibronectin.

Surprisingly, the authors discovered that several RNA-binding proteins, such as hnRNP K, hnRNP E1, FUS/TLS, Sm B and Sm D, preferentially bound to either talin, paxillin or vinculin in adherent cells. Not only did these proteins localize to SICs but so, too, did RNA. Neither, though, were present in mature focal adhesions. Finally — and perhaps most surprisingly — disrupting the function of hnRNP K, hnRNP E1 and FUS/TLS using antibodies increased the rate of fibroblast spreading. It's certainly something to talk about.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER de Hoog, C. L., Foster, L. J. & Mann, M. RNA and RNA binding proteins participate in early stages of cell spreading through spreading initiation centers. *Cell* **117**, 649–662 (2004)

FURTHER READING Aebersold, R. & Mann, M. Mass spectrometry-based proteomics. *Nature* **422**, 198–207 (2003)