STEM CELLS

Ignoring the stop sign

Stem cells have the capacity to continue dividing when other cells are quiescent, by ignoring cell-division stop signals. Reporting in *Nature*, Hannele Ruohola-Baker and colleagues now show that the microRNA (miRNA) pathway is required for stem cells to bypass the G1–S checkpoint at which the cell cycle would otherwise stop.

By impairing the RNase III enzyme Dicer-1 (Dcr-1) — which is essential for miRNA processing — in *Drosophila melanogaster* germline stem cells (GSCs), the authors found that the rate of egg chamber production was reduced, which reflected decreased progeny of GSCs. There were no abnormalities in the morphology, identity or maintenance of GSCs, but the frequency of cell division in *dcr-1* mutant GSCs was reduced.

So, might the *dcr-1* mutant cause a cell-cycle delay in GSCs? Ruohola-Baker and co-workers observed an increase in the number of *dcr-1* mutant GSCs that stained positive for cyclin E, a G1–S cyclin, and a decrease in the staining for cyclin A and cyclin B, which function at the G2–M transition, and for the mitotic marker phosphohistone-3. In addition, they showed that the number of *dcr-1* mutant GSCs in S phase was reduced compared with wild-type GSCs. Together, these findings imply that GSCs with a defective miRNA pathway are delayed in the cell cycle at the G1–S transition. This effect was specific for GSCs, as the cell divisions of other *dcr-1*-defective cell types were not affected.

In flies, the transition from G1 to S phase is negatively regulated by Dacapo (Dap), a homologue of the p21/p27 family of cyclin-dependent kinase (CDK) inhibitors. Dap traps the cyclin E–CDK2 complex in an inactive form. By reducing the level of Dap by ~50% in *dcr-1* mutant GSCs, the authors noted a partial rescue in GSC

MEMBRANE MICRODOMAINS

No rafts required

The T-cell–antigen-presenting-cell contact site is a highly organized complex of cell-surface receptors and associated intracellular signalling proteins that is known as the immunological synapse. Although the components of the immunological synapse are well defined, the mechanisms by which it forms are poorly understood. Now, a report published in *Cell* indicates that protein–protein interactions can direct the formation of discrete microdomains of receptors and signalling molecules after T-cell activation.

Previous studies have implicated lipid rafts and actin and myosin cytoskeletal networks as mediators of protein clustering at the immunological synapse. So, Douglass and Vale further investigated the mechanisms by which microdomains of signalling molecules form. They used both conventional confocal microscopy and single-molecule tracking to analyse the overall membrane distribution and single-molecule behaviour of green fluorescent protein (GFP)-tagged T-cell-signalling molecules, after the activation of Jurkat T cells by immobilized T-cell receptor (TCR)-specific antibodies.

In the absence of T-cell activation, individual molecules sometimes moved rapidly and at other times were static. However, the different signalling molecules showed distinct patterns of mobility: CD2 molecules were mostly immobile, with only occasional periods of rapid mobility, whereas both a marker of lipid rafts (the ten N-terminal amino acids of LCK (LCK10) fused to GFP) and a non-raft-associated protein (CD45) spent little time immobile. These patterns of diffusion indicate that immobilization was not due to lipid-raft formation. Consistent with this, after TCR crosslinking both raft-associated wild-type LAT (linker for activation of T cells) and a LAT mutant that cannot associate with lipid rafts but can mediate protein–protein interactions (LAT(C-S)) showed reduced mobility.

After TCR crosslinking, CD2 molecules were observed to cluster and to colocalize with LCK and LAT but not CD45. Colocalization of LCK and CD2 was not a result of LCK being a raft-associated protein, because the lipid-raft marker LCK10 did not colocalize with CD2. Furthermore, a raft-associated LAT mutant that cannot be phosphorylated (LAT(Y-F)) did not colocalize with CD2, which indicates that protein–protein interactions mediated by tyrosine-phosphorylated LAT mediate the colocalization of LAT and CD2. Consistent with this, CD2 clustering after TCR crosslinking was impaired in a cell line derived from Jurkat T cells that has severely reduced LAT expression, and this clustering could be restored by transfecting the cells with LAT but not LAT(Y-F).

Further single-molecule analysis indicated that CD45 was excluded from CD2-containing microdomains, whereas LCK, LAT and LAT(C-S) were often associated with these microdomains and, when associated, showed little mobility. Together, these studies indicate that LCK and LAT are preferentially trapped in the CD2-containing microdomains through protein–protein interactions, and that the association of these proteins with lipid rafts does not regulate this process. This led the authors to suggest that protein–protein interactions should be considered an early event in T-cell signalling and a mechanism of immunological-synapse formation.

Karen Honey, Associate Editor,
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References and links

ORIGINAL RESEARCH PAPER Douglass, A. D. & Vale, R. D. Single-molecule microscopy reveals plasma membrane microdomains created by protein–protein networks that exclude or trap signaling molecules in T cells. *Cell* **121**, 937–950 (2005)