RESEARCH HIGHLIGHTS

New ligases join the ubiquitylation cascade in repair

Histone ubiquitylation is an important component of the DNA double-strand break repair (DSBR) response. Previous work has identified a sequential cascade of the ubiquitin ligases RNF8 and RNF168, which are important for ubiquitylating histones H2A and H2AX, and for amplifying the ubiquitylation signals through K63-linked ubiquitin chains, respectively, at DSBs. These ubiquitylation events are crucial for recruiting and retaining additional regulators of the repair pathway at DSBs such as 53BP1. But precisely how these ubiquitylation events are restricted to break sites is not understood. New work from Lukas and colleagues (*Cell* **150,** 687–709, 2012) now provides insights into this process.

In an siRNA screen against known components of the human ubiquitinome, the authors identified two additional ubiquitin ligases, TRIP12 and UBR5, that act upstream of RNF168 to control its accumulation at DSBs. Depletion of the ligases led to enhanced accumulation of RNF168 on chromatin and a concomitant expansion of ubiquitin-enriched chromatin domains, including components of the repair pathway such as RAP80, BRCA1 and 53BP1. Whereas UBR5 was found to modestly affect levels of multiple ligases involved in DSBR, the TRIP12 effect was specific for RNF168. Finally, the authors found that levels of RNF168 are rate limiting in the pathway and increased accumulation of RNF168 seemed to enhance efficiency of repair.

The exact molecular mechanism underlying UBR5 and TRIP12 control of RNF168 levels awaits future study. SS

Mechanical control at cell–cell contacts

Cell adhesion and cortex tension are known to regulate cell–cell contact formation. Heisenberg, Paluch and colleagues now analyse the contributions of cell adhesion and cortex tension in contact formation and sorting of zebrafish progenitor cells (*Science* http://doi.org/jcp; 2012).

Building on previously published models of cell–cell adhesion and sorting, the authors developed a theoretical description of the shape of two progenitor cells adhering to each other. They then used dual micropipette aspiration assays to separate adhering progenitor cells from zebrafish embryos *ex vivo* and determined that cortex tension controls interfacial tension at the cell–cell contact, and thereby regulates cell–cell contact expansion. In contrast, cell adhesion was not involved in determining cell–cell contact size. Instead, the authors demonstrated that following mechanical separation of adhering cells, the linkage of cadherin to the actin cytoskeleton was crucial in limiting the mechanical resistance of adhesive bonds to pulling forces. The cytoskeletal anchoring of cadherins was further shown to be important for correct progenitor cell sorting within cell aggregates *in vitro*, and also during the segregation of progenitor cells in gastrulating zebrafish embryos *in vivo*. Thus, by combining theoretical, biophysical and live imaging experiments, the authors showed that cell adhesion is necessary to mechanically couple the cortices of adhering cells to support cell sorting. AIZ

Aurora A maintains embryonic stem cells

Although the transcription factors that control embryonic stem cell (ESC) self-renewal and pluripotency are well characterized, less is known about the upstream signalling pathways. Schaniel, Lemischka and colleagues now identify the mitotic kinase Aurka (Aurora A) to be necessary for ESC maintenance through suppression of p53 (*Cell Stem Cell* **11,** 179–194, 2012).

An shRNA screen of 104 protein kinases and phosphatases revealed that depletion of Aurka decreased mouse ESC renewal and expression of pluripotency markers, while promoting mesodermal and ectodermal differentiation. Further, high Aurka expression correlates with the undifferentiated state. The authors observed no major cell cycle or apoptotic defects in Aurka-depleted ESCs, suggesting that residual Aurka activity was sufficient for its normal proliferative and viability functions. Gene profiling of Aurka knockdown cells and subsequent gene set enrichment analysis indicated that Aurka negatively regulates p53 activity. Indeed, two Aurka phosphorylation sites were identified in p53, and a p53 mutant mimicking phosphorylation at one of these sites showed reduced reporter activity in ESCs. Aurka depletion increased known p53 targets associated with developmental processes, and expression of a phospho-mimic p53 mutant shifted gene expression from pluripotency- to differentiation-associated markers. Thus, Aurka maintains pluripotency through suppression of a p53-driven differentiation program.

Interestingly, the authors found that Aurka is also upregulated during reprogramming of mouse embryonic stem cells into induced pluripotent stem cells (iPSC), and that Aurka depletion impairs iPSC generation in a p53-dependent manner. CKR

Hippo regulates mitochondrial size

The Hippo pathway regulates cell proliferation and organ size. Banerjee and colleagues reveal that this pathway also regulates mitochondrial fusion and function in *Drosophila melanogaster* and in human cells (*Gene Dev.* http://doi.org/jcq; 2012).

In *Drosophila*, Hippo negatively regulates transcription by promoting Warts (Wts)-mediated phosphorylation and inhibition of the transcription factor Yorkie (Yki; known as YAP in mammals). The authors found that overexpression of Yki (YAP2), or mutations in Hippo or Wts, increased mitochondrial size. Electron microscopy revealed that expression of YAP2 or Yki elongated mitochondria in human or fly cells, indicating that the Hippo pathway might regulate mitochondrial size. Indeed, microarray analyses followed by chromatin immunoprecipitation (ChIP)-chip arrays showed that Yki bound to enhancer regions and promoted transcription of two key mitochondrial fusion genes, *Opa1* and *Marf*. Antioxidant and electron transport chain complex I genes were also bound by Yki and upregulated on Yki overexpression.

Intriguingly, RNA interference (RNAi)-mediated depletion of *Opa1* and *Marf* blocked tissue growth induced by Yki overexpression, suggesting that these proteins are key effectors of Hippo-mediated regulation of organ size. Yki activity is known to promote cell survival and proliferation; these phenotypes are also linked to mitochondrial fusion. The authors suggest that the Hippo–Yki pathway might stimulate tissue growth, promote cell proliferation and inhibit apoptosis through regulation of mitochondrial fusion. EJC

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