Caspase control: IAPs as NEDD8-E3 ligases

Inhibitor of apoptosis (IAP) proteins act as ubiquitin-E3 ligases and regulate caspase activity through ubiquitin-mediated proteasomal degradation; deubiquitylating enzymes (DUBs) can reverse the activity of ubiquitin and ubiquitin-like (UBL) proteins, such as NEDD8. In transgenic *Drosophila* expressing IAP antagonists to induce apoptosis, Broemer *et al.* have now systemically knocked down individual DUBs using RNAi and identified three NEDD8-specific proteases that, when knocked down, suppress cell death *in vivo* (*Mol. Cell* **40,** 810–812; 2010). Apoptosis was reduced in the null mutants of one of these genes, Deneddylase 1 (*DEN1*), and the effector caspase drICE was found to be neddylated *in vivo*, which suggested a role for NEDD8 in apoptosis. Interestingly, UV-mediated reduction in *Drosphila* IAP 1 (DIAP1) protein levels reduced neddylation of drICE and induced apoptosis, indicating that DIAP1 is a NEDD8-E3 ligase for drICE. Further investigation showed that DIAP1 neddylates drICE in a RING- and binding-dependent manner leading to reduced drICE proteolytic activity, and further mass spectrometry analysis identified nine lysine residues in drICE as sites for neddylation. As XIAP, a mammalian IAP, also promoted neddylation of caspase 7 in a RING-dependent manner, IAP-mediated neddylation seems to be evolutionarily conserved. This study demonstrates that IAPs can function as E3 ligases for NEDD8 as well as for ubiquitin and extends the complexity of IAP-mediated signalling. IO

Crumbs couples Hippo and Smads

Animal development requires integration of different signals, including cell density cues and morphogens: high cell density triggers the Hippo kinase cascade to inactivate the TAZ/YAP cofactors by cytoplasmic sequestration and TAZ/YAP also modulate the subcellular distribution of the Smad effectors of TGFβ signalling. Varelas *et al.* (*Dev. Cell* **19,** 831–844; 2010) now show that Hippo and TGFβ responses are coupled to cell density through the Crumbs cell polarity complex.

The authors demonstrate that the nuclear localization of activated Smads in cell culture and in the early mouse embryo depends on the subcellular distribution of TAZ/YAP. Under Hippo-activating conditions, such as high cell confluency, TGFβ fails to induce its transcriptional programmes, as the Smads remain cytoplasmic. Inhibition of Hippo signalling by depletion of TAZ/YAP or their upstream kinases restores TGFβ responsiveness. The authors link the effect of cell density on TGFβ activity to cell–cell junction formation and demonstrate that the interaction of TAZ/YAP with the fully assembled Crumbs polarity complex is required for the cytoplasmic localization of activated Smads. Depletion of Crumbs components blocks Hippo-induced Smad cytoplasmic sequestration and restores TGFβ responses connected to the epithelial–mesenchymal transition, such as induction of Snail1, thus demonstrating the functional integration of the Hippo and TGFβ pathways. AIZ

Follicle revolutions

A new form of morphogenetic movement is described by Haigo and Bilder, who show that elongation of developing *Drosophila* follicles is associated with rotation around the elongation axis (*Science* doi: 10.1126/science.1199424).

Drosophila eggs develop from multicellular follicles that are initially spherical but elongate dramatically along the antero–posterior (A–P) axis between stages 4 to 14 of oogenesis to form the oval egg shape. The authors tagged either the epithelium surrounding the follicle or germline nuclei with fluorescent markers and followed dissected follicles by confocal microscopy. They observed that follicles revolve around the A–P axis about three times between stages 5 and 9, which is when most of the elongation takes place. The integrin β_{PS} subunit, previously implicated in follicle elongation, was required for rotation as was Collagen IV. Haigo and Bilder also noticed that Collagen IV forms fibrils in the orientation of rotation. Acute disruption of collagen using collagenase caused rounding of elongated follicles, while blocking follicle rotation (using the integrin β_{PS} mutant, mys) disrupted Collagen IV matrix organization. Thus, the authors envisage a model where a treadmill-like migration of epithelial follicle cells creates a rotation movement. This is important for polarization of extracellular matrix fibrils, which in turn acts as a 'molecular corset' to constrain follicle shape. CKR

Kinetochores need active transcription

Centromeric chromatin contains the histone H3 variant CENP-A, on which kinetochores are assembled in mitosis. In primates, despite containing specific repetitive satellite DNA sequences, centromeric function is defined epigenetically and thought to depend on the pattern of histones and their modifications at centromeric loci, such as the presence of nucleosomes containing dimethylated H3K4 (H3K4me2), a mark usually associated with transcription. Earnshaw and colleagues (*EMBO J.* doi:10.1038/emboj.2010.329) probe the characteristics of centromeric chromatin using a synthetic human artificial chromosome (HAC), which has the ability to function as a neocentromere. They find that centromeric chromatin displays high levels of H3K36 methylation, a mark associated with transcription elongation, and also contains active RNA polymerase II and displays active transcription of the satellite sequences. To test the functional importance of active transcription of centromeres for kinetochore function, the authors tethered a fusion protein containing the lysine-specific demethylase LSD1 to the centromeric HAC to target only H3K4me2 in this region. Although depletion of H3K4me2 did not prevent kinetochore function in the short term, it eventually reduced active transcription of the centromeric region and the recruitment of the CENP-A chaperone HJURP, impairing CENP-A deposition and kinetochore stability. It remains to be resolved whether there is a direct role for transcription products in centromere function or whether an altered chromatin state, mediated by the transcription machinery, is important for CENP-A incorporation. NLB

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