by co-transfection of the tumour suppressor phosphatase and tensin homologue (PTEN), which promotes a decrease in FOXO1 phosphorylation. The increased transcriptional activity of FOXO1 was abolished in the presence of wild-type CDK2, but not by a kinase-inactive CDK2 mutant. Also, the inhibitory effect of CDK2 was diminished in a FOXO1 (Ser249, Ser298) double mutant. Together, these data indicate that the transcriptional activity of FOXO1 is repressed primarily by the CDK2-mediated phosphorylation of Ser249.

As residue Ser249 of FOXO1 is located adjacent to a nuclear localization motif, and as overexpression of CDK2 causes the cytoplasmic localization of wild-type FOXO1 but not of the phosphorylation-resistant S249A mutant, CDK2 phosphorylation is thought to induce the cytoplasmic localization of FOXO1 from the nucleus and thereby repress its transcriptional activity.

When Tindall and colleagues treated cells with a DNA-damaging agent, camptothecin or γ-irradiation, phosphorylation of FOXO1 at Ser249 was abolished. siRNA-mediated silencing of either the CHK1 or CHK2 kinase of the DNA-damage-mediated checkpoint pathway partially blocked the camptothecin-induced decrease in FOXO1 phosphorylation. These data indicate that DNA-damaging agents regulate FOXO1 by controlling CHK1- and CHK2-dependent checkpoint pathways. FOXO1-mediated cell death in response to DNA damage was observed both in p53-deficient and p53-containing cell lines, which implies that FOXO1 contributes to the apoptotic response to DNA damage independently of p53.

In summary, CDK2-mediated regulation of FOXO1 represents a novel pathway that links DNA damage to apoptosis. Interestingly, in cells in which FOXO1 was silenced, overexpression of FOXO3a or FOXO4 restored DNA-damage-induced apoptosis. It is therefore important to examine the roles of other FOXO proteins in the selective killing of cells in response to DNA damage.

Arianne Heinrichs

ORIGINAL RESEARCH PAPER Huang, H. *et al.* CDK2-dependent phosphorylation of FOXO1 as an apoptotic response to DNA damage. *Science* **314**, 294–297 (2006)
FURTHER READING Bartek, J. *et al.* Checking on DNA damage in S phase. *Nature Rev. Mol. Cell Biol.* **5**, 792–804 (2006)
WEB SITE
Donald Tindall's laboratory: http://mayoresearch.mayo.edu/mayo/research/staff/tindall_dj.cfm

was comparable to that observed when cells were treated with Rho or ROCK inhibitors, establishing ENDO180–Rho–ROCK–MLC2 as a functional pathway. Moreover, *ENDO180* siRNA or a ROCK inhibitor reduced the phosphorylation of three other ROCK substrates, LIM kinase-1 and -2, and myosin phosphatase-1.

Isacke and colleagues also show that ENDO180 is required for the spatial activation of Rho–ROCK–MLC2. Using cell lines that stably express an ENDO180 mutant that cannot be internalized, the authors demonstrate that ROCK cannot be recruited to focal adhesions. This shows that intracellular ENDO180-containing endosomes are required to activate Rho–ROCK.

ENDO180 is highly expressed in motile cells, particularly fibroblasts, and has an established role in collagen internalization. Bugge and colleagues have shown that mice undergoing polyomavirus-induced mammary carcinogenesis that also have a targeted deletion in ENDO180 have impaired tumour expansion, and therefore a reduced tumour burden. Isacke and colleagues suggest that this reduction in tumour burden could arise both from the inability of *ENDO180*-null fibroblasts to remodel the extracellular matrix and from impaired motility in the tumour. Indeed, targeting ENDO180 might benefit anticancer therapy by causing the retention of a tumour-inhibitory matrix.

Gemma Alderton, Associate Editor, Nature Reviews Cancer

ORIGINAL RESEARCH PAPER Sturge, J. *et al.* Endosomes generate localized Rho-ROCK-MLC2-based contractile signals via Endo180 to promote adhesion disassembly. *J. Cell Biol.* **175**, 337–347 (2006)
FURTHER READING Curino, A. C. *et al.* Intracellular collagen degradation mediated by uPARAP/Endo180 is a major pathway of extracellular matrix turnover during malignancy. *J. Cell Biol.* **169**, 977–985 (2005)

IN BRIEF

APOPTOSIS

Histone H2B deacetylation at lysine 11 is required for yeast apoptosis induced by phosphorylation of H2B at serine 10.

Ahn, S.-H. *et al. Mol. Cell* **24**, 211–220 (2006)

Histone modifications are known to regulate a number of cellular processes. In growing yeast, the histone protein H2B is acetylated on Lys11, whereas phosphorylation of the adjacent Ser10 residue has been linked to the induction of apoptosis. Ahn *et al.* now show that hydrogen peroxide treatment of growing cells first causes Lys11 to be deacetylated, followed by the subsequent phosphorylation of Ser10 and apoptosis. The deacetylase enzyme Hos3 mediates the first step, whereas phosphorylation depends on the kinase Ste20. The authors propose that a concerted series of histone modifications control the switch from cell growth to cell death.

DEVELOPMENT

Canonical notch signaling functions as a commitment switch in the epidermal lineage.

Blanpain, C. *et al. Genes Dev.* **20**, 3022–3035 (2006)

The formation of mammalian epidermis involves the differentiation and migration of basal-layer progenitor cells to suprabasal spinous cells. Blanpain and colleagues show that Notch signalling commits dividing progenitor cells to becoming spinous cells. Activated Notch intracellular domain (NICD) and its signalling partner RBP-J are both required to repress expression of progenitor-cell genes and to activate spinous-cell gene expression. Spinous-cell gene activation depends on the NICD–RBP-J target gene *HES1*, whereas progenitor-cell gene repression occurs through a *HES1*-independent mechanism.

SIGNAL TRANSDUCTION

Global, *in vivo*, and site-specific phosphorylation dynamics in signaling networks.

Olsen, J. V. *et al. Cell* **127**, 635–648 (2006)

Protein phosphorylation functions as a reversible switch in many signalling cascades. Using a high-throughput mass-spectrometry-based technique, the authors detected 6,600 protein phosphorylation sites in HeLa cells and monitored changes in phosphorylation during epidermal growth factor stimulation. Phosphorylation is modulated by at least twofold at 14% of phosphorylation sites and proteins that contain multiple phosphorylation sites are often regulated independently.

SUMOYLATION

Regulation of MBD1-mediated transcriptional repression by SUMO and PIAS proteins.

Lyst, M. J. *et al. EMBO J.* 26 Oct 2006 (doi:10.1038/sj.emboj.7601404)

Methylated DNA can serve as a binding site for methyl-CpG-binding domain (MBD) proteins, which, in turn, recruit co-repressor complexes that modify chromatin into an inactive state. Lyst *et al.* found that the activity of MBD1 can be regulated by sumoylation near its C terminus by two PIAS-family members, PIAS1 and PIAS3. Sumoylated MBD1 still binds to methylated DNA, but neither recruits the histone methylase SETDB1 nor silences gene expression in HeLa cells. MBD1 is proposed to function as a scaffolding platform on methylated DNA that can be regulated through sumoylation.