RESEARCH HIGHLIGHTS

CELL DEATH

Delicate decisions

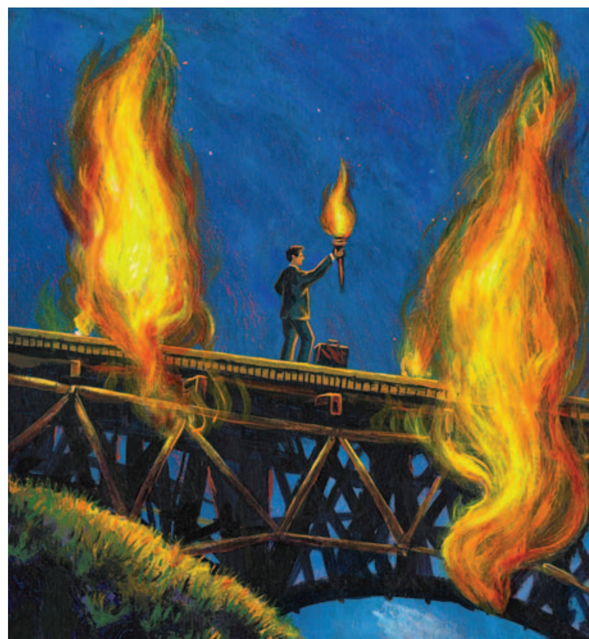
The anti-apoptotic BCL2-family member MCL1 can be induced by a number of growth factors and promote cell survival. However, how growth factors maintain MCL1 levels to prevent apoptosis has been unclear. Maurer *et al.* now report that the control of MCL1 stability by glycogen synthase kinase-3 (GSK3) is an important mechanism for the regulation of apoptosis by growth factors and AKT/protein kinase B (PKB) in haematopoietic cells.

Mitochondrial outer membrane permeabilization (MOMP) is a central point in the regulation of apoptosis, and leads to the release of cytochrome *c* and other pro-apoptotic proteins that reside in the mitochondrial intermembrane space. Although the phosphatidylinositol-3 kinase (PI3K)–AKT/PKB pathway has been shown to control MOMP, how apoptosis can be prevented is not fully understood.

To characterize the role of GSK3 in growth-factor-withdrawal-induced apoptosis, Maurer *et al.* investigated the function of GSK3 in haematopoietic cells, which undergo apoptosis after IL-3 withdrawal. The induction of apoptosis was also associated with the activation of GSK3β, and by using small-molecule inhibitors, the authors showed that the inhibition of GSK3 prevents IL-3-withdrawal-induced MOMP and apoptosis. Therefore, the inhibition of GSK3 by IL-3 is important for the prevention of apoptosis by the mitochondrial pathway.

But how does GSK3 regulate the apoptotic pathway? MCL1 is required to protect haematopoietic cells from apoptosis, which prompted the authors to investigate a potential interaction between GSK3 and MCL1. The modulation of the half-life of MCL1 by GSK3 activity and the identification of a conserved GSK3 phosphorylation site on MCL1 indicated that MCL1 could be phosphorylated by GSK3. Using mutation analysis, Maurer and colleagues showed that, both *in vitro* and *in vivo,* MCL1 is phosphorylated at Ser159 by GSK3 — this phosphorylation event was induced by IL-3 withdrawal and could be prevented by AKT/PKB or GSK3 inhibitors. Phosphorylated MCL1 was only detected upon inhibition of the proteasome, which indicated that the phosphorylation of MCL1 triggers increased proteasomal degradation.

The expression of an MCL1 phosphorylation-site mutant in IL-3-dependent cells revealed that non-phosphorylated MCL1 was more stable and conferred an increased protection from apoptosis compared with wild-type MCL1. But how does 'rescued' MCL1 — by inhibiting GSK3 after IL-3 depletion — contribute to the inhibition of MOMP? The authors investigated whether MCL1 does so by sequestering one of the obvious candidates, the pro-apoptotic BH3-only protein BIM. Immunoprecipitation studies showed that BIM was only associated with MCL1 in the presence of GSK3 inhibitors and when IL-3 was depleted, which indicated that MCL1 sequesters BIM, therefore preventing it from inducing MOMP.



The authors propose that the regulation of MCL1 by GSK3 is probably an important switch in the delicate life-or-death decisions made by immature haematopoietic cells during their maturation. The intriguing possibility that this PI3K–AKT/PKB–GSK3–MCL1 pathway might also regulate survival in other cell types remains to be investigated.

Ekat Kritikou

> “The intriguing possibility that this PI3K–AKT/PKB–GSK3–MCL1 pathway might also regulate survival in other cell types remains to be investigated.”

ORIGINAL RESEARCH PAPER Maurer, U. *et al.* Glycogen synthase kinase-3 regulates mitochondrial outer membrane permeabilization and apoptosis by destabilization of MCL-1. *Mol. Cell* **21**, 749–760 (2006)

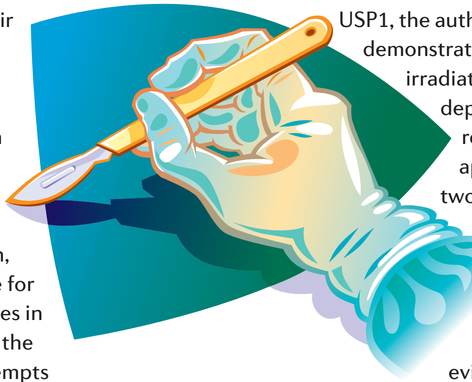
RESEARCH HIGHLIGHTS ADVISORS

GENEVIÈVE ALMOUZNI Institut Curie, Paris, France
JOAN S. BRUGGE Harvard Medical School, Boston, MA, USA
IVAN DIKIC Goethe University Medical School, Frankfurt, Germany
WILLIAM C. EARNSHAW University of Edinburgh, Scotland, UK
TOREN FINKEL National Institutes of Health, Bethesda, MD, USA
PAMELA GANNON Cell and Molecular Biology Online
YOSEF GRUENBAUM The Hebrew University of Jerusalem, Jerusalem, Israel
ULRICH HARTL Max Planck Institute, Martinsried, Germany
ELISA IZAURRALDE Max Planck Institute, Tübingen, Germany
STEPHEN P. JACKSON Wellcome Trust/Cancer Research UK Gurdon Institute, Cambridge, UK
JENNIFER LIPPINCOTT-SCHWARTZ National Institutes of Health, Bethesda, MD, USA
MATTHIAS MANN Max Planck Institute, Martinsried, Germany
NORBERT PERRIMON Harvard Medical School, Boston, MA, USA
NATASHA RAIKHEL University of California, CA, USA
ANNE RIDLEY Ludwig Institute for Cancer Research, London, UK
KAREN VOUSDEN Beatson Institute for Cancer Research, Glasgow, UK

DNA REPAIR

Making the cut

DNA-damage-repair pathways allow for the identification, excision and sometimes even the bypass of lesions to ensure DNA replication and cell survival. The regulation of these pathways is highly involved, and includes, among other mechanisms, post-translational modifications such as ubiquitylation. D'Andrea and colleagues, reporting in *Nature Cell Biology*, now demonstrate how auto-cleavage of a deubiquitylation enzyme is central in the regulation of translesion DNA synthesis (TLS), an important DNA-damage-repair pathway.



In specific cases of DNA damage, such as those that are induced by ultraviolet (UV) radiation, the best hope for cell survival lies in the bypass of the lesion, as attempts at repairing the lesion might result in prolonged cell-cycle arrest and eventual apoptosis. TLS, a pathway that involves specialized polymerases that can bypass lesions, is involved in some of these cases. TLS polymerases are recruited to DNA in times of damage by monoubiquitylated PCNA (proliferating cell nuclear antigen). These TLS polymerases are crucial in coping with lesions, but most are error prone and therefore not desirable under healthy replication conditions. So, in the absence of DNA damage, PCNA is deubiquitylated, through a previously unknown mechanism, to prevent the recruitment of error-prone TLS polymerases to DNA. But how is PCNA deubiquitylation regulated?

By carrying out protein-overexpression and small interfering RNA (siRNA)-mediated knockdown studies, D'Andrea and colleagues first demonstrated that PCNA is deubiquitylated by overexpressed USP1 (a deubiquitylation enzyme) and over-ubiquitylated in its absence. Next, they examined the effects of UV exposure on USP1 mutants. Western blots of wild-type USP1 revealed that the protein is cleaved immediately following a conserved C-terminal Gly–Gly motif. By contrast, when the catalytic domain is mutated, the degradation of mutant USP1 is inhibited. This indicates that USP1 is degraded through an auto-cleavage event.

In an *in vivo* siRNA knockdown of USP1, the authors demonstrated that UV irradiation of USP1-depleted cells resulted in an approximately two-fold increase in DNA-mutation frequency. The cumulative evidence supports a model in which UV radiation causes DNA damage and USP1 auto-cleavage. Subsequent to USP1 auto-cleavage, mono-ubiquitylated PCNA can no longer be deubiquitylated, and so recruits error-prone TLS polymerases that can bypass the UV-induced DNA lesions.

How UV exposure causes USP1 to auto-cleave, whether USP1 auto-cleavage has a role in DNA-damage sensing, and the possibility that auto-cleavage might be a recurring mechanism in the regulation of ubiquitylation and ubiquitin-like modifications all need further investigation. But, when it comes to DNA-damage repair, it seems that for cells to make the cut, sometimes they have to make the auto-cut.

Asher Mullard

ORIGINAL RESEARCH PAPER Huang, T. T. *et al.* Regulation of monoubiquitinated PCNA by DUB autocleavage. *Nature Cell Biol.* **8**, 341–347 (2006)
FURTHER READING Huang, T. T. & D'Andrea, A. D. Regulation of DNA repair by ubiquitylation *Nature Rev. Mol. Cell Biol.* **7**, 323–334 (2006)

Structure watch

TEAMWORK IN TRANSCRIPTION

The transmembrane receptor Notch mediates intercellular communication. Following ligand binding, Notch is cleaved and its intracellular domain (ICD) translocates to the nucleus. There, the ICD forms a transcription-activation complex with the DNA-bound transcription factor CSL and the coactivator protein Mastermind (MAM). In *Cell*, two groups have now furthered our understanding of this complex through structural studies. Blacklow and colleagues present the structure of a human complex that contains the ankyrin domain (ANK) of Notch1, CSL, DNA and Mastermind-like-1 (MAML1). Wilson and Kovall describe the structure of a *Caenorhabditis elegans* complex that contains the Notch ICD, CSL, DNA and MAM.

Both studies found that CSL and ANK together create a binding groove for MAM/MAML1, which explains why CSL and this region of Notch are both needed for MAM/MAML1 binding. The structure derived by Wilson and Kovall also contains the RAM domain of Notch, and the papers together provide insights into the assembly of the transcription-activation complex. The Notch RAM domain seems to interact with CSL first. ANK then binds to another region of CSL to create the composite surface for MAM/MAML1 binding. The binding of RAM, or the synergistic interaction of RAM and ANK with CSL, seems to induce a conformational change in CSL that might convert it from a repressor to an activator of Notch target genes.

REFERENCES Nam, Y. *et al.* Structural basis for cooperativity in recruitment of MAML coactivators to Notch transcription complexes. *Cell* **124**, 973–983 (2006) | Wilson, J. J. & Kovall, R. A. Crystal structure of the CSL–Notch–Mastermind ternary complex bound to DNA. *Cell* **124**, 985–996 (2006)

MODIFICATION REMOVAL

Specific Ser and Thr residues in nucleocytoplasmic proteins can be modified by *O*-linked *N*-acetylglucosamine (*O*-GlcNAc), and this modification can regulate processes such as the cell cycle and transcription. Two papers now give insights into how this modification is removed by describing crystal structures of close homologues of the human glycoside hydrolase *O*-GlcNAcase.

In *The EMBO Journal*, van Aalten and colleagues describe structures of an *O*-GlcNAcase from *Clostridium perfringens*, whereas, in *Nature Structural & Molecular Biology*, Vocadlo, Davies and co-workers present structures of an *O*-GlcNAcase from *Bacteroides thetaiotaomicron*. Both groups determined the structures of the native protein and of these proteins in complex with mimics of reaction intermediates. They found that *O*-GlcNAcase uses a variant of the substrate-assisted catalytic mechanism (the key catalytic residues are a tandem Asp–Asp pair), and showed that these proteins are suitable models for further studies of the function of human *O*-GlcNAcase.

REFERENCES Rao, F. V. *et al.* Structural insights into the mechanism and inhibition of eukaryotic *O*-GlcNAc hydrolysis. *EMBO J.* **25**, 1569–1578 (2006) | Dennis, R. J. *et al.* Structure and mechanism of a bacterial β-glucosaminidase having *O*-GlcNAcase activity. *Nature Struct. Mol. Biol.* **13**, 365–371 (2006)

CHROMOSOMES

Single is sometimes best

The centromere-specific histone variant CENP-A, which is known as CID in *Drosophila melanogaster*, and the centromere-associated kinetochore are crucial for the attachment of the microtubule spindle, congression to the metaphase plate and the subsequent chromosome separation in anaphase. A single centromere–kinetochore association per chromosome ensures accurate chromosome segregation during mitosis and meiosis. Gary Karpen and colleagues now show that the overexpression of CID leads to its mislocalization and the formation of functional ectopic kinetochores, which might provide a possible mechanism for genome instability during cancer progression.

The overexpression of CID caused a widespread mislocalization of CID in cultured cells, and led to growth defects, abnormal development and lethality in flies. Cytological analysis of fixed cells revealed several mitotic defects, including stretched, fragmented and lagging chromosomes during anaphase. In addition, time-lapse studies confirmed these results in live cells and also showed delays in mitosis and chromosome loss. The authors further established that these mitotic defects were distinct from those caused by the loss of endogenous centromere function or caused by the failure to separate sister chromatids. So, Karpen and colleagues concluded that the phenotypes that were observed are a direct result of the mislocalization of CID.

> ...these findings indicate that CID mislocalization leads to the formation of ectopic kinetochores that are probably functional.

Kinetochore formation is poorly understood, but is thought to depend on the presence of CID. Indeed, the Karpen team obtained several lines of evidence, which indicated that ectopic kinetochores are formed in response to mislocalized CID. First, several proteins that are associated with centromeric

MEMBRANE DYNAMICS

An alternative route

Marks and colleagues have discovered a novel method for sorting cargo into multivesicular bodies (MVBs). The well-known route for sorting to the intralumenal vesicles (ILVs) of MVBs involves protein ubiquitylation, HRS (hepatocyte-growth-factor-regulated tyrosine-kinase substrate) and the ESCRTs (endosomal sorting complexes required for transport). However, in *Developmental Cell*, they now describe an alternative route to ILVs for the melanosomal protein Pmel17.

During the biogenesis of melanosomes — the lysosome-related organelles of melanocytes in which melanin pigments are synthesized and stored — Pmel17 is incorporated into ILVs. At some time, it is cleaved in its lumenal domain to release a fibrillogenic fragment, and the resulting fibrils function as a matrix for melanin deposition as the melanosome matures.

To understand the role of ILVs in fibril formation, the authors dissected the molecular requirements for Pmel17 sorting to ILVs. They first showed that Pmel17 sorting was relatively insensitive to dominant-negative interference with the HRS–ESCRT pathway and to HRS depletion. In addition, using immunoelectron microscopy, they confirmed the localization of Pmel17 to ILVs in cells with disrupted HRS–ESCRT function. They also showed that ILVs still formed and Pmel17 was still sorted to them following the disruption of HRS–ESCRT function to an extent that was sufficient to block the sorting of ubiquitylated proteins to ILVs. Pmel17 therefore seems to be sorted to ILVs in an HRS- and ESCRT-independent manner.

So, what determinants are responsible for the sorting of Pmel17 to ILVs? To answer this question, Marks and co-workers analysed the movements of ectopically expressed wild-type and variant forms of Pmel17 in HeLa cells, which do not express endogenous Pmel17. Despite this, ectopic Pmel17 expression in HeLa cells still results in its accumulation in ILVs and in fibrils, which indicates that this sorting pathway is conserved in non-melanocytic cells.

> Marks and colleagues have discovered a novel method for sorting cargo into multivesicular bodies...

Using this approach, they showed that Pmel17 ubiquitylation is not required for its sorting to ILVs; mutating putative ubiquitylation sites in the cytoplasmic domain of Pmel17 did not disrupt its sorting to ILVs. They also showed that the cytoplasmic and transmembrane domains of Pmel17 could be replaced by those of an irrelevant protein with no detrimental effect on its sorting to ILVs. However, another construct that contained the Pmel17 cytoplasmic domain and the transmembrane and lumenal domains of the irrelevant protein was not sorted correctly, and the authors showed that the efficient sorting of Pmel17 to ILVs requires the two N-terminal lumenal subdomains

chromatin and inner- and outer-kinetochore proteins colocalized with the mislocalized CID. Second, the kinetochore-associated kinesin KLP59C, the dynein motor protein and the microtubule plus-end binding protein MAST colocalized at multiple, ectopic chromosome regions. Lastly, the authors observed microtubule attachments at ectopic sites that also contained CID and an outer-kinetochore protein.

Together, these findings indicate that CID mislocalization leads to the formation of ectopic kinetochores that are probably functional. The presence of multiple centromere–kinetochore associations per chromosome could therefore be responsible for the observed chromosome separation defects and the consequent cellular and organismal phenotypes. It will be interesting to investigate the prevalence of mislocalized CENP-A in human cancers to test this hypothesis.

Arianne Heinrichs

ORIGINAL RESEARCH PAPER Heun, P., Erhardt, S. *et al.* Mislocalization of the *Drosophila* centromere-specific histone CID promotes formation of functional ectopic kinetochores. *Dev. Cell* **10**, 303–315 (2006)

of Pmel17. To their knowledge, "...this is the first conclusive evidence for a role of a lumenal domain in directing cargo to ILVs."

In the final part of their study, Marks and colleagues showed that Pmel17 localization to ILVs is required for Pmel17 cleavage and subsequent fibril formation. This is the first time that localization to ILVs has been shown to provide a specialized environment for protein processing. This work has therefore identified a novel route to the ILVs of MVBs, and it might be that several ILV populations arise from different ESCRT-dependent and -independent pathways.

Rachel Smallridge

ORIGINAL RESEARCH PAPER Theos, A. C. *et al.* A lumenal domain-dependent pathway for sorting to intralumenal vesicles of multivesicular endosomes involved in organelle morphogenesis. *Dev. Cell* **10**, 343–354 (2006)

FURTHER READING Katzmann, D. J. No ESCRT to the melanosome: MVB sorting without ubiquitin. *Dev. Cell* **10**, 278–280 (2006)

WEB SITE

Mickey Marks' laboratory: http://www.med.upenn.edu/camb/faculty/cbp/marks.html

MITOSIS

The spindle matrix revisited

Chromosome segregation during mitosis is coordinated by the mitotic spindle, which requires the joint activities of microtubules, microtubule-binding proteins and chromosomes. Although it has been suggested that a structural scaffold — the so-called spindle matrix — tethers spindle-assembly factors (SAFs) and supports spindle assembly, the nature of this scaffold has remained elusive. Now, Yixian Zheng and colleagues report in *Science* that the intermediate-filament protein lamin-B is required for spindle assembly, and they propose a structural role for lamin-B in the spindle matrix.

Lamins have regulatory and structural functions in interphase nuclei, but previous studies indicated that a fraction of lamin-B might also associate with the mitotic spindle and therefore might have a role in mitosis. Zheng and colleagues showed, using immunofluorescence, that lamin-B associates with mitotic spindles in *Xenopus laevis* egg extracts and HeLa cells. Depletion of lamin-B by RNA interference in HeLa cells led to an increase in defects that are associated with spindle assembly and function, which indicates that lamin-B might function in spindle assembly. To rule out the possibility that the spindle defects were an indirect result of the perturbation of interphase functions, the authors carried out experiments in M-phase *X. laevis* egg extracts, and obtained similar results.

Next, the authors showed that lamin-B assembled into a matrix-like network during mitosis, and that this process depended on the presence of the GTP-bound form of the small GTPase Ran. Spindles were assembled in *X. laevis* egg extracts in the presence or absence of RanGTP, and the spindle microtubules were subsequently depolymerized with nocodazole. This revealed a lamin-B-specific fibrillar–granular matrix, which formed only in the presence of RanGTP. A possible explanation for the requirement for RanGTP is that the nuclear-import proteins importin-α and -β interfere with the assembly of the lamin-B matrix, and that RanGTP displaces importin-α and -β from proteins such as lamin-B that are required for matrix assembly.

Double-immunofluorescence analyses of the lamin-B matrix showed the presence of both lamin-B and SAFs (poly(ADP-ribose), NuMA, Eg5, XMAP215 and TPX2) in the same matrix. Although the depletion of lamin-B inhibited the formation of matrix structures that contained SAFs, the depletion of either Eg5 or XMAP215 still allowed the formation of lamin-B matrices that contained other SAFs. These findings indicate that lamin-B is required for the assembly of a spindle-associated matrix.

Zheng and co-workers were able to isolate the lamin-B matrix from M-phase egg extracts that were treated with nocodazole, and could indeed detect the presence of lamin-B and several SAFs. When incubated with pure tubulin, these isolated matrices caused the nucleation of microtubules, which tethered to the matrices. By contrast, no microtubules assembled in the absence of the matrices. Importantly, the matrices from which XMAP215 was absent were unable to promote microtubule assembly. This implies that the lamin-B matrices can promote spindle assembly by tethering SAFs.

Finally, the authors showed that lamin-B mutants that were unable to form proper interphase nuclear lamina disrupted spindle assembly. Also, the localization of SAFs on the microtubule structures in the matrices that contained mutant lamin-B was abnormal. Therefore, the proper assembly of the lamin-B matrix is essential for the organization and localization of SAFs on spindles during mitosis.

This work indicates that lamins might have nuclear structural roles not only in interphase, but also in mitosis, and sheds important new light on the elusive spindle matrix.

Arianne Heinrichs

ORIGINAL RESEARCH PAPER Tsai, M.-Y. *et al.* A mitotic lamin B matrix induced by RanGTP required for spindle assembly. *Science* **311**, 1887–1893 (2006)

In the news

STEM-CELL CONFLICTS

Stem cells have been in the news recently, because of several attempts to produce stem cells for therapeutic purposes. One study, published in *Nature*, reported the identification of a source of reprogrammable cells in the testes of adult mice.

This finding drew immediate attention because it promises, at least for men, a simple and more ethically acceptable method for harvesting therapeutic stem cells. These reprogrammable cells are derived from sperm-producing stem cells, but could they give rise to other cell types, as embryonic stem cells can?

Gerd Hasenfuss' team at the Georg August University of Göttingen, Germany, extracted, cultured and multiplied the cells 700-fold. They converted these cells into an embryo-like state in 4 out of 15 cases. *In vitro*, the converted cells gave rise to heart, brain and skin cells. Most importantly, when they were injected into an early embryo, they contributed to various organs of the resulting mouse.

But some researchers are sceptical. Takashi Shinohara, a mouse germ-cell expert at Japan's Kyoto University, was the first to isolate reprogrammable cells from mouse testes using young pups. In contrast to the findings of Hasenfuss' team, he found that stem cells that were injected into embryos were not integrated into various tissues. "Perhaps they have some different kind", he says, "but I don't think that type of cell exists." (*news@nature.com*, 29 March 2006).

Could these cells work as efficiently as stem cells that are derived from human embryos? "This could put the embryonic stem-cell people out of business," says Peter Donovan, a stem-cell expert at the University of California, Irvine, USA. "But it remains to be seen whether they work in humans." (*news@nature.com*, 29 March 2006).

Ekat Kritikou

CELL ADHESION

Anchors away!

"IMPs are expressed in various cancers, but whether the overexpression of IMPs in cancer cells might promote their invasive capacity remains to be seen."

The RNA-binding proteins IMPs (IGF-II mRNA-binding proteins) have been implicated in mRNA localization, nuclear export and translational control. Reporting in *The EMBO Journal*, Vikesaa and colleagues now show that two IMP proteins, IMP1 and IMP3, also have profound effects on cellular adhesion and invasion during development and cancer formation.

In an attempt to understand the cellular functions of the IMPs, Vikesaa *et al.* used RNA interference (RNAi) and selectively knocked down IMP1 and IMP3 in HeLa cells. Cells that were treated with small interfering RNAs against IMP1 and IMP3 became spindle-shaped, rounded and had fewer cellular extensions. Cell–cell contacts were severely reduced and the number of cells that were floating in the media indicated that IMP depletion resulted in cell detachment. Using confocal microscopy combined with a matrix-degradation assay, the authors also showed that the loss of IMPs almost completely disrupted the formation of invadopodia — actin-rich

MECHANISMS OF DISEASE

Under pressure

A new link between blood-pressure homeostasis and the dysregulation of transforming growth factor-β (TGFβ) has been reported in *Cell*. TGFβ has a key role in the development and pathophysiology of blood vessels, and increased levels of circulating TGFβ have been reported in hypertensive individuals. TGFβ and the secreted extracellular matrix (ECM) glycoprotein EMILIN1 (elastin microfibril interface-located protein-1) are co-expressed in vascular smooth-muscle cells of the aortic wall. Based on their analysis of *Emilin1*-knockout mice, Zacchigna *et al.* have shown that EMILIN1 is a negative regulator of TGFβ processing and activation, and they propose that this antagonism helps to explain hypertension in animals that lack EMILIN1.

By characterizing the cardiovascular structure and function of *Emilin1*-null mice, the authors found that blood-vessel diameter was reduced and both peripheral resistance and systemic blood pressure were increased. So what is the mechanism by which EMILIN1 controls vascular-cell behaviour? EMILIN1 is a multidomain protein that includes a cysteine-rich EMI domain. Zacchigna and colleagues reasoned that because other secreted proteins with cysteine-rich repeats are known to regulate growth-factor signalling, it was possible that EMILIN1 might modulate TGFβ activity in vascular cells through its EMI domain.

Experiments in *Xenopus laevis* embryos showed that EMILIN1 functions as an antagonist of TGFβ ligands; further *in vitro* studies in mammalian cells indicated that the EMI domain binds specifically to proTGFβ1, the inactive proprotein

"A new link between blood-pressure homeostasis and the dysregulation of [TGFβ] has been reported…"

structures that degrade and extend into the extracellular matrix during cell migration.

But how do IMPs regulate adhesion and invasion? Although the IMP proteins have been extensively studied, only a few RNA targets have been reported so far. So to identify novel factors and pathways that are regulated by IMP proteins and to dissect the mechanisms behind the phenotypic changes that were observed, the authors compared the gene-expression profiles of IMP RNAi-treated and control cells. They found that the levels of only 27 transcripts changed significantly — 7 transcripts were upregulated and 20 were downregulated. Strikingly, despite the small number of transcripts that were identified, 11 of the transcripts that were downregulated encoded for proteins that are involved in cellular adhesion and/or invasion.

Of these transcripts, the authors further characterized CD44, a hyaluronic-acid receptor that has been previously implicated in cell–matrix adhesion and the formation of invadopodia. Could CD44 be a direct target of IMPs, and could the reduction of *CD44* mRNA have a role in the loss of invadopodia in IMP-depleted cells? The *CD44* gene generates three transcripts by alternative polyadenylation and Vikesaa *et al.* not only showed that the selective knockdown of the longer transcript recapitulated the effect of IMP depletion and reduced the number of cells with invadopodia, they also found that IMP1 binds to and stabilizes this transcript.

These findings show that IMPs are involved in cell adhesion and the formation of invadopodia by regulating mRNA target genes that are involved in these processes. IMPs are expressed in various cancers, but whether the overexpression of IMPs in cancer cells might promote their invasive capacity remains to be seen.

Ekat Kritikou

ORIGINAL RESEARCH PAPER Vikesaa, J. *et al.* RNA-binding IMPs promote cell adhesion and invadopodia formation. *EMBO J.* **25**, 1456–1468 (2006)

of TGFβ, in the extracellular space. The precursor was therefore protected from proteolytic activation, and the subsequent production of mature TGFβ was prevented. But what is the *in vivo* significance of this interaction? The authors predicted that *Emilin1*-knockout mice would show increased TGFβ signalling in the vascular system, and they found supportive evidence by using several assays.

Zacchigna *et al.* have shown that EMILIN1, a glycoprotein that is associated with the ECM of blood vessels, can modulate TGFβ signalling, and they have "…identified a new mechanism of regulation of arterial blood pressure…". Their work highlights the importance of the ECM components of the vascular system and the modulation of TGFβ availability in the pathogenesis of hypertension, and could potentially lead to specific therapies that would be aimed at the origin of the disease. In addition, as the authors point out, their findings could be relevant beyond hypertension, as TGFβ is thought to have a role in many pathological conditions, including fibrosis, inflammation and cancer.

Sharon Ahmad

ORIGINAL RESEARCH PAPER Zacchigna, L. *et al.* Emilin 1 links TGF-β maturation to blood pressure homeostasis. *Cell* **124**, 929–942 (2006)
FURTHER READING Raman, M. & Cobb, M. H. TGF-β regulation by Emilin1: new links in the etiology of hypertension. *Cell* **124**, 893–895 (2006)

IN BRIEF

STEM CELLS

In vitro germline potential of stem cells derived from fetal porcine skin.

Dyce, P. W. *et al. Nature Cell Biol.* **8**, 384–390 (2006)

The authors isolated stem cells from fetal porcine skin, induced them to differentiate, and identified a subpopulation of cells that expressed several specific germ-cell markers, including the growth differentiation factor 9b (*GDF9b*) and deleted in azoospermia-like (*DAZL*) genes. Further differentiation caused follicle-like cell aggregates that secreted the steroid hormones oestradiol and progesterone and that responded to stimulation by gonadotropin. Some aggregates extruded large oocyte-like cells that expressed oocyte and meiosis markers and that, eventually, developed structures that resembled pre-implantation embryos. Together, these findings indicate that somatic stem cells that are derived from the later stages of fetal development can develop into germ cells *in vitro*.

TECHNOLOGY

3′ UTR seed matches, but not overall identity, are associated with RNAi off-targets.

Birmingham, A. *et al. Nature Methods* **3**, 199–204 (2006)

RNA interference is a powerful tool in research and therapeutic applications provided that the small interfering RNA (siRNA) that is used is potent and specific — that is, it does not cause 'off-target' gene silencing. Birmingham *et al.* applied an algorithm that is well suited for detailed alignment analysis of short sequences to a database of experimentally identified off-target genes, and found that the overall gene identity provides little insight into whether a gene will be affected by a given siRNA or not (except in the case of near identity). Instead, perfect matches between the hexamer or heptamer seed (positions 2–7 or 2–8 of the antisense strand) of an siRNA and the 3′ untranslated region (3′ UTR), but not the 5′ UTR or the open reading frame, were associated with off-target effects.

CELL MIGRATION

Analysis of cell migration using whole-genome expression profiling of migratory cells in the *Drosophila* ovary.

Wang, X. *et al. Dev. Cell* **10**, 483–495 (2006)

Systematic analysis of the transcriptional switch inducing migration of border cells.

Borghese, L. *et al. Dev. Cell* **10**, 497–508 (2006)

To identify new genes that are involved in cell migration, two groups carried out genome-wide expression profiling on purified border cells — a type of migratory cell that is present in the *Drosophila melanogaster* ovary. The transcription factor Slbo is required for these cells to become migratory, and this switch is poorly understood. So both groups compared the expression profiles of wild-type border cells with *slbo* mutants. Wang *et al.* identified 413 genes that were enriched in migratory cells, and 149 that were Slbo dependent. Many of the isolated genes are involved in cytoskeletal regulation and the secretory pathway. Borghese *et al.* found almost 300 genes that were significantly enriched in border cells; 28% were regulated by Slbo. As well as cytoskeletal regulators, they identified a group of 'muscle-like' genes that might enable cells to acquire muscle-like properties to invade neighbouring tissue.