AGEING

Connected clues?

> “…a potential link between acetyl-CoA pools, metabolism, ageing and histone modifications.”

Four new studies provide a potential link between acetyl-CoA pools, metabolism, ageing and histone modifications. This link might be mediated by sirtuin deacetylases, which have previously been implicated in ageing and metabolism.

Histone acetylation is important for many cellular processes including gene expression and DNA repair. Given that all known histone acetyltransferases use acetyl-CoA in the acetylation reaction, Takahashi *et al.* investigated the source of acetyl-CoA for global histone acetylation in yeast. They deleted genes in each of the different acetyl-CoA production pathways, but found that only one of them was required for bulk histone acetylation — the essential gene that encodes the acetyl-CoA synthetase Acs2. Indeed, a temperature-sensitive *acs2* mutant showed a marked decrease in global histone acetylation and had transcriptional defects when grown at the restrictive temperature. By targeting Acs2 to different subcellular localizations, the authors demonstrated that acetyl-CoA that was produced in the nucleus and cytoplasm existed in a single pool that was used for histone acetylation, whereas acetyl-CoA that was produced in the mitochondria formed an isolated pool that was unable to support histone acetylation. These findings therefore link a specific acetyl-CoA pool directly to global histone acetylation and chromatin-mediated cellular processes.

Interestingly, studies by Schwer *et al.* and Hallows *et al.* showed that the mammalian forms of Acs2, AceCS1 and AceCS2, are substrates for the sirtuin deacetylases SIRT1 and SIRT3, respectively. They demonstrated that AceCS1 and AceCS2 are activated by deacetylation. The subcellular localization of the acetyl-CoA synthetases was important for their regulation, as SIRT1 deacetylated AceCS1 in the cytoplasm, whereas SIRT3 controlled the activity of AceCS2 in mitochondria. Hallows *et al.* observed that AceCS2 expression led to a 10-fold increase in CO_2, whereas AceCS1 expression did not cause a change — indicating that the acetyl-CoA pools generated by AceCS1 and AceCS2 are distinct.

Another link between sirtuins, histone modifications and ageing has come from a study by Celic *et al.* They analysed the acetylation of histone H3 Lys56 (H3K56ac), a modification that occurs when cells pass through S phase and replicate their genome, and that subsequently disappears as the cell cycle progresses. The chromatin was hyperacetylated in the absence of the sirtuins Hst3 and Hst4, and mutant cells were hypersensitive to chemical agents and temperature and showed spontaneous DNA damage and chromosome loss. These findings indicate that sirtuins are important for controlling the level of H3K56ac, and that failing to deacetylate H3K56ac leads to genome instability, which could have implications for lifespan.

Intriguingly, sirtuins seem to have opposing effects: they increase acetyl-CoA production and they function as specific deacetylases. Could this indicate a homeostatic mechanism? These new clues will undoubtedly stimulate future studies of ageing and related disease states.

Arianne Heinrichs

ORIGINAL RESEARCH PAPERS Takahashi, H. *et al.* Nucleocytoplasmic acetyl-coenzyme A synthetase is required for histone acetylation and global transcription. *Mol. Cell* **23**, 207–217 (2006) | Schwer, B. *et al.* Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. *Proc. Natl Acad. Sci. USA* **103**, 10224–10229 (2006) | Hallows, W. C. *et al.* Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. *Proc. Natl Acad. Sci. USA* **103**, 10230–10235 (2006) | Celic, I. *et al.* The sirtuins Hst3 and Hst4p preserve genome integrity by controlling histone H3 lysine 56 deacetylation. *Curr. Biol.* **16**, 1280–1289 (2006)

MECHANISMS OF DISEASE

Receptor traffic — parkin rules

Parkinson's disease (PD), a progressive neurodegenerative disorder that causes tremors, stiffness and impaired balance and coordination, results from the loss of dopamine-producing neurons in the midbrain. Defects in the ubiquitin-proteasome system and protein aggregation in Lewy bodies have typically been associated with the development of PD. A study in *Nature Cell Biology* now indicates that a proteasome-independent ubiquitylation pathway also contributes to the aetiology of this disease.

Parkin encodes an E3 ubiquitin ligase that is inactivated in ~50% of early-onset PD cases. Edward Fon and colleagues showed that, in addition to targeting proteins for degradation, parkin can delay epidermal growth factor (EGF) receptor (EGFR) trafficking by ubiquitylating the endocytic scaffolding protein EPS15, and can promote signalling through phosphoinositide 3-kinase (PI3K)–Akt, an important pathway for neuronal survival.

The ubiquitin-like domain of wild-type parkin, but not of a PD-associated form, binds to the ubiquitin-interacting motif (UIM) of EPS15. This interaction is regulated by EGF, the addition of which transiently increased the formation of a parkin–EPS15–EGFR complex and EPS15 ubiquitylation. The authors propose that intramolecular UIM–ubiquitin binding not only decreases the capability of EPS15 to induce EGFR internalization, but also prevents EPS15 from interacting with parkin, possibly explaining the transient nature of the complex.

Overexpression of parkin specifically reduced EGF uptake in COS-7 cells. Consistently, the rate of EGFR internalization was faster in mouse embryonic fibroblasts from parkin-knockout mice compared with wild-type mice. Small-interfering-RNA-mediated EPS15 knockdown did not further decrease the rate of EGFR endocytosis, indicating that EPS15 is required for the effect of parkin on receptor internalization.

Last, the authors showed that by decreasing receptor internalization, parkin also regulates EGF signalling via the PI3K–Akt pathway. EGF-induced Akt phosphorylation in synaptosomes from parkin-knockout mice was remarkably reduced compared with wild-type synaptosomes. Due to the positive role of Akt in neuronal survival, these findings are indicative of a novel mechanism through which parkin could prevent neurodegeneration.

In addition, two studies in *Nature* have recently described a role for parkin in the maintenance of mitochondrial integrity in dopaminergic neurons. It will be interesting to determine whether particular parkin mutations affect specific aspects of parkin function. Therapeutic strategies that are aimed at these novel pathways could lead to better and more effective treatments for PD.

Monica Hoyos Flight, Editor,
Cell Migration Update

ORIGINAL RESEARCH PAPER Fallon, L. *et al.* A regulated interaction with the UIM protein Eps15 implicates parkin in EGF receptor trafficking and PI(3)K–Akt signalling. *Nature Cell Biol.* **8**, 834–848 (2006)
FURTHER READING Park, J. *et al.* Mitochondrial dysfunction in *Drosophila PINK1* mutants is complemented by *parkin*. *Nature* **441**, 1157–1161 (2006) | Clark, I. E. *et al. Drosophila pink1* is required for mitochondrial function and interacts genetically with *parkin*. *Nature* **441**, 1162–1166 (2006)
WEB SITE
Edward Fon's laboratory: http://www.mni.mcgill.ca/cfns.html#EdwardFon

IN BRIEF

APOPTOSIS

Mitochondrial rhomboid PARL regulates cytochrome *c* release during apoptosis via OPA1-dependent cristae remodelling.

Cipolat, S. *et al. Cell* **126**, 163–175 (2006)

OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion.

Frezza, C. *et al. Cell* **126**, 177–189 (2006)

Cytochrome *c* resides in the convoluted folds (cristae) of the mitochondrial inner membrane until it is released to amplify apoptosis. Two studies showed that cytochrome *c* mobilization from mitochondria is controlled by the rhomboid protease PARL and the dynamin-related protein OPA1. Cleavage of a membrane-bound form of OPA1 by mitochondrial PARL generates soluble OPA1, which forms oligomers that maintain the shape of the cristae and, thereby, the compartmentalization of cytochrome *c*. The pro-apoptotic BCL2-family protein BID disrupts OPA1 oligomers and widens cristae junctions, enabling cytochrome *c* release.

DNA REPAIR

Visualization of Rad54, a chromatin remodelling protein, translocating on single DNA molecules.

Amitani, I. *et al. Mol. Cell* **23**, 143–148 (2006)

Rad54 is a double-stranded DNA (dsDNA)-dependent ATPase that functions in dsDNA-break repair and homologous recombination. The authors developed an optically trapped DNA–bead complex to visualize the movement of Rad54 along single molecules of dsDNA. The translocation is rapid and processive, and Rad54 binds randomly along the dsDNA and moves either upstream or downstream with a velocity that depends on the ATP concentration.

MOLECULAR MOTORS

Single-molecule analysis of dynein processivity and stepping behaviour.

Reck-Peterson, S. L. *et al. Cell* **126**, 335–348 (2006)

Cytoplasmic dynein drives nearly all minus-end-directed motility in eukaryotic cells. Using a recombinant dimeric dynein protein, the Vale group showed the processive motion of dynein by structural and single-molecule analysis. Both dynein motor domains of the dimer are essential for its processivity, but the tail domains and associated subunits are not. Dynein primarily takes 8-nm steps, but shows considerable variability in its step size and direction, and its stepping behaviour is most reminiscent of myosin VI.

CYTOKINESIS

Assembly of the cytokinetic contractile ring from a broad band of nodes in fission yeast.

Wu, J.-Q. *et al. J. Cell Biol.* **174**, 391–402 (2006)

Wu *et al.* showed that the cytokinetic contractile ring in fission yeast arises from a broad band of small dots or nodes, rather than from a single myosin II progenitor as previously proposed. The anillin-like protein Mid1 is required to establish this band of nodes, which also contains other proteins, and actin polymerization stimulates contractile-ring formation.

Web watch

CELL SIGNALLING CONDENSED

• http://www.cellsignallingbiology.org

"The beauty of cell signalling", writes Michael J. Berridge, "is the way different pathways are combined and adapted to control a diverse array of cellular processes in widely different spatial and temporal domains." In the newly launched website, called Cell Signalling Biology, Berridge provides a large, comprehensive resource that discusses all of these aspects of cell signalling and integrates emerging cell-signalling findings.

The content is divided into 12 modules. Half of these focus on the components of cell-signalling pathways, with modules specifically dedicated to the on and off mechanisms of components and the spatial and temporal encoding of signalling information. The other half of the modules focus on how these cell-signalling pathways control the life cycle of cells, from cell proliferation through to cell differentiation and apoptosis, with a separate module dedicated to cell-signalling defects and disease. Within each module, content is further subdivided into more focused sections.

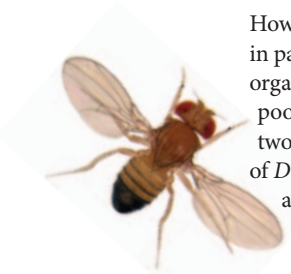
The text contains extensive linking to other pertinent sections, as well as to published research articles and reviews. An extensive collection of figures and tables complements the text excellently, and a split-screen setup allows for the convenient and helpful display of a figure or table alongside the text. In addition, a full-text searching function allows users to find relevant information quickly.

The content will be updated annually, and the website can be accessed through a personal or an institutional 12-month subscription or on a pay-per-view basis. But before you throw out your old cell-signalling text books, log on to the currently available free demonstration of this website and make sure that it fills all your cell-signalling-information needs.

Asher Mullard

DEVELOPMENT

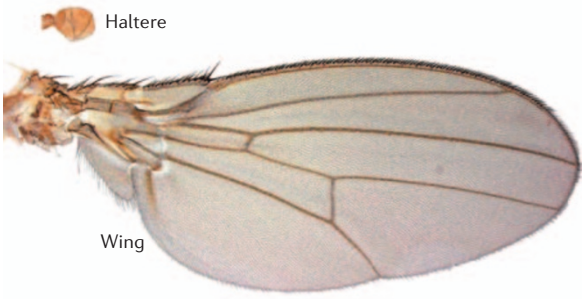
Controlling size



How organ size is controlled and, in particular, how homologous organs acquire different sizes, is poorly understood. By studying two homologous flight appendages of *Drosophila melanogaster* — wings and the much smaller halteres, which are needed for balance during flight — Crickmore and Mann have uncovered a mechanism by which the Hox 'selector' gene *Ultrabithorax* (*Ubx*) controls organ size. It functions by regulating the amount and distribution of the morphogen Decapentaplegic (Dpp).

The reason for examining these two organs is that all of the differences between wing and haltere development, including size, are controlled by the *Ubx* gene — when *Ubx* is removed from haltere cells, a wing forms instead. The authors first confirmed that *Ubx* limits the size of a haltere in a cell-non-autonomous manner, which indicates the involvement of morphogen-mediated pathways.

Crickmore and Mann then focused their attention on the morphogen Dpp, which promotes growth in both wings and halteres. Dpp is produced by cells that form a stripe along the middle of wings and halteres. Compared with wings, the width of the stripe of cells that expressed *dpp* in halteres was much narrower and expression was less intense. The authors also found differences in the pattern



Wild-type adult wing and haltere. Image courtesy of R. Mann, Columbia University, New York, USA.

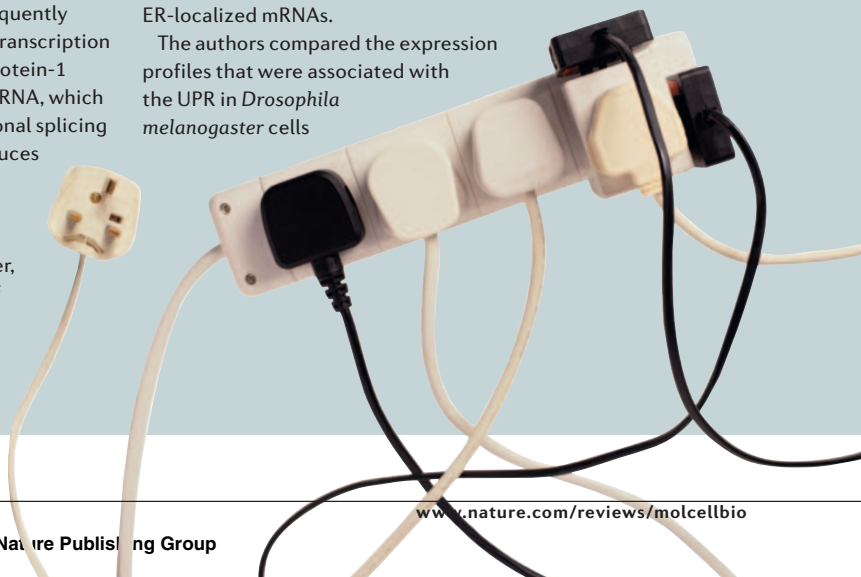
PROTEIN FOLDING

Avoiding overload

Inositol-requiring enzyme-1 (IRE1) — an endoplasmic reticulum (ER) transmembrane protein —senses the folding status of the ER. It detects misfolded proteins in the ER lumen and subsequently activates the cytosolic transcription factor X-box-binding protein-1 (XBP1) by cleaving its mRNA, which initiates an unconventional splicing reaction. XBP1 then induces the expression of genes with roles in ER-related processes including protein folding. However, this isn't the only role of IRE1 in the unfolded protein response (UPR) — a response that allows the ER to recover from the accumulation of misfolded proteins. As Hollien and Weissman now show in *Science*, IRE1 also has an XBP1-independent role that involves the degradation of ER-localized mRNAs.

The authors compared the expression profiles that were associated with the UPR in *Drosophila melanogaster* cells

> "However, this isn't the only role of IRE1 in the unfolded protein response…"



of the Dpp gradient between halteres and wings.

The gradient of Dpp could be modified by varying the expression pattern of the gene encoding the Dpp receptor, Thickveins (Tkv). When Dpp binds to Tkv, it triggers cell proliferation, but it also results in Dpp being captured for destruction. The authors observed that *tkv* expression around the source of Dpp was low in wings, which allowed Dpp to diffuse further and form two peaks of Dpp-signalling activity on either side of the stripe. However, *tkv* expression was high in all haltere cells, thereby trapping Dpp close to the source. Indeed, when *tkv* was overexpressed in wing cells, the Dpp gradient was narrow and wing size was reduced. By contrast, depletion of Tkv in haltere cells resulted in a broader gradient and increased haltere size.

So, how do Dpp production and mobility relate to *Ubx*? Dpp and Master of thickveins (Mtv) are both needed to repress *tkv* expression in the wing. However, the authors showed that Dpp functions as a repressor of *mtv* expression in *Ubx*-expressing haltere cells. Therefore, as only one repressor is active, *tkv* is de-repressed, which restricts the mobility of Dpp. The authors also noticed that in halteres, but not in wings, the Dpp-signalling profile coincides with that of Hedgehog (Hh). Given the overlapping activities and the fact that the transcription of *dpp* is activated by Hh signalling and repressed by Dpp signalling, they postulated that these conflicting inputs in the same cells might contribute to the reduced Dpp production that is observed in halteres compared with wings. This was indeed the case — so, *Ubx* reduces the production of Dpp by changing where Dpp signalling occurs.

On the basis of their results, the authors suggest the real possibility that "altering the shape and intensity of morphogen gradients may be a general mechanism by which selector genes affect tissue sizes in animal development."

Arianne Heinrichs

ORIGINAL RESEARCH PAPER Crickmore, M. A. & Mann, R. S. Hox control of organ size by regulation of morphogen production and mobility. *Science* **313**, 63–68 (2006)
FURTHER READING Stern, D. L. Morphing into shape. *Science* **313**, 50–51 (2006)

that had been depleted of IRE1 or XBP1 by RNA interference (RNAi). The comparison highlighted an unexpected XBP1-independent branch of the UPR in which IRE1 mediates the selective repression of ER-targeted mRNAs. The repressed targets include the mRNAs of plasma-membrane and secreted proteins that traffic through the ER, and the repression is rapid such that it can relieve the folding burden on the ER before the XBP1-dependent pathway comes into effect.

If this mRNA repression is initiated by an internal cleavage reaction such as the one that is mediated by IRE1, the resulting RNA fragments would become substrates for the housekeeping machinery that is involved in cytoplasmic 5′-to-3′ and 3′-to-5′ mRNA degradation. In support of such a cleavage reaction, the use of RNAi to deplete components of this machinery led to the IRE1- and UPR-dependent accumulation of 3′ and 5′ fragments of the mRNAs that are repressed by IRE1.

In terms of *cis* elements that are needed to target mRNAs to this degradation pathway, Hollien and Weissman showed that an ER-targeting signal sequence is crucial. They also found that degradation seems to occur during co-translational protein translocation. The authors therefore propose that: "A targeting mechanism based on direct peptide recognition or translational effects would potentially allow IRE1 to focus on messages that present the most immediate challenge to the translocation and folding machinery."

Rachel Smallridge

ORIGINAL RESEARCH PAPER Hollien, J. & Weissman, J. S. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science* **313**, 104–107 (2006)
FURTHER READING Ron, D. Stressed cells cope with protein overload. *Science* **313**, 52–53 (2006)
WEB SITE
Jonathan Weissman's laboratory: http://weissmanlab.ucsf.edu

Structure watch

START AS YOU MEAN TO GO ON

In *Nature Structural & Molecular Biology*, two papers now show that the core machinery that initiates DNA replication has a conserved structure in all domains of life (archaea, bacteria and eukarya). Initiators, which belong to the AAA+ (ATPases associated with various cellular activities) family of proteins, drive DNA remodelling at replication origins. Structural insights into initiators in bacteria and eukaryotes have now been provided by Berger, Botchan, Nogales and colleagues.

In the first paper, the Berger group describes a crystal structure of the initiator protein DnaA from *Aquifex aeolicus* bound to a non-hydrolysable ATP analogue. Rather than forming an oligomeric closed-ring structure, as has been seen for most AAA+ proteins so far, the DnaA monomers form an oligomeric right-handed superhelix. This structure indicates how DnaA binds and unwinds replication origins, and also indicates that other regulatory AAA+ proteins can bind to the exposed ends of the DnaA superhelix. In the second paper, Botchan, Nogales, Berger and co-workers present electron-microscopy reconstructions of the *Drosophila melanogaster* origin recognition complex (ORC). Docking a DnaA–'ATP' helical pentamer into the ORC reconstructions revealed a good fit, which indicates that a right-handed superhelix also forms the core of the eukaryotic initiation complex. Together with previous work in archaea, these studies indicate "…strong mechanistic commonalities in the ways that initiators engage and remodel replication origins…."

ORIGINAL RESEARCH PAPERS Erzberger, J. P. *et al.* Structural basis for ATP-dependent DnaA assembly and replication-origin remodeling. *Nature Struct. Mol. Biol.* **13**, 676–683 (2006) | Clarey, M. G. *et al.* Nucleotide-dependent conformational changes in the DnaA-like core of the origin recognition complex. *Nature Struct. Mol. Biol.* **13**, 684–690 (2006)

A DIMER OF DIMERS

p53 binds to DNA as a homotetramer to control the transcription of genes that mediate cellular stress responses. Although important insights into p53–DNA binding were provided over a decade ago by a structure of a p53 DNA-binding core domain (p53DBD) monomer bound to DNA, many questions remained. However, new insights into this interaction have now been provided by Marmorstein and colleagues in *The Journal of Biological Chemistry*.

The authors managed to obtain a crystal structure of a p53DBD dimer bound to DNA by using a crosslinking strategy. The overall protein fold and the DNA contacts are similar to those seen previously. However, this structure shows that the p53DBDs dimerize through their zinc-binding domains over the central DNA minor groove, and it seems that this dimerization interface is a hot spot for cancer-related mutations. Furthermore, the structure enabled the authors to model a dimer of p53DBD dimers bound to DNA, which indicates that dimer–dimer contacts are less frequently mutated in human cancers than intra-dimer contacts.

ORIGINAL RESEARCH PAPER Ho, W. C. *et al.* Structure of the p53 core domain dimer bound to DNA. *J. Biol. Chem.* **281**, 20494–20502 (2006)

SIGNAL TRANSDUCTION

Up close and personal

Cells respond to environmental and developmental cues by activating signal-transduction pathways that drive specific patterns of gene expression. The classic teaching is that signal kinases, the enzymatic components of these pathways, modulate gene expression from a distance, phosphorylating intermediaries that occupy and regulate target genes. A recent study in *Science*, however, challenges this view. Richard Young and colleagues show that, in *Saccharomyces cerevisiae*, activated signal kinases physically associate with their target genes, implying that kinases might function as intimate components of the transcriptional regulatory machinery.

The authors used chromatin immunoprecipitation and microarray analysis (ChIP-on-chip) to analyse the association of signal kinases with target genes in *S. cerevisiae*. They studied kinases from three signal-transduction pathways: the mitogen-activated protein kinase (MAPK) Hog1, the pheromone-responsive MAPKs Fus3 and Kss1, and the protein kinase A catalytic subunits Tpk1, Tpk2 and Tpk3. All the signal kinases, except Tpk3, colocalized with active target genes. But whereas Hog1 was recruited mainly to the promoter and, to a lesser degree, to the coding regions of its active target genes, Fus3, Kss1 and Tpk1 were recruited primarily to coding regions. Tpk2, on the other hand, localized to the promoter of its target genes, and its occupancy was unaffected by gene-expression status. Surprisingly, the adaptor protein of the pheromone-response pathway,

TUMORIGENESIS

Decidedly different

The aberrant expression of oncogenes induces tumour development, but it also triggers apoptosis or cell-cycle arrest. So, cooperating mutations are needed to overcome these barriers, but which is more cooperative: loss of apoptosis or loss of cell-cycle arrest?

Previous work from Gerard Evan's group has shown that the activation of a regulatable form of the MYC protein (MYC–ER^{TAM}) in the insulin-secreting islet β-cells of the pancreas (*pIns–Myc–ER^{TAM}* mice) results in the apoptosis of these cells. Inhibiting MYC-induced apoptosis through the expression of the anti-apoptotic protein BCL-X_L results in an increase in β-cell numbers. Other mutations, such as the loss of the tumour suppressors ARF or p53, also cooperate with MYC, but do they also work by suppressing MYC-induced apoptosis?

Evan's group crossed the *pIns–Myc–ER^{TAM}* mice with *Arf*$^{-/-}$ or *Trp53*$^{-/-}$ mice. Surprisingly, the *pIns–Myc–ER^{TAM}/Arf*$^{-/-}$ mice had higher rates of MYC-induced apoptosis than *pIns–Myc–ER^{TAM}* mice and higher rates of proliferation than the β-cells that expressed both MYC–ER^{TAM} and BCL-X_L. However, this increased proliferation in the *pIns–Myc–ER^{TAM}/Arf*$^{-/-}$ mice did not cause an expansion of the population owing to the high levels of apoptosis. β-cells from the *pIns–Myc–ER^{TAM}/Trp53*$^{-/-}$ mice showed an increased rate of proliferation similar to the *pIns–Myc–ER^{TAM}/ Arf*$^{-/-}$ mice, but rates of apoptosis were lower in this case than in *pIns–Myc–ER^{TAM}* mice.

The aberrant expression of MYC normally induces the expression of ARF, which leads to the stabilization of p53, expression of the anti-proliferative cyclin-dependent-kinase inhibitor p21 and the induction of apoptosis. The expression of p21 does not occur in *Arf* or *Trp53* knockout cells, which leaves MYC-induced proliferation unchecked, but apoptosis is not affected. These data imply several important points. First, that ARF limits the oncogenicity of MYC by inhibiting proliferation; second, that this is p53 dependent; and third, ARF does not limit the oncogenicity of MYC by inducing apoptosis. The lack of p21 expression also explains the increased proliferation in the *pIns–Myc–ER^{TAM}/ Trp53*$^{-/-}$ mice. The authors suggest that MYC-induced apoptosis is partially inhibited in the p53-deficient mice, because p53 is directly involved in inducing apoptosis by upregulating the expression of pro-apoptotic genes such as *Puma*, *Noxa* and *BAX*.

As ARF loss cooperated with MYC by increasing proliferation, and BCL-X_L cooperates by suppressing MYC-induced apoptosis, will the combination

Ste5, colocalized with Fus3 and Kss1 at the coding regions. Ste5 is known to interact with Fus3 and Kss1 at the plasma membrane, which indicates that adaptor proteins that usually mediate interactions at the cell periphery might well perform similar functions in the nucleus.

The localization of signal kinases to different regions of genes could reflect their preferential binding to proteins (such as transcription factors or chromatin modifiers) that associate with different genomic sites. This could, in turn, reflect novel and interactive roles of kinases at their target genes.

Shannon Amoils

ORIGINAL RESEARCH PAPER Pokholok, D.K. *et al.* Activated signal transduction kinases frequently occupy target genes. *Science* **313**, 533–536 (2006)
FURTHER READING Edmunds, J. W. & Mahadevan, L. C. Protein kinases seek close encounters with active genes. *Science* **313**, 449–451 (2006)

of ARF loss and BCL-X_L expression further increase the oncogenicity of MYC? When MYC was activated, *pIns–Myc–ER^{TAM}/RIP7–Bcl-x$_L$/Arf$^{-/-}$* mice rapidly developed invasive tumours in which apoptosis was suppressed. Because MYC-induced apoptosis is partially suppressed in the p53-deficient background, *pIns–Myc–ER^{TAM}/Trp53$^{-/-}$* mice also develop invasive tumours. However, the tumours remain histologically different — there is no apoptosis in the tumours of *pIns–Myc–ER^{TAM}/RIP7–Bcl-x$_L$/Arf$^{-/-}$* mice, but apoptosis persists in the tumours of *pIns–Myc–ER^{TAM}/Trp53$^{-/-}$* mice.

These results increase our understanding of how these pathways synergize in mouse models, but the relative importance of suppression of apoptosis and further increased proliferation is still unclear in the evolution and protracted development of human tumours.

Nicola McCarthy
Senior Editor, Nature Reviews Cancer

ORIGINAL RESEARCH PAPER Finch, A. *et al.* Bcl-xL gain for function and ARF loss of function cooperate oncogenically with Myc *in vivo* by distinct mechanisms. *Cancer Cell* **10**, 113–130 (2006)
FURTHER READING Pelengaris, S., Khan, M. & Evan, G. c-myc: more than just a matter of life and death. *Nature Rev. Cancer* **2**, 764–776 (2002)

APOPTOSIS

Model behaviour

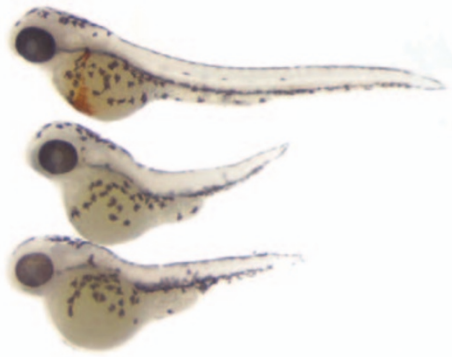
The zebrafish *Danio rerio* is a valuable model organism for studying various physiological processes. In two reports in *Cell Death & Differentiation*, Avi Ashkenazi and colleagues now demonstrate the potential use of zebrafish in the study of apoptotic pathways.

In the first paper, Ashkenazi and colleagues investigated the evolutionary conservation of the BCL2-protein family, which controls the intrinsic apoptosis pathway. They found that the zebrafish genome encodes homologues of most mammalian BCL2-family members, including the pro-apoptotic multi-BCL2-homology (BH)-domain proteins BAX and BAK, anti-apoptotic multi-BH-domain proteins (for example, BCL2 and MCL1) and pro-apoptotic BH3-only proteins (for example, BAD and PUMA). Overall, the multi-BH-domain proteins have the highest degree of homology to their mammalian counterparts, whereas the BH3-only proteins — except for their crucial BH3 domain — have diverged significantly from their mammalian counterparts.

Are these homologues functionally related to their mammalian counterparts? Indeed, ectopic embryonic expression of most zebrafish pro-apoptotic BCL2-family proteins resulted in caspase-3 processing and apoptotic death, and co-expression of the zebrafish anti-apoptotic BCL2-family proteins rescued embryos from apoptotic death. Furthermore, DNA-damage-induced apoptosis depended on zebrafish Puma and the Bax homologues. Ectopic expression of any of the anti-apoptotic BCL2-family homologues was sufficient to prevent DNA-damage-induced apoptosis.

The authors also showed that endogenous Mcl1a and Mcl1b, but not the BCL2-family homologue Blp2, are crucial for early zebrafish development. Mcl1a and Mcl1b protect embryos from death-ligand-induced apoptosis early in development, indicating that loss of anti-apoptotic members of the intrinsic pathway confers sensitivity to the extrinsic apoptotic pathway, which Ashkenazi and co-workers investigated in a separate report.

In contrast to the intrinsic apoptotic pathway, which exists in all metazoans, the extrinsic pathway is a more recent evolutionary development. For example, no tumour-necrosis factor (TNF) proteins have been identified in *Caenorhabditis elegans*, and TNF-ligand binding activates initiator caspases indirectly in *Drosophila melanogaster*. So, the zebrafish might



A wild-type embryo (top) and two embryos that have been injected with the TRAIL homologue Dl1b (bottom); all three have been stained to show that red blood cells are lost upon Dl1b overexpression. Image courtesy of P. Eimon, Genentech, San Francisco, USA.

provide an opportunity to study the extrinsic apoptotic pathway in a non-mammalian vertebrate.

The Ashkenazi team identified a single FasL homologue, four TNF-related apoptosis-inducing ligand (TRAIL) relatives, homologues of TNF receptors and other components of the extrinsic cell-death machinery. Immunoprecipitation experiments with tagged fusion proteins constructed from three of the TRAIL homologues showed that they bind the zebrafish haematopoietic death receptor (Hdr) and the ovarian TNF receptor (Otr). Ectopic expression of these ligands during embryogenesis induced localized apoptosis in erythroblasts and notochord cells. Knockdown of *hdr* or *otr* with antisense morpholino oligonucleotides blocked ligand-induced apoptosis, as did ectopic expression of the anti-apoptotic BCL2-family homologues.

So, the intrinsic and the extrinsic apoptotic pathways in zebrafish closely resemble their mammalian counterparts. Furthermore, the extrinsic pathway cooperates with the intrinsic pathway to trigger tissue-specific apoptosis following ectopic embryonic expression of TRAIL homologues. The authors propose that the zebrafish provides a promising model to investigate the function and mechanisms of apoptotic pathways in vertebrate tissues and during embryogenesis.

Rebecca Robey, Assistant Editor,
Cell Death & Differentiation

ORIGINAL RESEARCH PAPERS Kratz, E. *et al.* Functional characterization of the Bcl-2 gene family in the zebrafish. *Cell Death Diff.* 4 August 2006 (doi:10.1038/sj.cdd.4402016) | Eimon, P. M. *et al.* Delineation of the cell-extrinsic apoptosis pathway in the zebrafish. *Cell Death Diff.* 4 August 2006 (doi:10.1038/sj.cdd.4402015)

ENDOCYTOSIS

Enter to silence

> …exploiting this natural entry pathway might result in more effective and non-toxic approaches for dsRNA delivery.

To induce RNA interference (RNAi) silencing, exogenous double-stranded RNA (dsRNA) enters cells through an active and specific pathway that involves clathrin-mediated endocytosis, report Andino and colleagues in *Nature Cell Biology*. Although many metazoan cells can take up exogenous dsRNAs and use them to initiate gene silencing, until now the mechanism for this uptake was poorly defined.

The authors worked with cultured *Drosophila melanogaster* S2 cells and first showed that the uptake of dsRNAs from the culture was dependent on their length; dsRNAs of 200 or 1,000 base pairs (bp) could silence their target gene, whereas 21-bp small-interfering RNAs could not. Furthermore, low temperatures adversely affected uptake, which indicates that uptake is an active process.

Andino and co-workers used fluorescence microscopy to analyse the early phases of uptake. They found that dsRNA rapidly bound to the cell surface in a punctate pattern and was subsequently internalized but remained in punctate structures. Data from fluorescence microscopy and biochemical approaches showed that the uptake pathway discriminates between dsRNA and DNA, and also showed that dsRNA seems to accumulate in intracellular structures.

A genome-wide functional screen — an "RNAi of RNAi approach" — was used to identify components of the dsRNA-uptake pathway. The authors used a dsRNA library that targeted 7,216 *D. melanogaster* genes with known homologues in *Caenorhabditis elegans* and mammals, and they identified 23 genes that are specifically involved in exogenous dsRNA uptake and processing. Strikingly, many of these genes encode components of the endocytic pathway such as clathrin, and pharmacologically inhibiting this pathway blocked dsRNA uptake and gene silencing.

Knocking down orthologues of the identified genes in whole *C. elegans* organisms inactivated the RNAi response. This endocytic dsRNA-uptake pathway therefore seems to be evolutionarily conserved and is functionally relevant in intact organisms. As the main obstacle to RNAi therapeutic applications is delivery, Andino and colleagues propose that exploiting this natural entry pathway might result in more effective and non-toxic approaches for dsRNA delivery.

Rachel Smallridge

ORIGINAL RESEARCH PAPER Saleh, M.-C. *et al.* The endocytic pathway mediates cell entry of dsRNA to induce RNAi silencing. *Nature Cell Biol.* **8**, 793–802 (2006)
FURTHER READING Meister, G. & Tuschl, T. Mechanisms of gene silencing by double-stranded RNA. *Nature* **431**, 343–349 (2004)
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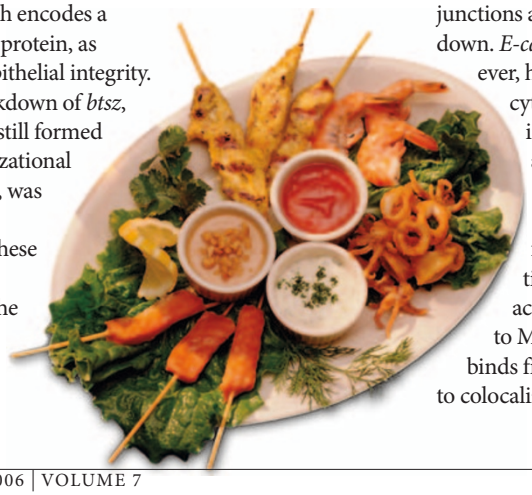
CYTOSKELETON

Nibbling away at actin organization

The actin organization that is responsible for the formation of an adherens junction — which, in turn, connects the actin cytoskeletons of two neighbouring cells — is mediated partly by Bitesize (Btsz). Reporting this finding in *Nature*, Pilot *et al.* explore the knockdown and binding partners of Btsz in *Drosophila melanogaster* embryos.

Using a microarray screen for genes that are involved in epithelial morphogenesis, Pilot *et al.* initially identified *btsz*, which encodes a synaptotagmin-like protein, as being involved in epithelial integrity. Following the knockdown of *btsz*, adherens junctions still formed and a crucial organizational protein, E-cadherin, was still recruited to the junctions. But, these junctions appeared fragmented under the microscope, were unstable, and led to a rapid collapse of the epithelial structure. A time-lapse analysis of the Btsz-deficient adherens junctions showed that E-cadherin localization along the adherens junctions was initially normal — that is, homogeneous — but subsequently became heterogeneous. These findings, therefore, indicate that Btsz is involved in the stabilization of E-cadherin rather than its localization.

> …Btsz affects the actin cytoskeleton through an E-cadherin-independent pathway…



Additional microscopy studies showed that the actin cytoskeleton was disorganized along adherens junctions as a result of *btsz* knockdown. *E-cadherin* knockdown, however, had less affect on the actin cytoskeleton. As these results indicate that Btsz affects the actin cytoskeleton through an E-cadherin-independent pathway, the researchers further examined the relationship between Btsz and actin. Btsz was shown to bind to Moesin — a protein that also binds filamentous actin — and to colocalize with Moesin and E-cadherin at the sites of adherens junctions in wild-type *D. melanogaster* embryos. But in *btsz*-knockdown or *btsz*-mutant embryos, Moesin localization to the necessary sites was diminished. Furthermore, the over-expression of a Moesin-orthologue mutant that lacked the actin-binding domain of Moesin resulted in defects that were similar to *btsz*-mutant embryos.

The authors' findings show that Btsz binds and localizes Moesin, and thereby affects actin cytoskeleton rearrangement, resulting in the stabilization of E-cadherin molecules, adherens junctions and epithelial organization. But, these Btsz findings leave us hungry for more. Particularly, do the seven mammalian orthologues of Btsz have a similar function in actin organization? And, as synaptotagmin-like proteins have also been linked to vesicle trafficking, is this process implicated in adherens-junction formation?

Asher Mullard

ORIGINAL RESEARCH PAPER Pilot, F. *et al.* Spatial control of actin organization at adherens junctions by the synaptotagmin-like protein Btsz. *Nature* **442**, 580–584 (2006)
FURTHER READING Fehon, R. Polarity bites. *Nature* **442**, 519–520 (2006)