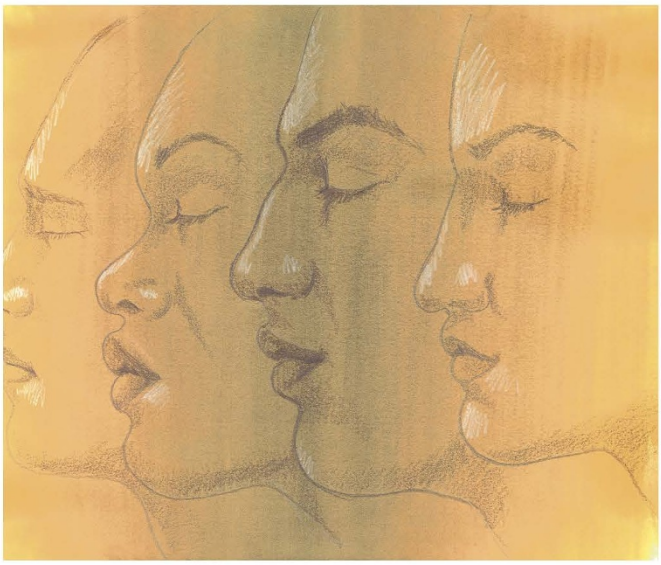
MICRORNA

Silence of the P bodies

Recent studies have indicated that microRNAs (miRNAs) can reduce the levels of their target transcripts and the expression of the translated proteins. Elisa Izaurralde and colleagues now provide further evidence that *Drosophila melanogaster* miRNAs silence gene expression by these two mechanisms, which both require the processing (P)-body component GW182.

Izaurralde and co-workers compared the expression profiles of GW182-depleted cells with those of argonaute-1 (AGO1)-depleted cells (AGO1 mediates gene silencing by miRNAs in *D. melanogaster*). The profiles were strikingly similar, which indicates that these proteins regulate a common set of mRNAs and are therefore likely to function in the same pathway.

> “The present findings place GW182 firmly in the miRNA-silencing pathway…”



By transfecting cells with a GW182 fusion construct that binds with high affinity to a luciferase reporter mRNA target, the authors demonstrated that GW182 silences expression of the bound transcripts. In cells that were depleted of AGO1, GW182 still silenced reporter expression, bypassing the requirement for AGO1, which indicates that GW182 functions downstream of AGO1. The authors also identified the N-terminal domain of GW182 as the AGO1-binding region.

Tethering of GW182 to the reporter mRNA caused a marked reduction in the levels of reporter mRNA, which could not account fully for the observed decrease in luciferase activity. This therefore indicated that GW182-mediated silencing can occur by two mechanisms — repression of protein expression and mRNA degradation. By monitoring the levels of reporter mRNA over time in the presence of GW182, the authors noticed a decrease in the half-life of the mRNA. The mRNAs also shortened slightly over time, which implied that deadenylation had occurred. Further tethering assays revealed that the CCR4–NOT deadenylase complex was required for GW182-mediated mRNA degradation. The DCP1–DCP2 decapping complex was similarly required for mRNA decay by GW182.

Using various reporter constructs that comprised endogenous *D. melanogaster* mRNA targets that are each inhibited by a corresponding miRNA, the authors showed that miRNAs trigger reductions in mRNA levels to different extents. As expected, target-mRNA levels were upregulated and expression levels increased in cells that were depleted of GW182. Silencing of one of the reporter transcripts occurred mainly at the translational level and, as a result, depletion of deadenylase or decapping components did not restore luciferase expression. It is currently unclear however how GW182 represses translation.

The present findings place GW182 firmly in the miRNA-silencing pathway; nevertheless, several questions remain open. Is miRNA-mediated translational repression the cause of mRNA degradation or are these two independent mechanisms? And what determines the degree of mRNA decay and the regulation of gene expression at the level of translation or mRNA stability?

Arianne Heinrichs

ORIGINAL RESEARCH PAPER
Behm-Ansmant, I. *et al.* mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes Dev.* 30 June 2006 (doi:10.1101/gad.1424106)

Web watch

RECIPES FOR RESULTS

- http://www.natureprotocols.com/

We've all spent frustrating hours searching through the methods sections of articles to find the best way to do our experiments. And we've leafed through cookbooks, trying to find that perfect recipe to impress that special someone. Now *Nature Protocols*, which launched in June 2006, has combined these two processes, switched the pinch of frustration for a helping of interactivity, and provided an online, comprehensive, recipe-like collection of biological and biomedical protocols.

Nature Protocols articles are commissioned and peer reviewed, and cover diverse subjects, guaranteeing that there is something for everyone. By the end of 2006, *Nature Protocols* aims to have over 400 published protocols. Alongside the commissioned protocols, the website presents the 'Protocols Network', in which anyone can freely upload their laboratory procedures.

Through an interactive forum, comments, advice and constructive criticism can be read, and written, for all of the protocols on the site, ensuring that protocols are up to date and that you are well informed. The website is searchable, and protocols are linked to other relevant procedures and grouped into categories to allow for easy browsing. And another helpful feature, the 'Tools & Reagents' subsection, details how to make up many commonly used reagents.

Access to the full content of *Nature Protocols* is available through an institutional site license, whereas the interactive access to the 'Protocols Network' is free. So whether you are having troubleshooting difficulties, or are designing your next ground-breaking experiment, log on to *Nature Protocols* and find the perfect protocol that will help you get the results you need to impress that special peer reviewer.

Asher Mullard

MECHANISMS OF DISEASE

VAV proteins get busy

> "Now, the role of VAV proteins is expanding, as two reports show that these proteins also have important functions in other tissues."

The function of VAV proteins has been extensively characterized downstream of immune-response receptors. Now, the role of VAV proteins is expanding, as two reports show that these proteins also have important functions in other tissues. Sauzeau *et al.* found that the loss of VAV3 promotes the gradual development of hypertension and cardiovascular dysfunction in mice, whereas a report by Hunter and colleagues indicates a role for VAV2 and VAV3 as regulators of angiogenesis *in vivo*.

The three members of the VAV family (VAV1–3) are guanine nucleotide-exchange factors (GEFs) for Rho-family GTPases, and they have similar regulatory and catalytic properties. By modulating the activities of Rho and Rac, they regulate the organization of the cytoskeleton. Despite the widespread localization of VAV2 and VAV3 in mouse tissues, most studies have focused on VAV function in the immune system.

To look for other functions of VAV3, Sauzeau and colleagues generated *Vav3*-knockout mice. These mice had high systolic and diastolic blood pressures. In addition, these mice had tachycardia and cardiovascular defects, reminiscent of human defects associated with hypertension. In an attempt to identify whether the renin–angiotensin system (RAS) or the endothelin system — two essential physiological circuits that are involved in hypertension — mediate

CYTOSKELETON

Measuring growth

In *Nature*, Dogterom and colleagues now describe a new optical-tweezers-based method that can be used to observe the assembly dynamics of individual microtubules at molecular resolution. Microtubules — a crucial part of the cytoskeleton in all eukaryotic cells — are dynamic protein polymers that are known to self-assemble from tubulin dimers. However, there has been a lack of information about the molecular events that underlie the growth and shrinkage of microtubules, because studies *in vitro* and *in vivo* have been limited to measurements of average growth and shrinkage rates.

> "…a new optical-tweezers-based method that can be used to observe the assembly dynamics of individual microtubules…"

To obtain information on microtubule dynamics at the resolution of single tubulin dimers (8 nm), Dogterom and co-workers developed a technique based on optical tweezers that allows dynamic microtubule plus ends to grow and shrink against a microfabricated barrier. A bead was attached to an axoneme — a rigid bundle of multiple stabilized microtubules — and the bead–axoneme construct was suspended in an optical trap near a barrier. Microtubule growth was initiated by the addition of tubulin and GTP, and microtubule growth was measured by monitoring bead displacement.

In the presence of tubulin and GTP, the authors observed 20–30-nm stepwise increases in microtubule length. This is significantly larger than the 8-nm size of a tubulin dimer, which indicates that microtubule assembly might not always occur through the addition of individual dimers. When the microtubule-associated protein

the *Vav3*$^{-/-}$ defects, the authors measured the levels of several molecules. They found high levels of renin, angiotensin-converting enzyme and angiotensin II, which indicates that the RAS system contributes to the development of the cardiovascular dysfunction.

Although the molecular mechanism of *Vav3*$^{-/-}$-mediated hypertension remains to be investigated, Sauzeau and colleagues also found that the levels of the catecholamines (adrenaline, noradrenaline and dopamine) of the sympathetic nervous system (SNS) were elevated from the time of birth in the plasma of *Vav3*$^{-/-}$ mice. Treatment with a non-selective β-adrenergic-receptor inhibitor blocked the development of hypertension, tachycardia and heart fibrosis, indicating that the *Vav3*$^{-/-}$ cardiovascular defects are due to an SNS-dependent stimulation of RAS.

The second report highlights a potential mechanism for Vav protein function in another non-haematopoietic process: angiogenesis. Using a yeast two-hybrid screen, Hunter *et al.* identified VAV3 as a binding partner of the EphA2 receptor tyrosine kinase, which is an important regulator of angiogenesis. Deletion-mutant analysis in conjunction with immunoprecipitations showed that both VAV2 and VAV3 are recruited to phosphorylated EphA2 receptors in mammalian cells after treatment with the EphA2 ligand ephrin-A1.

Morphological analysis in mouse embryonic fibroblasts showed that *Vav2- Vav3*-deficient cells have impaired spreading on ephrin-A1-coated surfaces. Furthermore, activation of VAV2 and VAV3 by the EphA2 receptor induced Rac1 GTPase activity and cell migration of endothelial cells. Based on the defects of *Vav2- Vav3*-deficient cells, the authors then examined *Vav2*$^{-/-}$ *Vav3*$^{-/-}$ mice and found that loss of both VAV2 and VAV3 is required for ephrin-A1-mediated angiogenic remodelling *in vivo*.

Not bad for a family of proteins that was thought 'only' to function in the immune system. The analysis of the *Vav2*$^{-/-}$ *Vav3*$^{-/-}$ mutant mice should provide further insights into the functions of these molecules in various tissues.

Ekat Kritikou

ORIGINAL RESEARCH PAPERS Sauzeau, V. *et al.* Vav3 proto-oncogene deficiency leads to sympathetic hyperactivity and cardiovascular dysfunction. *Nature Med.* 11 June 2006 (doi:10.1038/nm1426) | Hunter, S. G. *et al.* Essential role of Vav family guanine nucleotide exchange factors in EphA receptor-mediated angiogenesis. *Mol. Cell. Biol.* **26**, 4830–4842 (2006)

XMAP215 was included in the assay, the growth was markedly enhanced such that rapid length increases of 40–60 nm were observed. These observations indicate that "small tubulin oligomers are able to add directly to growing microtubules and that XMAP215 speeds up microtubule growth by facilitating the addition of long oligomers." In the future, it will be interesting to use this technique to investigate how other types of microtubule-associated protein regulate microtubule dynamics.

Rachel Smallridge

ORIGINAL RESEARCH PAPER Kerssemakers, J. W. J. *et al.* Assembly dynamics of microtubules at molecular resolution. *Nature* 25 June 2006 (doi:10.1038/nature04928)
FURTHER READING Howard, J. & Hyman, A. A. Dynamics and mechanics of the microtubule plus end. *Nature* **422**, 753–758 (2003)
WEB SITE
Institute for Atomic and Molecular Physics, Dutch Foundation for Fundamental Research on Matter: http://www.amolf.nl

IN BRIEF

DNA REPLICATION

Live-cell imaging reveals replication of individual replicons in eukaryotic replication factories.

Kitamura, E. *et al. Cell* **125**, 1297–1308 (2006)

The authors developed a new assay to study the dynamics of DNA replication in single cells using time-lapse microscopy. Their findings support the long-standing hypothesis that sister replication forks that are generated from the same origin remain associated with each other in a replication factory during replication. In addition, the formation of replication factories was found to depend on Cdc6, which is required for DNA replication initiation. The authors therefore propose that replication-factory formation is a consequence of DNA replication.

DNA REPAIR

Histone modification-dependent and -independent pathways for recruitment of checkpoint protein Crb2 to double-strand breaks.

Du, L.-L. *et al. Dev. Cell* **20**, 1583–1596 (2006)

Live immunofluorescence-microscopy studies were combined with genetic analysis to investigate the role of the *Schizosaccharomyces pombe* protein Crb2, a mediator of the DNA-damage response. Du and co-workers show that Crb2 relocalizes to sites of DNA double-strand breaks and that this relocalization requires the modification of histones H2 and H4. However, neither of these modifications is required for Crb2 to carry out its mediator function. A second recruitment mechanism is independent of histone modifications, and such dual recruitment mechanisms are thought to be a common feature of DNA-damage-checkpoint mediators.

MORPHOGENESIS

Coordinated cell-shape changes control epithelial movement in zebrafish and *Drosophila*.

Köppen, M. *et al. Development* **133**, 2671–2681 (2006)

In this study, the authors show that the process of spreading the outer epithelium of the zebrafish embryo over the yolk cell surface and the underlying cells requires the localized recruitment of actin and myosin-2 in the yolk cytoplasm. This process also requires the Ste20-like kinase Msn1, an orthologue of *Drosophila melanogaster* Misshapen. Similarly, these three proteins are needed during dorsal closure in *D. melanogaster*. Together, these data hint at the existence of a conserved mechanism that coordinates cell-shape changes during epithelial morphogenesis.

NUCLEAR TRANSPORT

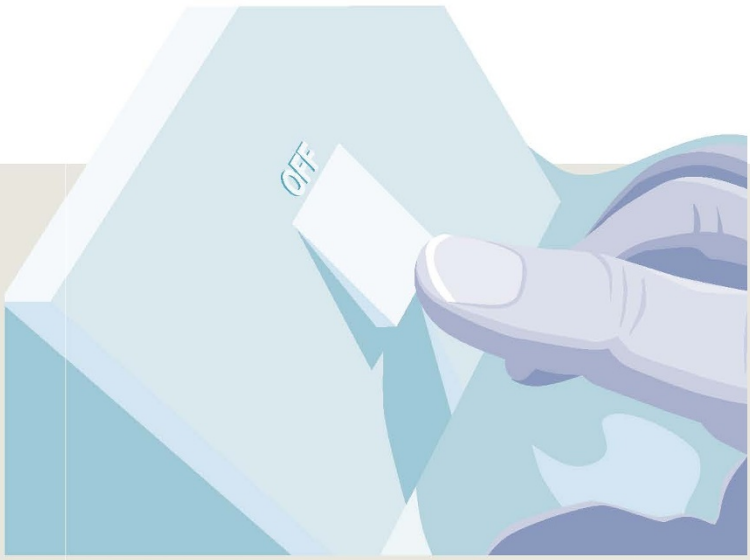
Flexible phenylalanine–glycine nucleoporins as entropic barriers to nucleocytoplasmic transport.

Lim, R. Y. H. *et al. Proc. Natl Acad. Sci. USA* **103**, 9512–9517 (2006)

The nature of the selective barrier-gate of nuclear pore complexes (NPCs) is poorly understood. About 30% of the nucleoporins, which make up the NPC, contain a domain that is enriched in Phe–Gly (FG) repeats. Tethering of the FG domain of nucleoporin Nup153 to the surface of so-called nanodots showed that this domain is unfolded and flexible. Atomic force microscopy analysis showed that it forms brush-like conformations, which function as a repulsive entropic barrier at the nuclear pore.

CANCER

p53 turns on the energy switch



More than 50 years ago, Otto Warburg showed that cancer cells downregulate their aerobic respiratory activity and preferentially use glycolytic pathways to generate energy. But how does this change occur in cancer cells? Two reports now show that the tumour suppressor p53, one of the most frequently mutated genes in human cancers, regulates respiratory and glycolytic pathways through its direct transcriptional targets.

Although changes in the mitochondrial cytochrome oxidase c (COX)-complex activity and subunit composition have been reported in cancer cells, the mechanisms that control these changes remain unclear. Matoba and colleagues investigated the role of p53, one of the usual suspects commonly altered in cancers, in the assembly and activity of the COX complex. Measurements of aerobic respiration in liver mitochondria preparations from wild-type as well as $p53^{+/-}$ and $p53^{-/-}$ mutant mice showed that the loss of p53 was associated with a significant decrease in oxygen consumption and COX enzymatic activity, as previously shown for p53-deficient cells. p53-deficient cells generate significantly higher levels of lactate, which indicates that these cells generate more energy through glycolysis.

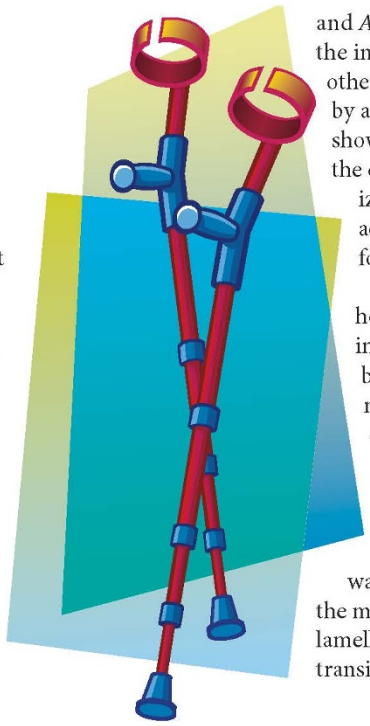
> “…p53, one of the most frequently mutated genes in human cancers, regulates … glycolytic pathways through its direct transcriptional targets.”

How does p53 mediate this effect on mitochondrial respiration? SCO2, which is required for the assembly of the mitochondrial DNA-encoded COX II complex, was identified as a possible regulator of this change by looking at a serial analysis of gene expression (SAGE) database of potential p53 target genes. p53 expression induced *SCO2* mRNA expression, and SCO2 protein levels decreased in p53-deficient mouse liver mitochondria and human cell lines. The expression of wild-type SCO2 in $p53^{-/-}$ cells increased mitochondrial oxygen consumption. Finally, disruption of the *SCO2* locus in human cells could phenocopy the p53-deficient cells — the cells displayed a decrease in oxygen consumption and an increase in glycolytic activity, with total ATP production remaining unaffected.

In a second report, Bensaad *et al.* identified TIGAR (TP53-induced glycolysis and apoptosis regulator), a novel p53 target, which regulates glycolysis and protects against oxidative stress. The sequence similarity between TIGAR and the bisphosphatase domains of the different isoforms of the enzyme PFK-2/FBPase-2 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase) prompted the authors to investigate whether TIGAR might also function as a fructose bisphosphatase and regulate the levels of fructose-2,6-bisphosphate (Fru-2,6-P_2), which stimulates glycolysis. *TIGAR* expression in cells was associated with a concomitant decrease in

CYTOSKELETON

A modifier of motility



The arginylation of β-actin regulates the actin cytoskeleton and cell motility, report Kashina and colleagues in *Science*. The post-translational addition of an Arg residue to the N terminus of a protein is important for embryogenesis, cardiovascular development and angiogenesis. However, until now, the molecular effects of arginylation, which is catalysed by Arg–tRNA protein transferase-1 (ATE1), and the proteins that are arginylated *in vivo* were largely unknown.

Actin undergoes N-terminal processing *in vivo* in such a way that it could become a target for arginylation. By analysing the actin isoforms in whole-cell lysates, the authors showed that Arg can be added to residue Asp3 of β-actin. They also estimated that ~40% of β-actin is arginylated *in vivo*.

So what effect does this modification have? Arginylation has been proposed to mark proteins for degradation, but the authors found that β-actin stability was unaffected by this modification. Furthermore, by comparing immunoprecipitations from wild-type and $ATE1^{-/-}$ cells, they found that the interactions of β-actin with other proteins were unaffected by arginylation. However, they showed that arginylation affected the capability of actin to polymerize; in $ATE1^{-/-}$-cell extracts, actin filaments clustered and formed filamentous aggregates.

Next, the authors analysed how arginylation affects the intracellular functions of β-actin by comparing the morphology, motility and actin cytoskeleton of wild-type and $ATE1^{-/-}$ cells. In the absence of arginylation, the intracellular β-actin distribution was altered and lamella formation was disrupted, which reduced the motility of $ATE1^{-/-}$ cells. These lamella defects could be rescued by transiently transfecting $ATE1^{-/-}$ cells

Fru-2,6-P_2 levels and inhibition of glycolysis, whereas small interfering RNA (siRNA)-mediated knockdown of *TIGAR* resulted in an increase in both Fru-2,6-P_2 levels and glycolytic rates.

To examine how inhibition of glycolysis by TIGAR could confer a survival advantage, the authors then investigated whether modulation of glycolytic rates could have an effect on the apoptotic sensitivity of cells. siRNA-mediated knockdown of *TIGAR* in several cell lines led to an enhanced sensitivity to apoptosis following p53 activation. Next, Bensaad *et al.* showed that TIGAR regulates the levels of intracellular reactive oxygen species (ROS) and selectively inhibits ROS-mediated apoptosis. The authors propose that TIGAR might modulate the apoptotic response, allowing cells to survive following stress signals that can be reversed or repaired.

These two proteins, SCO2 and TIGAR, provide important insights into the coordinate regulation of aerobic respiration, glycolysis and apoptosis by p53. Whether they can be specifically targeted in human cancers remains elusive.

Ekat Kritikou

ORIGINAL RESEARCH PAPERS Matoba, S. *et al.* p53 regulates mitochondrial respiration. *Science* **312**, 1650–1653 (2006) | Bensaad, K. *et al.* TIGAR, a novel regulator of glycolysis and apoptosis, is induced by p53. *Cell* 14 July 2006 (doi:10.1016/j.cell.2006.05.036)

with a 'permanently arginylated' β-actin construct.

Kashina and co-workers have therefore shown that arginylation can have wide-ranging effects on the molecular and cellular levels through a single protein target. They propose that the arginylation of β-actin adds bulky positive charges to actin filaments to prevent them from aggregating. This would facilitate the assembly of a loose actin network at the leading edge and regulate lamella formation in motile cells.

Rachel Smallridge

ORIGINAL RESEARCH PAPER Karakozova, M. *et al.* Arginylation of beta actin regulates actin cytoskeleton and cell motility. *Science* 22 June 2006 (doi:10.1126/science.1129344)

WEB SITE

Anna Kashina's laboratory: http://www.med.upenn.edu/camb/faculty/cbp/kashina.html

NUCLEAR TRANSPORT

Active on the spot

The regulation of mRNA nuclear export is crucial for accurate gene expression in eukaryotes and requires the remodelling of mRNAs into ribonucleoprotein complexes (mRNPs). The DEAD-box helicase Dbp5 is essential for mRNA export, but its precise role is poorly understood. Two reports in *Nature Cell Biology* now show that, in *Saccharomyces cerevisiae*, Dbp5 requires the nuclear pore complex (NPC)-associated protein Gle1 and inositol hexakisphosphate ($InsP_6$) for its local activation at the nuclear pore.

Karsten Weis and colleagues demonstrated that Dbp5 is an ATP-dependent single-stranded-RNA-binding protein, and confirmed previous findings that its ATPase activity probably requires one or more cofactors. One such cofactor turned out to be the cytoplasmic NPC component Gle1, as shown by the Weis group and, in another report, by Susan Wente and colleagues. Both groups measured the catalytic efficiency of Dbp5, which increased significantly in the presence of Gle1. In addition, Weis and co-workers characterized a *gle1* allele with a temperature-sensitive growth defect that was deficient for Dbp5 activation *in vitro* and for mRNA export *in vivo*, which confirmed that Gle1 is an essential cofactor of Dbp5.

The Wente group had previously uncovered a correlation between inositol polyphosphates and mRNA export. Genetic analysis by Wente and colleagues showed that a double mutant that was defective for $InsP_6$ production and for either Gle1 or Dbp5 was lethal. This supports the hypothesis that $InsP_6$ is required for an mRNA-export pathway that requires Gle1 and Dbp5. They also showed that the overexpression of Dbp5 rescued the growth defect of a conditional double mutant that was defective for efficient Gle1 localization and for $InsP_6$ production, and, importantly, rescued the mRNA export defects of this mutant.

Both groups demonstrated that Gle1 binds to $InsP_6$ and found that the addition of $InsP_6$ further increased the Gle1-mediated activation of the ATPase activity of Dbp5. Two-hybrid analysis and *in vitro* binding studies showed that $InsP_6$, Gle1 and Dbp5 are required for optimal binding. Weis and co-workers identified dominant suppressor mutations of Dbp5 and Gle1 that rescued the growth defect associated with a lack of $InsP_6$ production.

These data provide evidence for the spatial control of mRNA export by the activation of Dbp5 at the cytoplasmic side of the nuclear pore complex. Future studies will hopefully elucidate the precise role of Dbp5 in remodelling mRNPs during nuclear export *in vivo*.

Arianne Heinrichs

ORIGINAL RESEARCH PAPERS Weirich, C. S. *et al.* Activation of the DExD/H-box protein Dbp5 by the nuclear-pore protein Gle1 and its coactivator $InsP_6$ is required for mRNA export. *Nature Cell Biol.* 18 June 2006 (doi:10.1038/ncb1424) | Alcázar-Román, A. R. *et al.* Inositol hexakisphosphate and Gle1 activate the DEAD-box protein Dbp5 for nuclear mRNA export. *Nature Cell Biol.* 18 June 2006 (doi:10.1038/ncb1427)

FURTHER READING Cole, C. N. & Scarcelli, J. J. Unravelling mRNA export. *Nature Cell Biol.* **8**, 645–647 (2006) | Rocak, S. & Linder, P. DEAD-box proteins: the driving forces behind RNA metabolism. *Nature Rev. Mol. Cell Biol.* **5**, 232–241 (2004)

Technology watch

DETECTING DYNAMICS

A new live-cell-imaging approach that gives insights into the dynamic nature of protein-interaction networks in intact cell nuclei is now described by Day and colleagues in *Nature Methods*. This approach combines the use of photoactivated green fluorescent protein (PA-GFP) and fluorescence resonance energy transfer (FRET) microscopy, and has been named photoquenching FRET (PQ-FRET).

The transcription factor CCAAT/enhancer-binding protein-α (C/EBPα) surprisingly localizes to regions of heterochromatin that are usually associated with transcriptional silencing and are marked by heterochromatin protein-1α (HP1α). The authors therefore developed PQ-FRET to define the interaction between HP1α and C/EBPα. By photoactivating PA-GFP–HP1α, they could monitor the mobility of this protein. Furthermore, using PA-GFP–HP1α as a photoactivatable FRET acceptor and cyan fluorescent protein (CFP)–C/EBPα as a FRET donor, they could quantify the dynamic interaction between HP1α and C/EBPα. They showed, for the first time, that there is a dynamic association of HP1α and C/EBPα in regions of heterochromatin. They have also provided an assay that can directly measure a protein's mobility, its exchange within macromolecular complexes and its interactions with other proteins in living cells.

REFERENCE Demarco, I. A. *et al.* Monitoring dynamic protein interactions with photoquenching FRET. *Nature Methods* **3**, 519–524 (2006)

SEEING INSIDE

The optical absorption of biological tissues is strongly associated with physiological status. Despite this, current high-resolution optical imaging techniques such as confocal microscopy do not detect optical absorption directly. Furthermore, optical scattering prevents these techniques from penetrating deeper than ~1 mm beneath the surface of tissues. However, in *Nature Biotechnology*, Wang and co-workers now describe a functional photoacoustic microscopy (fPAM) system that measures optical absorption directly and has been shown to have an imaging depth of more than 3 mm in live animals.

The irradiation of biological tissues with a short-pulsed laser induces wideband ultrasonic waves that are known as photoacoustic waves. fPAM detects absorbed photons ultrasonically through the photoacoustic effect. The authors have shown that their fPAM system has several *in vivo* imaging applications. For example, they showed that it can be used to image angiogenesis, which is potentially useful for understanding tumour growth and metastasis, for diagnosing cancers and for evaluating therapies. They also showed that fPAM allows the vessel-by-vessel mapping of the oxygen saturation of haemoglobin, which permits the monitoring of, for example, oxygen consumption by tumours. This technique, which is safe for human subjects, is therefore likely to have applications in clinical and basic research.

REFERENCE Zhang, H. F. *et al.* Functional photoacoustic microscopy for high-resolution and noninvasive *in vivo* imaging. *Nature Biotechnol.* 25 June 2006 (doi:10.1038/nbt1220)

CELL SIGNALLING

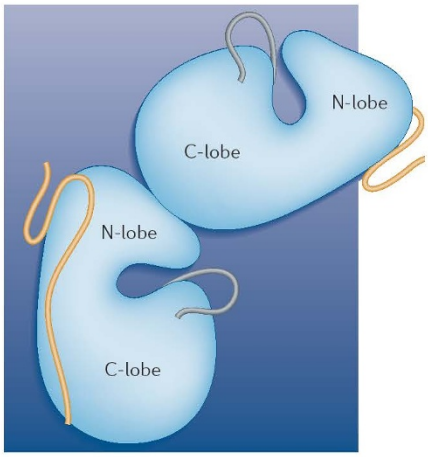
Asymmetry in action

Epidermal growth factor (EGF) causes its receptor, EGFR, to dimerize and — through a previously unknown mechanism — become activated to effect downstream signalling, cell growth, proliferation and differentiation. Although EGFR is crucial for healthy cell growth, its mutant variants are linked to several forms of cancer. Zhang, Kuriyan and colleagues examined the structural mechanisms of EGFR activation and report their findings in *Cell*, providing insights for cancer treatments.

Following crystallography screens, these researchers studied two forms of active-conformation EGFR kinase-domain dimers. The first dimer was symmetrical, with the two kinase domains interacting in a mutual head-to-tail fashion. By contrast, the second dimer was asymmetrical, with the C-lobe of one monomer (B) nestled against the N-lobe of the other monomer (A) (see figure). Kuriyan and colleagues proposed that, in the asymmetrical dimer, the C-lobe of monomer B forces the allosteric activation of monomer A, in a manner that is similar to the activation of cyclin-dependent kinases by cyclins.

To test this idea, specific residues that are central to the symmetrical dimer interface were mutated. These mutations had no effect on the phosphorylation of residues that are indicative of EGFR activation. The fact that mutations of the dimer interface do not affect activation indicates that the symmetrical dimer is not involved in the activation process. But, when a similar mutational study was carried out on residues of the asymmetrical dimer interface, the phosphorylation of the same 'EGFR-activity-indicative' residues was almost completely abrogated, indicating that this dimer interface is crucial for EGFR activation.

To check that the abrogation of EGFR activity was not due to misfolding, two models were tested.



Representation of the asymmetrical dimer of the EGFR kinase domain that has been shown by Zhang *et al.* to be involved in the activation of EGFR. Adapted from figure 6 of the highlighted paper.

A monomer with a mutation in its C-lobe interface region should be able to be activated, but not function as an activator. Conversely, a monomer with a mutation in its N-lobe interface region should be able to function as an activator, but be activation resistant. Co-transfectional studies with mutated EGFR showed that these predictions were correct.

Furthermore, the researchers solved the crystal structure of a form of the EGFR kinase-domain dimer that was mutated in the C-lobe face of the dimer interface. As expected, this mutant displayed an inactive conformation, confirming the important role of the dimer interface in triggering the active conformation of the kinase. This inactive structure also explains the activating effects of two sets of EGFR mutations that are commonly found in human lung cancers.

The EGFR signalling pathway is crucial in normal development, and its malfunction, as shown by cancer-causing mutations of EGFR, is disastrous. Zhang *et al.* describe a mechanism of allosteric activation that allows greater levels of regulation than standard activation by *trans*-phosphorylation. They also show that, although symmetry is thought to be beautiful, in the case of EGFR, it's asymmetry that turns things on.

Asher Mullard

ORIGINAL RESEARCH PAPER Zhang, X. *et al.* An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell* **125**, 1137–1149 (2006)

STEM CELLS

Nanog: the gift of choice

Although mouse embryonic stem (ES) cells were first isolated 25 years ago, many important questions about these remarkable cells remain unanswered. For instance, it's well known that ES cells can 'donate' pluripotency to somatic cells after ES–somatic cell fusion; however, the identity of the ES cell-derived factors that reset the epigenome of the somatic cell is unknown. Austin Smith and colleagues now reveal that the transcription factor Nanog is crucial for this reprogramming process.

Nanog is a homeodomain protein that is expressed in early embryos and that maintains pluripotency in ES cells. But does Nanog also have a role in the transfer of pluripotency?

To answer this question, Smith and co-workers first showed that overexpression of Nanog markedly increased the yield of pluripotent hybrid colonies formed by fusion of ES cells with neural stem (NS) cells. In the presence of high levels of Nanog, conversion of ES–NS cell hybrids to ES cell colonies was as efficient as that of ES–ES cell fusions.

In a second series of experiments, the authors showed that high levels of Nanog increased the numbers of hybrid colonies formed by fusion of ES cells with more differentiated cells, such as thymocytes or fibroblasts. In both cases, however, fewer colonies were produced compared with the ES–NS cell fusions. Smith and colleagues suggest that these lower hybrid yields could reflect fundamental differences in the epigenome of cells at different developmental stages. Chromatin modifiers might be relatively inactive in ES cells, giving rise to an epigenome that responds readily to transcriptional regulators, endowing the cell with diverse developmental options. As cells differentiate, the addition of repressive chromatin modifications might render the epigenome less malleable, progressively restricting lineage options and ease of reprogramming.

> "...Nanog cooperates with other as-yet-unknown ES cell factors to orchestrate pluripotency."

Finally, transfection of NS cells with a Nanog transgene had no effect on the NS cell phenotype, indicating that Nanog cooperates with other as-yet-unknown ES cell factors to orchestrate pluripotency.

Shannon Amoils

ORIGINAL RESEARCH PAPER Silva, J., Chambers, I., Pollard, S. & Smith, A. Nanog promotes transfer of pluripotency after cell fusion. *Nature* **441**, 997–1001 (2006)

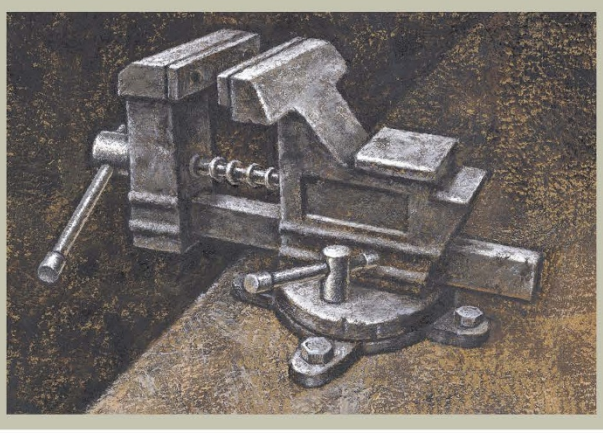
NEUROTRANSMISSION

Clamping down on exocytosis

> "...a reversible clamping protein, known as complexin, provides the crucial link."

Neurotransmitter release is well known to depend on calcium-triggered exocytosis of synaptic vesicles. But what is the mechanism that couples synaptic vesicle fusion and calcium? Giraudo and colleagues report that a reversible clamping protein, known as complexin, provides the crucial link.

SNARE proteins on the presynaptic and vesicle membranes form a complex that mediates vesicle fusion in response to an increase in intracellular calcium that is detected by the calcium-sensing protein synaptotagmin. Complexin is known to bind to exocytotic SNARE proteins, and genetic deletion of complexin and synaptotagmin results in a similar phenotype. Complexin could therefore be a prime candidate to function cooperatively with SNARE proteins and synaptotagmin in calcium-mediated vesicle fusion.



Giraudo and colleagues assessed the effects of ectopically expressed SNARE proteins on the cell surface, which normally lacks the SNARE complex, to understand the role of complexin in regulating fusion. Introducing recombinant human complexin to this 'flipped' SNARE assay abolished, or 'clamped', fusion. By contrast, synaptotagmin, when added on its own or in the presence of calcium, had neither a blocking nor a stimulating effect on fusion, indicating that it is neither a clamp nor a fusion protein. When complexin and synaptotagmin were incubated together, fusion remained blocked, but the addition of calcium in these conditions led to a full recovery.

These data are consistent with a model in which the fusion machinery is constitutively active, but is clamped by complexin during the basal state. This clamping is reversed in response to a trigger for secretion — the binding of calcium to synaptotagmin — thereby allowing fusion to take place.

Although *in vivo* studies will be required to confirm the clamping role of complexin in neurotransmission, this elegant work nevertheless sheds light on a key mechanism of neuronal communication that has until now remained elusive.

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ORIGINAL RESEARCH PAPER Giraudo, C. G. *et al.* A clamping mechanism involved in SNARE-dependent exocytosis. *Science* 22 June 2006 (doi:10.1126/science.1129450)
FURTHER READING Rizo, J. & Südhof, T. C. SNAREs and Munc18 in synaptic vesicle fusion. *Nature Rev. Neurosci.* **3**, 641–653 (2002)