

# HIGHLIGHTS

## HIGHLIGHTS ADVISORS

### JOAN S. BRUGGE

HARVARD MEDICAL SCHOOL,  
BOSTON, MA, USA

### PASCALE COSSART

INSTITUT PASTEUR, PARIS,  
FRANCE

### GIDEON DREYFUSS

UNIVERSITY OF PENNSYLVANIA,  
PHILADELPHIA, PA, USA

### PAMELA GANNON

CELL AND MOLECULAR  
BIOLOGY ONLINE

### JEAN GRUENBERG

UNIVERSITY OF GENEVA,  
SWITZERLAND

### ULRICH HARTL

MAX-PLANCK-INSTITUTE,  
MARTINSRIED, GERMANY

### NOBUTAKA HIROKAWA

UNIVERSITY OF TOKYO, JAPAN

### STEPHEN P. JACKSON

WELLCOME/CRC INSTITUTE,  
CAMBRIDGE, UK

### VICKI LUNDBLAD

BAYLOR COLLEGE OF  
MEDICINE, HOUSTON, TX, USA

### TONY PAWSON

SAMUEL LUNENFELD RESEARCH  
INSTITUTE, TORONTO, CANADA

### NORBERT PERRIMON

HARVARD MEDICAL SCHOOL,  
BOSTON, MA, USA

### THOMAS D. POLLARD

THE SALK INSTITUTE,  
LA JOLLA, CA, USA

### JOHN C. REED

THE BURNHAM INSTITUTE,  
LA JOLLA, CA, USA

### KAREN VOUSDEN

NATIONAL CANCER INSTITUTE,  
FREDERICK, MD, USA

### JOHN WALKER

MRC DUNN HUMAN NUTRITION  
UNIT, CAMBRIDGE, UK

## GENE EXPRESSION

# Be specific

How do you begin to comprehend the complex relationship between chromatin remodelling and transcription? Although many ATP-dependent chromatin-remodelling proteins have been identified, their function in transcription remains poorly defined due to the inherent difficulties in assaying this process. In a recent report in *Nature*, however, Tjian and colleagues describe the identification of a chromatin-remodelling protein complex that is specifically required for ligand-activated transcription from chromatin templates.

To search for transcription cofactors, the authors set up an *in vitro* transcription assay containing chromatin templates that were under the control of a nuclear hormone receptor. Starting with a set of purified proteins that are sufficient to drive transcription from 'naked' DNA, the authors asked which additional factors were required to drive transcription from a chromatin template.

They found an activity in human nuclear fractions that strongly potentiated ligand-dependent transcription from chromatin templates. Purification of this cofactor showed that it was a large multi-protein complex of the SWI/SNF family of chromatin-remodelling proteins, called SWI/SNF-B or polybromo- and BRG1-associated factor-containing complex (PBAF). The members of the SWI/SNF protein family use BRG1 or hBRM as their central ATPase, and this family is the largest

of the four families of chromatin-remodelling proteins.

SWI/SNF complexes share many common subunits, but BAF180, which contains six bromodomains, is unique to PBAF. The bromodomain motif has previously been shown to selectively bind to acetylated histone tails, and this binding could be important in targeting the cofactor complex to chromatin.

Tjian and colleagues wanted to know if other ATP-dependent chromatin-remodelling complexes could mimic the transcriptional activation activity shown by PBAF in the *in vitro* assay. They compared the effects of PBAF, SWI/SNF-A (which shares considerable similarity to PBAF) and ACF (a mechanistically different chromatin-remodelling protein complex) in their assay, and showed that although these proteins could all remodel the chromatin template, only PBAF efficiently activated transcription.

The authors also observed this effect using a class of transcription activators other than nuclear receptors, which showed that chromatin-remodelling proteins are not functionally interchangeable and that PBAF may have a function in transcription that is not limited to its chromatin-remodelling activity.

The finding that PBAF is required for nuclear-receptor-mediated transcription from chromatin templates is the first documented example of a specifically acting chromatin-remodelling protein complex. How PBAF



activates transcription remains unclear, but the *in vitro* assay used by Tjian and colleagues has provided a valuable insight into the functional specificity that can exist among the highly related complexes involved in gene regulation.

Rachel Smallridge

## References and links

**ORIGINAL RESEARCH PAPER** Lemon, B. *et al.* Selectivity of chromatin-remodelling cofactors for ligand-activated transcription. *Nature* **414**, 924–928 (2001)

**FURTHER READING** Flaus, A. & Owen-Hughes, T. Mechanisms for ATP-dependent chromatin remodelling. *Curr. Opin. Genet. Dev.* **11**, 148–154 (2001) | Havas, K. *et al.* ATP-dependent chromatin remodeling activities. *Cell Mol. Life Sci.* **58**, 673–682 (2001)

## WEB SITES

Encyclopedia of Life Sciences:

<http://www.els.net/>

Chromatin remodelling and histone modification in transcription regulation

Robert Tjian's laboratory:

<http://mob.berkeley.edu/faculty/BMB/tjianr.html> |

<http://www.hhmi.org/research/investigators/tjian.html>

## WEB WATCH

In its own little world 

- <http://www.imb-jena.de/RNA.html>

If you're looking for information about RNA, head for the RNA World Website. Set up and maintained by the Institute for Molecular Biology in Jena, Germany, this site does a sterling job of bringing together RNA-related web resources to make the search for relevant information much easier. The site has been updated fairly recently, although this is more than can be said for several of the entries that it links to — probably the only major disadvantage of the site. However, divided into six subcategories, the site should still have something for everyone.

Take, for example, the Books and Tutorials subcategory. For non-specialists, there are five tutorials on ribosomes that provide general structural and functional overviews using a combination of clear diagrams and text. Among other useful topics selected for tutorials are RNA splicing, RNA and DNA as catalysts, and RNA interference (RNAi). The timely RNAi tutorials provide not only an overview of this approach, but also offer informative protocols.

Valuable information is also available within the Software subcategory. Here, various software is available, including programs that predict RNA structures and RNA movies, which “create the impression of an RNA molecule exploring its own 2D structure space”. Within ‘Sequences, Secondary Structures and Other’, you can gain access to the RNA Editing Web Site, whereas in ‘Databases and Web Tools’, you can learn all about the geometrical features of DNA — from A to Z.

Katrin Bussell

## CYTOSKELETON

## Branching out

The actin related proteins Arp2 and Arp3, together with five other protein subunits, form the Arp2/3 complex, which promotes polymerization of G-actin into F-actin and branching of these filaments to form an actin filament network. Pollard and colleagues have now determined the structure of the bovine Arp2/3 complex at 2.0 Å resolution, while Welch's team have reconstituted the human complex by expressing the subunits using a baculovirus expression system. Both studies give a clear insight into the nucleation and branching functions of the Arp2/3 complex.

The crystal structure shows that the seven subunits of the Arp2/3 complex are arranged into a ‘kidney’-shaped flat ellipsoid. At the core of this complex, Arp2 and Arp3 are cradled by a C-shaped clamp formed by a stable heterodimer of the p20 and p34 subunits, which associate through their long carboxy-terminal  $\alpha$ -helices. Welch and colleagues showed that this heterodimer is critical for the integrity of the complex. The crystal structure revealed that the p40 (bovine) subunit is a seven-blade WD40  $\beta$ -propeller, which strengthens the top and bottom of the clamp, while p16 and p21 are globular subunits that are found at opposite edges of the complex.

The three-dimensional structures of Arp2 and Arp3 are similar to that of actin, being bilobed. However, in contrast to actin, ATP is absent, so their central clefts are open. Furthermore, Arp2 and Arp3 are rotated 180° around the filament axis relative to each other, compared with two neighbouring actin subunits. In this conformation, they are unable to nucleate a new filament.

So, Pollard and colleagues propose that activation of the Arp2/3 complex involves a rotation of the two halves of the complex to re-orient Arp2 and Arp3 so that they resemble an actin dimer in conformation, which favours nucleation. They also suggest that Wiskott–Aldrich Syndrome protein (WASP) — and its relatives — favours nucleation by promoting this active conformation. ATP binding to the Arp subunits might also contribute to activation.

In support of these findings, Welch's team showed that subcomplexes of the Arp2/3 complex that lack both Arp3 and p21 showed no nucleation activity, even when nucleation-promoting factors such as ActA and WASP were added. Subcomplexes missing only p21 retained nucleating activity in the presence of ActA or WASP, which is consistent with a crucial role of Arp3 as a nucleation template. Adding ActA or WASP to subcomplexes lacking p16 and p41 (human) only weakly stimulated nucleation, indicating that they have an important function in the structural organization of the nucleation site, inducing conformational changes during nucleation, or binding to ActA or WASP.

Does the crystal structure give us any clues as to how Arp2/3 promotes branching? The structure of branches shows multiple contacts between the Arp2/3 complex and the side of the ‘mother’ filament. Pollard's group suggest that the p40 (bovine) subunit — which contains a helix in a loop between two of its blades — might provide one of these anchors. Welch and colleagues showed that the purified p34/p20 heterodimer co-sedimented with F-actin, indicating that it also binds to actin filaments. Furthermore, they showed that p34/p20 could also crosslink actin filaments, although the structures did not fully resemble the typical ‘Y’ branches that the complete complex can form, implicating other subunits in branching. This is consistent with previous work showing that p34 can be chemically crosslinked to actin. So, further studies are needed to determine all of the subunits that are required for branching. Another challenge will be to fit depolymerizing factors, capping proteins and other actin-binding proteins into the equation.

Katrin Bussell

 **References and links**

**ORIGINAL RESEARCH PAPERS** Robinson, R. C. *et al.* Crystal structure of Arp2/3 complex. *Science* **294**, 1679–1684 (2001) | Gournier, H. *et al.* Reconstitution of human Arp2/3 complex reveals critical roles of individual subunits in complex structure and activity. *Mol. Cell* **8**, 1041–1052 (2001)

**FURTHER READING** Volkman, N. *et al.* Structure of Arp2/3 complex in its activated state and in actin filament branch junctions. *Science* **293**, 2456–2459 (2001)

**WEB SITE**

Matthew Welch's laboratory: <http://mcb.berkeley.edu/labs/welch/>





## Poised for action

When cells enter mitosis, several processes are put on hold — presumably to ensure that all resources are channelled towards the successful completion of division. One abandoned process is RNA polymerase II (RNAPII)-dependent transcription. But how does the cell manage to efficiently pick up where it left off after division?

The dogma has long been that, during chromosome condensation, most transcription factors and RNAPII are displaced. Then, after division, cells get back to work and re-initiate the transcription of previously active genes. Intuitively, this seems like a very inefficient way of doing things, and recent work from the Levens lab has hinted that mitotically silenced genes might retain a ‘bookmark’, which allows them to remember that they were active before mitosis. Now, reporting in *Nature Cell Biology*, Christova and Oelgeschläger unveil the identity of such a bookmark. They show that some basal transcription factors remain bound during mitosis, keeping the promoters of previously active genes poised for post-mitotic action.

One candidate for the putative bookmark was the transcription factor TFIID — it was known, for example, that the complexes formed between TFIID and promoter DNA are extremely stable *in vitro*. A further hint was that TFIID and another transcription factor, TFIIB, associate with mitotic chromosomes *in vitro* on immunoblots.

To test whether any of these factors remain bound during mitosis, the authors carried out chromatin immunoprecipitation experiments. First, they looked at association of these factors in different types of promoter in asynchronous cells. Consistent with expectations, they saw that the TATA-binding protein (TBP), TAF<sub>II</sub>100 and TFIIB were associated with active, but not inactive, RNAPII promoters.

They then compared this occupancy with that in mitotic cells, and

found that TFIIB and TAF<sub>II</sub>100 occupancy were essentially unaffected. Although TBP showed a partial decrease, in view of the fact that TBP is required for TFIIB binding, the authors concluded that both TFIID and TFIIB can remain bound to previously active promoters during mitosis.

One possibility, then, is that genes remain poised for transcription, with a stalled RNAPII sitting at the promoter. To test this, the authors looked at RNAPII promoter occupancy in asynchronous and mitotic cells. In contrast to TFIIB and TFIID, they saw that RNAPII falls off the promoter during division.

This suggests that the TFIIB and TFIID that remain bound cannot initiate the formation of transcriptional complexes. Previous work has suggested that binding of negative cofactors such as the transcriptional repressor NC2 might alter the activity of promoter-bound transcription factors. So does binding of NC2 correlate with the mitotic silencing? Surprisingly, the authors found that on entry into mitosis, NC2 dissociated from some previously active genes, indicating that NC2 does not act as a general mitotic repressor of transcription.

Intriguingly, these results are reminiscent of recent studies in yeast, showing that transcription factors can remain bound to condensed, transcriptionally silent heterochromatin. One striking difference, though, is that RNAPII can also remain bound in the case of yeast heterochromatin. Whether these complexes are mechanistically similar remains to be seen, but one intriguing model, say the authors, is that “mitotic TFIID–promoter complexes may serve to maintain transcriptional competence of genes, perhaps by excluding nucleosomes from core promoter DNA regions, and may facilitate rapid reactivation of transcription upon exit from mitosis”.

Alison Schults

Associate Editor, *Nature Cell Biology*

### References and links

**ORIGINAL RESEARCH PAPER** Christova, R. & Oelgeschläger, T. Association of human TFIID–promoter complexes with silenced mitotic chromatin *in vivo*. *Nature Cell Biol.* **4**, 79–82 (2002)

**WEB SITE**  
Oelgeschläger laboratory:  
<http://www.mrci.ac.uk/egr/egrh.html>



### MEMBRANE DYNAMICS

## Touché!

Can the Golgi form *de novo*, or does it need a stable template? This simple question is at the heart of an intense debate, which, despite alternating victory claims from the two camps, seems to have no end in sight.

The origin of the debate lies in the discovery that, under certain experimental conditions — for example, treatment with the fungal metabolite brefeldin A (BFA) — Golgi enzymes return to the endoplasmic reticulum (ER). About a year ago, Graham Warren’s group reported that not all Golgi proteins return to the ER — a subset, dubbed Golgi matrix proteins, are left behind and form a scaffold around which the Golgi can reassemble. But Brian Storrie’s and Jennifer Lippincott-Schwartz’s groups now report in *The Journal of Cell Biology* that the entire Golgi apparatus, including matrix proteins, cycles in interphase — so the existence of a stable template is unlikely.

Storrie and colleagues tested the effect of an ER exit block, and specifically Warren’s experimental protocol (BFA-induced dispersal of the Golgi followed by washout in the presence of an ER exit block induced by a GTP-restricted Sar1 mutant), on the distribution of 12 different Golgi proteins. They reproduced Warren’s results but, when they used higher concentrations of the GTP–Sar1 mutant, all 12 proteins, including matrix proteins, left the Golgi. Similarly, using a photobleaching technique, Lippincott-Schwartz and colleagues found that none of the four types of Golgi proteins — enzymes, cycling proteins, coats and matrix proteins — are stably associated with this organelle; they all cycle between the Golgi and the ER or cytosol.

But BFA-induced Golgi dispersal is known to leave behind some peripheral structures, called Golgi remnants. So, if the whole Golgi cycles, what are these Golgi remnants? Lippincott-Schwartz and colleagues show that these structures contain Sec13, a component of COPII coats, so they are probably ER exit sites.

Although both groups have shown convincingly that no Golgi proteins are stably associated with this organelle, they have not actually shown that a Golgi can form *de novo* from ER. But this stimulating debate has been with us for so long that it would almost be a shame to see it end.

Raluca Gagescu

### References and links

**ORIGINAL RESEARCH PAPERS** Miles, S. *et al.* Evidence that the entire Golgi apparatus cycles in interphase HeLa cells: sensitivity of Golgi matrix proteins to an ER exit block. *J. Cell Biol.* **155**, 543–555 (2001) | Ward, T. *et al.* Maintenance of Golgi structure and function depends on the integrity of ER transport. *J. Cell Biol.* **155**, 557–570 (2001)

## IN BRIEF

## TECHNIQUE

Profiling the global tyrosine phosphorylation site by Src homology 2 binding.

Nollau, P. & Mayer, B. J. *Proc. Natl Acad. Sci. USA* **98**, 13531–13536 (2001)

The reversible process of tyrosine phosphorylation creates binding sites for Src-homology-2 (SH2) domain-containing proteins. Here, the authors describe a far-Western blot technique for profiling the global tyrosine phosphorylation state in cell protein extracts, which involves probing membranes containing immobilized, electrophoresed proteins with purified recombinant glutathione-S-transferase-SH2-domain peptides. As this approach relies on protein–protein interactions, it could be extended to any protein-domain interaction.

## ENDOCYTOSIS

Regulation of membrane-type matrix metalloproteinase 1 activity by dynamin-mediated endocytosis.

Jiang, A. *et al. Proc. Natl Acad. Sci. USA* **98**, 13693–13698 (2001)

Membrane-type matrix metalloproteinase 1 (MT1-MMP) has an important function in remodelling the extracellular matrix, but how the activity of this cell-surface enzyme is controlled remains poorly understood. In this study, Jiang *et al.* show that this control might be mediated through endocytosis. The authors found that the expression of MT1-MMP on the cell surface is regulated through its cytoplasmic domain by dynamin-mediated internalization into clathrin-coated pits.

## CELL ADHESION

Taking cell–matrix adhesions to the third dimension.

Cukierman, E. *et al. Science* **294**, 1708–1712 (2001)

Most of our knowledge regarding the function of focal and fibrillar adhesions comes from studies of fibroblasts on two-dimensional (2D) tissue culture substrates, but fibroblast morphology and migration differ significantly within three-dimensional (3D) substrates. This report shows that cell adhesions in 3D cultures differ in structure, function and signalling compared to their 2D counterparts, implying that current 2D methods might not be entirely appropriate for studying cell–substrate adhesions.

## CELLULAR MICROBIOLOGY

Extensive surface diversity of a commensal microorganism by multiple DNA inversions.

Krinos, C. M. *et al. Nature* **414**, 555–558 (2001)

Few studies have investigated how commensal organisms in the human intestine evade the host's immune response. Krinos *et al.* now show that *Bacterioides fragilis* alters its surface antigenicity by producing at least eight different capsular polysaccharides — the most observed in a bacterium to date. Polysaccharide expression is subject to phase variation controlled by invertible promoter sequences upstream of the polysaccharide biosynthesis loci. This diversity might also have a role in the pathogenicity of *B. fragilis*.

## DNA REPAIR

## Damage limitation

If you've ever felt the pain of sunburn, you'll be convinced that the body puts up a feeble fight against exposure to the sun. But sunburn, in fact, is the best response, as it is the cue for the body to defend itself against any potentially harmful effects. A report by Schwarz and colleagues in *Nature Cell Biology* identifies an added complexity to this response, and their findings could pave the way to new areas of therapeutic intervention.

Exposure to ultraviolet-B (UVB) radiation induces the apoptotic cell death of keratinocytes, which leads to the appearance of sunburn cells (SCs) in the epidermis. The discovery that p53 is involved in this process led scientists to believe that the formation of SCs was a protective mechanism to destroy cells with irreparable DNA damage that could lead to skin cancer.

But does that mean the body just destroys cells and makes no effort to repair the DNA? Further studies suggested this might not be the case, as cytokines such as interleukin-1 were found to influence UV-induced apoptosis, although the mechanism was unknown.

So Schwarz and colleagues investigated the effects of various cytokines on UVB-induced apoptosis. They found that the immunomodulatory mediator interleukin-12 (IL-12) can inhibit this process in keratinocytes, as exposing epithelial cells to IL-12 *in vitro* before UVB-exposure significantly reduced apoptosis. This was due to a reduction of UVB-induced DNA damage, but this effect was not immediate — it occurred only after a period of time, which the authors suspected could be linked to the induction of DNA repair.

They tested this by looking for single-strand breaks created during nucleotide excision repair (NER) — the main repair mechanism for UVB-induced DNA damage in mammalian cells — using a 'comet' assay. This showed that IL-12 treatment enhances comet length, which meant that IL-12 might induce NER.

The researchers confirmed this by studying *Xpa*-knockout mice, which are severely deficient in NER. These mice had a higher number of SCs compared with wild-type mice that had received the same dose of UVB; and although IL-12 reduced SC numbers in UVB-irradiated wild-type mice, it had no effect on SCs in the knockout mice. This was further confirmed when cells taken from both a patient with xeroderma pigmentosum (which is caused by defects in various components of the NER) and a healthy control were exposed to UVB in the presence or absence of IL-12. IL-12 reduced UVB-induced DNA damage in the healthy cells but not in the cells from the patient.

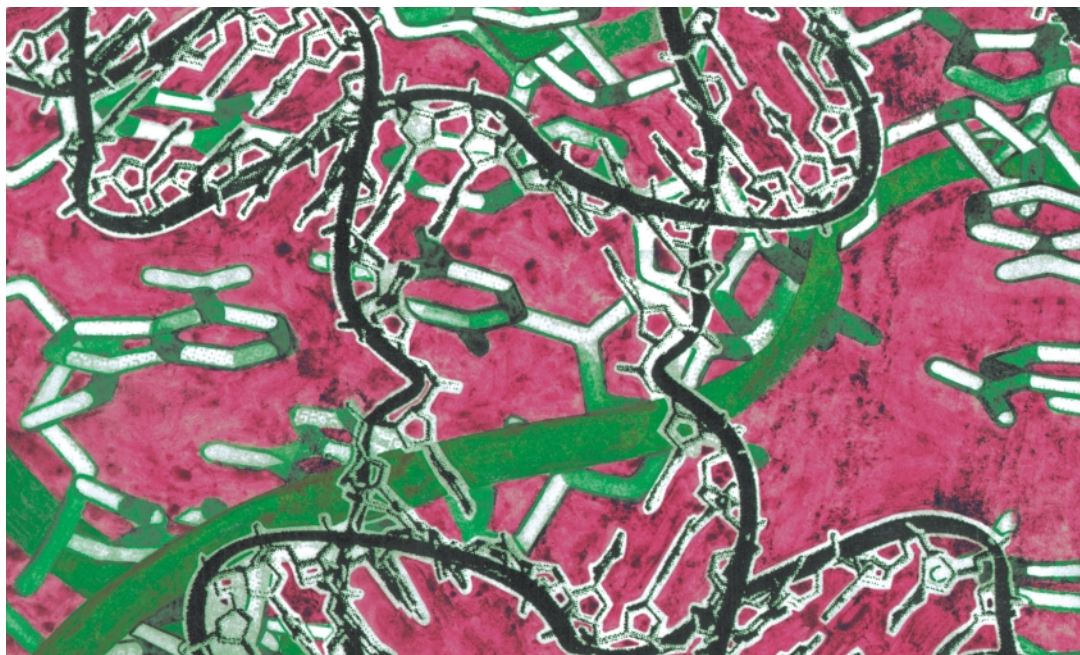
So, this is the first demonstration that cytokines can protect cells from apoptosis in response to DNA damage from UVB by switching on the DNA repair machinery. It remains to be seen whether overexpression of IL-12 could reduce both the number of SCs in humans and the risk of UV-induced skin cancer. But it does offer the exciting therapeutic possibility that topically applied IL-12 could help prevent this disease.

Simon Frantz

## References and links

**ORIGINAL RESEARCH PAPER** Schwarz, A. *et al.* Interleukin-12 suppresses ultraviolet radiation-induced apoptosis by inducing DNA repair. *Nature Cell Biol.* **4**, 26–31 (2002)





DNA REPLICATION

## New Year's resolution

One resolution that's always broken is the conversion of Holliday junctions into linear duplex products. These four-stranded DNA crossover structures (see illustration), which are formed during DNA recombination and repair, are 'resolved' by the simultaneous introduction of breaks into two opposite strands. Several structure-specific endonucleases — 'resolvases' — are known to do this job in prokaryotes, but similar activities have been surprisingly difficult to pin down in eukaryotic cells.

Enter MUS81. Two new papers show that this endonuclease is an essential component of a resolvase activity in fission yeast and human cells. What's more, MUS81 seems to have evolved specifically to cope with the problems caused by stalled replication forks.

Fission yeast *mus81* was first described as a protein that associates with the replication checkpoint kinase *cds1*. It is related to the XPF subunit of the XPF-ERCC1 complex — a structure-specific endonuclease involved in nucleotide excision repair. Because XPF acts as part of a complex, Paul Russell and colleagues wondered whether the same might be true for *mus81*, and they report in *Cell* that it is.

Using two different approaches, Russell and co-workers identified *eme1* (for 'essential meiotic endonuclease 1') as a new binding partner for *mus81*. Genetic analy-

ses revealed that *mus81* and *eme1* act in the same pathway of resistance to UV damage, and that they are also required during meiosis. These studies indicated that the meiotic defect might be due to failure of the chromosomes to segregate properly, which could, in turn, be due to unresolved recombination intermediates — such as Holliday junctions.

To test this possibility, the authors expressed a bacterial Holliday junction resolvase called RusA in *mus81* mutants. This was enough to correct the meiotic defect in most cases, and it was dependent on the endonuclease activity of RusA. Consistent with this, the predicted endonuclease active site of *mus81* was found to be essential for activity.

Finally, Russell and colleagues used synthetic Holliday junctions (made by annealing oligonucleotides) to show that an affinity-purified *mus81-eme1* complex acts as a resolvase *in vitro*. Like the previously characterized bacterial resolvases, *mus81-eme1* introduces paired incisions on opposing strands of the X-structure to form linear duplex products. However, unlike them, it cuts only 5' to a double-strand/single-strand junction — bacterial resolvases have no such requirement.

Russell's group next teamed up with Clare McGowan and co-workers to clone the human homologue of *mus81*. They report in *Molecular Cell*

that, like the yeast protein, human MUS81 interacts with CDS1, a checkpoint kinase. Levels of MUS81 are increased in cells that are exposed to DNA-damaging agents ( $\gamma$ -irradiation or UV) and to hydroxyurea, which interrupts DNA replication. Given that some UV-induced lesions are also thought to block progression of replication forks, the observed increase in MUS81 could, say the authors, indicate "a role in cellular responses to blocked DNA replication".

The authors next asked whether, like its yeast counterpart, MUS81 has a structure-specific endonuclease activity. They incubated synthetic junctions with MUS81 immunoprecipitates from HeLa cells, and observed the formation of defined cleavage products. The pattern of cleavage was consistent with the hypothesis that MUS81 cleaves on opposite strands, close to the double-strand/single-strand junction. But as MUS81 elutes from a gel-filtration column at a higher molecular weight than the monomeric protein, the authors believe that it probably functions as a heterodimer. Identifying its partner — possibly a human *eme1* homologue? — will be one of the next steps.

Alison Mitchell

### References and links

**ORIGINAL RESEARCH PAPERS** Boddy, M. N. *et al.* Mus81-Eme1 are essential components of a Holliday junction resolvase. *Cell* **107**, 537–548 (2001) | Chen, X.-B. *et al.* Human Mus81-associated endonuclease cleaves Holliday junctions *in vitro*. *Mol. Cell* **8**, 1117–1127 (2001)

**FURTHER READING** Lilley, D. M. & White, M. The junction-resolving enzymes. *Nature Rev. Mol. Cell Biol.* **2**, 433–443 (2001)

**WEB SITE**  
Russell laboratory:  
<http://www.scripps.edu/mb/russell/>

## HIGHLIGHTS

### IN THE NEWS

#### To clone or not to clone?

"Storm over human embryo" was the headline that greeted Advanced Cell Technology's (ACT) announcement that they had cloned the first human embryo (*The Sun*, 28 November 2001).

The study showed that human embryos could be developed by somatic cell transfer. The somatic nuclei showed evidence of reprogramming, returned to the pronuclear state and divided into six cells, like a normal fertilized human egg.

Michael West, chief executive of ACT, stressed that the cloned embryo would be used for therapeutic reasons only, saying, "we took extreme measures to ensure that a human clone could not result from this technology" (*The Guardian*, 26 November 2001).

The response to this study was profound. John Gearhart, editorial advisor to the journal that published the study, resigned. The experiment, "was in his judgement a failure and should not have been published" (BBC News, 3 December 2001). The claim was vigorously defended by ACT.

But there were further ramifications from the research. The UK government rushed through legislation to close a legal loophole allowing people like Severino Antinori to carry out his attempts to help childless couples clone themselves. The European Commission said it opposed the research and would not finance any similar projects (BBC News, 26 November 2001).

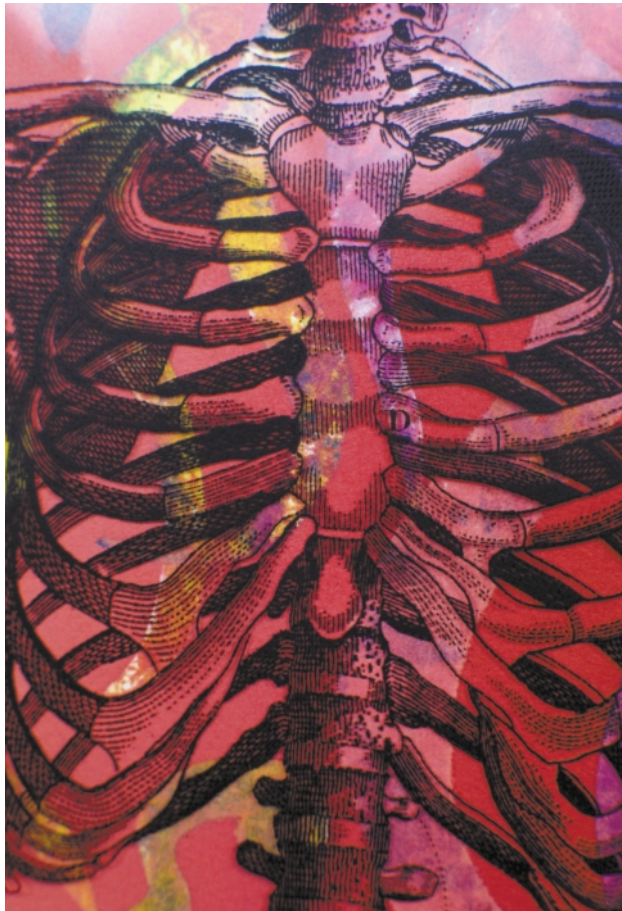
However, the US Senate refused to ban human cloning research, as opponents stressed that the continuation of studies could lead to new disease treatments.

*The Sun* agreed, saying the benefits would outstrip the fears if we stay sensible. "Laws must be passed to stop full-scale cloning of a human being. Science is not evil. Neither are medical scientists. Indeed, many are unsung heroes".

Simon Frantz

## CELL SIGNALLING

## From Wingless to boneless



Human bone mass is the result of a fine balancing act between bone deposition — carried out by bone-forming cells called osteoblasts — and degradation by the osteoclasts. In later life, as bone catabolism supersedes anabolism, the result is often a loss of bone mass (osteoporosis). To prevent this condition, we need to know more about the processes that underlie bone formation, and a report in *Cell* by Matthew Warman and co-workers now takes us a step closer, revealing an intriguing link to the Wingless (Wnt) signalling pathway.

Warman, along with Yaoqin Gong and an international group of clinicians and scientists, started by searching for the gene behind osteoporosis-pseudoglioma syndrome (OPPG) — an autosomal-recessive disorder in which patients have very low bone mass. They studied 28 affected families, and identified six different homozygous frameshift or nonsense mutations in the low-density lipoprotein receptor-related protein-5 (*LRP5*) gene.

Next the authors looked at expression of mouse *Lrp5* messenger RNA during embryogenesis by *in situ* hybridization on developing skeletal elements. They detected expression in osteoblasts; interestingly, in pluripotent cell lines induced into the osteoblastic lineage by exogenous

growth factors, the authors also found increased expression of LRP5. And this could, they speculate, indicate a role for LRP5 in terminal osteoblastic differentiation.

To delve deeper into the function of LRP5, Warman and co-workers used what is already known about its relatives. The mouse, *Xenopus laevis* and *Drosophila melanogaster* paralogues of LRP5 are involved in the Wnt signalling cascade, so the authors examined the effects of expressing various Wnt proteins *in vitro*.

To do this they used alkaline phosphatase (ALP) as a marker of osteoblast differentiation in two pluripotent mesenchymal cell lines. Whereas WNT3a — which participates in the canonical Wnt signalling pathway — could induce ALP activity in both lines, WNT5a and WNT4 (which use other signalling pathways) could not. WNT3a-induced ALP activity was also reduced when dominant-negative forms of LRP5 that lack the cytoplasmic tail were expressed in these cell lines. Moreover, induction of ALP by WNT3a could be blocked by coexpression with a dominant-negative form of Dishevelled, which acts downstream of WNT3a in the canonical pathway.

Finally, Warman and colleagues studied the effect of culturing bone explants in conditioned media from cells expressing the secreted form of

## CELL SIGNALLING

## Closing the gap

Cell signalling and membrane transport have traditionally been separate fields of cell biology. But they seem to converge more and more, as illustrated by a report in *Science* that describes a new GTPase-activating protein (GAP) for  $G\alpha_s$  subunits of heterotrimeric G proteins that seems to also function as a sorting nexin in controlling the lysosomal degradation of epidermal growth factor receptors (EGFRs).

$G\alpha_s$  subunits come in many flavours, and each of them seems to be regulated by a specific GAP of the regulator of G-protein signalling (RGS) family. Farquhar and colleagues set out to find the RGS protein that regulates  $G\alpha_s$  — a polyvalent  $G\alpha$  subunit that controls various cellular responses, including cell growth, hormone secretion, and learning

and memory. They searched databases for proteins with an RGS domain, and found a sequence that contained such a domain as well as a Phox domain, so they called the protein RGS-PX1.

They showed that RGS-PX1 interacts specifically and directly with  $G\alpha_s$ , and that it acts as a GAP for  $G\alpha_s$  *in vitro*. They also confirmed that the RGS domain of RGS-PX1 attenuates  $G\alpha_s$  signalling in HEK293 cells and neonatal rat cardiac membranes.

So, the RGS domain of RGS-PX1 seemed fully functional. What about the Phox domain? Farquhar and colleagues wondered whether RGS-PX1 could function as a sorting nexin — a family of proteins characterized by a Phox domain and involved in membrane transport. They overexpressed RGS-PX1 in HEK293 cells and studied its effect on EGFR sorting. Whereas in control cells, ligand-bound EGFR was rapidly sorted to lysosomes and degraded, in RGS-PX1-expressing cells, its degradation was markedly delayed, which

resulted in sustained activation of downstream targets of EGFR.

Farquhar and colleagues also determined that, like other Phox domains, the PX1 domain could bind to specific phosphoinositides, including phosphatidylinositol 3-phosphate, which is abundant in endosomes. So they tested the localization of RGS-PX1 and found that it is indeed associated with early endosomes, as it colocalized with the early endosomal marker EEA1 in COS-7 cells (see picture).

So, RGS-PX1 is both a GAP for  $G\alpha_s$  and a sorting nexin involved in sorting of EGFR. This new link between membrane transport and cell signalling might help us to understand how membrane transport compartmentalizes signal transduction and, conversely, how signal transduction regulates membrane transport.

Raluca Gagescu

## References and links

ORIGINAL RESEARCH PAPER Zheng, B. *et al.* RGS-PX1, a GAP for  $G\alpha_s$  and sorting nexin in vesicular trafficking. *Science* **294**, 1939–1942 (2001)

LRP5, which could act as a decoy receptor. In three independent experiments they showed that these explants had lower bone mass than did explants cultured in media from cells expressing the wild-type, non-secreted form of LRP5. The authors additionally found that carriers of OPPG mutations also have reduced bone mass compared with non-carrier controls, suggesting that the activity of LRP5 is dosage sensitive.

These impressive results not only implicate LRP5 in the acquisition of bone mass, but they provide a clue as to how it does this. Given that LRP5 is expressed in several different tissues, one surprise is that the phenotypic effects of the mutation seem to appear only in the skeleton and the eye. This could mean that the functions of LRP5 are redundant or that it binds to other ligands too — questions that will need to be answered if pharmacological modulation of LRP5 is to be used in the fight against osteoporosis.

Alison Mitchell

#### References and links

**ORIGINAL RESEARCH PAPER** Gong, Y. *et al.* LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* **107**, 513–523 (2001)

**FURTHER READING** Tamai, K. *et al.* LDL-receptor-related proteins in Wnt signal transduction. *Nature* **407**, 530–535 (2000)

#### WEB SITES

NIH osteoporosis and related bone diseases:  
<http://www.osteoporosis.org/>  
Warman laboratory:  
<http://genetics.gene.cwru.edu/bone/>

#### SIGNAL TRANSDUCTION

## Chop and change

CD44 is an important cell-surface adhesion molecule. It is expressed in most human cell types and has been implicated in many physiological and pathological processes, such as cell migration and the regulation of tumour cell growth and metastasis. To mediate these processes, CD44 must be able to transduce numerous intracellular signals but how this occurs has remained unclear. Now, in *The Journal of Cell Biology*, Okamoto and co-workers report the identification of a novel CD44 signalling pathway.

It was previously shown that the extracellular ectodomain of CD44 can be proteolytically cleaved by membrane-associated metalloproteinases (MMPs) to produce soluble CD44 and membrane-bound CD44 ectodomain cleavage products. As this cleavage regulates the cell-migration function of CD44, Okamoto and colleagues investigated how proteolysis could affect other CD44 functions.

By inducing calcium influx or by using 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treatment, the authors induced the activation of MMPs in human glioma cells. Using immunoblot analysis, they observed the expected CD44 ectodomain cleavage products, but, at a later timepoint, they also observed a smaller fragment of CD44, which corresponded to the intracellular domain (ICD). They showed that, after MMPs act to generate ectodomain cleavage products, further cleavage by intracellular proteases produces CD44ICD.

So what does CD44ICD do? In transiently transfected cells, Okamoto and co-workers showed that tagged CD44ICD is localized to the nucleus. This nuclear localization was also demonstrated for endogenous CD44ICD. Using a luciferase reporter, the authors showed that CD44ICD can enhance transcription that is mediated through the TPA-responsive element (TRE), and that CD44ICD translocation to the nucleus is essential for this enhancement. MMP inhibitors

block CD44-dependent transcription enhancement and the enhancement is not observed when CD44 is mutated to remove the intracellular proteolytic cleavage site. The authors therefore concluded that sequential proteolytic cleavage of CD44 and release of CD44ICD is essential for CD44-dependent transcription enhancement.

Using GAL4 transactivation assays, the authors showed that CD44ICD alone is unlikely to act as a transcription factor. So, they tested the hypothesis that it affects other transcription factors — c-Fos and c-Jun — or transcriptional coactivators — CREB-binding protein (CBP) and p300 — involved in TRE-mediated transcription. CD44ICD did not affect GAL4-c-Fos- or GAL4-c-Jun-induced transcription from a GAL4-dependent promoter, but it did enhance transcription by GAL4-CBP and GAL4-p300. Whether the CD44ICD transactivation mediated through CBP/p300 occurs by a direct or indirect interaction remains to be determined.

To identify the endogenous gene targets of CD44ICD, the authors compared HeLa cells transfected with either a control plasmid or one encoding CD44ICD. The *CD44* gene contains TRE sequences in its promoter region, and Okamoto and co-workers found that CD44ICD induces *CD44* expression. They propose that this CD44ICD-induced *CD44* transcription promotes the rapid turnover of CD44 that is required for cell migration.

Signalling pathways are usually thought to involve interactions between cell-surface proteins and cytoplasmic proteins, which, in turn, regulate gene transcription. Here, however, Okamoto and colleagues have shown that CD44 bypasses a step in this pathway, as CD44ICD itself can activate gene transcription. In addition to identifying a novel CD44 signalling pathway, this paper has highlighted an important functional link between proteolytic processing of cell-surface adhesion molecules and transcriptional activation in the nucleus.

Rachel Smallridge

#### References and links

**ORIGINAL RESEARCH PAPER** Okamoto, I. *et al.* Proteolytic release of CD44 intracellular domain and its role in the CD44 signaling pathway. *J. Cell Biol.* **155**, 755–762 (2001)

**FURTHER READING** Pure, E. & Cuff, C. A. A crucial role for CD44 in inflammation. *Trends Mol. Med.* **7**, 213–221 (2001) | Bajorath, J. Molecular organization, structural features, and ligand binding characteristics of CD44, a highly variable cell surface glycoprotein with multiple functions. *Proteins* **39**, 103–111 (2000) | Lesley, J. & Hyman, R. CD44 structure and function. *Front. Biosci.* **3**, D616–D630 (1998)

#### WEB SITE

CD44: [http://www.ncbi.nlm.nih.gov/prov/guide/1621804819\\_g.htm](http://www.ncbi.nlm.nih.gov/prov/guide/1621804819_g.htm)

