In the news

HITTING JUNK

Repeating experiments is crucial for data evaluation. However, one does not expect having to re-evaluate all the genome-wide RNA interference (RNAi) screens that were carried out in *Drosophila melanogaster* cell cultures.

The groups of Philip Beachy (Johns Hopkins University School of Medicine, USA) and Norbert Perrimon (Harvard Medical School, USA) did exactly that. Unexpectedly, they found that off-target effects (OTEs) can occur in RNAi experiments using long double-stranded RNA (dsRNA) sequences.

OTEs have been recognized as a problem for RNAi screens that are carried out using short RNAs. However, genome-wide *D. melanogaster* libraries contain dsRNAs that consist of hundreds of bases, which were not thought to produce OTEs. "Initially, no one in the field … had seen an off-target effect, but as more screens were done, we realized these things could happen", said Perrimon (*The Scientist*, 11 Sep 2006).

Suspicion arose when Yong Ma, a postdoctoral researcher in the Beachy laboratory, attempted to validate seven promising candidates for novel components of the Wingless pathway that were identified using a library of more than 21,000 dsRNAs in *D. melanogaster* cells. Ma found that all candidates contained short regions of homology with *armadillo*, a well known gene of this pathway. Looking back at another published RNAi screen on the Wingless pathway, Ma realized that ~60% of the negative regulators that were identified shared a repeated trinucleotide sequence, CAN, that is found in only 5% of the library.

Perrimon found evidence of OTEs in a retrospective analysis of 30 RNAi screens. The false positive rate is variable. Whether false or suspect, according to Perrimon "… these need to be repeated with new dsRNAs, which we are doing now" (*The Scientist*, 11 Sep 2006).

Ekat Kritikou

CHROMATIN

The yin and yang…

The high-mobility group A (HMGA) proteins are non-histone chromatin proteins that are most well known as transcriptional activators and as proliferogenic and tumorigenic agents. Scott Lowe and colleagues now identify a surprising new role for HMGA proteins as promoters of cellular senescence.

Senescence is a growth-arrest programme that prevents uncontrolled cellular proliferation and that is thought to counteract tumour formation. Senescent cells have a typical appearance in which heterochromatin accumulates in nuclear bodies that are known as senescence-associated heterochromatic foci (SAHF). These foci are thought to represent repressive chromatin environments that prevent the activation of proliferogenic genes.

Lowe and colleagues analysed the chromatin composition of senescent fibroblasts and found that HMGA1 and HMGA2 associate with the chromatin fraction of these cells. Unexpectedly, HMGA proteins are enriched at SAHF, and ectopic

MEMBRANE TRAFFICKING

Kiss and patch up

The cell lining of the gastrointestinal tract is continually damaged by mechanical stresses and scratching by partially digested food as it traverses the gut. Potentially, this could have fatal consequences for the cells, but the digestive tract has evolved two defence mechanisms: the formation of membrane patches that plaster over holes in the membrane, and the secretion of a lubricating mucus that cushions the membrane against further abrasions. Miyake *et al.* have now shown that during cell injury, both of these processes are rapidly activated to protect the gastrointestinal tract.

Miyake *et al.* monitored mucus secretion using fluorescently labelled lectins that bind to the glycoproteins found in mucus granules. Wounding the mucosal cells, by repetitively drawing them up through a syringe, induced the release of lectins into the surrounding medium. The amount of lectin secretion increased with the amount of cell injury but was inhibited in the absence of calcium, which is known to be an important regulator of membrane-fusion events.

> "…membrane resealing occurs as a consequence of mucus secretion."

The authors developed a second method for assessing plasma membrane resealing after wounding. Cells were incubated with a lipophilic fluorescent dye that labels the plasma membrane. When cell membranes were punctured by a laser insult, the cytoplasm was quickly labelled by the dye in the absence of calcium. In the presence of calcium, no intracellular labelling was observed, which indicated that the plasma membrane was rapidly resealed following injury.

By combining these two techniques, Miyake *et al.* conclusively showed that membrane resealing occurs as

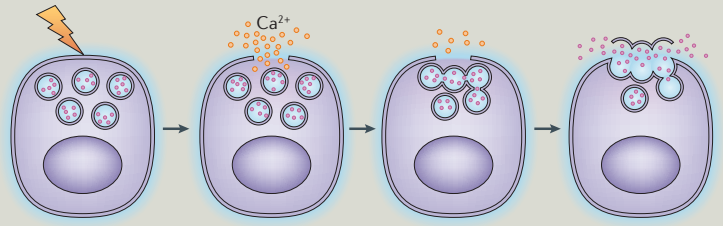
expression of HMGA1 or HMGA2 in cycling fibroblasts promoted cell-cycle arrest and the accumulation of SAHF-like foci. Further studies showed that these proteins cooperate with the tumour suppressor p16^{INK4a} to promote SAHF formation and proliferative arrest. On the other hand, disruption of HMGA function by competitive DNA-binding assays or by the knockdown of *HMGA1* or *HMGA2* resulted in the disappearance of SAHF, confirming that these proteins are integral structural components of these heterochromatic domains.

In normal cells, HMGA proteins are renowned gene activators that bind to DNA and create a transcriptionally permissive 'open' chromatin environment. So, it is reasonable to assume that they might promote senescence by activating senescence-associated genes. In fact, microarray experiments indicated that, in senescent fibroblasts, HMGA proteins function as transcriptional repressors, downregulating proliferation-associated genes such as the E2F target genes *CDC2* and *cyclin A*. Taken together with their predilection for SAHF, these data indicate that, during senescence, HMGA proteins limit transcription by chromatin-mediated repression.

These findings indicate that the function of HMGA proteins is modulated, and the authors point out that this modulation might resemble that of histone proteins. HMGA proteins, similar to histones, are subject to post-translational modifications such as acetylation, phosphorylation and methylation, and opposing HMGA functions might be determined by the specific patterns of these modifications. The revelation that HMGA1 and HMGA2 have a putative role in tumour suppression as well as in oncogenesis highlights the importance of considering cellular context when analysing the roles of proteins, and provides important insights into the complex machinery that orchestrates senescence.

Shannon Amoils

ORIGINAL RESEARCH PAPER Narita, M. *et al.* A novel role for high-mobility group A proteins in cellular senescence and heterochromatin formation. *Cell* **126**, 503–514 (2006)



Coupling of mucus secretion and membrane repair. Adapted from figure 7 in the highlighted paper.

a consequence of mucus secretion. After injury, cells that lacked cytosolic staining by the lipophilic dye were abundantly labelled on their membranes by fluorescent lectins, indicating that mucus secretion had occurred. Furthermore, cells that survived plasma-membrane disruption were depleted of intracellular mucus, which was instead secreted. Therefore, it seems that an increase in calcium promotes the secretion of mucus-laden vesicles. It is the 'spent' vesicle membranes themselves that subsequently remain on the plasma membrane and patch over the hole.

Importantly, when these experiments were repeated in segments of rat colon, the same conclusions were reached. This work has raised some key questions. For example, how is calcium involved in mucus secretion and membrane resealing? And, could these 'healing' processes be defective in pathological conditions of the gastrointestinal tract, as has been demonstrated to be the case for skeletal muscle?

James Pickett

ORIGINAL RESEARCH PAPER Miyake, K. *et al.* Disruption-induced mucus secretion: repair and protection. *PLoS Biol.* **4**, e276 (2006)
FURTHER READING Bansal, D. *et al.* Defective membrane repair in dysferlin-deficient muscular dystrophy. *Nature* **423**, 168–172 (2003)

IN BRIEF

CELL CYCLE

Inhibition of centrosome protein assembly leads to p53-dependent exit from the cell cycle.

Srsen, V. *et al. J. Cell Biol.* **174**, 625–630 (2006)

The authors followed cell-cycle progression after inhibiting centrosome assembly by knocking down the expression of two centrosome-associated proteins, pericentriolar material-1 (PCM1) and pericentrin. Cells failed to enter S phase, yet cells that lacked p53 did not arrest. In addition, inhibiting the p38 mitogen-activated protein kinase rescued cell-cycle progression in the absence of functional centrosomes. Together, this indicates that defective centrosome assembly activates a p53-dependent checkpoint, which requires the p38 stress pathway.

NUCLEAR TRANSPORT

Karyopherin-mediated import of integral inner nuclear membrane proteins.

King. M. C. *et al. Nature* **442**, 1003–1007 (2006)

How inner nuclear membrane (INM) proteins are targeted to the INM is poorly understood. But, King *et al.* now provide evidence that the mechanism might be similar to that of soluble proteins. INM proteins have sequences that resemble 'classic' nuclear localization signals. Like nuclear import of soluble proteins, INM-protein import requires Ran GTPase and nuclear transport factors called karyopherins. Specific components of the nuclear pore complex (NPC), termed nucleoporins, also contribute to this process, which implies that the NPC might be adapted to allow the passage of INM proteins.

CELL POLARITY

CYK-4/GAP provides a localized cue to initiate anteroposterior polarity upon fertilization.

Jenkins, N. *et al. Science* **313**, 1298–1301 (2006)

Sequential functioning of the ECT-2 RhoGEF, RHO-1 and CDC-42 establishes cell polarity in *Caenorhabditis elegans* embryos.

Motegi, F. & Sugimoto, A. *Nature Cell Biol.* **8**, 978–985 (2006)

Two reports provide insight into how sperm entry initiates cell polarity in the *Caenorhabditis elegans* one-cell embryo. Jenkins and colleagues showed that the Rho GTPase-activating protein CYK-4 is enriched in sperm, and that paternally donated CYK-4 is essential for polarity along the anterior–posterior axis. The small GTPase RhoA (also known as RHO-1) and the guanine nucleotide-exchange factor ECT-2 are also needed for polarity. They promote myosin light-chain (MLC) activation, which is required for actomyosin contractility. By contrast, CYK-4 inhibits MLC activation and thereby actomyosin contractility. So, sperm entry in the posterior cortex downregulates the actomyosin network locally, and the differential activation of MLC creates a contractile actomyosin gradient. Motegi and Sugimoto showed that ECT-2 somehow gets excluded from the posterior cortex where the sperm enters. This causes the asymmetric distribution of RhoA/RHO-1, which generates an actomyosin gradient to the anterior cortex. This mechanism might work together with the CYK-4 signal to reduce RhoA/RHO-1 activity and establish a contractility gradient that initiates polarity.