

## 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.

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

“...membrane resealing occurs as a consequence of mucus secretion.”

