IN THE NEWS

Identical twins express their differences

Identical twins are not as similar as they might appear. In fact, according to a recent report in *Proceedings of the National Academy of Sciences*, the genes expressed by twin siblings can differ markedly. And, as monozygotic twins share a common genotype, these differences are due to epigenetic variation — specifically, differing patterns of histone and DNA modifications.

The study conducted by a multinational team of scientists quantified the global DNA methylation content and histone acetylation levels in samples from 40 pairs of monozygotic twins ranging in age from 3 to 74 years.

Remarkably, 35% of the twin pairs showed significant differences in these epigenetic marks, and the older the twins and the more disparate their lifestyles or medical histories, the greater the differences.

Importantly, a 50-year-old twin pair with the greatest difference in DNA methylation and histone acetylation levels varied markedly in their gene expression profiles, whereas the gene expression profiles of a 3-year-old twin pair were almost identical. This provides some of the strongest evidence so far for the impact of the environment on gene expression.

As lead author Mario Fraga points out,"Most people had the hypothesis that changes in DNA methylation are effected by the environment … This is the first time that somebody has demonstrated that this is the case." (*news@nature.com*, 4 July 2005).

Arturas Petronis, research scientist at the University of Toronto, Canada, comments that the study has proved its worth by "…quantifying how genetically identical individuals could differ in gene expression on a global level due to epigenetics." (*The Scientist*, 7 July 2005).

It is hoped that future studies will reveal the mechanisms responsible for these epigenetic differences.

Shannon Amoils

GENE EXPRESSION

What lies at the core

Small interfering RNAs (siRNAs) can target the RNA interference (RNAi) machinery to homologous chromosomal regions where it induces chromatin modifications and transcriptional silencing. Two groups now report that, in fission yeast, RNA polymerase II (pol II) is required for RNAi-mediated chromatin modifications and gene silencing.

In fission yeast, transcription at the centromeric regions generates siRNAs that are loaded into the RNAi targeting machinery. To determine whether RNAi-mediated chromatin modifications require homologous transcripts, Robin Allshire and colleagues constructed a yeast strain (Ter$^+$) that contained a modified *ura4* gene. This construct contained a transcription terminator immediately upstream of a gene region that is homologous to a synthetic hairpin RNA (which is processed into siRNAs by the RNAi machinery). A second strain (Ter-M5) contained a mutation in this terminator, which allowed read-through, resulting in 75% full-length transcripts.

In Ter$^+$ cells, truncated transcripts of the *ura4* gene were detected, both in the presence and absence of hairpin RNA. In Ter-M5 cells, however, the presence of hairpin RNA resulted in the loss of full-length *ura4* transcripts. In addition, the authors detected dimethylation on K9 of histone 3 (H3K9me2) on the Ter-M5 *ura4* gene, but only in the presence of hairpin RNA. So, transcription is required for the gene repression and chromatin modification that is triggered by the presence of siRNAs.

Why is transcription essential for RNAi-mediated chromatin modification and gene silencing? Could it be that an RNA polymerase is required to allow access of siRNAs to the template DNA/homologous region? Possibly — however, Allshire and co-workers found that transcription mediated by bacteriophage T7 polymerase did not allow RNAi-mediated chromatin modification and gene silencing, which implies

CANCER

A bad influence

Signals that encourage the delinquent behaviour of tumour cells can originate from the surrounding environs as well as from cancer cells themselves. Stromal matrix metalloproteinases (MMPs), including MMP3/stromelysin-1, are upregulated in many breast cancers and can induce transformation in cultured mammary epithelial cells and in transgenic mice. Here, Radisky *et al.* reveal the molecular details by which this occurs, and implicate Rac1b as the main culprit in this process.

MMP3 brings about epithelial–mesenchymal transition (EMT), which increases cellular motility and invasiveness, a key trait of tumour cells. The process requires changes in the actin cytoskeleton and, although the activities of RhoA and Cdc42 were unaffected by MMP3, the authors found that a previously identified splice variant of Rac1 — Rac1b — was upregulated and, indeed, was necessary for MMP-induced motility.

Could Rac1b mediate the pleiotropic effects that are caused by MMP3 and, if so, how? Previous studies have shown that active Rac can stimulate the production and release of mitochondrial superoxide into the cytoplasm, which can damage cells, potentiate tumour progression and be further converted into other destructive reactive oxygen species (ROS). Treating cells with MMP3 or expressing Rac1b increased cellular ROS levels, and this increase was inhibited by expressing dominant-negative Rac1. Transfecting cells with superoxide dismutase-2 (SOD2), a mitochondrial enzyme that reduces superoxide levels, inhibited MMP3-induced cell scattering, which indicates that MMP3- or Rac1b-induced mitochondrial superoxide production is required for EMT. Similarly, *N*-acetyl cysteine (NAC), a ROS quencher, inhibited the MMP3-induced downregulation of an epithelial marker and the upregulation of a mesenchymal marker and, consequently, blocked motility and invasion. Radisky *et al.* saw that MMP3-induced ROS also caused cellular DNA damage and genomic instability, which, again, could be induced independently by the ROS hydrogen peroxide and inhibited by NAC.

The expression of many genes, including the transcription factor Snail, changes during EMT. Snail expression was induced by MMP3, but was also stimulated independently by increasing ROS levels or by expressing Rac1b. Conversely, NAC blocked Snail induction. Snail, when expressed