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

that a specific RNA polymerase is required.

Next, the authors constructed a strain with a truncated C-terminal domain (CTD) in the largest subunit of RNA pol II (Rpb1). This strain, which did not have a substantial general defect in transcription, was unable to silence the expression of two centromeric marker genes and showed decreased levels of H3K9me2 associated with centromeric repeats. In addition, the Allshire team showed that pol II immunoprecipitated with the RNAi component Argonaute 1 (Ago1), and that this association required an siRNA-loaded RNAi complex. Also, Ago1 associated with centromeric chromatin, and this depended on pol II transcription.

In a complementary report, Yota Murakami and colleagues also showed the requirement for pol II in RNAi-mediated heterochromatin formation. They identified a fission yeast mutation, which they mapped to the second largest subunit of pol II (Rpb2). The mutant showed an accumulation of centromeric transcripts, and a loss of heterochromatic histone modifications and siRNAs. This indicates that pol II might be required for the production of siRNAs. However, the Allshire group did not observe an effect on siRNA generation, and instead proposes that the role of pol II transcription lies downstream — in mediating the RNAi-dependent chromatin modifications.

These findings provide yet another example of the core role of pol II in coordinating transcription with other RNA-processing events. Future studies will no doubt try to establish the precise links between pol II transcription in RNAi-dependent chromatin modification and transcriptional silencing.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPERS Kato, H. *et al.* RNA polymerase II is required for RNAi-dependent heterochromatin assembly. *Science* 9 June 2005 (doi:10.1126/science.1114955) | Schramke, V. *et al.* RNA-interference-directed chromatin modification coupled to RNA polymerase II transcription. *Nature* 19 June 2005 (doi:10.1038/nature03652)

in mouse mammary epithelial cells, was sufficient to induce EMT and cell scattering. So MMP3 induces Rac1b expression, which, in turn, increases cellular ROS levels, upregulating Snail and causing EMT. So, by inducing Rac1b, MMP3 can lead normal cells astray.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Radisky, D. C. *et al.* Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature* **436**, 123–127 (2005)

STRUCTURE WATCH

Complex contacts

The covalent attachment of SUMO (small ubiquitin-related modifier)-family proteins to target proteins is known be involved in various cellular processes. In DNA mismatch repair, uracil/thymine DNA glycosylase (TDG) releases thymine or uracil from G•T and G•U mismatches and remains stably bound to the resulting abasic site until it is transferred to the next enzyme in the repair pathway. SUMO conjugation to TDG promotes its release from the abasic site, and the 2.1-Å-resolution crystal structure of the central region of human TDG conjugated to SUMO-1 shows how this occurs.

The structure, described by Shirakawa and colleagues in *Nature*, is comprised of two domains — the catalytic core domain of TDG and a SUMO-containing domain that consists of SUMO-1 and the C-terminal region of TDG. No significant structural rearrangements are induced in the TDG core domain or in SUMO-1 by SUMO conjugation. The interesting feature is the complex interface, which contains a covalent interaction (the conjugation site) and non-covalent interactions (an intermolecular β-sheet pairing). The formation of these interactions causes an α-helix of TDG, which is located between them, to protrude from the surface of the complex, such that it would sterically clash with bound DNA. Both the covalent and non-covalent contacts seem to be essential for TDG dissociation from DNA. This work has provided insights into how SUMO modifications function, and has indicated how SUMO proteins might interact with other proteins in a non-covalent manner.

REFERENCE Baba, D. *et al.* Crystal structure of thymine DNA glycosylase conjugated to SUMO-1. *Nature* **435**, 979–982 (2005)

The workings of a family

RNase H is a sequence-nonspecific endonuclease that cleaves RNA strands in RNA–DNA hybrids. It is a member of a superfamily of nucleotidyl transferases that includes transposase and Holliday junction resolvase, and the enzymes of this family have important roles in many processes. However, little is known regarding their mechanism of substrate recognition and metal-ion-dependent catalysis. Despite continuous efforts to gain structural insights, there hasn't been a structure of RNase H bound to its RNA–DNA substrate — until now. In *Cell*, Yang and co-workers describe structures of RNase H from *Bacillus halodurans* with and without substrate.

In the complex, the RNA–DNA hybrid has a mixed A and B conformation. RNase H specifically recognizes the A form of RNA and the B form of DNA, and probably selects and stabilizes this mixed conformation to enable it to distinguish the hybrid from double-stranded RNA. The enzyme specifically targets the RNA strand for cleavage through direct contacts with its 2′-OH groups. There are two Mg^{2+} ions in the active site of the complex, and comparing the available structures of members of the family indicates a conserved two-metal-ion-dependent catalytic mechanism. In nucleases, the ions are asymmetrically coordinated and have separate roles in nucleophile activation and transition-state stabilization. In transposases, these ions are symmetrically coordinated and swap roles to activate a water molecule and a 3′-OH group alternately for successive DNA cleavage and transfer using a ping-pong mechanism.

REFERENCE Nowotny, M. *et al.* Crystal structures of RNase H bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis. *Cell* **121**, 1005–1016 (2005)