IN THE NEWS

Double trouble

Scientists are constantly striving to understand the molecular events that predate the onset of cancer.

Now, a study in *Science* has shown that an increase in the number of immature cells in the lining of the bowel predisposes to the development of colon cancer. This novel phenotype was seen in mice that had been genetically manipulated to express twice the normal amount of the imprinted gene insulin-like growth factor *(Igf)2*.

In humans, loss of imprinting of *IGF2* is associated with several tumour types, including colorectal cancer. Surprisingly, in the mutant mice, doubling the amount of this growth factor did not affect the turnover of intestinal cells but instead seemed to block their maturation, which increased the numbers of immature cells in the gut lining. Importantly, mice that had both defective *Igf2* imprinting and an additional tumour-promoting mutation developed twice as many bowel tumours as mice with the tumour-promoting mutation alone. This indicates that the immature bowel cells are a 'hot-spot' for neoplastic transformation.

This study shows how a combination of epigenetic and genetic misfortune can have catastrophic results. As co-author Christine Iacobuzio-Donahue explains: "In the mice with a double dose of IGF2, everything is pretty normal except for the extra precursor cells … But when the genetic mutation is present, too, we found a clear cost for what otherwise appears to be a benign effect of extra IGF2." (*myDNA.com*, 25 February 2005).

Andrew Feinberg, main author, is excited by the prospect that "…this discovery should expand attention in colon cancer to earlier events, situations present well before tumors appear." (*bio.com*, 24 February 2005). The hope is that this will lead to effective cancer prevention strategies.

Shannon Amoils

EPIGENETICS

RB1 goes global

The retinoblastoma 1 (RB1) protein is a well-studied tumour suppressor protein. It regulates the transcription of cell cycle genes through physical interaction with the E2F family of transcription factors and by recruiting chromatin-modifying enzymes to specific promoters. A new study by Blasco and colleagues in the April issue of *Nature Cell Biology* now shows that RB1 also maintains constitutive pericentric and telomeric heterochromatin — the inactive form of chromatin — with the potential of non-selectively repressing gene expression. This adds a new and more global tool to the repertoire of tumour-suppressor functions of RB1.

To address the role of the RB1 family — which comprises RB1, retinoblastoma-like 1 (RBL1) and RBL2 — in genome stability and chromosome structure, the authors used mouse embryonic fibroblasts (MEFs) from single-, double- (DKO) and triple-knockout (TKO) animals. After several passages, aneuploid cells were already evident in DKO and TKO MEFs, and after 20 passages, TKO cells established tetraploidy. These chromosome segregation defects were confirmed by fluorescence *in situ* hybridization (FISH) analysis, which showed chromosomes with four sister chromatids ('butterfly chromosomes').

Investigating changes in the chromatin structure of TKO cells in more detail, Blasco and colleagues found reduced DNA methylation, increased acetylation of histone 3 (H3), and decreased tri-methylation of histone 4 at lysine 20 (H4K20). As hypermethylation of DNA and histones, as well as the hypoacetylation of histones, are hallmarks of constitutive heterochromatin, the authors investigated the effect of RB1 proteins on pericentric and telomeric heterochromatin. Indeed, they found decreased DNA methylation and trimethylation of H4K20 at pericentric heterochromatin in TKO cells. Using

a dominant-negative mutant of E2F, the authors excluded a possible involvement of transcriptional changes caused by the absence of an RB1–E2F interaction.

As tri-methylation of H4K20 at pericentric chromatin is known to be mediated by the histone methyltransferases (HMTases) Suv4-20h1 and Suv4-20h2, the authors focused on potential interactions of these enzymes with RB1. First, they showed

SIGNALLING

Reassessing Ras routes

It's easy to group all isoforms of a certain protein under the same umbrella. But a closer look at the Ras GTPases H-ras, N-ras and K-ras reminds us that they generate isoform-specific biological outputs, and that this is influenced by their differential localization at the plasma membrane (PM) and subcellular membranes. This, in turn, reflects their C-terminal lipid modifications: after farnesylation, AAX proteolysis and methylation of the farnesylated cysteine, H-ras and N-ras are palmitoylated, which enables them to localize to the PM and Golgi membranes; by contrast, K-ras bypasses the secretory pathway and localizes to the PM.

Both the PM and the Golgi are sites of active Ras signalling, but how this compartmentalized localization and activity of palmitoylated Ras isoforms is accomplished has remained unclear. Work by Rocks *et al.*, reported in *Science*, now shows that a constitutive de- and re-palmitoylation cycle maintains the specific compartmentalization.

Golgi-localized Ras was thought to comprise nascent proteins trafficking to the PM. But by inhibiting protein synthesis, and thereby removing nascent proteins, the authors showed that H-ras and N-ras still localized at the Golgi. Moreover, photobleaching and photoactivation studies showed that palmitoylated Ras cycled between the PM and the Golgi, and that Golgi Ras was replenished by retrograde transport of PM-localized Ras. Using a hexadecylated version of N-ras — HDFar — that could not undergo de- and re-palmitoylation, the authors showed that this localization wasn't mediated by clathrin, caveolae or cholesterol, but that de- and re-palmitoylation events were required. Microinjected HDFar localized nonspecifically throughout the membrane system.

The authors also noticed that the kinetics of H-ras and N-ras trafficking were different. H-ras is palmitoylated on two cysteines, whereas palmitate moieties are added to only one cysteine in N-ras. N-ras trafficked faster than H-ras, so Rock *et al.* studied monopalmitoylated H-ras mutants. C181S and C184S H-ras had an increased preference for Golgi localization relative to wild-type H-ras, and showed faster PM–Golgi exchange. By developing a new assay to compare the dynamics of Ras activation at the PM and Golgi, Rock *et al.* showed H-ras to be rapidly and transiently activated at the PM in response to growth factor stimulation, and to have a delayed onset but to be sustained at the Golgi. By contrast, active H-ras C184S and N-ras were detectable

that a lack of RB1 didn't alter the expression levels of Suv4-20h. By overexpressing fluorescently tagged HMTases they confirmed that these enzymes were properly recruited to constitutive heterochromatin in TKO cells. These overexpressed HMTases could rescue the decreased H4K20 tri-methylation, so the authors concluded that this phenotype was the result of decreased Suv4-20h activity, indicating that RB1 proteins stabilize H4K20 tri-methylation by these HMTases. Finally, the authors showed direct binding of RB1, RBL1 and RBL2 to Suv4-20h1 and Suv4-20h2 *in vitro*, but not *in vivo*.

Altogether, this new study impressively shows that RB1 proteins are not only transcriptional repressors of specific target genes, but also have a role in the assembly of constitutive heterochromatin. Loss of RB1 therefore not only results in de-repression of specific promoters, but also in the global loss of a repressive chromatin state. Given this, RB1 seems to have made a quantum jump from a specific to a global tumour suppressor.

Markus Wagner,
Nature Cell Biology

References and links

ORIGINAL RESEARCH PAPER Gonzalo, S. *et al.* Role of the Rb family in stabilizing histone methylation at constitutive heterochromatin. *Nature Cell Biol.* 6 Mar 2005 (doi:10.1038/ncb1235)

FURTHER READING Liu, H. *et al.* New roles for the RB tumor suppressor protein. *Curr. Opin. Genet. Dev.* **14**, 55–64 (2004)

WEB SITE

María Blasco's laboratory: http://www.cnio.es/ing/programas/prog102.asp

much sooner at the Golgi. Total inhibition of palmitoylation blocked trafficking from the PM to the Golgi and therefore blocked Ras activation.

So, rapid exchange of palmitoylated Ras isoforms at the PM and Golgi is driven by de- and re-palmitoylation cycles. De-palmitoylation confers an equal distribution between the cytosol and membranes. Re-palmitoylation, which enables stable membrane anchorage, occurs at the Golgi; from here, Ras is redirected to the PM in the exocytic pathway. As the authors' findings of such a cycle extended to other proteins that have been reported to localize to the PM and the Golgi, they propose that this process has a universal role in subcellular distribution.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Rocks, O. *et al.* An acylation cycle regulates localization and activity of palmitoylated Ras isoforms. *Science* 11 Feb 2005 (doi:10.1126/science.1105654)

FURTHER READING Hancock, J. F. Ras proteins: different signals from different locations. *Nature Rev. Mol. Cell Biol.* **4**, 373–385 (2003)

IN BRIEF

DNA REPAIR

Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage.

Falck, J. *et al. Nature* 2 Mar 2005 (doi:10.1038/nature03442)

The phosphoinositide-3-kinase-related protein kinase (PIKK)-family members ATM, ATR and DNA-PKcs are activated in response to DNA damage and recruited to sites of DNA damage by individual partner proteins. Falck *et al.* have now identified a conserved interaction motif in the C terminus of each partner protein — Nbs1, ATRIP and Ku80, respectively — that is essential for the recruitment of these PIKKs as well as for the downstream signalling events.

CHROMOSOME SEGREGATION

Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2.

Hauf, S. *et al. PLoS Biol.* **3**, e69 (2005)

Shugoshin prevents dissociation of cohesin from centromeres during mitosis in vertebrate cells.

McGuinness, B. E. *et al. PLoS Biol.* **3**, e86 (2005)

During mitosis, sister chromatids are held together by cohesin, which is cleaved in anaphase, triggering chromatid segregation. However, most cohesin is removed from the chromosome arms, but not from the centromere, before anaphase. One study now shows that phosphorylation of the cohesin subunit SA2 is required for cohesin removal from chromosome arms in early mitosis. So what safeguards centromeric cohesin? Shugoshin, according to a second study, as this protein seems to protect centromeric SA2 from phosphorylation to prevent premature chromatid segregation.

PLANT BIOLOGY

RNA polymerase IV directs silencing of endogenous DNA.

Herr, A. J. *et al. Science* 3 Feb 2005 (doi:10.1126/science.1106910)

Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation.

Onodera, Y. *et al. Cell* 10 Feb 2005 (doi:10.1016/S0092867405001510)

Plants encode catalytic subunits of a fourth RNA polymerase, Pol IV, the function of which has remained unknown, until now. Data from two studies now indicate that Pol IV causes small interfering (si)RNA-mediated gene silencing and facultative heterochromatin formation. These events are associated with RNA-directed DNA methylation, which requires siRNAs for targeting specific *de novo* DNA-methylation events. The production of siRNAs might be mediated by a mechanism that involves Pol IV, although its precise role is yet to be determined.