NEWS & VIEWS

CANCER GENOMICS

Small RNAs with big impacts

Paul S. Meltzer

Although they are tiny, microRNAs can have large-scale effects because they regulate a variety of genes. These minuscule molecules are now definitively linked to the development of cancer.

During the past few years, molecular biologists have been stunned by the discovery of hundreds of genes that encode small RNA molecules[1]. These microRNAs (miRNAs) — 21 to 25 nucleotides in length — are negative regulators of gene expression. The mechanisms by which they work are similar in plants and animals, implying that they are involved in fundamental cellular processes[2]. As cancer is essentially a consequence of disordered genome function, one might expect these regulatory molecules to be involved in the development of this disease. Indeed, there are hints that the levels of some miRNAs are altered in cancer[3,4]; there is also evidence that an miRNA regulates the cancer-promoting *ras* gene[5]. Three studies in this issue[6–8] change the landscape of cancer genetics by establishing the specific miRNAs expressed in most common cancers, and investigating the effects of miRNAs on cancer development and cancer genes.

The initial product of an miRNA gene (Fig. 1) goes through several processing steps before it is exported from the nucleus to the cytoplasm. One strand of the resulting double-stranded RNA is then incorporated into the 'RNA-induced silencing complex' (RISC). RISC can target protein-coding messenger RNAs (mRNAs) either for inhibition, by blocking their translation into protein, or destruction (as in RNA interference). Base pairing between the miRNA and its complementary target mRNA gives the process its specificity.

The choice between translational inhibition and destruction is thought to be governed by the degree of mismatch between the miRNA and its target mRNA, with degradation being the outcome for best-matched targets. Because miRNAs can inhibit the translation of imperfectly matched targets, it is possible that each miRNA may target multiple genes, and that several miRNAs may regulate a given target. The interplay between mRNA and miRNA is vital for the normal regulation of gene expression, but its role in disease is just beginning to be investigated.

The expression of miRNAs appears to be highly regulated according to the cell's developmental lineage and stage. Lu *et al.* (page 834)[6] find that aspects of this specificity are maintained in cancer: by measuring the expression of 217 human miRNAs in cancer samples, they found that the pattern of miRNA expression varies dramatically across tumour types. Remarkably, the expression pattern of this small set of miRNAs defines the cancer type better than expression data from 16,000 mRNAs. As might be expected from the role of some miRNAs in development, the miRNA profiles of tumours are in accord with the tumours' developmental history; tumours derived from tissues with a common embryonic precursor (such as gastric, colon and liver cancers, which are all derived from the embryonic endoderm) share similar miRNA expression patterns. Leukaemias are clearly separate from solid tumours and, strikingly, are subgrouped according to their underlying genetic abnormalities.

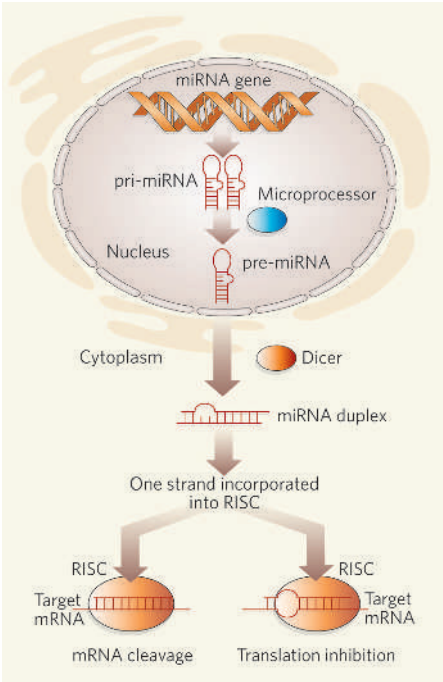

Figure 1 | MicroRNA production. The precursor of an miRNA (pri-miRNA) is transcribed in the nucleus. It forms a stem–loop structure that is processed to form another precursor (pre-miRNA) before being exported to the cytoplasm. Further processing by the Dicer protein creates the mature miRNA, one strand of which is incorporated into the RNA-induced silencing complex (RISC). Base pairing between the miRNA and its target directs RISC to either destroy the mRNA or impede its translation into protein. The initial stem–loop configuration of the primary transcript provides structural clues that have been used to guide searches of genomic sequence for candidate miRNA genes.

These observations could improve the diagnosis of poorly defined cancers with unknown origins, allowing better-informed choices for treatment. They also promise to shed light on the regulatory circuits that malfunction during tumorigenesis. The expression of miRNAs seems to be lower in cancers than in normal tissues — consistent with the possibility that reduced miRNA expression leads to a cancer-specific block that halts the normal development of cells. This may allow the cells to continue to divide and grow, unlike their mature counterparts. Small RNAs can easily be measured from the formalin-fixed tissue specimens used routinely in hospital pathology laboratories; so potential miRNA-based diagnostics could fit simply into the standard hospital workflow.

He *et al.* (page 828)[7] examine an old puzzle: the accumulation of extra copies of a chromosome 13 fragment (13q31–32) in certain human lymphomas (cancers derived from immune cells). The region of amplification contains a gene called c13orf25 (ref. 9), which turns out to encode the precursors of seven miRNAs. He *et al.* establish that the c13orf25 miRNAs are indeed overexpressed in lymphoma cells that have extra copies of c13orf25.

To investigate the biological consequences of this miRNA overexpression, they employ a genetically engineered mouse model in which lymphoma development is driven by a cancer-promoting gene (oncogene) called *myc*. The authors infected blood-forming cells from these mice with a retrovirus carrying a portion of the miRNA cluster and then transplanted the cells back into recipient lymphoma-prone mice. Compared with control mice injected with a virus lacking the miRNAs, there was a

striking decrease in the latency of leukaemia development (from 3–6 months to 51 days) and an increase in the frequency of the cancer in the mice (from about 30% to 100% of animals). The mechanism for these effects remains unclear, and it is uncertain which components of the c13orf25 miRNA cluster are responsible. Nonetheless, this study emphatically nominates this miRNA cluster as the first candidate non-coding oncogene.

The connection between miRNAs and the *myc* oncogene is also examined by O'Donnell *et al.* (page 839)[8]. This gene encodes the transcription regulator c-Myc, the overexpression of which is frequent in cancer. To explore the effects of c-Myc on miRNAs, O'Donnell *et al.* use a lymphoma cell line carrying a *myc* gene that can be switched on by treating the cells with an 'inducer'. They find that increasing the expression of c-Myc leads to increased expression of six miRNAs. Remarkably, two of these are encoded by the c13orf25 cluster and the remainder are encoded by two related clusters on chromosomes 7 and X. O'Donnell *et al.* confirm that c-Myc binds to a candidate regulatory site in c13orf25.

A predicted target of two of the encoded miRNAs is the transcription factor E2F1, which is itself a critical regulator of the cell cycle. O'Donnell *et al.* demonstrate that this prediction is correct, and that expression of E2F1 is only affected if the miRNA target site is present in the E2F1 mRNA. E2F1 and c-Myc are known to induce each other's expression. In the absence of other controls, this could set up a positive-feedback loop leading to overexpression of both genes — with disastrous consequences for normal cell-cycle regulation. O'Donnell *et al.* propose that by negatively regulating E2F1, miRNAs induced by c-Myc could dampen the runaway effect, fine-tuning the dynamics of E2F1 action during the cell cycle.

Questions remain, of course. What regulates the expression of miRNAs? What are the targets of each miRNA? Do miRNAs act mainly to 'fine-tune' gene expression or more often as binary on/off switches? Given that each miRNA may regulate numerous targets, it is possible that thousands of protein-coding genes could be regulated by a few hundred miRNAs. Candidate miRNA target genes can be identified by bioinformatics approaches, but a great deal of experimental work remains to be done in validation.

We need to find out which of the biological pathways underlying cancer are regulated by miRNAs, but the complexity of the problem is underlined by the dual roles for the same miRNAs identified in two of this week's reports[7,8]. In one context, the c13orf25 cluster can act as an oncogene; in another it seems to antagonize the effects of different oncogenes, acting like a classic tumour-suppressor gene. Sorting out the miRNA regulatory networks will be challenging, but is vital to explain the nuanced regulation of gene expression essential to the growth, development and survival of multicellular organisms. ■

Paul S. Meltzer is at the Cancer Genetics Branch, National Human Genome Research Institute, 50 South Drive, Bethesda, Maryland 20892-8000, USA.
e-mail: pmeltzer@nhgri.nih.gov

1. Lagos-Quintana, M., Rauhut, R., Lendeckel, W. & Tuschl, T. *Science* **294,** 853–858 (2001).
2. Bartel, D. P. *Cell* **116,** 281–297 (2004).
3. Calin, G. A. *et al. Proc. Natl Acad. Sci. USA* **101,** 11755–11760 (2004).
4. Eis, P. S. *et al. Proc. Natl Acad. Sci. USA* **102,** 3627–3632 (2005).
5. Johnson, S. M. *et al. Cell* **120,** 635–647 (2005).
6. Lu, J. *et al. Nature* **435,** 834–838 (2005).
7. He, L. *et al. Nature* **435,** 828–833 (2005).
8. O'Donnell, K. A., Wentzel, E. A., Zeller, K. I., Dang, C. V. & Mendell, J. T. *Nature* **435,** 839–843 (2005).
9. Ota, A. *et al. Cancer Res.* **64,** 3087–3095 (2004).

ORGANIC CHEMISTRY

Fast reactions 'on water'

Jaap E. Klijn and Jan B. F. N. Engberts

Efficient reactions in aqueous organic chemistry do not require soluble reactants, as had been thought. A newly developed 'on-water' protocol is characterized by short reaction times, and the products are easy to isolate.

Water is unique. The chemistry of living organisms depends on its combination of unusual properties, and it is difficult to imagine life in the absence of the aqueous medium[1]. For synthetic organic chemistry, however, it is less important, because water is traditionally not a popular choice of solvent.

There are two main reasons for this. Functional groups in the organic molecule may themselves react with water and, more importantly, most organic molecules are nonpolar, and so hydrophobic and generally highly insoluble in water. It is therefore assumed that a mixture of water and one or more nonpolar organic reactants will usually have low reaction rates and low yields of the desired products. As a group led by Barry Sharpless reports in *Angewandte Chemie*[2], however, this assumption does not hold for a variety of organic reactions in the 'on water' approach that they have pioneered.

There was already compelling evidence that many organic reactions are faster in water than in organic solvents, but that evidence was based on reaction kinetics in very dilute, homogeneous solutions (in which the reactants are in the same phase). Breslow and Rideout[3] were the first to show that the otherwise solvent-insensitive Diels–Alder reactions — which are among the most useful reactions in organic chemistry, often used for the synthesis of six-membered rings — may be greatly accelerated in water. Similar results were obtained for other types of simple (uni- and bimolecular) organic reactions. Subsequent mechanistic studies have established that this behaviour results from enforced hydrophobic interactions and stabilization of the activated complex by hydrogen-bond formation. Although synthetic applications evolved from these studies[4], there were invariably limitations that stemmed from lack of solubility. And scant attention was paid to the kinetics of organic transformations under heterogeneous aqueous conditions, when the reactants are in different phases.

Sharpless's group[2] now shows that several uni- and bimolecular reactions are greatly accelerated when carried out in vigorously stirred aqueous suspensions (Fig. 1). The reactions include the important classes known as cycloadditions, ene reactions,

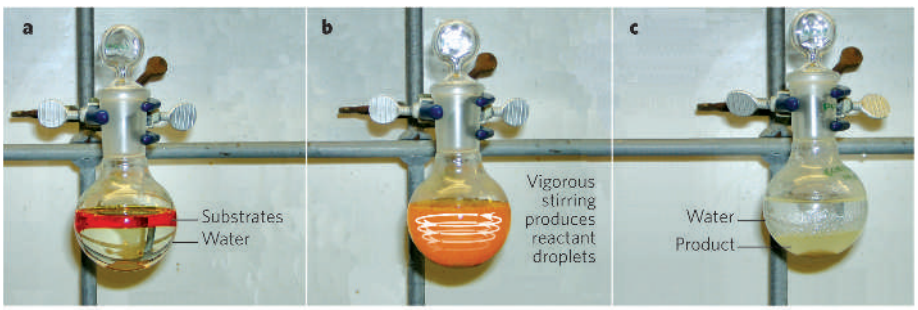

Figure 1 | The 'on water' protocol, as developed by Sharpless and colleagues[2]. **a**, The organic substrates are initially floating on top of the water. **b**, The mixture is stirred vigorously, which disperses the reactants as small droplets and leads to a large increase in the surface area between the reactants and the aqueous phase. **c**, The reaction product either precipitates as a solid or floats on top of the water as an oil, either of which can be isolated easily. The mechanism of the rate acceleration has yet to be clarified, but it probably depends on the increase in interfacial area.