Silencing is golden

RNA interference in research and medicine

A natural defence against viruses, RNA interference has gone from scientific curiosity to invaluable research tool in just five years – and could soon be making its debut in the clinic.

While petunias are attractive flowers, and petunia horticulture is big business, their relevance to medical research has, until recently, been minimal. But a strange phenomenon first seen in petunias is turning out to be one of biology's most exciting techniques of recent years.

The petunia story dates back to the late 1980s when a US team led by Rich Jorgensen was manipulating the plant genetically in order to produce more deeply coloured flowers. Their idea was to add extra copies of the gene for chalcone synthase, one of the key enzymes in the pathway of pigment synthesis. Instead of the deep purple flowers they expected, however, the researchers found that many of the flowers were white or variegated. Further investigation showed that the introduced gene and the resident chalcone synthase gene were transcribed normally, but somehow all the chalcone synthase messenger RNA (mRNA) was being destroyed.

Completely independently, researchers using the nematode worm *Caenorhabditis elegans* had long been exploiting a similar phenomenon. Particular genes could be conveniently inactivated in worms by the injection of antisense RNA – artificial RNA molecules whose sequence was complementary to the normal mRNA found in the cell. It was assumed that the antisense RNA and mRNA formed a double-stranded molecule like the DNA double helix, and this prevented the mRNA from being translated into protein.

One curious observation, however, did not fit this simple picture: when artificial sense RNA was injected, it also inactivated genes. Since sense RNA has exactly the same sequence as mRNA, it should not form a double-stranded molecule. So how was it exerting its effects?

In 1998, Andrew Fire and Craig Mello provided an explanation for this puzzling observation when they injected worms with sense RNA, antisense RNA and (most importantly) a mixture of both. They found that the mixture had a much more potent silencing effect than either of the single strands alone. They termed this phenomenon RNA interference (RNAi). As even tiny amounts of double-stranded RNA injected into the gut could completely shut down the corresponding gene, they surmised that silencing mediated by sense RNA could be caused by small amounts of contaminating double-stranded RNA.

Further experiments showed that RNAi could be induced not only by the injection of double-stranded RNA, but also by feeding worms on bacteria making double-stranded RNA or soaking them in a double-stranded RNA solution. The silencing lasted a long time, affecting not only the treated worms but also their first generation of offspring.

The discovery of RNAi in the worm soon led others to try the technique in different organisms. It was then realized that the variegated petunias might be evidence of the same process. Later, it was found that this type of gene silencing could be induced not only by artificially introduced genes but also by viruses, if the virus carried a copy of a plant gene. Importantly, most plant viruses have RNA genomes and use a double-stranded RNA intermediate in their replication cycle. Could it be that double-stranded RNA was the trigger for gene silencing in plants as well as for RNAi in the worm?

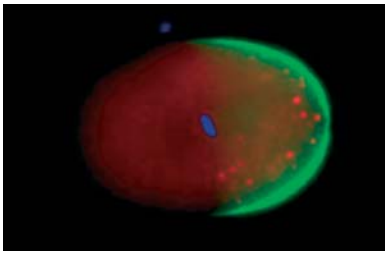
Double trouble

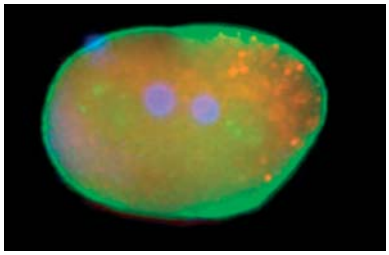
The answer appears to be yes, but raised another perplexing question: how does the presence of double-stranded RNA trigger the complete elimination of the corresponding mRNA, even at minuscule doses?

Gradually, through the combined efforts of researchers working on plants, worms and fruit flies, the picture became clearer. Several groups independently discovered small double-stranded RNA molecules in all cells affected by silencing, and further common factors were identified as the genes required for RNAi were sequenced and found to encode similar proteins. The following two-stage model of RNAi has emerged from such studies (Figure 1).

The *initiation* of silencing requires double-stranded RNA. Sometimes this can be produced when inserted genes are transcribed or when viruses replicate, or it can be introduced artificially. When double-stranded RNA enters the cell, it is recognized and cut up into pieces by an enzyme called DICER, which moves along the double-stranded RNA and releases fragments, 21–23 bp long with short overhangs (short interfering RNAs, siRNAs). In some poorly understood way, these siRNAs are thought to be able to copy themselves and magnify the silencing effect.

The *activation* of silencing occurs when the siRNA assembles with certain proteins to form what is known as an





Left: RNAi identified a new gene involved in cell polarity. In a normal embryo, *PAR-2* (stained green) is found only at the posterior; in the mutant, it is distributed throughout the embryo.
J Ahringer

RNA-induced silencing complex (RISC) – essentially, a sequence-specific mRNA-eating machine. The siRNA is unwound during this assembly process and one of the strands is discarded. The remaining strand is then able to recognize complementary mRNA molecules in the cell. Once a matching mRNA is found, the RISC cuts it into pieces and releases it, before moving on to find further mRNAs of the same sequence.

Applications of RNAi

The specificity, efficiency and potency of RNAi make it an attractive tool for analysing the function of genes. Thanks to large-scale sequencing projects, scientists now have access to thousands of genes but have little idea what most of them do. The most straightforward way to establish a gene's function is to mutate it, making it inactive, and then see what happens to the mutant cell or organism. RNAi can do the same kind of thing, but is quicker and easier, allowing functional analysis on a much larger scale. Box 1 describes recent, Wellcome Trust-funded studies of gene function in *C. elegans*, which involved the RNAi analysis of nearly 17 000 genes.

Another advantage of RNAi is that it can be used in situations where traditional mutation analysis cannot be used, such as human cells. It was initially thought that RNAi did not work in mammals because most mammalian cells show a non-specific response to the presence of double-stranded RNA – more or less all of protein synthesis shuts down, making it difficult to identify the gene-specific effects of RNAi. It has become clear, however, that the non-specific response only occurs in the presence of double-stranded RNA molecules over 30 bp long, so researchers got round the problem by making their own siRNAs and introducing these directly into the cell to achieve the specific RNAi effect. Functional analysis has now been carried out in over 30 human cell lines. For example, RNAi silencing of the *TSG101* gene showed that its product is essential for budding of HIV-1 viral particles from infected cells.

Moreover, as well as research, RNAi may also be valuable therapeutically. For example, the ability of siRNAs to discriminate between very closely related sequences could be used to silence disease-specific versions of genes while leaving any normal copies unharmed. Many human disorders and cancers are caused by mutations and rearrangements that could be targeted by RNAi. Chronic myeloid leukaemia is caused by a rearrangement of genetic material that fuses together genes found on chromosomes 9 and 22. The fusion gene, which causes cells to proliferate, can be silenced effectively by RNAi in cell culture. Similar studies have been carried out successfully against a mutant hormone receptor and the mutant gene that causes spinobulbar muscular atrophy. While RNAi drugs are a long way off, this is a new and exciting area for medical research to explore. *RMT*

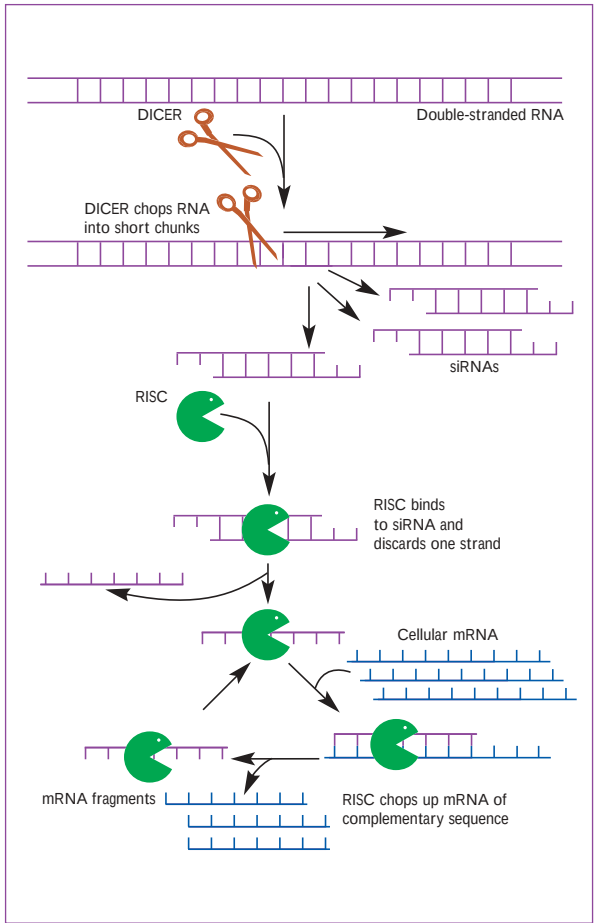


Figure 1: Triggered by double-stranded RNA, two enzyme complexes, DICER and RISC, combine to degrade messenger RNA.

RNAi in the worm

Wellcome Senior Research Fellow Julie Ahringer of the Wellcome Trust/Cancer Research UK Institute and Department of Genetics, Cambridge, has developed a method of carrying out RNAi screens of almost the entire *C. elegans* genome.

Dr Ahringer's team began by creating 17 000 strains of bacteria each of which makes a particular double-stranded RNA molecule corresponding to a worm gene. Together these bacteria represented 86 per cent of the genes in the *C. elegans* genome. Systematic RNAi analysis was carried out by feeding groups of worms with each bacterial strain. Over 1500 of the targeted genes showed reproducible RNAi effects, which were divided into three broad classes: nonviable (RNAi causes the worms to die), slow growth, and viable with defects in growth and development. About a third of these genes were already well characterized, but over 1000 had no biological function assigned to them before the project began.

Interestingly, 30 of the genes identified in the screen were close relatives of known human disease genes, so it is possible that this example of genome-wide analysis will provide new disease models and useful insights into human gene function. The bacterial strains are themselves a valuable resource that can be re-used, distributed and augmented for future global RNAi studies in the worm.

More recently Dr Ahringer and colleagues at Harvard University used RNAi to search systematically for genes affecting fat metabolism. They identified 305 gene inactivations that reduced body fat and 112 that increased fat storage – in one experiment identifying more than 400 genes that presumably play some role in fat metabolism. Again it is highly likely that many of these genes will have similar roles in humans.